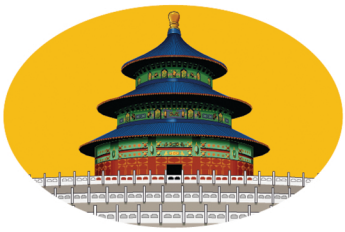
ISSN 0014-2980 · EJIMAF 49 (Supplement S3) · Vol.49 · Supplement S3 · October 2019

European Journal of Immunology

Supplement S3



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European Journal of Immunology

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Beijing, China

European Journal of Immunology Volume 49, Suppl. 3, October, 2019

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Eur. J. Immunol. 2019. 49 (Suppl. 3): 1–2223 *Abstracts*
DOI: 10.1002/eji.201970400

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DOI: 10.1002/eji.201970400

Antigen Processing and Presentation

O385

Antigens can be cross-presented by human blood dendritic cells from live and not only dead cells

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Antigens (Ag) from apoptotic HIV-1-infected cells are cross-presented by human conventional and plasmacytoid dendritic cells (cDC, pDC) to $CD8^+$ T cells. We have previously shown that Ag from live cells are also cross-presented by human monocyte-derived DC and by murine bone-marrow-derived DC, leading to protection of mice from tumoral challenge. We tested whether human blood DC would also cross-present antigens from live cells.
As Ag donor cells, we used HLA-$A2^-$, HIV-infected H9 cells, kept alive by caspase inhibitor Z-VAD. As effector $CD8^+$ T cells, we derived anti-gag_{77-85} clones from HLA-$A2^+$ HIV-infected patients. Direct viral presentation was prevented by saquinavir. DC were immunomagnetically and FACS-sorted to >95% purity, from HLA-$A2^+$ or $^-$ donor buffy coats. $CD8^+$ T cell IFN-γ intracellular production was assessed by flow cytometry.
CDC2 stimulated by LPS cross-presented HIV-Gag from live infected H9 cells. PDC cross-presented Gag from live infected H9 cells even without microbial stimulation and induced higher IFN-γ production than cDC. As DC are known to become cytotoxic in the presence of HIV, we tested whether they in fact cross-presented from live cells only after killing them. Indeed, in the same culture conditions, pDC induced up to 25% Ag donor cell death through caspase- and non-caspase related mechanisms, but cDC2 did not.
This crosspresentation from live cells has been underestimated in the past, but it will be further studied as a tool to eradicate HIV reservoirs with low HIV expression levels, or residual and metastatic live tumor cells.
Support: ANRS, ANR-10-LABX-62-IBEID, INSERM, CNRS, Université de Paris

O388

Autophagy protein ATG5 regulates CD36 expression and anti-tumor MHC class II antigen presentation in dendritic cells

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Macroautophagy/autophagy has been implicated in cytoplasmic and viral antigen presentation on major histocompatibility complex (MHC) class II molecules. However, the role of autophagy in the presentation of phagocytized tumor-associated antigens *in vivo* remains unclear. Following the administration of apoptotic tumor cells and *in vivo* chemotherapy, mice with a dendritic cell-specific deletion of *Atg5*, a key autophagy gene, exhibit reduced $CD4^+$ T-cell priming but not $CD8^+$ cytotoxic T-cell priming. Interestingly, *Atg5*-deficient dendritic cells have an elevated expression of scavenger receptor CD36 and show excessive lipid accumulation. *Atg5*-deficient dendritic cells increased CD36-dependent phagocytosis of apoptotic tumor cells. CD36 blockade ameliorates elevated phagocytosis and increases $CD4^+$ T-cell priming in dendritic cells; intratumoral CD36 blockade inhibits tumor growth. Our results demonstrate that *Atg5* is required for proper antigen phagocytosis and presentation to MHC class II via modulation of CD36 in dendritic cells and may be a future therapeutic target for anti-tumor therapy.

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DOI: 10.1002/eji.201970400

O389

Association of the variant Val379Ile of the transporter associated with antigen presentation 2 (TAP2) gene with high risk of cervical lesions

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HPV causes cervical lesion and the viral clearance depends on elicit cellular immune response. The presence of polymorphisms in the transporter associated with antigen presentation 2 (TAP2) gene may contribute to the impairment in antigen presentation to immune system.
Objective: to compare the frequencies of TAP2 alleles (rs1800454:G→A, Val→Ile) and protein levels in women infected or not with HPV, having or not cervical lesion and aneuploidy.
Methods: 194 women from a cohort set in Recife, Brazil, were studied (Ethical clearance CAAE 51111115900005190). DNA from cervical samples were used to HPV detection and typing, and to TAP2 polymorphism study by PCR and sequencing. Aneuploidy was measured by flow-cytometry. Biopsies of 23 lesions and 25 adjacent clean area were stained with TAP2-antibodies for protein quantification. Comparison between groups was performed using Fisher´s exact test, and Mann-Whitney test was used when appropriated.
Results: Genotypes and alleles of the Val379Ile genetic variant wasn't associated with HPV infection nor cervical intraepithelial neoplasia. However, the GG genotype (p=0.0499) was overrepresented in patients with low-grade squamous intraepithelial lesion (L-SIL) when compared with high grade (H-SIL). Group of women carrying GA+AA genotypes presented higher risk for aneuploidy, which causes genetic instability (P=0.0329; OR=3.75). High TAP2 levels was observed in tissue adjacent to lesion in women harboring the AA in comparison to who harbor the GG genotype (p=0,002).
Conclusions: Carriers of the TAP2 Val379Ile variant are likely to express high levels of TAP2 protein in cervical lesion and to develop cellular aneuploidy, which is a proxy of cancer progression.

O390

New participants and pathways in immune receptor trafficking.

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Expression of immune receptors at the cell surface is a key determinant of immunity. Understanding mechanisms that govern receptor expression patterns enables exploitation of receptors in settings of immunotherapy or vaccination. One mechanism by which receptor expression is controlled is ubiquitination. Ubiquitination regulates the cell surface display of MHC II (major antigen presenting molecule) and CD86 (co-stimulatory molecule). We have identified ubiquitin codes (ubiquitin chain linkage types) associated with MHC II isolated from primary murine immune cells. Second, we have identified a new role for MHC II ubiquitination in the regulation of dendritic cell homeostasis that involves a surprising intersection between innate and adaptive immunity. Third, we have performed a genome-wide CRISPR/Cas9 screen to identify new molecular participants in ubiquitin-mediated immune trafficking. Finally, we have analysed trafficking of dendritic cell receptors of significant interest for antibody-targeted vaccination, including C-type lectin DEC-205. We have identified new

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DOI: 10.1002/eji.201970400

genes that regulate both the expression and the internalisation of DEC-205 from the cell surface. In summary, our analysis provides significant advances to understanding how immune receptors are trafficked inside cells with important consequences for immunity.

O391

ER-Golgi dynamics is altered in MHC Ib restricted T cells due to ERAAP-deficiency

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The peptide repertoire presented by classical MHC Ia molecules is critical for immune surveillance of intracellular pathogens and cancer. The presentation of this normal peptide repertoire is regulated by ERAAP, the ER aminopeptidase associated with antigen processing. Changes in ERAAP function cause profound changes in the presented peptides that could cause autoimmunity or alter the efficacy of cancer immunotherapy. We have found an unusual CD8+ T cell subset termed “QFL-T cells” that recognize ERAAP-deficient cells presenting the QFL ligand. The QFL ligand contains a highly conserved FL9 peptide presented by Qa-1^b, a non-classical MHC Ib molecule and is recognized by semi-conserved alpha beta TCRs on QFL-T cells. To determine the functional differences in the QFL specific CD8-T cells in wild-type versus ERAAP-deficient mice, we carried out RNA-seq transcriptome analysis of these cells (n=3 each). We identified 77 genes showing significant differential expression. Interestingly, genes involved in ER and Golgi dynamics were among the most differentially expressed. This suggests a potential feedback transcriptional regulation of these genes due to loss of ERAAP activity as well as an unexpected impact on antigen processing pathways.

LB001

The peptide presentation of MHC I molecules from domestic animals, i.e. chicken and dogs

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Background: MHC molecules are found in all jawed vertebrates and are known to present peptides to T lymphocytes. We determined the molecular bases of chicken MHC I BF2*1201 bound to influenza virus peptides and dog MHC I DLA-88*50801 bound to canine distemper virus peptides, respectively.
Methods: Peptide presentations by chicken and canine MHC class I were structurally determined through X-ray crystallography. The binding affinity was determined by thermostability measurements.
Results: We found that BF2*1201 binds many peptides that hang out of the groove at the C terminus. The classical class I molecules of mammals have a nearly invariant Tyr (Tyr84 in humans) that coordinates the peptide C terminus, but all classical class I molecules outside of mammals have an Arg in that position in common with mammalian class II molecules. This invariant Arg residue switches conformation to allow peptides to hang out of the groove of BF2*1201. by thermostability Meanwhile, the crystal structures of DLA-88*50801 reveal diversified anchoring modes of the binding peptides in the groove. Flexible and large pockets composed of both hydrophobic and hydrophilic residues can accommodate pathogen-derived peptides with diverse anchor residues.
Conclusions: These results further our understanding of chicken and canine T-cell immunity through peptide presentation of MHC class I and shed light on the molecular basis for vaccine development for infectious diseases in the important domestic animals.
Keywords: Canine, chicken, MHC I, peptide

DOI: 10.1002/eji.201970400

P0001

Oncoprotein SND1 promotes immune escape from immune surveillance through inhibiting antigen presentation of MHC class I molecules

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Understanding the MHC class I heavy chain(HC) maturation in tumor microenvironment is important since peptides loaded onto MHC class I molecules are the base for tumor antigen presentation generating immune responses. Here we show that a previously known oncoprotein, SND1, profoundly facilitates immune evasion from tumor immune microenvironment through inhibition of antigen presentation and that this effect is mediated by downregulation of MHC-I(HC) molecule. **Loss of function of SND1 in either melanoma or colon cancer from subcutaneously tumor-bearing immune competent wild type mouse results in growth inhibition and promotes tumor inflammation with more cytotoxic CD8+T, CD4+T, and dendritic cells.** Genetic ablation of SND1 in OVA-expressed tumors on OT-I mice induces sufficient antigen presentation to cytotoxic CD8+T cells and enhances anti-tumor immunity.
MHC-I(HC) is preferably hijacked by SN domain of SND1 from being normally assembled at an early stage of ER processing, which is independent of glycosylated modification, thereby leading MHC-I(HC) to the proteasome-pathway of ER-associated degradation(ERAD) promoted by the AAA ATPase p97/Valocin-containing protein (VCP) and sensitizing tumor to the diminished immune surveillance. Thus we reveals SND1 as a probable candidate exploiting ERAD components that determines the fate of MHC-I(HC) maturation and orchestrates a cancer-favored immune microenvironment. Our results propose the blockade of SND1-MHC-I(HC) axis in tumors as a viable option for immune system against cancer.
Keywords: MHC-I, heavy chain, SND1, cytotoxic CD8+T, ERAD

P0002

Binding of CLIP to the PBR of MHC class I molecules in grass carp

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The MHC class II-associated invariant chain (Ii) binds to MHC class II molecules and participates in peptide exogenous antigen presentation, during which, the CLIP (class II-associated Ii peptide) of Ii occupies the groove within PBR (peptide binding region) of the class II molecules. Our previous studies showed that grass carp Ii could bind to MHC class I molecules. In this paper, we focused on the functional region in MHC class I molecules bound with the CLIP. The results showed that the CLIP could also co-localize with MHC Iα, although it more widely distributed in the cells than Ii in eukaryotic expression under Confocal laser scanning microscope (CLSM), the both co-localization molecules also formed the complexes found in Co-immunoprecipitation and Western blot. Moreover, the CLIP could bind to the MHC $I\alpha_1\alpha_2$, which lacked cytoplasm, transmembrane and immunoglobulin-like domains and had only PBR in prokaryotic expression, observed by Pull-down tests and Western blot. However, this binding of the CLIP to MHC $I\alpha_1\alpha_2$ showed a competitive characteristics. The Ii, which had combined with MHC $I\alpha_1\alpha_2$, could not prevent CLIP also binding to the MHC $I\alpha_1\alpha_2$. Similarly, the MHC $II\beta_1$, the PBR of MHC class II molecule β chain, could not prevent MHC $I\alpha_1\alpha_2$ binding to CLIP in complexes CLIP-MHC $II\beta_1$. All these results suggest that the CLIP could stably bind to PBR of MHC class I molecules, such as its binding to MHC class II molecules.

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DOI: 10.1002/eji.201970400

P0003

UHRF1 promotes renal cell carcinoma progression through epigenetic regulation of TXNIP

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UHRF1 is an important epigenetic regulator that belongs to the UHRF family. Overexpression of UHRF1 has been found in many kinds of tumors, and its overexpression is associated with poor prognosis and short survival in certain cancer types. However, its function in renal cell carcinoma (RCC) is not clear. Here, we report that RCC tumor tissues had obviously higher UHRF1 expression than normal renal tissues. Downregulation of UHRF1 by siRNA or shRNA in RCC cell lines resulted in decreased cell viability, inhibited cell migration and invasion, and increased apoptosis. UHRF1 knockdown RCC xenografts also resulted in obviously inhibited tumor growth *in vivo*. After down-regulation of UHRF1 in RCC cells, the expression of TXNIP was up-regulated. Additionally, after UHRF1 and TXNIP were simultaneously down-regulated, cell viability and cell invasion increased, while cell apoptosis decreased compared with UHRF1 single down-regulated cells. We also showed that UHRF1 could recruit HDAC1 to the TXNIP promoter and mediate the deacetylation of histone H3K9, resulting in the inhibition of TXNIP expression. Our results confirm that UHRF1 has oncogenic function in RCC, and UHRF1 may promote tumor progression through epigenetic regulation of TXNIP. UHRF1 might be used as a therapeutic target for RCC treatment.

P0004

Failure in generation of a GILT dependent epitope is responsible for lack of anti-insulin Ab in GILT knock-out mice

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The γ-IFN-inducible lysosomal thiol reductase (GILT) is the only thiol reductase localized in lysosome with its optimal activity at low pH, and is expressed in all professional APCs. GILT is critical to processing protein Ag by catalyzing disulfide bond (S-S) reduction, thus facilitating unfolding of S-S in protein Ag for further processing. Demonstrated previously, GILT-/- splenocytes were defective in processing several S-S containing Ags. However, the role of GILT in Ab response to S-S containing Ag has not been examined. Here we report that after immunization, GILT-/- mice failed to generate Ab to an exogenous Ag, bovine insulin (bIns), which contains A and B chains with two interchain and an intrachain S-S. In contrast, Ab responses to several other model protein Ags were comparable in both GILT-/- and B6 mice. The deficiency of Ab response to bIns in GILT-/- mice was due to a lack of generating an epitope during processing bIns by GILT-/- APCs. The epitope contains two cystines that are involved in formation of both interchain and intrachain S-S, and is located within the first 15 aa in the A chain of bIns. Consequently, CD4 T cells from immunized GILT-/- mice specifically showed a reduced proliferative response to bIns. Immunized GILT-/- mice also had a diminished numbers of bIns specific germinal center B cells. Our results demonstrated that GILT function is indispensable in generation of Ab against bIns in vivo and elucidated a cellular mechanism underlying the selective deficiency of Ab response to bIns in GILT-/- mice.

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DOI: 10.1002/eji.201970400

P0587

The establishment of a fluorescently traceable immunogen expression system for antibody development and screening

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Background: OX40, a hot target in immunotherapy, is a type I transmembrane protein with very low expression level, thus the overexpression system for OX40 is indispensable for its antibody screening and for the establishment of cell immunization system which will benefit the development of antibodies with high clinical value.
Methods: The extracellular domain of OX40 (the region where the immunogen is located) and the 49aa transmembrane segment (PDGFR origin) were synthesized and constructed into pcDNA3.1 (+) vector followed by insertion of GFP into the construct by homologous recombination. The expression and subcellular localization of GFP OX40 chimeric protein were identified by the means of WB, ICC and FC.
Results: The sequencing data showed that the OX40 plasmid was constructed correctly. When 2.5 µg of plasmid and 8 µl of PEI were used, the transfection efficiency was the highest, reaching 90%. The target protein of 60 kDa was detected by WB. ICC assay showed that GFP OX40 chimeric protein was correctly localized on the cell membrane. And the FC data showed that the same population of cells were both GFP and anti-OX40 positive.
Conclusion: The fluorescently traceable expression system for OX40 was established successfully. Since the extracellular domain of OX40 can be easily replaced by the extracellular domains from any transmembrane protein, **the system can be widely used for developing and screening antibodies with high clinical value especially for the immunogens which are transmembrane proteins.**
Keywords: OX40; fluorescently traceable; GFP; transmembrane protein

P0588

PPT1 controls the balance between viral resistance and T cell crosspriming in dendritic cells

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Conventional type 1 dendritic cells (cDC1s) are inherently resistant to many viruses, but paradoxically possess less acidic phagosomes that enable antigen retention and cross-presentation. We report that palmitoyl-protein thioesterase 1 (PPT1), which catabolizes lipid-modified proteins in neurons, is highly expressed in cDC1s. PPT1-deficient DCs are more susceptible to vesicular stomatitis virus (VSV) infection, and mice with PPT1 deficiency in cDC1s show impaired response to VSV. Conversely, PPT1-deficient cDC1s enhance crosspriming of tissue-resident KLRG1^{+} effectors and memory T cells, and lead to rapid clearance of tumors and Listeria monocytogenes. Mechanistically, PPT1 protects steady-state DCs from viruses by promoting antigen degradation and endosomal acidification via V-ATPase recruitment. After DC activation, quick downregulation of PPT1 is likely to facilitate cross-presentation, production of costimulatory molecules and inflammatory cytokines. Thus, PPT1 acts as a molecular rheostat that allows cDC1s to crossprime efficiently without compromising viral resistance. These results suggest potential therapeutics to enhance cDC1-dependent crosspriming.

DOI: 10.1002/eji.201970400

P0589

NLRC5: a central player in the MHC class I pathway

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MHC class I and class II molecules play critical roles in the activation of the adaptive immune system by presenting antigens to CD8+ and CD4+ T cells, respectively. Although it was long known that an NLR (nucleotide-binding domain, leucine-rich-repeat containing) protein, CIITA (MHC class II transactivator) is a master regulator of MHC class II gene expression, the mechanism of MHC class I gene transactivation had been much less known. We identified another NLR protein, NLRC5 as an MHC class I transactivator (CITA). NLRC5 represents a critical regulator for the transcriptional activation of MHC class I genes and other genes involved in the MHC class I antigen presentation pathway. NLRC5 is a critical component of human cancer immunity through the recruitment and activation of tumor killing CD8+ T cells. We discuss the molecular function and mechanism of CITA/NLRC5 in the MHC class I pathway and its role in cancer.

P1183

The changes of B cell-activating factor and a proliferation- inducing ligand in serum of children with Henoch-schonlein purpura nephritis

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Objective: To investigate the changes of B cell-activating factor (BAFF) and a proliferation- inducing ligand (APRIL) in serum of children with Henoch- Schonlein purpura nephritis (HSPN), and to explore their role in the pathogenesis of children HSPN.
Methods: A total of 28 children with HSPN who were before treatment were selected 16 children with henoch-Schonlein purpura (HSP group) and 20 health children (control group) were selected as control. The clinical data were collected. Serum levels of BAFF and APRIL were measured by adopting enzyme - linked immunosorbent assay (ELISA) .
Results: 1. The serum levels of BAFF in HSPN children were significantly lower than those in the HSP group and healthy control group.[HSPN group (0.728±0.340) ng/mL, HSP group (1.276±0.459) ng/mL, healthy control group (1.285±0.299) ng/mL , F = 23.715, P = 0.000]. Moreover, the serum levels of BAFF in before treatment group were significantly lower than in after treatment group. [before treatment group (0.652±0.354) ng/mL, after treatment group (0.860±0.262) ng/mL , P< 0.05). 2. Changes of serum APRIL level The serum levels of APRIL in HSPN and HSP children were both significantly higher than those in healthy control group, 3. The serum levels of APRIL were positively correlated with serum IgA(P< 0.05).
Conclusions: The level of serum BAFF decreased and APRIL increased in children with HSPN, which was related to the degree of renal involvement. It suggests that BAFF and APRIL may be related to the pathogenesis of HSPN in children

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DOI: 10.1002/eji.201970400

P1184

The role of innate immune triggers for the induction of poxviral vectored cytotoxic T cell responses

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Modified vaccinia virus Ankara (MVA) is a highly attenuated virus strain which was used to eradicate smallpox. Currently, MVA is under investigation as a promising vaccine vector because of its high safety properties and the ability to induce efficient cytotoxic T cell responses. During primary MVA infection cytotoxic T cells are induced via direct as well as cross-presentation of antigen by dendritic cells (DCs). However, the innate immune triggers which license DCs for cross-presentation during MVA infection remain elusive. The detailed understanding of the mechanisms which allow DCs to induce efficient cytotoxic T cell responses may improve the development of MVA-based vaccines. By analyzing the transcriptome of MVA-infected cells, we were able to obtain target genes which might trigger cross-presentation in DCs. We chose the most promising genes and generated cell lines which were deficient for a particular gene using the CRISPR/Cas9 system as gene editing approach. Among our target genes were genes involved in cell death as well as pattern recognition receptors. These cells will be characterized for their ability to induce cross-presentation by analyzing T cell activation and DC maturation *in vitro*. However, we already observed in supernatant transfer experiments that the innate immune trigger which activates cross-presenting DCs is cell-associated and cytokines only play a minor role during this process. Furthermore, we will analyze the role of cross presentation on CD8 T cell responses during infection with MVA as well as vaccinia virus *in vitro* and *in vivo* exploiting BATF3 deficiency.

P1185

Splenic CD169+ marginal zone macrophages cross-prime CD8+ T cells in vivo independently of dendritic cells using a vacuolar processing pathway

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Cross-priming of CD8+ T cells is of critical importance in triggering CD8+ T cell responses against pathogens and tumors. It is commonly thought that conventional type 1 dendritic cells (cDC1) cross-prime with unequalled efficacy, conferred to them by a proteasome and TAP-dependent pathway. Here we present data challenging the unique cross-priming efficacy of both the cDC1 subset and the proteasome/TAP-dependent pathway. Splenic CD169 macrophages are strategically placed in the marginal zone for capture of blood-borne pathogens. However, they are considered unable to directly cross-present and to depend on antigen transfer to cDC1 for cross-priming. We have developed a protocol for purification of CD169 macrophages. Extensive characterization shows that these cells display morphology, phenotype and gene expression distinguishing them clearly from cDC1, cDC2, plasmacytoid DC and red pulp macrophages. In vitro, CD169 cells cross-present soluble, receptor-targeted and phagocytosed antigen with equal or better efficiency than cDC1. Remarkably, this is achieved through a vacuolar, BFA and epoxomicin-insensitive pathway. Using intravital biphoton microscopy, 3D light sheet imaging and antigen targeting to CD169, we show that CD169 macrophages establish long-lasting antigen-dependent contacts with cognate CD8+ T cells that result

DOI: 10.1002/eji.201970400

in T cell activation in situ and generation of triple-positive effector and memory cells. Transfer of antigen-loaded CD169 cells reconstitutes in vivo cross-priming as well as transfer of cDC1 cells. Therefore, splenic CD169 macrophages represent a second set of myeloid cells equipped for highly efficient cross-priming. The biological role of cross-priming by these cells and their cooperation with cDC1 cells are under investigation.

P1777

Investigating the development and function of CD8$^+$ invariant NKT cells with humanized mouse models

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NKT cells are a group of innate T cells restricted by MHC class I-like molecule, CD1d. The major group of NKT cells, invariant NKT (iNKT) cells, possess potent anti-tumor function as demonstrated in both in vitro and in wild-type mice. Nevertheless the major iNKT cell ligand, a-galactosylceramide (a-GalCer), showed limited efficacy in multiple cancer immunotherapy clinical trials. One of the major mechanisms for the sharp difference in therapeutic efficacies between human and mice is the difference of iNKT cell compositions in the two species, including a human-specific population of CD8$^+$ iNKT cells. To build more relevant preclinical models for iNKT cell-based cancer immunotherapies, we humanized the CD1d/iNKT cell antigen presentation system by expressing human CD1d and human iNKT cell TCRa chain in mice. Remarkably, we can clearly detect a CD8$^+$ iNKT cell population in our humanized mice. We specifically investigated the ontogeny of these CD8$^+$ iNKT cells by addressing how CD1d/iNKT cell antigen presentation differ in our humanized mice from that in wild-type mice. We discovered that the development of CD8$^+$ iNKT cell is mostly due to the lower affinity between CD1d/lipid and iNKT TCR during the antigen presentation. The lower affinity leads to a transcriptional program different from that in CD4+ iNKT cells, featuring transcriptional factors such as Eomes. Functionally, CD8$^+$ iNKT cells are Th1-biased in cytokine production and possess potent direct anti-tumor function. We are continuously working on the optimal activation of different subsets of iNKT cells for eventual best efficacy of iNKT cell-based cancer immunotherapies.

P1778

CD40 at the interface of Leishmania-Macrophage interaction

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The parasite and its host co-evolved interacting with each other; while the host marshaled immune attacks to eliminate the parasite, the parasite evaded the host immune response to ensure its survival. CD40, a costimulatory receptor expressed on antigen-presenting cells, plays significant roles in the interactions between *Leishmania*, a protozoan parasite that causes the disease leishmaniasis, and its host- the macrophages. We observed the following: [1] the virulence of the parasite was expressed as receptor partitioning on the cell membrane; [2] CD40 signal switching between counteractive signaling modules; [3] differential induction of counteractive effector functions. As single amino acid substituted CD40-binding ligands evoked different responses, it is possible that the messages encrypted in the ligand instruct the receptor to be partitioned into different domains of the cell membrane and evoke differential immune responses. Our works show for the first time how an initial receptor-ligand interaction can decide the signaling processes and effector responses.

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DOI: 10.1002/eji.201970400

P1779

Identification of T-cell autoantigenic peptides in rheumatoid arthritis as new targets for an antigen-specific dendritic cell-based therapy

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease, initiated and perpetuated by CD4+ T cells that recognize autoantigens presented by dendritic cells (DCs). Although several candidates have been proposed, T-cell autoantigenic peptides responsible for triggering autoimmunity remain poorly characterized. This knowledge is essential for designing DCs-based therapies to restore self-tolerance. In this work we aimed to identify immunodominant T-cell epitopes derived from synovial proteins to be used for tolerogenic DCs (Tol-DCs) loading.
Methods: Peptides bound to major histocompatibility complex (MHC)-class II molecules, either from synovial fluid-pulsed DCs, synovial tissue resident-DCs, or a cell-free system, were isolated and sequenced by mass spectrometry. Selected peptides were synthetized and used for stimulation of peripheral blood mononuclear cells isolated from 18 RA patients and 8 healthy donors. Reactivity of CD4+ T cells was evaluated by flow cytometry based on the expression of several activation markers and pro-inflammatory cytokines. Monocyte-derived Tol-DCs and matured-DCs were generated from 5 RA patients, loaded with interest peptides, and co-cultured with autologous CFSE-stained CD4+ T cells. Proliferation and cytokine production were evaluated by flow cytometry.
Results: Twelve peptides were selected, of which six (derived from calreticulin, vimentin, fibronectin, and fibrinogen), displayed a greater immunostimulatory capacity on RA-CD4+ T cells, and showed to be specific for RA. Priming with Tol-DCs reduced the expression of pro-inflammatory cytokines by autoreactive CD4+ T cells.
Conclusion: We identify six new T-cell epitopes that could be relevant for RA development, whose pro-inflammatory responses can be modulated by Tol-DCs, supporting their application as antigen-specific therapeutic strategy.

LB123

Tim-3 binding protein Bat3 controls dendritic cell function by regulating metabolic quiescence via ER homeostasis

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DOI: 10.1002/eji.201970400

Tim-3 is an inhibitory molecule expressed on terminally differentiated effector T cells where it induces T cell exhaustion/dysfunction. Despite the name Tim3, and an extensive body of literature on Tim3 in T cell biology, this molecule is most abundantly expressed by Dendritic cells, but its function on DCs has not been well characterized. We previously demonstrated that Bat3 is an adapter protein that binds to the cytoplasmic tail of Tim-3 to restrain Tim-3 function in the absence of appropriate ligands. Using genetic deletion models, we now show that Bat3 additionally acts as a critical regulator of DC function. Lack of Bat3 in CD11c+ cells results in a defect in DC maturation. Moreover, mice with conditional deletion of Bat-3 in DCs are profoundly resistant to induction of CNS autoimmunity. Mechanistically, upon activation by toll-like receptor (TLR) ligands, Bat3 deficient DC demonstrate prolonged ER stress. This fosters a shut-down of energy metabolism with impaired mitochondrial respiration upon LPS challenge, together resulting in defects in the maturation of Bat3 deficient DCs. We further demonstrate that inhibition of ER stress response in Bat3-deficient DCs partially rescues the abnormal phenotypes of DC activation via restoring homeostasis of ER, and energy metabolism. Taken together, our study identified that Tim-3 binding protein Bat3 not only directly suppresses Tim-3 signaling but acts as a crucial signaling hub in DCs by regulating DC activation and promoting normal immune homeostasis by coordinating ER stress response and metabolic homeostasis of DCs.

P2372

Dendritic-tumor fusion cells combined with rapamycin induce tumor-specific immune response

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Rapamycin an mTOR inhibitor that mediates the CD8+ T cell effector and memory responses. This study evaluated whether dendritic cell (DC)/B16 fusion cells plus rapamycin improve the anti-tumor activity of T lymphocytes. Compared with rapamycin and DC/B16 single treatment, combined treatment was more efficient in inducing T cell proliferation, generating CD8+CD44+ memory T cells and IFN-γ producing T cells. Rapamycin combined with DC/B16 activated T cells to specifically kill B16 cells but not H22 cells. The intravenous administration of unstimulated T cells as well as T cells stimulated by rapamycin and DC/B16 (alone or in combination) reduced tumor volume; prolonged animal survival; decreased the proportion of CD4+CD25+CD127-Tregs in the spleen and the proportion of myeloid-derived suppressor cells (MDSCs) in the spleen, tumor and bone marrow; inhibited tumor cell proliferation and induced tumor cell apoptosis. Additionally, the most evident anti-tumor activity was detected in animals given T cells stimulated by rapamycin and DC/B16. Our findings suggest that T cells stimulated by rapamycin and DC/B16 specifically kill melanoma B16 cells possibly through reducing the populations of Tregs and MDSCs, thereby inhibiting tumor cell proliferation and inducing tumor cell apoptosis.

P2373

Quantitative prediction of class I MHC/epitope binding affinity using QSAR modeling derived from amino acid structural information

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The activation of T cell immune responses, which relies on peptide antigens transported by TAP and bound to major histocompatibility complex (MHC) molecules, is recognized by T cell receptors (TCR). The quantitative prediction of MHC-epitope binding affinity can facilitate epitope screening and reduce cost and experimental efforts greatly. In this study, a comprehensive quantitative prediction method of binding affinity was established using quantitative structureactivity relationship (QSAR) modeling

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DOI: 10.1002/eji.201970400

derived from amino acid physicochemical information. Firstly, the epitope was characterized by a set of amino acid physicochemical parameters. Secondly, the structural variables were optimized by the stepwise regression (STR). Finally, the robust quantitative models with were built by multiple linear regressions (MLR) for 31 MHC Class I subtypes. The normalized regression coefficients (NRCs) of QSAR model could demonstrate the mechanism of interaction of MHC, epitope, and TCR very well. The contribution of amino acid at each position of epitope, which was calculated by NRC, could determine which one was favorable for binding affinity or not. Therefore, the quantitative models established by STR-MLR could be used to guide virtual combinational design and high throughout screening of CTL epitope. Besides, they have many advantages, such as definite physiochemical indication, easier calculation and explanation, and good performances.

P2374

A novel HLA-A2-restricted altered MUC1 epitope induce more CTLs against gastric cancer

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Previous studies have proved MUC1 is tumor associated antigen, its expression is an indication of prognosis in many tumor types, MUC1 is non-mutated antigen overexpressed in maglignant transformed cells. As self-antigen, MUC1 without strong immunogenicity, but altered peptide of MUC1 has been considered as an attractive peptide vaccine for treatment of malignant tumors. The altered peptide with amino acid replacements in their sequences would enhance its immunogenicity and result in specific cytotoxic T lymphocyte effectively cross-recognize tumor expressed wild-type peptide. Here, we explore a novel HLA-A2 restricted altered peptide of MUC1 that can induce MUC1-specific anti-Stomach cancer. The altered peptide of MUC1 showed superior binding ability compared to wild-type peptide. In addition, the altered peptide was better than wild-type peptide on the part of immunological efficacy in vitro as measured by IFN-γ release and cytotoxicity assays. In contrast, peptide M1.1 that has been proved as a very promising anti-tumor epitope in various tumors were not able to generate strong lysis to tumor cell in this study. Our results indicate the altered peptide is a new HLA-A2-restricted CTL epitope that can be incorporated into vaccine or adoptive cell therapy for patients with STAD.

P2375

Near-infrared II phototherapy induces deep tissue immunogenic cell death and potentiates cancer immunotherapy

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Deep and inner parts of solid tumors lack lymphocytic infiltration and are subjected to various immune escape mechanisms. The reversion of immunosuppression deep within the tumor is vital in clinical cancer therapy, however remains a huge challenge. In this work, we have demonstrated the use of a near-infrared II light to trigger immunogenic cancer cell death more homogeneously and deeper in solid tumors, thereby enabling effective tumor control and metastasis prevention. Specifically, photothermal transducers with similar components, structures, and photothermal conversion efficiencies, but different absorptions in the red light, NIR(I), and NIR(II) biowindows were constructed by controlling self-assembly of AuNPs on fluidic liposomes. *In vitro*, photothermal treatments induced

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DOI: 10.1002/eji.201970400

immunogenic cell death (ICD) accompanied by the release of damage-associated molecular patterns (DAMPs) regardless of wavelengths of incident lasers. *In vivo*, NIR(II) light resulted in more homogenous release and distribution of DAMPs in the deeper parts of tumors. With the induction of ICD, NIR(II) phototherapy simultaneously triggered the innate and adaptive immune responses. In the cancer vaccination experiments, cancer cells that underwent NIR(II)-induced ICD enabled efficient tumor control with 5/8 of the mice remaining tumor free. Additionally, the NIR(II) photothermal treatment in combination with checkpoint blockade therapy exerted long-term tumor control over both primary and distant tumors. Finally, systematically administrati 2D polypyrrole nanosheets as a transducer, we achieved striking therapeutic effects against whole-body tumor metastasis *via* synergistic photothermal-immunological response.

P2376

PEST-containing nuclear protein regulates cell proliferation, migration, and invasion in lung adenocarcinoma

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Lung cancer is the leading cause of cancer-related mortality worldwide. PEST-containing nuclear protein (PCNP) has been found in the nucleus of cancer cells. Whether PCNP plays a role in the growth of lung adenocarcinoma is still unknown. In the present study, the results indicated that the level of PCNP in lung adenocarcinoma tissue was significantly higher than that in corresponding adjacent non-tumor tissue. Over-expression of PCNP promoted the proliferation, migration, and invasion of lung adenocarcinoma cells, while down-regulation of PCNP exhibited opposite effects. PCNP over-expression decreased apoptosis through up-regulating the expression levels of phospho (p)-signal transducers and activators of transcription (STAT) 3 and p-STAT5 in lung adenocarcinoma cells, whereas PCNP knockdown showed opposite trends. PCNP overexpression enhanced autophagy by increasing the expression levels of p-phosphatidylinositol 3-kinase (PI3K), p-Akt, and p-mammalian target of rapamycin (mTOR) in lung adenocarcinoma cells, however an opposite trend was observed in the sh-PCNP group. In addition, overexpression of PCNP showed the tumor-promoting effect on xenografted lung adenocarcinoma, while PCNP knockdown reduced the growth of lung adenocarcinoma via regulating angiogenesis. Our study elucidates that PCNP can regulate the procession of human lung adenocarcinoma cells via STAT3/5 and PI3K/Akt/mTOR signaling pathways. PCNP may be considered as a promising biomarker for the diagnosis and prognosis in patients with lung adenocarcinoma. Furthermore, PCNP can be a novel therapeutic target and potent PCNP inhibitors can be designed and developed in the treatment of lung adenocarcinoma.

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DOI: 10.1002/eji.201970400

P2377

Siglec-G negatively regulates antigen cross-presentation of dendritic cells by inhibiting formation of MHC class I-peptide complex

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Antigen-presenting cells (APCs) can endocytose exogenous antigens and cross-present them to naïve $CD8^+$T lymphocytes by MHC class Ⅰ pathway, which called “cross presentation”, that is an essential process for immunological defense against viruses, intracellular bacteria and tumors. Splenic $CD8a^+$ Dendritic cells (DCs) are considered to be the most potent at cross-presentation, but the details of the mechanisms made them different from $CD8a^-$ DCs on cross-presentation remain unknown. In this study,we showed that the expression of a lectin family member, Siglec-G, on DCs was upregulated by infection, and Siglec-G expression was significantly lower in $CD8\alpha^+$ DCs than $CD8\alpha^-$ DCs. Siglec-G deficient mice generated more antigen-specific CTLs to inhibit *Listeria monocytogenes, Mycobacterium bovis* bacillus Calmette-Guérin and tumor growth. Next we found that Siglec-G expressed on DCs phagosomes recruited the phosphatase SHP-1 to inhibit the activation of NOX2 on phagosomes, which resulted in excessive hydrolysis of exogenous antigens, led less expression of MHC class Ⅰ-exogenous antigen peptide complex for cross-presentation on DCs. Therefore, Siglec-G plays a critical role in negatively regulating DCs to cross-present antigen in initiation of antigen-specific $CD8^+$T cell response. The data add the insight to the molecular mechanism of cross presentation in infection and immunity.

P2378

Increased translocation of antigens to endosomes and TLR4 mediated endosomal recruitment of TAP contribute to nicotine augmented cross-presentation

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Cross-presentation by dendritic cells (DCs) requires surface molecules such as lectin, CD40, langerin, heat shock protein, mannose receptor, mediated endocytosis, the endosomal translocation of internalized antigen, and the relocation of transporter associated with antigen processing (TAP). Although the activation of α7 nicotinic acetylcholine receptor (α7 nAchR) up-regulate surface molecule expression, augment endocytosis, and enhance cross-presentation, the molecular mechanism of α7 nAchR activation-increased cross-presentation is still poorly understood. In this study, we investigated the role of mannose receptor in nicotine-increased cross-presentation and the mechanism that endotoxins orchestrating the recruitment of TAP toward endosomes. We demonstrated that nicotine increase the expressiones of mannose receptor and Toll-like receptor 4 (TLR4) via PI3K-Akt-mTOR-p70S6 pathway. Both endosomal translocation of mannose receptor-internalized antigens and TLR4 signaling are necessary for nicotine-augmented cross-presentation and cross-priming. Importantly, the recruitment of TAP toward endosomes via TLR4-MyD88-IRAK4 signaling contributes to nicotine-increased cross-presentation and cross-activation of T cells. Thus, these data suggest that increased recruitment of TAP to Ag-containing vesicles contributes to the superior cross-presentation efficacy of α7 nAchR activated DCs.

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DOI: 10.1002/eji.201970400

Immune Receptor Signaling

O313

NDR2 promotes the antiviral immune response via facilitating TRIM25-mediated RIG-I activation in macrophages

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Retinoic acid-inducible gene I (RIG-I), a pivotal cytosolic sensor, recognizes viral RNAs to initiate antiviral innate immunity. However, posttranslational regulation of RIG-I signaling is not well understood. We report here that nuclear Dbf2-related kinase 2 (NDR2) functions as a crucial positive regulator of the RIG-I-mediated antiviral immune response. Overexpression of NDR2 or its kinase-inactive mutants potentiates RNA virus-induced production of type I interferons and proinflammatory cytokines and dampens viral replication. NDR2 conditional knockout mice ($Lysm^{+}NDR2^{f/f}$) show an impaired antiviral immune response. Mechanistically, NDR2 directly associates with RIG-I and TRIM25, thus facilitating the RIG-I/TRIM25 complex and enhancing the TRIM25-mediated K63-linked polyubiquitination of RIG-I, which is required for the RIG-I-mediated antiviral immune response. Furthermore, NDR2 expression is notably down-regulated in peripheral blood from respiratory syncytial virus-infected patients and in virus-infected macrophages. Collectively, these findings provide insights into the function of NDR2 in antiviral immunity and its related clinical significance.

O314

C-terminal additions to insulin B-chain fragments create super-agonists for T cells in mouse and human type-1 diabetes

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In type 1 diabetes (T1D), CD4 T cells respond to the insulin B:9-23 peptide in both mice and humans. We previously showed that C-terminal amino acids changes to this peptide are required for proper binding to MHC and recognition by T cells. Here structures of mouse and human T cell receptors (TCRs) complexed with these modified peptides bound to IA^{g7} or HLA-DQ8 show how the amino acid substitutions create super-agonists for the T cells. We also show how fusions between fragments of B:9-23 and of proinsulin C-peptide create chimeric peptides with activities like the modified peptides. We propose transpeptidation as a mechanism to accomplish this in vivo. Our data reveal the striking similarities between mouse and human insulin specific T cells in recognizing MHCII-insulin ligands and provide an explanation for how diabetogenic T cells escape negative selection in the thymus but find their modified target antigen in the pancreas to cause T1D.

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DOI: 10.1002/eji.201970400

O315

TCR-induced PLC-γ1 sumoylation regulates the microcluster assembly and physiological function of PLC-γ1

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The SUMO modification system plays an important role in T cell activation, yet how sumoylation regulates TCR-proximal signaling remains largely unknown. We show here that Phospholipase C-γ1 (PLC-γ1) is conjugated by SUMO1 at K54 and K987 upon TCR stimulation and that K54 sumoylation is pivotal for PLC-γ1-mediated T cell activation. We further demonstrate that TCR-induced K54 sumoylation of PLC-γ1 significantly promotes the formation of PLC-γ1 microclusters and the association of PLC-γ1 with the adaptor proteins SLP76 and Gads, but only slightly affects the phosphorylation of PLC-γ1 on Y783, which determines the enzyme catalytic activity. Moreover, upon TCR stimulation, the SUMO E3 ligases PIASxβ and PIAS3 both interact with PLC-γ1 and cooperate to sumoylate PLC-γ1, facilitating the assembly of PLC-γ1 microclusters. Together, our findings reveal a critical role of PLC-γ1 K54 sumoylation in PLC-γ1 microcluster assembly that controls PLC-γ1-mediated T cell activation, suggesting that sumoylation may have an important role in the microcluster assembly of TCR-proximal signaling proteins.

O316

Competitive CD226/TIGIT signaling balances macrophage polarization and treg production in graft-versus-host disease

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Graft-versus-host disease (GVHD) is the most common complication in allogeneic hematopoietic stem cell transplantation. Advances in the understanding of costimulatory molecules have substantially contributed to the knowledge of the pathophysiology, treatment, and prevention of allograft rejection. TIGIT and CD226 are two competitive ligands for the receptor CD155, and CD226/TIGIT-CD155 interaction is an important costimulatory/coinhibitory signaling mechanism in immune tolerance induction. In this study, monoclonal antibodies (mAbs), recombinant CD226-Fc or TIGIT-Fc, were employed to manipulate CD226/TIGIT signaling in a GVHD model using Foxp3-Cre (WT) and CD226 fl/fl Foxp3-Cre (CKO) mice. In the TIGIT-Fc-treated mice, GVHD symptom occurrence and GVHD-related mortality were delayed compared with those in the control mice. Further, tissue damage in the liver and small intestine reduced with minimal lymphocytic infiltration, and the percentage of $CD8^+$IFN-γ^+ and $CD8^+$ granzyme B^+ cells significantly decreased. Moreover, treatment with TIGIT-Fc, even after the onset of GVHD, ameliorated symptoms and prolonged survival. TIGIT-Fc also inhibited $CD8^+$ T-cell activation in vitro, and this was dependent on the presence of CD155 on BMDCs and on IL-10 production. In addition, TIGIT/CD155 ligation triggered Erk phosphorylation and STAT3 nuclear translocation. In the GVHD model, the expression of CTLA-4 and TIGIT on CD226 CKO donor Tregs decreased and Glut1 expression increased. The mice in the CD226 CKO donor group had increased survival rates and lower GVHD clinical scores. Taken together, these data indicate that CD226/TIGIT-CD155 signaling plays an important role in the development of GVHD and is a potential therapeutic target for the treatment of acute GVHD.

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DOI: 10.1002/eji.201970400

O317

SUMO E2 conjugating enzyme Ubc9 regulates T cell activation by interaction with immune adaptor SLP-76

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While the post-transcriptional modification with small ubiquitin-related modifiers (SUMO), namely SUMOylation, is one of important mechanisms that regulate a series of cellular key processes, the role for SUMOylation linked to a function of T cells has remained elusive. We have previously shown SUMO E2 conjugating enzyme Ubc9 binds to immune adaptor ADAP, and is selectively required for the integrin-mediated T cell adhesion but not for TCR proximal signaling responses or TCR-induced IL-2 transcription. Here, we demonstrate for the first time that Ubc9 interacts with and SUMOylates SLP-76 in TCR signaling, an immune adaptor that integrates and propagates the T cell activation signal emanating from TCR/CD3 complex. TCR stimulation promotes SLP-76-Ubc9 binding, accompanied by an elevated level in SLP-76 SUMOylation. In addition, SLP-76 and Ubc9 synergies to potentiate the TCR-induced IL-2 transcription and production, which depends on the SUMOylation of SLP-76. Moreover, while not required for TCR proximal signaling events, the Ubc9 mediated SUMOylation of SLP-76 is required for TCR-induced assembly of NFAT-Ubc9 complex for IL-2 transcription. These results suggest that Ubc9 can modulate the function of SLP-76 in T cell activation both by direct interaction and by SUMOylation of the SLP-76, and that Ubc9-SLP-76 module may act as a novel regulatory layer on the controls of T cell activation.

O318

Innate receptor TREM-2 expressed on T cell surface interacts with CD3zeta/Zap70 complex to activate pro-inflammatory Th1 responses

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Triggering receptors expressed on myeloid cells 2 (TREM-2) is a pattern recognition receptor on innate immune cells that negatively modulates inflammatory response. However, the role of TREM-2 in *in vivo* models of infection and inflammation remains controversial, indicating that TREM-2 may execute a distinct function in other immunocytes. In the present study, we demonstrated that in both humans and mice, TREM-2 expression on $CD4^+$ T cells was induced by *Mycobacterium tuberculosis* (*Mtb*) infection, and positively associated with T cell activation and effector memory phenotype. To be noted, unlike the observation in myeloid cells that TREM-2 signals though the adaptor molecule DAP12, in $CD4^+$ T cells, TREM-2 interacted with CD3zeta/Zap70 complex by virtue of its transmembrane domain (especially the K173 site), leading to STAT1/4 activation, T-bet transcription

and Th1 polarization. In addition, *in vivo* infection of reconstituted *Rag2*$^{-/-}$ mice (with TREM-2-deficient vs WT, or TREM-2^{+} vs TREM-2^{-} CD4^{+}T cells), revealed that TREM-2 promoted the Th1-mediated host defense against *Mtb* infection. More importantly, TREM-2 induction on CD4^{+} T cells was also found in sepsis patients with distinct infections and in mice with DSS-induced colitis, favoring pro-inflammatory Th1 responses. Taking together, these findings identify a novel role of TREM-2 in evoking pro-inflammatory Th1 responses, which may provide potential therapeutic targets for infectious and inflammatory diseases.
Keywords: Triggering receptors expressed on myeloid cells 2; CD3zeta; Th1 response; inflammation; infection

O319

Tmod1 affects the immune functions and biophysical properties of Dendritic cells through TLR4 signaling pathway and cytoskeleton remodeling

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Several evidences show that tumor-derived suppressive cytokines deteriorate DCs' immune functions through remodeling their F-actin cytoskeleton. But the underlying mechanism is still elusive. Tropomodulin1 (Tmod1), a cytoskeleton-binding protein, regulates and stabilizes actin filaments lengths and cytoskeleton architecture, which involves in the regulations of the morphology, formation of neural dendrites and biophysical properties of cells. Our previous studies found that mature DCs (mDCs) had a higher expression of Tmod1 than immature DCs (imDCs). Therefore, it's hypothesized that Tmod1 maybe involve in the modification of DCs' functions. We found that Tmod1-deficient mDCs failed to express enough MHC-II, CD80/86, CD40 and CCR7 on their cell surface and secrete enough inflammatory cytokines such as IL-6 and IFN-γ while increasing the secretion of anti-inflammatory cytokines IFN-β and IL-10, which leaded to their deficient antigen-presenting ability. Furthermore, Tmod1 knockout DCs showed impaired migration ability, higher osmotic fragility and lower Young's modulus. The underlying mechanisms are due to the inhibitions of the TLR4-mediated NF-κB and p38 MAPK singling pathway and the activation of the IRF3 signaling pathway, as well as the disturbed reorganization of the F-actin cytoskeleton including less F-actin content and shorter dendritic pseudopodia. Our results provide a new insight on the functions of Tmod1 which can affect the DCs' immune functions and biophysical properties through regulating the TLR4-mediated singling pathways and cytoskeleton remodeling.
Key Words: Tmod1, dendritic cells, immune functions, biophysical properties, cytoskeleton.

O320

Mesangial cell proliferation upon receptor tyrosine kinase Axl activation is mediated by the PI3K-Akt-mTORC1 pathway

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Glomerulonephritis (GN) is a critical condition in many forms of kidney diseases. Mesangial cell proliferation is often a manifestation of the GN. Receptor tyrosine kinase Axl contributes to the

DOI: 10.1002/eji.201970400

clearance of apoptotic cells and maintenance of immune homeostasis. Previously, we have shown that Axl contributes to GN by promoting mesangial cell proliferation and small molecular inhibition of Axl ameliorates nephrotoxic serum-induced GN in mice. In this study, we investigated the signaling cascade induced by Axl activation in renal mesangial cells. We found that Axl stimulation led to the activation of mTORC1 complex, followed by the ribosomal protein S6 phosphorylation, and subsequent Bcl-xl upregulation. Targeted inhibition of Akt and mTOR diminished mTOR and downstream S6 phosphorylation. However, inhibition of mTOR decreased NFκ-B activity and inhibition of NFκ-B also reduced mTOR phosphorylation, indicating a reciprocal regulation of mTOR and NFκ-B under Axl activation in mesangial cells. Interestingly, Axl-mediated phagocytosis of apoptotic cells was observed only in mesangial cells, but not in the similarly treated peritoneal macrophages that also bearing Axl on the surface. However, a weak calcium signaling was recorded in mesangial cells upon Axl stimulation. Our data suggest that Axl mediated mTOR activation and apoptotic cell engulfment seem to be mesangial cell specific and targeting Axl in GN is likely to be a fruitful therapeutic strategy.

LB039

Potential antimicrobial actions of nano-curcumin, suggest a pleasant study area

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Curcuma longa L. (Zingiberaceae family) and its polyphenolic compound curcumin have been subjected to a variety of antimicrobial investigations due to extensive traditional uses and low side effects. The promising results for antimicrobial activity of curcumin made it a good candidate to enhance the inhibitory effect of existing antimicrobial agents through synergism. The use of the curcumin nanoparticle has improved main problems of curcumin such as low solubility, instability, poor bioavailability, and rapid metabolism in infection disease. Indeed, different investigations have been done to increase the antimicrobial activity of curcumin. This review aims to summarize previous antimicrobial studies of nano-curcumin towards its application in the future studies as a natural antimicrobial agent.

P0005

Identification of chicken CAR as the cellular attachment receptor of egg drop syndrome virus (EDSV) and crystal structure of EDSV fiber head

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Egg drop syndrome virus (EDSV) is the only member of the avian group III *Aviadenovirus* within the family *Adenoviridae*. Until now, EDSV virus-cell interactions are poorly understood, and the cellular receptor is still unknown. In the present study, we demonstrate that chicken coxsackievirus and adenovirus receptor (CAR) is a cellular attachment receptor for EDSV based on three lines of evidences. And then, we determined the crystal structure of the EDSV fiber head to gain further insight into the receptor-binding property of EDSV. Firstly, knockdown of the chicken CAR remarkably impaired virus infection in the EDSV permissive DEF cells. And the EDSV fiber head was shown to specifically interact with DEF cells by flow cytometry analysis. Secondly, confocal, co-immunoprecipitation, and pull-down analysis validated that the fiber head specifically recognized and interacted with CAR. In inhibition assays, soluble fiber head or CAR protein significantly inhibited EDSV infection in a dose-dependent manner. Finally, overexpression of CAR resulted in an increase

DOI: 10.1002/eji.201970400

in the binding of EDSV virions to both permissive and nonpermissive cells. Therefore, these results indicate that CAR is a cellular attachment receptor for EDSV. Furthermore, the atomic structure of the fiber head of EDSV was determined at 2.74 Â resolution. Structure comparison reveals that the avian adenovirus may interact with the receptor in a unique fashion. Taken together, the results presented here are helpful for further exploring the pathogenesis related to the interaction between EDSV and host cells, and may be used for vaccine development and intervention strategies against EDSV infection.

P0006

Profiling the pattern of the human T-cell receptor γδ complementary determinant region 3 repertoire in patients with lung carcinoma via high-throughput sequencing analysis

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γδ T cells recognize tumor-associated stress antigens via TCR γδ and play important roles in the antitumor immune response. In this study, we characterized the pattern of the human TCR γδ complementary determinant region 3 (CDR3) repertoire in patients with lung carcinoma (LC) via high-throughput sequencing. The results showed that the diversity of CDR3δ was significantly reduced, and that of CDR3γ was unchanged in LC patients compared with healthy individuals; in addition, LC patients shared significantly more CDR3δ sequences with each other. The CDR3 and N-addition length distribution did not significantly differ between LC patients and healthy individuals. In addition, the CDR3 repertoire tended to use more Vδ2 and fewer Vδ1 germline gene fragments among LC patients. Moreover, we found a combination of four TCR γδ repertoire features that focus on CDR3δ and can be used as a biomarker for LC diagnosis. Our research suggests that the TCR γδ CDR3 repertoire changed in LC patients due to the antitumor immune response by γδ T cells in vivo, and these changes primarily focus on the amplification of certain tumor-specific CDR3δ clones among patients. This study demonstrates the role of γδ T cells from the TCR γδ CDR3 repertoire in tumor immunity and lays the foundation for elucidating the mechanism underlying the function of γδT cells in antitumor immunity.

P0007

Structure and function of MyD88 in Northeast Chinese lamprey (Lethenteron morii)

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Toll-like receptors (TLRs) are an important type of pattern recognition receptor (PRR) in innate immunity. They activate innate immunity by recognizing pathogen-associated molecular pattern (PAMP). Myeloid differentiation factor 88 (MyD88) is a key adaptor of Toll-like receptors (TLR). In the typical mammalian TLR signaling pathway, MyD88 activates downstream nuclear factor κB (NF-κB) and IFN regulatory factor (IRF) via the MyD88-dependent signaling pathway after binding to TLRs. Nevertheless, MyD88 and its mediated signaling pathways remain to be studied in primitive vertebrates. Lampreys are the most ancient group of vertebrates and the bridge between invertebrates and vertebrates. In this study, MyD88 was cloned and identified from *Lenthenteron morii*. Bioinformatics analysis showed that LmMyD88 has the typical Toll/IL-1R domain (TIR) and death domain (DD) of the MyD88 family. Phylogenetic tree showed that the homology of LmMyD88 was consistent with the phylogenetic status of lampreys. Subcellular localization results showed that

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DOI: 10.1002/eji.201970400

LmMyD88 was expressed in nucleus, cytoplasm and other parts. Real time PCR analysis showed that the expression of LmMyD88 in the adult tissues increased significantly after infection by *Pseudomonas aeruginosa*. The dual luciferase reporter assay showed that LmMyD88 activated NF-κB downstream of TLR signaling pathway. Based on these results, we confirmed that the LmMyD88 was a constitutive and inducible adaptor that mediates immune defense, and trace the innate immunity function of MyD88 to primitive vertebrates. This study suggested that LmMyD88 might play an important role in the innate immune signal transduction process of *Lenthenteron morii*.
Keywords: *Lethenteron morii*; MyD88; innate immunity

P0008

Tropomodulin1 inhibits TLR4 endocytosis in macrophages

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As now known, the cytoskeleton and the Toll like receptor (TLR) are functionally coupled, which in turn affects the phagocytosis and activation of macrophage (Mφ), but the specific details are still elusive. Tropomodulin1 (Tmod1) is a cytoskeleton-binding protein that caps the pointed end of F-actin, which regulates and stabilizes actin filaments lengths and cytoskeleton architecture. Here, we constructed Tmod1 knockout mice and studied the role of Tmod1 in the function of Mφ to exert immune functions. The results showed that the Tmod1 was upregulated in LPS-treated Raw264.7 cells. Compared with control cells, Tmod1 overexpressed Raw264.7 cells showed less colocalization between TLR4 and early endosome antigen 1(EEA-1), lysosome-associated membrane protein 1(LAMP-1), Golgi matrix protein 130 kD (GM130) after LPS treatment. In Tmod1 overexpressed cells, the expression levels of surface CD14 was higher than those of control, while TLR4 downstream pathway IRF3's activation was downregulated, and the gene expression levels of IL-6 and IFN-β respectively were increased and decreased, the inflammation and clinical manifestations of Tmod1 knockout mice were severer than those of wild type mice, indicating that Tmod1 inhibited the TLR4-mediated endocytosis of Mφ and exaggerated the inflammatory reaction. It could be concluded that cytoskeleton-binding protein functionally coupled with TLR in Mφ.
Keywords: macrophage, LPS, TLR4, endocytosis, Tmod1

P0009

TCR repertoire analysis of PD-1$^-$ or PD-1$^+$ tumor infiltrating lymphocytes in colorectal cancer and breast cancer: TCR property affects their phenotype

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Adoptive T cell therapy using tumor-specific T cells or T cell receptor (TCR)-modified T cells is the promising next-generation immunotherapy. So far, the major source of tumor reactive T cells is PD-1$^+$ tumor-infiltrating lymphocytes (TILs). In contrast, PD-1$^-$ TILs have been paid little attention. Here we analyzed TCRβ repertoire of PD-1$^-$ and PD-1$^+$ CD8$^+$ TILs derived from colorectal cancer and breast cancer. To this end, we single cell-sorted the PD-1$^-$ or PD-1$^+$ CD8$^+$ TILs and analyzed their TCRβ repertoire at single cell levels. Approximately 40 to 60% of PD-1$^+$ population consisted of oligoclonal

DOI: 10.1002/eji.201970400

population in colorectal cancer and breast cancer. On the other hand, approximately 37% of PD-1$^-$ population consisted of oligoclonal population in colorectal cancer, whereas 14% of them were oligoclonal in breast cancer. In primary colorectal cancer, TCR repertoires of PD-1$^-$CD8$^+$ TILs and PD-1$^+$CD8$^+$ TILs were hardly overlapped. Importantly, clonally expanded PD-1$^+$CD8$^+$ TIL clones in primary tumors were found to be PD-1-positive in the metastases, and clonally expanded PD-1$^-$ CD8$^+$ TIL clones in primary tumors were found to be PD-1-negative in the metastases. These results pose a possibility that characteristics of TCR determines the fate of TILs whether they become PD-1$^+$ or PD-1$^-$ in the tumor microenvironment. We found that a TCR obtained from clonally expanded TILs in colorectal cancer reacted with a patient-derived tumor cells. Further functional analysis of TCRs of TILs may lead us to understand the regulatory mechanisms of PD-1 expression on TILs, which may contribute to tumor immunotherapy.

P0010

Role of CD99 and its ligand in pro-inflammatory cytokine regulation

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Over the last several decades, ligands of several leukocyte surface molecules involving T cell regulation have been identified. Uncovering these ligands is essential for understanding the precise immunoregulation mechanism. CD99 has been reported to be involved in T cell regulation. Interaction of CD99 and its ligand involvement in the immunoregulation has been postulated. In this study, recombinant CD99 proteins were produced and used as a tool for determining the role of CD99 and its ligand interaction. Interaction between recombinant CD99 and its ligands up-regulated the production of pro-inflammatory cytokines IL-6 and TNF-α, but not IFN-g, in anti-CD3 monoclonal antibody activated T cells. The cytokine alteration was not observed in unstimulated T cells indicating the cytokine up-regulation required the signal from T cell activation. The recombinant CD99 proteins, however, did not affect either CD25, CD69 or MHC class II expression or T cell proliferation. Furthermore, the CD99 ligands were demonstrated to be expressed on monocytes, NK cells and dendritic cells, but not on B and T cells. Our results indicated the existence of CD99 ligands on leukocyte surface. Interaction between CD99 and its ligand involves in the regulation of pro-inflammatory cytokine production. This information may lead to the development of a new strategy for the treatment of inflammatory diseases.

P0590

Krüppel-like factor 2 inhibits hepatocarcinogenesis through negative regulation of the Hedgehog pathway

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. The most important reason for the occurrence of HCC is hepatitis C or B infection. Moreover, genetic factors play an important role in the tumorigenesis of HCC. Here, we demonstrated that Krüppel-like factor 2

DOI: 10.1002/eji.201970400

(KLF2) expression was downregulated in HCC samples compared with adjacent tissues. Additionally, KLF2 was shown to inhibit the growth, migration and colony-formation ability of liver cancer cells. Further mechanistic studies revealed that KLF2 can compete with Gli1 for interaction with HDAC1 and restrains Hedgehog signal activation. Together, our results suggest that KLF2 has potential as a diagnostic biomarker and therapeutic target for the treatment of HCC.
Keyword: HDAC1; Hedgehog signaling; Krüppel-like factor 2; hepatocellular carcinoma; intrahepatic metastasis

P0591

Identification of a novel key regulator of T cell activation by genome-wide CRISPR/Cas9 screening

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T cell activation is triggered by the interaction of T cell receptors (TCR) and antigens, through a series of signaling events, leading to the production and secretion of cytokines. Efforts in decades have identified many molecules involved in TCR signaling pathway with classical genetic approaches. With the advent of CRISPR/Cas9, researchers can comprehensively analyze of genes involved in T cell activation. To address this, we established a human T lymphocyte reporter cell line, Jurkat-pIL2-eGFP, to monitor the production of interleukin 2 (IL-2), which is a hallmark of T cell activation. We perform a genome-wide loss of function screening by infected reporter cell with CRISPR/Cas9 knockout lentivirus library and sort the cells that cannot be activated by CD3/CD28 stimulation. Next generation sequencing is employed to identify genes involved in TCR signaling pathway, some of which are previously uncharacterized after three rounds of selection. Further identification and functions of the regulators of TCR signaling will be studied. This CRISPR/Cas9 based platform represents a robust approach to identify novel genesin T cell activation which could be targets for the treatment of cancer and inflammation disease.

P0592

Dengue virus-2 infection induces autophagy in human endothelial cells via the AMPK/ERK/mTOR signaling pathway

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Dengue virus (DENV) infection is a global public health problem. Dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS) may cause the death of patients with severe disease and dengue virus infection. At present, the pathogenic mechanism of DENV infection is unclear. Therefore, in the present study, we investigated the pathogenic mechanism of DENV-2 infection associated with autophagy signaling AMPK/ERK/mTOR in human umbilical vein endothelial Cells (HUVECs) and related signaling molecules. The results showed that autophagic vesicle formation was induced in HUVECs infected with DENV-2. DENV-2 infection inhibited mTOR but activated ERK1/2, the expression of ERK1/2 was downregulated after treatment with UO126 (a MAPK kinase inhibitor), while the phosphorylation level of mTOR was restored. U0126 inhibited the phosphorylation of TSC2 but not that of AMPK, compound C (an AMPK inhibitor) inhibited the phosphorylation of ERK1/2 and TSC2, and the protein level of LC3-II, while restoring the phosphorylation level of mTOR. The results of co-

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DOI: 10.1002/eji.201970400

immunoprecipitation showed that ERK1/2-AMPK and ERK1/2-TSC2 interacted. In conclusions, DENV-2 infection induced a complete autophagy reaction in HUVECs via the AMPK/ERK/mTOR signaling pathway. The present study provides an important theoretical basis for controlling DENV-2 infection and to further study its pathogenesis.
Key words: dengue virus type 2, autophagy, autophagy, signaling pathway

P0593

Lck self-feedback regulation underlies digital responses of T cells

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T cells are essential components of adaptive immune system, and play important roles in the elimination of pathogens, tumor surveillance and autoimmune disease. Therefore, it is of great importance to understand how T cells are activated and regulated.
Nuclear factor of activated T cells（NFAT）was initially identified as an important inducible nuclear factor. Recently, growing evidences have proved that many cytokines regulated by NFAT in an all-or-nothing manner(digital response). This indicated that NFAT activation may be digital, but the underlying mechanism has not yet been elucidated. Our work focused on the digital nuclear translocation of NFAT. We used a real-time imaging method to study the temporal dynamics of NFAT activity and built a mathematic model to screen the key signaling networks responsible for NFAT digital activation. We found that Lck positive self-feedback regulation underlies the digital activation of NFAT and multiple positive feedback regulations have synergetic effect.

P0594

HVEM expression in intestinal epithelial cells mediates intestinal homeostasis

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The intestinal epithelium localizing between the body core and the intestinal lumen is a single cell layer consisted of diverse epithelial cells with a large number of intraepithelial lymphocytes embedded. The epithelium forms a barrier that is essential for not only protection against pathogens from the lumen but also for absorbing nutrients and providing tolerance to self-antigens.Herpes virus entry mediator (HVEM) has been demonstrated to play a crucial role in the clearance of invasive pathogens in the intestinal tract, but its role at steady state remained unknown. We show here that epithelial cell expression of HVEM is important for the homeostasis of the small intestine. Mice with an epithelial-specific deletion of the gene encoding HVEM (*Hvem$^{\Delta IEC}$*) had significantly decreased TCRγδ^{+}CD8αα^{+} and TCRαβ^{+}CD8αα^{+} natural intraepithelial lymphocytes (IEL) in the proximal SI and decreased patrolling capability of IEL. Interestingly, mice with deletion of LIGHT, the member of TNF superfamily, also had decreased natural IEL. Furthermore, *Hvem$^{\Delta IEC}$* mice exhibited increased goblet cell hypertrophy and hyperplasia, crypt hyperplasia, villous atrophy as they aged, and they showed a severe defect in fucosylation of cell surface proteins, even at younger ages.Thus, HVEM plays a critical role in maintaining the homeostasis of the small intestine. Supported by the NIH MIST U01 grant.

DOI: 10.1002/eji.201970400

P1186

Abnormality in the T cell repertoire contributes to immunodeficiency in patients with DNA repair/methylation defects

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DNA damage response and methylation is important during antigen receptor recombination, creates a diverse repertoire in the developing lymphocytes, but how their defects relate to T cell repertoire and phenotypic heterogeneity of immunodeficiency remains obscure. **We studied the TCR repertoire in patients with mutation in different genes (NHEJ, *ATM, DNMT3B* and *ZBTB24*) and uncovered distinct characteristics of repertoire diversity.** We propose that early aberrancies in thymus T cell development predispose to the heterogeneous phenotypes of the immunodeficiency spectrum. **Shorter CDR3 lengths in ATM deficient patients, resulting from a decreased number of nucleotide insertions** during VDJ recombination in the pre-selected TCR repertoire, as well as the increment of CDR3 tyrosine residues, **lead to the enrichment of pathology associated TCRs**, which may contribute to the phenotypes of ATM deficiency. Furthermore, patients with *DNMT3B* and *ZBTB24* mutations who exhibit discrepant phenotypes present longer CDR3 lengths and a reduced specificity for known common antigens.

P1187

Molecular and clinical characterization of CD163 expression via large-scale analysis in glioma

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The expression and function of CD163 in glioma are not fully understood. In this report, we collected totally 1323 glioma samples from the Chinese Glioma Genome Atlas (CGGA) dataset, including 325 RNA-seq data and 301 mRNA microarray data, and 697 glioma samples from The Cancer Genome Atlas (TCGA) dataset to characterize the molecular and clinical features of CD163 in glioma by conducting a large-scale study. We found that CD163 expression was positively associated with the grade of malignancy of glioma. CD163 expression was up-regulated in IDH wild-type glioma and mesenchymal subtype. Gene ontology analysis suggested that CD163-related genes were more involved in immune response and angiogenesis in glioma. Moreover, CD163 showed a positive relationship with stromal and immune cell populations. Kaplan-Meier curves analysis revealed that higher CD163 expression indicated significantly poor survival in glioma and glioblastoma multiforme (GBM). Pearson correlation analysis revealed that CD163 was robustly associated with the immune checkpoints and other macrophage markers. These results demonstrated that CD163 predicts poor prognosis in glioma patients. Additionally, combination of CD163 and immune checkpoints may impair angiogenesis and reverse dysfunctional phenotypes of T cells, which suggest that CD163 may be a promising biomarker and target for immunotherapeutic strategies.

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DOI: 10.1002/eji.201970400

P1188

CCDC134 facilitates T cell activation and inflammatory responses by regulating T-cell receptor signaling

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Available evidence indicates that CCDC134 (coiled-coil domain containing 134), a cytokine-like molecule served as a potential member of the γ_c cytokine family, is involved in anti-tumor process, by augmenting $CD8^+$ T cell-mediated immunity. Modulation of surface T cell antigen receptor (TCR) expression is an important mechanism for the regulation of immune responses against invading pathogens and tumor cells. However, whether CCDC134 is involved in this process remains to be defined. Here, we delete *Ccdc134* gene in early stage of T-cell development by crossing *Ccdc134*$^{fl/fl}$ mice with Lck-cre transgenic mice and analyze the *in vivo* consequences of *Ccdc134* deficiency in T cells. *Ccdc134*$^{-/-}$ mice show a reduction in both $CD4^+$ and $CD8^+$ T cell subsets, and *Ccdc134*$^{-/-}$ T cells resulting from *in vitro* T-cell activation under different TCR-ligation conditions show lower activation and proliferation levels than *Ccdc134*$^{+/+}$ T cells, supporting the relevance of Ccdc134 in the induction of proper T-cell proliferative responses. Moreover, *Ccdc134* deficiency resulted in elevated TCR expression on T cells after CD3 stimulation, and attenuates TCR signaling pathways and impairs T cell activation, rendering mice refractory to T cell-mediated inflammatory responses. Taken together, these findings provide insight into CCDC134 as a positive regulator of TCR-proximal signaling, as well as into the functional consequences of *Ccdc134* deficiency on ongoing T-cell responses, which includes attenuation of T cell-mediated proliferative and inflammatory responses.

P1189

CD226 deficiency promotes megakaryocyte maturation and platelet hyperactivity

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CD226 is a key activation receptor T cell and NK functions. However, the role of CD226 as a platelet membrane glycoprotein is largely unknown. In this study, we used constitutive CD226 gene knockout (KO) mice as a model to investigate the function and mechanism of action of CD226 in megakaryocyte (MK) maturation and platelet activation. Although CD226 deficiency did not affect MK polyploidization or platelet granule abnormalities, increased numbers of MKs were detected in the splenic and femoral bone marrow of CD226 KO mice. Particularly, CD226 KO mice had a more extensive open canalicular system than wild-type (WT) mice, suggesting increased surface area for interactions upon activation. Platelets of CD226 KO mice had a similar lifespan and moderately increased mature platelet ratios in comparison with WT mice platelets. We also showed that CD226 KO mice display increased number of platelets, reduced bleeding time, and enhanced response in platelet aggregation. In addition, no changes were observed in CD62P, also known as P-selectin; CD49b, also known as integrin alpha-2; and CD226 associated molecule integrin lymphocyte function-associated antigen 1 (LFA-1) that may contribute to platelet adhesion and activation. The reduced bleeding time indicated the lack of a nitric oxide (NO) effect in vivo. Taken together, these results reveal a novel mechanism for the development and activation of platelets mediated by the adhesion molecule CD226.

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DOI: 10.1002/eji.201970400

P1190

The phosphorylation kinetics of the key regulators in T cell receptor signaling pathway

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T cell receptor (TCR) signaling pathway regulates T cell homeostasis and adaptive immune responses. Clinically, the activation of TCR is indispensable to the success of T cell-based immunotherapy. The engagement of TCR phosphorylates immunoreceptor tyrosine-based activation motifs (ITAM) on CD3, which is followed by a protein phosphorylation cascade. Protein phosphorylation, regulated by kinases and phosphatases, changes protein conformation and determines protein function. A better understanding of TCR signaling phosphorylation events is crucial to both decipher the T cell regulation mechanism and boost the efficacy of T cell immunotherapy. Here, we developed a panel of monoclonal antibodies that target several of the TCR signaling regulators. Using these antibodies, we performed a kinetic analysis of the TCR signaling pathway. Jurkat T cells were cross-linked by soluble anti-CD3 and anti-CD28 antibodies, and the phosphorylation kinetics of the key TCR signaling regulators, such as phospho-ZAP-70 and phospho-PLCg1, were evaluated by intracellular flow cytometry (ICFC) and western blot (WB). We show that TCR signaling activation induced by cross-linking CD3 and CD28 is a relatively transient event and the optimal activation of the key TCR signaling regulators varies from 1 to 15 minutes. In addition, we demonstrate the activation of TCR signaling in different primary T cell subsets. Our data profiled the phosphorylation kinetics of the key regulators in TCR signaling pathway.

P1191

Ras GTPase isoforms (H, K and N) trafficking in CD40-activated cellular responses

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Ras, a small cellular GTPase, is known as the 'molecular switch' of the cell and a key regulator of cellular responsiveness. Because of the G-domain structural identityof the three isoforms H-, K- and N-Ras, the Ras isoforms are conventionally considered as a single entity. Referring to embryonic lethality of K-Ras but not of H-/N-Ras deficient mice, we hypothesized that the Ras isoforms are functionally non-redundant. We show that inhibition of N-Ras or targeting its activator significantly reduces *Leishmania major*infection and restores the host-protective T cell response in a susceptible host such as in BALB/c mice whereas silencing of K-Ras or H-Ras increases the infection in a resistant host such as C57BL/6 mice. In *Leishmania major*infection, N-Ras expression increased but K-Ras expression decreased. Increased expression but reduced activity of H-Ras in these macrophages is due to its entrapment in the Golgi suggesting altered trafficking of Ras isoforms in *Leishmania*-infected macrophages. We report that Ras isoforms exhibit activator and effector specificities. While H-/K-Ras are activated by Ras-GRP and Vav, N-Ras is activated by SOS-1/2. Silencing of these Ras-GEFs reduces the activation of downstream signaling effector molecules Raf-1 and PI-3K, accordingly. Clearly, our data show a link between intracellular Ras trafficking and Isoform-specific regulation of antigen-specific immune response. The observations provide a scientific rationale for Ras isoform-directed immunotherapy.
Key words- GTPase; Ras; CD40; GEFs; Signaling; isoforms

DOI: 10.1002/eji.201970400

P1780

PKC mediates tlr2 induced differential response

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Toll like receptors(TLR),which are also known as pathogen recognition receptors(PRRs), act as innate immune sensor. TLRs have the ability to form dimer among themselves for the transmission of cellular signal. Though other TLRs form homodimer among themselves,TLR2 can associate with TLR 1 and TLR 6 resulting in IL 10 and IL 12 synthesis respectively. But the intracellular mediators of TLR2 induced differential response are not known. Here in, using three different ligands of TLR 2-Pam3CSK4(TLR2/1),PGN(TLR2/2) and FSL-1(TLR2/6), we are going to show that Protein Kinase C(PKC), which is a serine-threonine kinase, acts as a intracellular mediator of this TLR 2 induced differential responses in peritoneal macrophages. For this purpose, PKC phosphorylation has been monitored at different doses of TLR 2 ligands. We have measured IL 10 and IL 12 secretion under PKC inhibitor treated and TLR 2 stimulated condition from macrophages. We have also shown the impact of PKC inhibitors in TLR 2 signaling in the context of *Leishmania* infection. Leishmaniasis is a protozoan disease caused by *Leishmania* parasites having three forms-visceral, cutaneous and mucosal. Here, we have done parasite load kinetics of *Leishmania major*,causative agent of cutaneous Leishmaniasis, in PKC inhibited and TLR2 stimulated macrophages.

P1781

CD40 signalling cascades induced by differential CD40-CD40L interaction

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CD40-CD40L signalling has a pivotal role during immune response, homeostasis, antibody class switching and tolerance by initiating Th1 or Th2 type of response by regulating different cytokines and co-stimulatory / inhibitory molecules. The molecular mechanism underlying the dichotomy of CD40 signalling initiated by CD40L is unknown. In this study, 41 mer peptides were generated using the wild type CD40L mouse sequence and the selected mutant residues were between the region D117 to N157. These wild type and mutant peptides were examined for gain or loss of function in comparison with wild type using Leishmania infected macrophages as a model. Peritoneal derived mice macrophages when treated with mutant peptides showed differential activation of signalling intermediates along with transcription factor. The observed activation led to production of IL-10 & IL-12 accompanied by NO production. These effects observed by mutant peptides perpetuated in the clearance of leishmania from macrophages aiding the survival & proliferation of T-cells. This effect was further supported by the observed changes in the membrane lipid cholesterol and HMGCoA and Ceramide Synthase. **When these peptides were reconstituted in leishmania infected CD40L$^{-/-}$ mice they could contain the infection as observed by decreased footpad thickness, lymph node weight, parasite burden, and a decrease in the IL-10 & T_H17 population with a parallel increase in IL-12, IFN-γ, NO & T_H1 subsets. Mutation at residues K141 V & K142V of CD40L resulted in higher activation of CD40 receptor which could comprehend the leishmania infection both *invitro* & *invivo*.**

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DOI: 10.1002/eji.201970400

P1782

Plasmodium infection inhibits the expansion and activation of MDSCs and Tregs in the tumor microenvironment in a murine Lewis lung cancer model

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A major challenge in the development of effective cancer immunotherapy is the ability of tumors and their microenvironment to suppress immune cells through immunosuppressive cells such as myeloid - derived suppressor cells (MDSCs) and regulatory T cells (Tregs). We previously demonstrated that *Plasmodium* infection promotes innate and adaptive immunity against cancer in a murine Lewis lung cancer (LLC) model. Here we found that *Plasmodium* infection significantly reduced the proportions of MDSCs and Tregs in the lung tumor tissues of the treated mice by downregulating their recruiting molecules and blocking cellular activation pathways, an effect which was mediated through exosome-like vesicles. Importantly, $CD8^+$ T cells isolated from the tumors of *Plasmodium*-treated mice exhibited significantly higher levels of granzyme B and perforin and remarkably lower levels of PD-1. These results reveal for the first time, the effects of *Plasmodium* infection on the expansion and activation of MDSCs and Tregs with a consequent elevation of $CD8^+$T cell-mediated cytotoxicity within the tumor microenvironment and hold great promise for the development of effective immunotherapeutic strategies.

P1783

Explore new cancer immunotherapy targets in T cell signaling pathway using proteomic and genetic approaches

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Recent success of T cell-based cancer immunotherapies highlights the importance of further understanding molecular mechanisms in the regulation of T cell responsiveness. Here, we performed a genome-wide CRISPR screen and developed a novel proteomic technology to identify genes that regulate T cell activation upon anti-T cell receptor (TCR)
stimulation. Our screen confirmed many of the known regulators in proximal T cell signaling. Moreover, we identified a previously uncharacterized gene named FAM49B, which acts as a negative regulator in T cell activation. Our study suggests that genome-wide CRISPR screening is a powerful means to identify key regulators of TCR signaling. The same strategy could be applied to CD28-mediated costimulatory signaling or PD-1-mediated coinhibitory signaling. The unbiased approach presented here may allow us to identify new therapeutic targets for cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P1784

TCR-induced sequential proteolysis of L-selectin (CD62L) by ADAM17 and presenilin 1

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The lymph node homing receptor L-selectin is proteolytically shed in response to TCR engagement. Loss of cell surface L-selectin is thought to redirect activated T-cells away from lymph nodes to sites of infection. However, we have shown that activated T-cells re-express L-selectin before exiting lymph nodes and use L-selectin to locate to virus-infected tissues (Mohammed et al, 2016, doi:10.1016/j.celrep.2015.12.0900). We considered other roles for L-selectin proteolysis and found that ADAM17-dependent proteolysis of L-selectin promotes clonal expansion of virus-specific T-cells (Mohammed et al, 2019, doi:10.1038/s41598-019-41811-z). This led us to hypothesise that the ADAM17 generated membrane-retained fragment of L-selectin has intracellular signalling potential. The aim of this study was to determine the fate of the membrane-retained fragment of L-selectin. Using human T leukaemic cells expressing V5-His cyto-tail tagged L-selectin, we found that, following cleavage by ADAM17, the membrane-retained fragment of L-selectin was subject to presenilin 1 (PS1) dependent cleavage. To identify L-selectin binding partners, Co^{2+} magnetic beads were bound to the V5/His tag at the carboxy terminus of L-selectin in pull-down assays. We found that full length L-selectin forms a complex with ADAM17 in unactivated T-cells which, on TCR-activation, leads to rapid cleavage of L-selectin and dissociation of the L-selectin-ADAM17 complex. The membrane-retained fragment of L-selectin then binds nicastrin, the substrate recognition component of γ-secretase, as well as PS1 in the active site, resulting in rapid cleavage.
Future studies will isolate the PS1 cleavage product of L-selectin, determine its signalling capacity and role in the rapid expansion of T-cells following virus infection

P1785

Role of leukotriene B4 receptor (LTB4R) in neutrophil extracellular trap formation

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Neutrophil extracellular trap (NET) formation is a novel antimicrobial mechanism wherein neutrophils are activated in response to pathogenic infections to release their chromatin along with antimicrobial proteins forming a net like structure for trapping and killing the invading pathogens. The underlying mechanism for NETs formation is not well characterized. LTB4R regulates NADPH oxidase activity which is an essential component of the NET formation along with some basic functions of the neutrophils. The aim is to investigate if LTB4R mediated signaling has a role in regulation of NETs formation. Briefly, For NETs induction, neutrophils isolated from cord blood of newborns and HL-60 derived surrogate neutrophils were treated with LPS (1µg/ml) for 5 hrs. Inhibition studies were done using LY223982 (LTB4R antagonist) and LTB4R siRNA. Confocal microscopy, chromatin release, ROS generation and neutrophil elastase (markers of NET induction) estimations were done. Western blotting for Rap1 a/b, pERK1/2, B-Raf, and CitH3 was performed.We observed significantly increased expressions of LTB4R, histoneH3, Rap1a/b, pERK1/2, NE, extracellular DNA release, ROS generation upon NETs induction which were reversed by inhibition of LTB4R. LTB4R signaling regulates NETs formation via activation of Rap1a/b- Raf-ERK1/2 cascade.

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DOI: 10.1002/eji.201970400

P2379

Dual-DNA aptamer selected against CD3 and CD105 with biological function

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The objective of this study was to develop a dual-DNA aptamer to improve T lymphocytes activation and target anti-tumor effects. The 293T-CD3 oligonucleotide aptamer was obtained by 15 rounds of screening. CD3-4´s Kd value was only 18.56±1.49 nM, which has a favorable affinity. CD3-4 and 293T-CD3 cells can be combined at 4℃ and 37 ℃. It was confirmed that CD3-4 aptamer can produce better biological effects in vivo and in vitro. The bispecific nucleic acid aptamer bsaCD3-CD105 promoted the proliferation of T cells and induced T lymphocytes to kill HepG2 cells. bsaCD3-CD105 promotes the anti-tumor effect of T cells. It provides a new strategy for the prevention and treatment of tumors with potential clinical application value.

P2380

Formation of functional IFN-γ receptor is required for efficient activation of macrophage innate response against intracellular bacterial infection

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Interferon-gamma (IFN-γ) plays critical functions in immune response to intracellular bacteria and tumor cells. Functional IFN-γR is composed with α (IFN-γR1) and β (IFN-γR2) subunits, the cytoplasmic β subunit is induced and translocates to plasma membrane for joining the α subunit upon pathogen stimulation, which is a critical step to trigger intracellular signals through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway during innate immune response. The mechanisms involved in driving IFN-γR2 membrane translocation remains largely unclear. The mice deficient in E-selectin, an endothelial cell-specific cell adhesion molecule, were unexpectedly found displaying impaired innate activation of macrophages upon *Listeria monocytogenes* infection, while had increased level of circulating IFN-γ in serum. We further found that the inflammatory macrophages from mice deficient in E-selectin or EFhd2, which is a calcium-binding protein identified association with IFN-γR2, had less surface IFN-γR2 and impaired IFN-γ signaling JAK-STAT pathway. Mechanically, cytoplasmic IFN-γR2 at tyrosine 289 IS phosphorylated by activated BTK kinase elicited by extrinsic E-selectin engagement, which facilitates EFhd2 binding and promoting IFN-γR2 trafficking from Golgi to cell membrane. IL-2Rβ and TLR4 was also observed of E-selectin-dependent membrane translocation, which indicates broader this molecule's relevance in regulation of receptor membrane expression. Our findings identify a new crosstalk between E-selectin and immune cells in promoting

DOI: 10.1002/eji.201970400

assembly of functional cytokine receptors as an important new layer in innate activation and cytokine signaling.

P2381

Expressions of P2X7 purinoceptor and mast cell tryptase in patients with inflammatory bowel disease

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Objective: To explore the expressions of P2X7 purinoceptor and mast cell tryptase and analyze their correlation in the intestinal tissue and peripheral blood of patients with inflammatory bowel disease (IBD).
Methods: A total of 80 active IBD patients in the Second Affiliated Hospital of Zhengzhou University from December 2016 to March 2018 were collected. Their paraffin-embedded specimens, serum samples and results of fecal calprotectin(FC) were collected. The normal intestinal mucosa tissues from surgical specimens of 40 colorectal cancer patients were collected as tissue control, blood samples from 40 healthy volunteers were collected as serum control. The expression of P2X7 purinoceptor in intestinal mucosa were detected by immunohistochemistry, and the level of tryptase in serum were measured by ELISA.
Results: Compared with control group, the P2X7 purinoceptor in UC group was significantly increased (c^2=7.062, P=0.008; c^2=18.983, P< 0.05; c^2=32.216, P< 0.05). And the severe UC group was higher than mild UC group(c^2=7.242, P=0.007). The P2X7 purinoceptor in CD group was significantly increased(c^2=5.223, P=0.022; c^2=5.223, P=0.022; c^2=11.161, P=0.001). There was no statistical difference between CD groups. And the expression of tryptase in IBD group was significantly increased (P< 0.001). The severe IBD group was higher than mild and moderate IBD group. In addition, the expression of P2X7 purinoceptor was positively correlated with tryptase (UC: r=0.872, P< 0.05; CD: r=0.866, P< 0.05). And the expression of P2X7 purinoceptor was positively correlated with fecal calprotectin(r=0.543, P< 0.05).
Conclusion: P2X7 purinoceptor may participate in the development of IBD by activating mast cell to release tryptase.

P2382

Lentivirus-mediated over-expression of let-7b microRNA suppresses hepatic fibrosis in the mouse infected with Schistosoma japonicum

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Transforming growth factor-β (TGF-β) signaling pathway is documented to participate in liver fibrosis *via* multifactorial mechanisms. microRNA Let-7b (Let-7b) has been proved to alleviate cell fibrosis through regulating TGF-β receptor I (TβRI), but whether it is involved in Schistosomiasis liver fibrosis (SLF) has not been determined. In the present, SLF mice model was used to investigate Let-7b´s function and mechanism in SLF. We found that hepatic let-7b expression was continuously declined in SLF, accompanied by the induction of TGF-β pathway molecules (TGF-β1, TβRI), profibrogenic mediators (α-SMA, colla I), and Th1/Th2 cells response factors (IFN-γ, IL-4). When recombinant Lentivirus of let-7b (Lenti-let-7b) was transfected into S. japonicum-infected mice, the mice hepatic

DOI: 10.1002/eji.201970400

fibrosis was distinctly ameliorated, and TGF-β1, TβRI, α-SMA, and colla I expressions were remarkly decreased, mice serum IL-4 and IFN-γ levels were reduced. Similarly, over-expression of let-7b down-regulated the expression of TβRI in THP-1 cells transfected with let-7b mimics, while TβRI was up-regulated after treated with let-7b inhibitor. These findings suggested that let-7b is a negative regulator to SLF through downregulating TβRI, and inhibits Th1 and Th2 type cell immune response. This provides a novel potential therapeutic strategy for SFL prevention.
Keywords: Let-7b miRNA; Schistosomiasis liver fibrosis (SLF); Suppress; TβRI

P2383

Curcumin inhibits NF-kB maybe through PI3K pathway

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Objective: To investigate whether Curcumin inhibits NF-KB through PI3K pathway.
Methods: HepG2 cells were divided into two groups. When the cell fusion degree was 70%-80%, one group was not treated, and the other group was co-cultured with 80 uM Curcumin. After 24 hours, the levels of expression of PI3K, NF-kB protein and mRNA were detected by Western and QPCR.
Results: Compared with the untreated HepG2 cells, the expression both of PI3K, NF-kB protein and mRNA of HepG2 cells co-cultured with Curcumin were significantly inhibited and the levels of PI3K and NF-kB protein and mRNA in the Curcumin co-culture group were significantly lower than those in the untreated HepG2 cells ($p < 0.01$).
Conclusion: Our results indicate that Curcumin can significantly down-regulate the expression of PI3K and NF-kB in the levels of mRNA and protein. Curcumin down-regulate NF-kB effect of liver cancer cell growth maybe through PI3K pathway.

P2384

High indoleamine 2,3-dioxygenase is correlated with microvessel density and worse prognosis in breast cancer

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Indoleamine 2,3-dioxygenase (IDO) catalyzes the breakdown of the essential amino acid tryptophan into kynurenine, which makes it a key player in cancer immunotherapy. However, IDO has received a lot of attention due to its non-immune function, including regulation of angiogenesis. The purpose of this study was to investigate the effects of IDO on microvessel density (MVD) and to explore its prognostic role in breast cancer. Our results in breast cancer specimens showed that IDO expression was positively correlated with CD105 (MVD-CD105)-labeled MVD. The expression of IDO and MVD-CD105 were correlated with the initial TNM stage, histological grade and metastasis of tumor drainage lymph nodes (TDLNs). univariate analysis showed that patients with IDO expression or high MVD levels had a poorer prognosis than those without IDO expression or low levels of MVD. In the multivariate analysis, late TNM stage (stage III) was significantly associated with a shorter 5-year survival rate of PFS. And we examined the expression of IDO mRNA in breast cancer cell lines,and estimated IDO-enzyme activity. The results showed that the IDO expressed by MCF-7 cells has functional activity and can metabolize tryptophan. Human umbilical vein endothelial cells (HUVEC) treated with MCF-7 cell supernatant proliferated and expressed CD105, and the phenomenon was

DOI: 10.1002/eji.201970400

inhibited by IDO inhibitor 1-MT. These results suggest that IDO can promote angiogenesis in breast cancer and may be a potential target to inhibit angiogenesis.

P2385

Combination of atorvastatin and probucol enhances autophagy, leading to improvement in atherosclerotic plaque

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Background: Atorvastatin combined with probucol may improve atherosclerotic plaques, but it is unclear whether its effect is associated with autophagy, which can affect plaque stability.
We aimed to investigate the effect of atorvastatin combined with probucol on atherosclerotic plaques mediated by regulating autophagy levels.
Methods: Apolipoprotein-E-deficient (ApoE-/-) mice fed a high-fat diet were randomly divided into three groups (n = 8/each group): Control, Statin, and Combination (statin+probucol). Blood lipid levels were measured, and Hematoxylin and Eosin, and Orcein staining was conducted for plaque area and elastic fiber assessments. Immunohistochemical staining was performed using MOMA-2 and α-actin antibodies. Western blotting was performed to determine LC3Ⅱ/Ⅰ, P62, and Beclin-1 expression levels.
Results: The Combination group had fewer macrophages and higher percentage areas of elastic fibers in the plaque than the Control group. The Statin and Combination groups had more smooth muscle cells in the plaque than the Control group. Compared with the Control group, LC3Ⅱ/Ⅰ and Beclin-1 expressions were increased and P62 expression was decreased in the Combination group.
Conclusions: Atorvastatin combined with probucol could enhance autophagy more effectively than atorvastatin alone, which might be a potential mechanism for the improved atherosclerotic plaque.

P2386

Effect of wumei pills on inflammatory factors in type 2 diabetic rats based on TLRs/NF-κB signaling pathway

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Objective: Numerous studies have shown that activation of the NF-κB signaling pathway induces the expression of pro-inflammatory cytokines and accelerates the development of insulin resistance. Based on TLRs/NF-κB signaling pathways,this study investigated the regulatory effect of Wumei Pill on rats with type 2 diabetes model.
Methods: :Ten of the 60 rats were randomly selected as normal groups,and the remaining 50 were induced by a combination of streptomyzocoris abdominal injection after treated with high-sugar and high-fat emulsion for 8 weeks.The random average is divided into model group,metformin group, High, medium and low dose groups of Wumei Pills.The expressions of NF-κBp65 and TLR4 in colon were detected by Western-bloting, and the expressions of serum insulin, TNF-α and IL-10 were detected by ELISE.
Results: Compared with the normal group, the fasting blood glucose of the model group was increased (P< 0.01), the expression of NF-κBp65,TLR-4 and TNF-α increased(P< 0.01).The level of INS and IL-10 decreased (P< 0.01).Compared with the model group, the fasting blood glucose of Wumei Pills in high, middle and low groups and metformin group decreased(P< 0.01), NF-κBp65,TLR-4 and TNF-α decreased(P< 0.01).INS and IL-10 increased (P< 0.01).

DOI: 10.1002/eji.201970400

Conclusion: Wumei Pill can regulate fasting blood glucose and insulin levels in type 2 diabetic rats,reduce the expression of NF-κBp65 and TLR-4, decrease the level of inflammatory factor TNF-α, and promote the production of anti-inflammatory factor IL-10, thereby achieving the prevention and treatment of type 2 diabetes.

P2387

LncRNA HAND2-AS1 inhibits proliferation and promotes apoptosis of chronic myeloid leukemia cells by sponging with micRNA-1275

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Objective: The long noncoding RNA HAND2-AS1 is down-regulated and microRNA-1275 (miR-1275) is up-regulated in many types of cancers. However, the expressions of them and the relationship between HAND2-AS1 and miR-1275 in chronic myeloid leukemia (CML) remain unknown.
Materials and methods: Bone marrow samples from 30 CML patients and 10 healthy donors were collected, HAND2-AS1 and miR-1275 were detected by RT-PCR.
Results: Results showed that HAND2-AS1 was significantly downregulated than healthy control ($p< 0.05$), HAND2-AS1 expressions on stages of AP and BP were much lower than that of CP ($p< 0.05$). The miR-1275 expression was significantly upregulated than healthy control ($p< 0.05$), and the expressions in stages of accelerated phase (AP) and blast phase (BP) were much higher than that in the stage of chronic phase (CP) in CML($p< 0.05$). Furthermore, HAND2-AS1 was negatively correlated with miR-1275 in CML but not in healthy control ($p< 0.05$). After lentiviral HAND2-AS1 transfection, the HAND2-AS1 expression was significantly up-regulated while miR-1275 was significantly down-regulated($p< 0.05$). Furthermore, the mRNA and protein levels of Bcl-2, MMP-2 and MMP-9 were significantly down-regulated($p< 0.05$), while the expression of Caspase-3 was significantly up-regulated($p< 0.05$). Luciferase reporter assay showed that HAND2-AS1 was a target gene of miR-1275. After treating with miR-1275 inhibitor, HAND2-AS1 was significantly upregulated ($p< 0.01$) and cell proliferation was inhibited ($p< 0.01$).
Conclusions: HAND2-AS1 was downregulated and miR-1275 was upregulated in CML, and HAND2-AS1 inhibited proliferation and promoted apoptosis of CML cells by sponging with micRNA-1275, which might be a novel therapeutic target for CML.

P2388

Homoharringtonine contributes to imatinib sensitivity in chronic myeloid leukemia cell lines

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Objective: The purpose was to investigate the mechanism responsible for homoharringtonine (HHT) contributing to imatinib sensitivity in a chronic myeloid leukemia (CML) cell line.
Method: We established cell lines from a patient with CML at the time of first diagnosis and relapsed phase, and designated them as NPHA1 and NPHA2, respectively. Stable underexpressing EphB4 cells (NPHA2-EphB4-sh) were obtained. Leukemia cell lines were incubated by HHT. The activated signal proteins in cells were tested by Western blot.
Result: The EphB4 was overexpressed in IM-resistant NPHA2 in comparison with the NPHA1 cell line, but the expression of EphB4 mRNA and protein significantly decreased in knockdown NPHA2-EphB4-sh cells compared with NPHA2 and NPHA1 (P< 0.001) cell lines. NPHA2-EphB4-sh cells were sensitive to IM (IC50 0.93 mg/L), and NPHA2 showed IM resistance (IC50 5.45 mg/L) (P< 0.001).

DOI: 10.1002/eji.201970400

However, co-stimulation with HHT+IM decreased IC50 of NPHA2 cells to 1.17 mg/L (P< 0.001). Meanwhile, phospho-Rac1/cdc42 was significantly increased in NPHA2 cell compared to NPHA2-EphB4-sh (P< 0.001). HHT blocked the expressions of EphB4/RhoA.
Conclusions: The overexpression of EphB4 contributed to IM resistance in CML line cells. EphB4/RhoA may be a new marker of IM resistance. HHT contributed to IM gained more treatment advantages than IM alone by blocking EphB4/RhoA pathways.

P2389

Study of EphB4 mediated the resistance to dasatinib in chronic myeloid leukemia cell line

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Objective: It is still a tough problem for newly dasatinib (DAS)-resistance in the treatment of DAS. To determine the role and mechanism of EphB4 in DAS resistance in advanced chronic myeloid leukemia (CML), we explored the EphB4-mediated apoptotic and matrix microenvironment pathway in human CML and K562 cell lines.
Method: Heparinized bone marrow samples were obtained from enrolled five patients (2015-2017, newly diagnosed, identified as A to E and visits identified by number) at initial diagnosis (A1-E1) and in the DAS-resistance advanced phase (A2-E2). Meanwhile, highly DAS-resistant cells, named K562-R cells, were obtained from K562-W cells with increasing concentrations of DAS. Stable under-expressing EphB4 cells (K562-R-EphB4-sh) were obtained from K562-R cells by RNA interference. K562-W, K562-R and K562-R-EphB4-sh cells (10^8) were respectively injected subcutaneously on the dorsal surface of BALB/C nude mice to establish the xenografts models.
Result: The result showed that the mRNA/protein of EphB4 was overexpressed in the DAS-resistant A2-E2 in comparison with the A1-E1 human cell lines. Further, compared with K562-R cells, the expressions of EphB4 and p-Rac1/Cdc42 protein/mRNA were significantly downregulated in K562-R-EphB4-sh cells (F=618.11, P< 0.001). K562-R cells showed the highest DAS resistance (IC50 10.54±0.67 μg/ml), but K562-R-EphB4-sh cells became sensitive to DAS (IC50 1.02±0.1 μg/ml, F=828.35, P< 0.001). The expression of EphB4/p-RhoA/MCL-1 protein was gradually increased in the stimulating of EphrinB2-Fc, which partly made K562-R-EphB4-sh cells restore sensitivity to DAS (4.18±0.30μg/ml).
Conclusion: The present study found that a new DAS resistance pathway of EphB4 overexpression, which induced the resistance to DAS by activating RhoA/ROCK1/PTEN/MCL-1 signaling.

P2390

TAK1 is a druggable kinase for diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common form of lymphoma, and up to 30% DLBCL patients eventually died by using first-line chemotherapy regimens. Currently, Bruton tyrosine kinase (BTK) inhibitor (ibrutinib) is one of the most promising medicine in clinical trials for DLBCL, to which about 25% of patients with relapsed or refractory DLBCL are responsive. Thus, it is urgent to discover new druggable targets for DLBCL, especially for patients who are unresponsive to first-line chemotherapy and ibrutinib. Here, we found that MAP 3K7 (TAK1) is required for DLBCL survival. Inhibition of TAK1 by small molecule 5Z7 or genetic silence could massively induce deaths of DLBCL cells. Mechanistically, TAK1 inhibition could

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DOI: 10.1002/eji.201970400

dramatically reduce the nuclear factor kappa B (NF-κB) activity. Notably, ibrutinib-resistant DLBCL cells also respond to TAK1 inhibition. Database analysis showed that high expression of TAK1 in patients with DLBCL shows poor survival. A subtype of DLBCL patients showed that high expression of both TAK1 and BTK1 is poorly responsive to the current chemotherapy. Moreover, DLBCL cell line with high expression of both TAK1 and BTK1 is resistant to Dox. Simultaneously targeting TAK1 and BTK not only increases cellular toxicity of individual drug but also enhances the sensitivity to Dox. Taken together, we provide convincing evidence to show that kinase TAK1 is a druggable target in DLBCL.

P2391

BRD4 inhibitor tends to target BTK for suppression of ABC-DLBCL

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Activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) is the most common DLBCL lymphoma with an extremely poor 5-year survival. It features a chronic active BCR/NFκB signaling, which is required for survival of ABC-DLBCL and therefore serves as a promising therapeutic target. Here, we revealed that ABC-DLBCL cells are sensitive to BRD4/super-enhancer inhibitors such as JQ1 and I-BET-762. Mechanically, JQ1 and I-BET-762 inhibit the chronic active BCR/NFκB signaling through reduction of BTK transcription. JQ1 and I-BET-762 have minimal effect on other components in BCR/NFkB signaling. The inhibitor effect of JQ1 on BTK transcription is independent on super-enhancer, but likely due to disruption of BRD4 binding within BTK gene. BRD4 inhibition has synergic effect with BTK inhibitor Ibrutinib. Moreover, BRD4 inhibition can induce massive cell death in Ibrutinib-resistant ABC-DLBCL cells. These findings suggest a novel molecular mechanism by which JQ1 and I-BET-762 serve as an alternative approach to target BTK. Simultaneously targeting multiple mechanisms in BCR/NFκB signaling might increase therapeutic efficiency and overcome resistance in clinic. BRD4 inhibition might be a promising strategy for treatment ABC-DLBCL especially for Ibrutinib-resistant ABC-DLBCL.

P2392

Aggravation of helicobacter pylori stomach infections in stressed military recruits

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Objective: To investigate the effect of military stress on immune response and Helicobacter pylori stomach infections.
Methods: In this prospective, observational study, the Symptom Checklist-90 questionnaire was completed by military recruits before and following a 3-month basic training programme. H. pylori immunoglobulin (Ig)G levels, C^{14}-urea breath-test values and levels of cortisol, catecholamine, and certain humoral and cellular immune responses were measured before and after the basic training.
Results: For 60 military recruits, somatization, depression and paranoid ideation scores were significantly increased after, compared with before, basic training. Post-training H. pylori IgG detection revealed three additional cases of H. pylori infection. Post-training C^{14}-urea breath-test values were significantly higher compared with before training - thus suggesting higher levels of H. pylori colonization in the stomach. Post-training cortisol and catecholamine levels were increased, while serum IgG levels were decreased; complement component (C)3 and C4 levels remained unchanged. Post-training $CD4^+$ and $CD8^+$ T-cell percentages and the $CD4^+/CD8^+$ ratio were significantly reduced

DOI: 10.1002/eji.201970400

compared with before training. Serum interleukin (IL)-2 levels were lower and IL-10 levels were higher following training and there was a significant decrease in the IL-2/IL-10 ratio.
Conclusion: Military stress may reduce humoral and cellular immune responses and may aggravate the severity of H. pylori infection.

P2393

MS4A6D promotes NLRP3 inflammasome activation through inducing calcium mobilization in macrophages

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MS4A6D is a novel member of the MS4A superfamily (membrane-spanning 4-domain family, subfamily A) but its expression, distribution and biofunction are unclear. We here showed that *Ms4a6d*-deficient (*Ms4a6d*$^{-/-}$) mice are resistance to chemical induced acute colitis and attenuate the severity of lipopolysaccharide (LPS) caused endotoxic shock. MS4A6D restricts on tissue resident macrophages and dendritic cells. Functionally, MS4A6D not only acts as a store-operated calcium (SOC) channel that mediates extracellular Ca^{2+} influx upon endoplasmic reticulum (ER) calcium store-depletion, but also interacts with spleen tyrosine kinase (Syk) to activate phospholipase C-γ, which induces inositol 1,4,5-trisphophate (IP_3) biosynthesis and stimulates ER Ca^{2+} release under inflammatory conditions. Therefore, MS4A6D mediated Ca^{2+} mobilization amplifies the assembly, caspase-1 activation and IL-1β secretion following NLRP3 inflammasome stimuli, whereas, *Ms4a6d*$^{-/-}$ macrophages have opposite effects. These combined data highlight MS4A6D as a prospective target for treating NLRP3-associated inflammatory disorders.

Innate Receptors and Signaling

O105

Listeria hijacks host cell mitophagy through a novel mitophagy receptor to evade killing

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Cells utilize mitophagy to remove damaged or unwanted mitochondria to maintain homeostasis. Here we report that intracellular bacterial pathogen *Listeria Monocytogenes* (L.m) exploits host mitophagy to evade killing. We found that L.m induced mitophagy in macrophages through the virulence factor *Listeriolysin O* (LLO). We discovered that NLRX1, the only Nod-like receptor (NLR) family member with a mitochondria targeting sequence, contained an LC3-interacting region (LIR) and directly associated with LC3 through the LIR. NLRX1 and its LIR motif were essential for L.m-induced mitophagy. Either NLRX1 deficiency or mitophagy inhibitor increased mitochondrial reactive oxygen species production and consequently suppressed L.m survival. Mechanistically, L.m or LLO induced NLRX1 oligomerization to promote its LIR binding to LC3 for mitophagy induction. Our study identifies NLRX1 as a novel mitophagy receptor and discovers a previously unappreciated strategy by pathogens to hijack a host cellular homeostasis system for their survival.

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DOI: 10.1002/eji.201970400

O106

A novel terminal uridyltransferase controls uridylation and degradation of Regnase-1 mRNA to regulate TLR4-triggered inflammation

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The innate immune response is an important component of host defense against pathogenic infection. However, different levels of regulatory mechanisms, including post-transcriptional regulation, are needed to elaborately regulate the inflammatory response without causing harmful health effects. A novel terminal uridyltransferase (TUT) is known to control the fate and stability of RNAs by adding uridine tails to their 3' ends, but its exact function in regulating the innate immune response remains elusive. Here, we reveal that TLR4 activation induces this novel TUT expression, which in turn selectively regulates the production of a subset of cytokines, including Interleukin-6 (IL-6). The TUT mediates IL-6 expression by controlling Regnase-1 mRNA stability. Mechanistically, TLR4 activation causes the TUT to bind directly to the stem-loop structure on the 3'UTR of Regnase-1, prompting uridylation and degradation of Regnase-1 mRNA. Consistently, Regnase-1 mRNAs possess uridylated 3' ends and shorter poly(A) tails in TUT-proficient macrophages after TLR4 activation, whereas Regnase-1 transcripts in *Tut*$^{-/-}$ cells carry long poly(A) tails without uridylation. Together, our findings reveal the functional role of the TUT in sculpting TLR4-driven responses by modulating the mRNA stability of inflammatory mediators.

O107

Glycoprotein (Gn) and nucleocapsid protein (NP) of hantaviruses manipulate autophagy flux to restrain host innate immune responses

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Hantavirus infection, which causes severe zoonotic diseases with high mortality rates in humans, has become a global public health concern. The explicit mechanisms of how hantaviruses regulate the host autophagy pathway and the functions of this modulation during infection remain elusive. Here, we demonstrate that Hantaan virus (HTNV), the prevalent prototype of the hantavirus in Asia, can restrain innate immune responses by manipulating host autophagy flux. HTNV induces complete mitophagy at the early stage of infection but incomplete autophagy at the late stage, and it is highly involved with the viral glycoprotein (Gn) and nucleocapsid protein (NP), respectively. Gn translocates to mitochondria and interacts with the Tu translation elongation factor mitochondrial (TUFM), recruiting microtubule-associated protein 1 light chain 3B (LC3B) and promoting mitophagy. Gn-induced mitophagy inhibits type I interferon (IFN) responses by degrading the mitochondrial antiviral signaling protein (MAVS), resulting in increased viral replication at the early stage of infection. Additionally, we found that NP competes with Gn for binding to LC3B, which inhibits Gn-mediated autophagosome formation, and interacts with the synaptosome-associated protein 29 (SNAP29), which prevents sensitive factor attachment protein receptor (SNARE) protein-mediated autophagosome-lysosome fusion. Thus, NP disturbs the autophagic degradation of Gn, promoting viral packaging and propagation at the late stage of infection. Both in *vivo* and in *vitro* experiments show that inhibiting autophagy at the early infection stage can enhance host IFN responses and restrict HTNV replication. These findings highlight how hantaviruses repurpose host autophagy and evade innate immune responses for their life cycle and pathogenesis.

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DOI: 10.1002/eji.201970400

O108

Identification and characterization of vaccinia virus proteins which inhibit autophagy

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Autophagy is crucial to fight against intracellular pathogens, like viruses by participating in induction of immune responses (e.g.: innate via type I IFN expression or adaptive via MHC-I and -II antigen presentation). During infection with highly attenuated Modified Vaccinia Virus Ankara (MVA), LC3 lipidation is strongly induced. Autophagy is required for antigen presentation via MHC-II in MVA-infected DCs to activate $CD4^+$ T cells. We observed that autophagy was inhibited during infection with virulent strain Western Reserve (WR). Molecular mechanisms behind MVA-induced or WR-inhibited autophagy are poorly characterized. We demonstrated that UV-inactivated WR has lost its ability to inhibit activation of the autophagy cascade indicating the requirement of viral gene expression. We aim to identify and characterize genes that are counteracting the above mentioned pathway during infection with the virulent strain. For identification we used a siRNA screening library derived from strain WR. DsRed-LC3-eGFP expressing HeLa cells were used as an autophagy reporter cell line that is suitable for FACS and confocal microscopy detection. Analysis of LC3 lipidation during vaccinia siRNA library screening showed us different results depending on whether cells were infected with the virulent WR alone or together with MVA as co-infection. This outcome could be explained by differences in the genomic repertoire of the two virus strains with MVA having lost or truncated multiple genes. To investigate this hypothesis, we created MVA-knock in and WR-knock out viruses for identified target genes that could play a role in the inhibition of autophagy during WR infection.

O109

HER2 disarms cellular damage surveillance via AKT1-mediated perturbation of the STING signalosome

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Sensing cytosolic DNA through the cGAS-STING pathway constitutes a widespread innate immune mechanism to monitor cell damage and microbe invasion. Evading this surveillance is crucial in tumorigenesis, but the process remains largely unexplored. Here, we found that the receptor tyrosine kinase HER2/ErbB-2/Neu potently inhibits cGAS-STING signaling and prevents cancer cells from cytokine secretion, entering senescence, and undergoing apoptosis. HER2, but not the related receptor EGFR, associates strongly with STING and recruits AKT1 to directly phosphorylate TBK1, which preventing the TBK1-STING association and TBK1 K63-linked ubiquitination, thus inactivating the STING signalosome. Unexpectedly, we observed that DNA sensing robustly activates the HER2-AKT1 axis, resulting in negative feedback. Accordingly, genetic or pharmacological targeting of the HER2-AKT1 axis augments DNA sensing, antiviral response, and damage-induced cellular senescence and apoptosis, and governs STING-mediated antiviral and antitumor immunity in mice. Thus, our findings reveal a critical function of the oncogenic pathway in innate immune sensing and unexpectedly connect HER2-AKT1 signaling to surveillance of cellular damage and antitumor immunity.

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DOI: 10.1002/eji.201970400

O110

A division of labour for the type Ⅰ Interferon and apoptosis induction after viral infection

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A cell-to-cell variability is often observed even in isogenic cell populations in response to uniform physiological stimuli. Upon viral infection, mammalian cells trigger antiviral innate immune responses such as type Iinterferon (IFN) production and apoptosis, although a cell-to-cell variability of these responses has been elusive. In this study, by real-time monitoring of IFN production and apoptosisat a single cell level, we found that viral infection or its mimic, cytoplasmic double stranded RNA stimulation, induced IFN production without apoptosis in some cells and induced apoptosis without IFN production in other cells within the clonal fibroblasts, irrespective of the level of stimulation.Surprisingly, very few cells triggered both responses, suggesting that IFN and apoptosis responses are regulated in a mutually exclusive manner.Notably, members of the IAP family were highly expressed and played a key role in preventing apoptosis in the IFN-expressing population. Our study therefore reveals an unexpected division of labour between infected host cells, which may contribute to protection of IFN-expressing cells from being deprived by apoptosis.

O111

NOD2 inhibits tumorigenesis and increases chemosensitivity of hepatocellular carcinoma by targeting LKB1/AMPK/mTORC1 axis

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NOD2 is a recognized innate immune sensor which can initiate potent immune response against pathogens. However its role in cancer is not well understood. Here we investigated the role of NOD2 in the development of hepatocellular carcinoma (HCC) and our data indicated a tumor suppressor role of NOD2 in the progression of HCC. We demonstrated that NOD2 deficiency promoted hepatocarcinogenesis in DEN/CCL4 induced HCC mice model and xenograft tumor model. We further demonstrated that NOD2 significantly inhibited the proliferation, invasion and colony formation capabilities of HCC cells. Clinical investigation showed that NOD2 expression was completely lost or significantly downregulated in clinical HCC tissues, and loss of NOD2 expression was significantly correlated with advanced disease stages. Further investigation showed that NOD2 inhibited malignant behaviors and enhanced chemosensitivity of HCC cells through activating LKB1/AMPK/mTORC1 signaling pathway by directly binding with AMPK-LKB1 complex. AMPK was the intracellular energy sensor and we verified that NOD2 exerted its anti-tumor effect through AMPK pathway mediated energy switch from glycolysis to oxidative phosphorylation. Thus,these findings provided a direct link between NOD2, a well-known innate immune sensor, and AMPK, a potent protein kinase mainly associated with regulation of metabolism; and it indicated a novel metabolic regulatory role of NOD2 in HCC cells.
Funding: This study was supported by the National Natural Science Foundation of China (No.81672391) and the Major Innovation Project of Shandong Province (No.2018CXGC1217).

DOI: 10.1002/eji.201970400

O112

B7H6, an immunoligand for natural killer cell receptor NKp30, not only activates NK cells, but also demonstrates inhibitory effects on tumor cell proliferation and migration

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B7 homologue 6 (B7H6) is a ligand for the NK cell activating receptor NKp30. Current research suggests that this ligand is selectively expressed on several cancer types, thus making it an important molecule to be studied. B7H6 binding to NKp30 results in NK cell-mediated killing of B7H6-expressing tumor cells. However, it is also interesting to note that the intracytoplasmic domain of B7H6 contains an ITIM along with SH2 and SH3 motifs, suggesting this ligand may be involved in intracellular signaling pathways. To investigate this idea, we used B7H6 positive representative cervical cancer cell lines (SiHa and HeLa), which were stimulated with recombinant soluble NKp30, and evaluated inhibitory effects using the impedance RTCA system for cell proliferation, scratch method for cell migration, and cytometry for apoptosis. After the addition of soluble NKp30 to the B7H6-expressing tumor cells, we found significantly decreased tumor cell proliferation, as well as a decrease in migration rate. We also showed an elevated level of soluble B7H6 in the cell supernatant, which may be a strategy of escaping from NK cell cytotoxicity. In conclusion, we find that the ligand B7H6 may have a previously unknown inhibitory effect on tumor cells and may prove an interesting target for cancer therapy.

O377

Redox homeostasis maintained by GPX4 licenses STING activation in innate immunity during viral infection

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Optimal activation of innate immunity is crucial for controlling infectious diseases and tumors. Stimulator of interferon genes (STING) is a central receptor of innate immunity, which senses the second messenger, cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) (cGAMP) produced by the key DNA sensor cyclic GMP-AMP synthase (cGAS). cGAS recognizes cytosolic pathogen-derived DNA or self-DNA from genomic DNA damage, and then activates STING-dependent signaling. cGAS-STING activation triggers the production of type I interferons and proinflammatory cytokines, and plays fundamental roles in host against viral infection and mediating DNA damage-induced inflammation. Redox homeostasis is a vital component of a physiological cellular steady state. However, how intracellular redox state regulates STING signaling remains unclear. Here we show that cellular redox homeostasis maintained by glutathione peroxidase 4 (GPX4) is required for STING activation. GPX4 deficiency or inhibition enhances cellular lipid

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DOI: 10.1002/eji.201970400

peroxidation during viral infection and thus specifically inhibit cGAS-STING dependent expression of type I interferons. Concordanatly, GPX4 deficiency or inhibition inhibits HSV-1 induced innate antiviral immune responses and promotes HSV-1 replication *in vivo*. Mechanistically, GPX4 inactivation promotes the production of lipid peroxidation, such as 4-HNE, which promotes carbonylation of STING and inhibits its translocation from endoplasmic reticulum (ER) to Golgi complex. Our results demonstrate how lipid peroxidation, a physiological process during stress (such as viral infection), specifically atteunates cGAS-STING dependent innate immune response, and suggest GPX4 licensed STING activation by maintaining cellular redox homeostasis of lipid. Our work uncovers a novel mechanism of STING activation and suggests a new approach for modulating STING-dependent immunopathologies.

O378

Leishmania donovani modulates host macrophage polarization and innate immune response via lipophosphoglycan elicited TLR2 mediated TPL2/COT signaling switch for parasite survival

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TLR receptors of macrophage are essential in immunogenic response to *Leishmania donovani* infection. TLR2 and TLR4 are extensively involved in the entire process. Although pathways supporting switch in TLR2 responses during *L.donovani* infection and contribution of its antigenic molecules in the process is unknown. Our work discusses how *L.donovani* subtly regulate the macrophage polarization and influence the signaling behavior of TLR2 elicited by its antigenic molecule lipophosphoglycan (LPG) to establish infection. Initial in-vivo study reveals gradual increase in M2 type macrophage polarization during *Leishmania donovani* infection establishment. TLR1,2 and 6 silencing profile depicts slight increase in T-reg population on TLR2 silencing compared to infected non silenced BALB/c mice model. Role of TLR2 was further corroborated as TLR2 blocking hindered ERK phosphorylation without affecting phospho-P38 level. TPL2/COT inhibition too impacted ERK phosphorylation, leaving phospho-P38, phospho-Akt level undeterred citing TPL2 as signaling switch during infection. *Leishmania* surface molecule, LPG stimulation induces TLR2 expression. Whereas masking of LPG affects TLR2 signaled anti-inflammatory response. The involvement of TLR2-LPG is further confirmed via computational approach. Their interaction and molecular dynamics studies confirmed the length of phosphoglycan core of LPG actively modulating the ligand-receptor binding strength as well as TLR2 anti-inflammatory signaling for parasite survival.Thus *L.donovani* regulates macrophage polarization and initiates TLR2 mediated signaling via TPL2/COT with its surface antigenic molecule LPG, where the metacyclic LPG (phosphoglycan repeats) -TLR2 interaction pattern is significant in the pathogenesis.

O379

Sirtuin 5 promotes antiviral response by demalonylating DDX3

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DOI: 10.1002/eji.201970400

RIG-I-like receptors (RLRs) sense viral dsRNA or ssRNA in the cytosol to facilitate IFN-I expression through adaptor protein MAVS, in which the molecular mechanism remains further to be elusive. Here, we show that Sirtuin5 (SIRT5) promotes antiviral response and signaling by functional screening of Sirtuin family. SIRT5 deficiency attenuates antiviral innate immune in vivo and in vitro viral infection model. Mechanical screening revealed that SIRT5 interacted with DDX3 and demalonylated DDX3 on lysine, which increased the RIG-I and MAVS complex formation. Mutant lysine 118 on DDX3 increased IFNβ production and abrogated SIRT5 overexpression function in antiviral response. Thus we found a novel positive regulation mechanism by SIRT5 in anti-viral innate immunity responses and provided new potential targets of antiviral drugs.

O380

The otubain YOD1 suppresses aggregation and activation of the signaling adaptor MAVS through LYS63-linked deubiquitination

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MAVS is a critical adaptor required for activating an innate antiviral immune response against viral infection. The activation of MAVS requires modification of the Lys63-linked ubiquitination and formation of prion-like aggregates. However, the molecular mechanisms regulating MAVS activity remain largely obscured. In this study, we identified a deubiquitinase YOD1, also known as a member of the ovarian tumor family, as a negative regulator of MAVS activation in both human and murine cells. YOD1 was recruited to mitochondria to interact with MAVS through its UBX and Znf domains after viral infection. Subsequently, YOD1 cleaved the K63-linked ubiquitination and abrogated the formation of prion-like aggregates of MAVS, which led to attenuation of IRF3, P65 activation, and IFN-β production. Knockdown of YOD1 potentiated IRF3 and P65 activation, IFN-β production, and antiviral innate immune response to RNA virus. Our findings thus provided, to our knowledge, novel insights into the regulatory cascade of the cellular antiviral response through YOD1-mediated K63-linked deubiquitination and aggregation of MAVS.

O381

TLR5 signaling in murine bone marrow induces hematopoietic progenitor cell proliferation and improves survival from radiation

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Bacteria flagellin is a potent stimulator of innate immune responses by activating toll-like receptor 5 (TLR5) and Nod-like receptor C4 (NLRC4), along with NF-κB activation and several inflammatory cytokines production. Mice treated with flagellin are protected against several foreign challenges including virus infection, inflammation and radiation. The exact mechanism remains to be investigated.

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DOI: 10.1002/eji.201970400

In the current project, we have studied the mechanism how flagellin protect mice from radiation-caused death. When mice were treated with flagellin, within 24 hours, we observed a 3-fold increase of proliferation of bone marrow $Lin^{-}Sca1^{+}Kit^{+}$ cells (LSK cells) that are enriched in hematopoietic stem cells (HSCs) and multipotent progenitor cells (MPPs). Treatment of mice bone marrow cells ex vivo with flagellin also induced proliferation of LSK cells. Using TLR5 and NLRC4 KO mice, we found the increase of LSK cells proliferation is dependent on TLR5 but not NLRC4. Further analysis showed MPPs rather than HSCs proliferation were induced by flagellin. Flagellin treatment stimulated LSK cells proliferation by inducing a 10-fold increase in type 3 MPP (MPP3), a distinct myeloid-biased MPP subset. Adoptively transfer of FACS-sorted flagellin-induced MPP3 cells along with limited number of whole bone marrow cells to lethally irradiated mice dramatically increased the survival rate of the bone marrow transplanted mice compared with the group without MPP3. Further analysis showed that such cells predominantly repopulated the neutrophil compartment. These findings suggest flagellin-induced bone marrow MPP3 cells can induce neutrophil production and mobilization, help cure radiation-caused neutropenia and improve survival after radiation.

O382

$P2X_7$ is a high affinity receptor for ATP in antigen presenting cells and triggers the inflammation associated with hypertension

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The $P2X_7$ receptor is a nucleotide-gated ion channel. It is generally thought that the $P2X_7$ receptor has low affinity for ATP so that millimolar ATP is widely used to study $P2X_7$-mediated actions in immune cells. However, **the studies of ATP-$P2X_7$ affinity were mostly performed in $P2X_7$ transfected cell lines, which may not reflect the biological activity of $P2X_7$ in antigen presentation cells (APCs).** Hypertension is accompanied with low-grade systemic inflammation and T cells appear critical in disease progression. We found that **hypertensive mice have increased antigen specific T cell responses. A root cause for this is plasma ATP levels, which rise to about 3 µM after the onset of hypertension and stimulate APC upregulation of CD86.** Three micromolar ATP could efficiently upregulate CD86 expression and IL-1β secretion in APCs and these effects could be abrogated by $P2X_7$ antagonists but not antagonists against other purinergic receptors. However, unlike the effect of millimolar ATP on $P2X_7$, prolonged exposure to 3 µM ATP did not make APCs permeable to larger molecules nor leads to cell death. In vitro, 3 µM ATP triggers Ca^{2+} influx in dendritic cells and macrophages but not T cells nor APCs derived from $P2X_7$-deficient mice. Mechanistic analyses unveil that **APCs contain high amount of lysophosphatidylcholine which increases the sensitivity of $P2X_7$**. Pharmacological or genetic ablation of $P2X_7$ activity suppressed the T cell overactivity and blood pressure increase associated with hypertension. Thus, **$P2X_7$ is a high affinity receptor for ATP in APCs and mediates the inflammation associated with hypertension.**

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DOI: 10.1002/eji.201970400

O383

Plant defensin γ-thionin modulates the TLR2 pathway and histone deacetylase activity in bovine mammary epithelial cells infected with Staphylococcus aureus

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Defensins constitute one of more important groups of host defense peptides. Besides their antimicrobial activity, defensins have immunomodulatory properties, which have been mainly described for mammal defensins, but similar effects for plant defensins remain unknown. Previously, we showed that the defensin γ-thionin from *Capsicum chinense* reduces the *Staphylococcus aureus* internalization into bovine mammary epithelial cells (bMECs) while induces Toll-like receptor 2 (TLR2) and modulates the inflammatory response. Using this model, the objective of this work was to analyze the effect of γ-thionin on TLR2 pathway in bMECs infected with *S. aureus* and to determine if it could modulate epigenetic marks. First, we analyzed the activation status of MAPKs related to TLR2 pathway by flow cytometry. According to the results, pretreated-bMECs with γ-thionin (100 ng/ml) reduced the basal activation of p38 and ERK1/2 (~3 fold), but JNK and ERK1/2 activity was increased (~1.5-fold). Also, infected bMECs induced p38 but this effect was reversed by γ-thionin; whereas ERK1/2 activity was reduced by infection but stimulated by γ-thionin. Interestingly, γ-thionin reduced the activation of FAK and AKT kinases ~50% (related to bacterial internalization). Furthermore, γ-thionin induced the activation of transcriptional factors from inflammatory response in bMECs, highlighting EGR, E2F-1, AP-1 and MEF; which were turn off by bacteria. All these effects were related to the chromatin modulation due that γ-thionin induced the activation of histone-deacetylases (HDACs, ~50%) at 12 h in infected bMECs. Altogether, these results demonstrated by the first time that a plant defensin interferes with inflammatory signaling pathways in mammalian cells.

O384

USP18 regulates the antiviral innate immunity through promoting the K63-linked polyubiquitination of MAVS

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During the infection of RNA viruses, the activation of MAVS, a key adaptor molecule in RLR signaling, is particularly important for antiviral signaling transduction. However, the molecular mechanisms regulating the activation of MAVS is not completely understood. Ubiquitination, with its versatile functions associated with multiple types, plays a central role in modulating the type I interferon response. Previously, our lab reported that E3 ubiquitin ligase TRIM31 catalyzes the K63-linked polyubiquitination of MAVS, promoting its aggregation and ensuing activation. In this study, we identify a deubiquitinase USP18 facilitating the interaction between TRIM31 and MAVS. We report that (i) Overexpression of USP18 significantly enhances the K63-linked polyubiquitination and aggregation of MAVS, rather than the stability of MAVS; (ii) Depletion of USP18 in THP-1 or mouse peritoneal macrophage cells affect the expression and production of type I interferons following the infection of SeV or EMCV, suggesting a positive role of USP18 in modulating antiviral response. (iii) USP18 binds with MAVS instead of other signaling molecules in RLR pathway. More importantly, this interaction occurs in mitochondria and is elevated upon viral infection; (iv) The enzymatic activity of USP18 is dispensable for the increase of K63-linked polyubiquitination of MAVS, which is abrogated by

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DOI: 10.1002/eji.201970400

knockdown or knockout of TRIM31. (v) USP18 also interacts with TRIM31 *in vitro* and *in vivo*, and the interaction between TRIM31 and MAVS is enhanced in the presence of USP18. These results indicate that there are multiple levels of regulation on the activation of MAVS, emphasizing its importance in innate antiviral signaling.

LB002

CD89 acts as an innate receptor for bacteria and protects from sepsis

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Direct bacterial recognition by innate receptors is crucial for bacterial clearance. This finding shows that the IgA receptor CD89 is a major innate receptor that directly binds bacteria independently of its cognate ligands IgA and c-reactive protein. This binding is only partially inhibited by serum IgA and induces bacterial phagocytosis by $CD11c^+$ dendritic cells and monocytes and/or macrophages, suggesting a physiological role in innate host defense. Blood phagocytes from common variable immunodeficiency patients bind, internalize, and kill bacteria in a CD89-dependent manner, confirming the IgA independence of this mechanism. In vivo, CD89 transgenic mice are protected in two different models of sepsis: a model of pneumonia and the cecal ligation and puncture (polymicrobial model of infection).These data identify CD89 as a first-line innate receptor for bacterial clearance before adaptive responses can be mounted. Fc receptors may emerge as a class of innate receptors for various bacteria with pleiotropic roles.

LB040

STING-mediated IFI16 degradation negatively controls type I interferon production and p53-dependent apoptosis

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Gamma-interferon-inducible protein-16 (IFI16), a key DNA sensor, triggers downstream STING-dependent type I interferon (IFN-I) production and p53-dependent apoptosis. However, it is still unclear how to negatively regulate IFI16 to avoid excessive IFN-I production and autoimmunity. Here we find that STING directly interacts with IFI16 and facilitates IFI16 degradation via the ubiquitin-proteasome pathway by recruiting E3 ligase TRIM21. We have also identified the key sites which lead to STING-mediated IFI16 ubiquitination and degradation. Comparing to IFI16-WT, higher level of viral DNA-triggered IFN-b was detected in the cells transfected with IFI16-K3/4/6R, an IFI16 mutant resistant to degradation. In addition, overexpression of STING significantly reduces Ser392-phosphorylated p53 and apoptosis in *WT* U2OS cells rather than in *IFI16*$^{-/-}$ cells, suggesting that STING-mediated IFI16 degradation suppresses p53-dependent apoptosis, and thus may promote DNA virus-induced tumorigenesis. Our study has described that STING-mediated negative feedback regulation of IFI16 controls both IFN-I production and p53-dependent apoptosis.

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DOI: 10.1002/eji.201970400

LB076

Signaling of infected macrophages through Clec4e: an innovative strategy to restrict the survival of *Mycobacterium tuberculosis*

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Host-directed therapies are gaining considerable impetus following the observation that there is an enhancement in the emergence of drug-resistant strains of pathogens due to antibiotics therapy. Therefore, there is an urgent need to exploit alternative and novel strategies directed to host molecules to successfully restrict the infections. The C-type lectin receptor (Clec4e) and Toll-like receptor (TLR-4) expressed by the host cells are the first line defense system to encounter pathogens. Therefore, for the first time, we exploited the role of signaling of macrophages through Clec4e in association with TLR-4 ($C_4.T_4$) in restricting the proliferation *Mtb*. Intriguingly, we observed significant improvement in the host immunity and decline in the bacterial load in the lungs of *Mtb*-infected mice and Guinea pigs. Further, intracellular killing of *Mtb* was achieved with 10-fold lesser dose of isoniazid or rifampicin in conjunction with $C_4.T_4$ than the drugs alone. Activation of NF-κB, phosphorylation of PI3K, STAT-1 and lysosome biogenesis, resulted in repressed *Il-10* and *Il-4* genes expression and enhanced autophagy. For the first time, the present finding unveils the novel role of Clec4e in inducing autophagy. This study suggests a novel immunotherapy involving Clec4e in conjunction with TLR-4 in restricting the survival of *Mtb* through autophagy.

P0011

The cyclopeptide astin C specifically inhibits the innate immune CDN sensor STING

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cGAS-STING signaling is essential for innate immunity. Its misregulation promotes cancer or autoimmune and autoinflammatory diseases, and it is imperative to identify effective lead compounds that specifically downregulate the pathway. We report here that astin C, a cyclopeptide isolated from the medicinal plant Aster tataricus, inhibits cGAS- STING signaling and the innate inflammatory responses triggered by cytosolic DNAs. Moreover, mice treated with astin C are more susceptible to HSV-1 infection. Consistently, astin C markedly at- tenuates the autoinflammatory responses in Trex1/BMDM cells and in Trex1/ mouse autoimmune disease model. Mechanistically, astin C specifically blocks the recruitment of IRF3 onto the STING sig- nalosome. Collectively, this study characterizes a STING-specific small-molecular inhibitor that may be applied for potentially manipulating the STING- mediated clinical diseases.

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DOI: 10.1002/eji.201970400

P0012

CD38 regulate LPS induced acute kidney injury by TLR4-NFκB-IFN-γ signaling pathway

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Gram-negative bacterial sepsis accounts for up to 50% worldwide sepsis that causes hospital mortality. Acute kidney injury (AKI), a common complication of Gram-negative bacterial sepsis, is caused by Toll-like receptor 4 (TLR4) activation. Lipopolysaccharide (LPS) is an endotoxin in Gram-negative bacteria and is recognized specifically by TLR4, which initiates innate immune response. Also, TLR4 signaling pathway activation is essential in response to LPS infection. CD38 is one of well-known regulators of innate immunity, whose dysregulation contributes to sepsis. Many studies have proved that an attenuated Gram-positive bacterial induces sepsis in CD38-blocking model. However, the pathogenesis of Gram-negative bacterial-induced sepsis in $CD38^{-/-}$ mice model remains unclear. The aim of this study is to investigate whether kidney injury is still attenuated in LPS-induced $CD38^{-/-}$ sepsis model and identify the potential mechanism. We assess the severity of kidney injury related to proinflammatory cytokines expression (IFN-*γ*, TNF-*α*, IL-1*β* and IL-6) in WT and $CD38^{-/-}$ mice. Our results showed more aggravated kidney damage in $CD38^{-/-}$ mice than WT mice, accompanied with an increase of pro-inflammatory cytokines expression. In addition, compared with $CD38^{-/-}TLR4^{mut}$ mice, we found an increase of TLR4 expression and mRNA expression of these cytokines in the kidney of $CD38^{-/-}$ mice, although only increased IFN-*γ* level was detected in serum. Taken together, these results demonstrated that increased TLR4 expression in $CD38^{-/-}$ mice could contribute to the aggravation of AKI might through boosting the production of IFN-*γ*.

P0013

IFI16 regulates HTLV-1 replication through promoting HTLV-1 RTI-induced innate immune responses

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Interferon (IFN)-inducible protein 16 (IFI16) regulates human immunodeficiency virus replication by inducing innate immune responses as a DNA sensor. Human T-lymphotropic virus type 1 (HTLV-1), a delta retrovirus family member, has been linked to multiple diseases. Here, we report that IFI16 expression is induced by HTLV-1 infection or HTLV-1 reverse transcription intermediate (RTI) ssDNA90 transfection. IFI16 overexpression decreases HTLV-1 protein expression, whereas IFI16 knockdown increases it. Furthermore,
the knockdown of IFI16 is followed by impaired innate immune responses upon HTLV-1 infection. In addition, IFI16 forms a complex with ssDNA90 and enhances ssDNA90-triggered innate immune responses. Collectively, our data suggest a critical role for IFI16 during HTLV-1 infection by interacting with HTLV-1 RTI ssDNA90 and restricting HTLV-1 replication.

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DOI: 10.1002/eji.201970400

P0014

Modulation of cellular immune response by OASL1 during virus infection via enhancing the MDA5 and MAVS interaction

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Virus infection induces type I interferons (IFNs) that in turn exert their pleiotropic effects through inducing a large number of interferon-stimulated genes (ISGs). The IFN-induced 2',5'-oligoadenylate synthetases-like (OASL) have been identified as a member of the ISGs characterized by the ability to regulate the innate immunity signaling pathway. In this study, we characterized the *tOASL1* of the Chinese tree shrew (*Tupaia belangeri chinensis*), a small mammal genetically close to primates and has the potential as animal model for viral infections. Overexpression of tOASL1 significantly inhibited DNA virus and RNA virus replications in tree shrew primary renal cells. Interestingly, the antiviral activity of tOASL1 are independent of the canonical RNase L -dependent pathway. Furthermore, coimmunoprecipitation assays revealed that tOASL1 interacted with MDA5 and MAVS and further positively regulating the type I IFN signaling via stabling the MDA5 and MAVS interaction. Collectively, our results revealed a novel mechanism by which tOASL1 contributes to host antiviral responses by enhancing the MDA5 and MAVS interaction.

P0015

MicroRNA-33/33* inhibit the aggregation and activation of MAVS through AMPK-mediated mitophagy in antiviral innate immunity

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Upon viral infection, RIG-I-like receptors (RLRs) sense virus dsRNA and activate the mitochondrial adaptor MAVS to form prion-like aggregates, to further elicits the innate antiviral response.Antiviral innate immunity serves as a prominent part of host defense against pathogens and must be tightly regulated at the cellular level. Being a vital orchestrator in cholesterol homeostasis, microRNA-33/33* (miR-33/33*) have been widely investigated in cellular energy metabolism. However, whether they have a role in antiviral innate immunity is largely unknown. Here we reported that VSV stimulation decreased the expression of miR-33/33* and their host gene *srebf-2* through an interferon dependent manner in macrophages. MiR-33/33* negatively regulated antiviral innate immune response where overexpression Overexpression of miR-33/33* resulted in an impaired innate antiviral signalinginnate immunity, enhancing virus load and morbidity whereas attenuating Type I interferons production both *in vitro* and *in vivo*. Notably, we found that miR-33/33* specifically prevented the mitochondrial signaling adaptor MAVS from forming prion-like aggregates by targeting the cellular energy sensor AMPK, which resulted in the perturbations of a mitophagy mediated mitochondrial repair process, process. This impaired mitochondrial homeostasisthus disturbing mitochondrial homeostasis and finally impeding inhibited MAVS activation. Our findings establish miR-33/33* as a negative modulator of the RNA virus triggered innate immune response and identify a previously unknown regulatory mechanism between mitochondrial homeostasis and the innate immune response.

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DOI: 10.1002/eji.201970400

P0016

Structural basis for the oligomerization of innate immune receptor MDA5

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MDA5 is an important pattern-recognition receptor in the innate immune system, capable of sensing viral RNA and activating immune signals, protecting people from various infections and diseases. People will be affected by encephalomyocarditis virus infection when MDA5 disorders, and the over-activation of MDA5 will produce lupus and other autoimmune diseases. Ubiquitination is essential for the oligomerization and activation of the MDA5. However, due to the lack of structural information, the molecular mechanism of oligomerization of MDA5 remains unclear. This subject aims to solve the structures of MDA5 CARD domain with and without ubiquitin, combining functional experiments to elucidate the molecular mechanism of oligomerization of MDA5 and activation of downstream signaling pathways. The crystal structure of MDA5 CARD1 domain has been solved. MDA5 2CARD and CARD2 domain proteins are purified and crystals are growing. The state of the 2CARD domain protein without ubiquitin was detected by static light scattering and negative dyeing electron microscopy. Then, the interaction between MDA5 CARD domain and ubiquitin will be detected *in vitro*, and the structural analysis of the complex and related functional experimental studies will be performed. These studies will provide more structural information for understanding MDA5 antiviral signal activation mechanism.

P0017

The regulatory and mechanistic study of β-arrestin 2 in anti-viral immune responses

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Infectious diseases induced by viruses cause more than 14 million deaths every year, which raise an urgent requirement to study their pathogenesis. Previous studies have suggested that β-arrestin 2 could regulate GPCR signaling and immune responses through multiple ways, while its role in virus infection remains unclear. Our experiments showed that the expression of β-arrestin 2 decreased during HSV-1 and VSV infection. β-arrestin 2 could increase the production of cytokines, type Ⅰ interferon, and reduce the intracellular viral loads. Meanwhile, the survival rate of β-arrestin 2 knock out mice are much higher than wild type mice after virus infection. Mechanically, β-arrestin 2 interacted with TBK1, TRAF3, cGAS and promote the activation of type Ⅰ interferon pathway. β-arrestin 2 promoted k63-linked ubiquitination of TBK1 and TRAF3, which induced the activation of TBK1 and TRAF3, while had no effect on k48-linked ubiquitination. AS for cGAS, β-arrestin 2 facilitated k63-linked ubiquitination of cGAS, while cGAS induced the degradation of β-arrestin 2 during late stage of infection, which suggest the role of cGAS in maintaining the homeostasis of β-arrestin 2. In this project, we have investigated the antiviral function of β-arrestin 2 at molecular, cellular, animal levels. These results will provide a new molecular basis for infection and immunity study, and open a new window for the treatment of infectious diseases and the development of novel drugs, which has important application prospects and values.

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DOI: 10.1002/eji.201970400

P0018

Viral infection upregulated PRMT6 expression promotes immune escapes by interrupting TBK1-IRF3 signal complex formation

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Host defend against viral infection by producing type-I interferon, in which the TBK1-IRF3 signal complex formation has a central role in antiviral innate immunity to DNA and RNA virus. Protein arginine methyltransferases (PRMTs) play diverse biological roles and are specifically involved in immune cell development and inflammation. However, their role in antiviral innate immunity has not been elucidated. Here, we screened the antiviral function of nine mammalian PRMTs by RNA-I and detecting the IFN-β expression and found that the inhibitory role of PRMT6 in antiviral innate immune response. PRMT6 protein levels was upregulated upon viral infection. PRMT6-deficient mice exhibited enhanced antiviral innate immunity with increased IFN-β production and viral clearance. The TBK1-IRF3 signal complex formation was increased, subsequently IRF3 activation and type-I interferon production were also enhanced in PRMT6 deficient macrophages. Mechanistically, PRMT6 could directly bind IRF3. Viral infection enhanced the binding of PRMT6 to IRF3 and inhibited the interaction between IRF3 and TBK1. Which was independent on PRMT6 methyltransferase activity. Thus, PRMT6 inhibits antiviral innate immunity by sequestering IRF3, thereby blocking TBK1-IRF3 signal complex formation. Our work demonstrates a methyltransferase activity independent role for PRMTs. It also identifies a negative regulator of the antiviral immune response, which may protect the host from the damaging effects of an overactive immune system and/or be exploited by viruses to escape immune detection.

P0019

Human milk oligosaccharides and its acid hydrolysates show immunomodulatory effects via TLR signaling

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Human milk oligosaccharides (hMOs) have beneficial immune effects but the mechanisms of action are not well-understood. Here we study Toll-like receptor (TLR) signaling of the hMOs 2'-FL, 3-FL, 6'-SL, LNnT, and hMO's acid hydrolysates LNT2, and their effect on cytokine production in human macrophages.
The effects of 2'-FL, 3-FL, 6'-SL, LNT2, and LNnT were studied on TLRs signaling with reporter cell lines. TLR activation was observed for 3-FL and LNT2. 3-FL activated TLR2 and LNT2 activated all TLRs. In an inhibition assay, 2'-FL, 6'-SL, and LNnT inhibited TLR5 and 7, while 3-FL inhibited TLR5, 7, and 8. 6'-SL showed a synergistic effect on ssRNA40-induced TLR8 activation. In addition, we measured hMO-induced cytokine production in THP1 macrophages. IL-10 and TNFα were induced by LNT2, and the effects were NF-κB dependent, while the other hMOs had minor effects. From the results we can conclude that the effects of hMOs and its acid hydrolysates on TLR signaling and immunomodulation of macrophages are structure dependent.

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DOI: 10.1002/eji.201970400

Understanding how and which hMO modulate immunity, contributes to the future design of hMO containing products with predictable beneficial effects in specific target groups.

P0020

Differential platelet receptor expression for viral capture (DC-SIGN) regulates plasma leakage in patients with dengue infection?

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Introduction: Platelet activation and cells expressing DC-SIGN receptors have been reported to play a major role in dengue infection. The present study assesses the expression of the DC-SIGN receptor on platelet surface in dengue patients and its association plasma leakage by hematocrit.
Material and methods: This was an analytical cross-sectional study carried out in JIPMER hospital, Puducherry. 35 patients with dengue infection (cases) and 36 patients with non dengue acute other febrile illness (controls) were recruited. Hematological parameters on admission and Platelet rich plasma was assessed for DC-SIGN using BD FACS Calibur™ on: admission, day 3 & discharge.
Results: Subjects were divided into groups based on the gender specific mean value of hematocrit. We observed a decreased expression of DC-SIGN in cases compared to controls on admission in both groups. An increasing trend, though not significant, was observed in expression of DC-SIGN in both groups in cases and in patients with higher hematocrit, DC-SIGN expression remain suppressed along the course of disease.
Conclusion: Our results suggest that DC-SIGN, which is a receptor for viral capture might be prolongedly suppressed in dengue patients with plasma leakage. This could be part of protective response from the host to prevent platelet activation and subsequent endothelial damage.

P0021

Fine-tuning of type I interferon response by STAT3-PLSCR2 axis

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Type I interferon (IFN-I) is a pluripotent cytokine that modulates innate and adaptive immunity. We have previously shown that STAT3 suppresses IFN-I response in a manner dependent on its N-terminal domain (NTD), but independent of its DNA-binding and transactivation ability. Using the yeast two-hybrid system, we have identified phospholipid scramblase 2 (PLSCR2) as a STAT3 NTD-binding partner and a suppressor of IFN-I response. Overexpression of PLSCR2 attenuates ISRE-driven reporter activity, which is further aggravated by co-expression of STAT3. Moreover, PLSCR2 deficiency enhances IFN-I-induced gene expression and antiviral activity without affecting the activation or nuclear translocation of STAT1 and STAT2 or the assembly of ISGF3 complex. Instead, PLSCR2 impedes promoter occupancy by ISGF3, an effect further intensified by the presence of STAT3. Moreover, palmitoylation of PLSCR2 is required for its binding to STAT3. Loss-of-function mutation of PLSCR2 in the palmitoylation motif blocks its interaction with STAT3 and for the suppressive activity. Together, these results define the role of a novel STAT3-PLSCR2 axis in fine-tuning IFN-I response.

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DOI: 10.1002/eji.201970400

P0596

Glycolipid iGb3-triggered CD1d reverse signaling feedback amplifies innate immune responses via Pyk2 activation

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CD1d molecules are MHC class I-like transmembrane glycoproteins which present lipid antigens to NKT cells, and play critical roles in antimicrobial responses and antitumor immunity. Apart from the well known function of lipid antigen presentation, it has been implicated the intracellular reverse signaling of CD1d may also exert nonclassical biological functions. Nevertheless, the role of lipid antigen presenting molecule CD1d in innate immune responses is intriguing to be explored. Here we found CD1d deficiency attenuated the inflammatory innate response of host and CD1d deficient mice were more susceptible to bacterial infection. TLR-triggered production of proinflammatory cytokines and type I interferon was also impaired in CD1d deficient APCs. TLR activation in macrophages induced metabolic changes of glycosphingolipids (GSLs) profile. Among these changed GSLs, glycolipid antigen isoglobotrihexosylceramide (iGb3), a potent CD1d-presented self-antigen, was markedly produced after TLR ligation. Exogenous added iGb3 could promote TLR-initiated innate response in APCs, while ablation of endogenous iGb3 production impaired TLR-induced cytokine production. More importantly, cytoplasmic CD1d was required for iGb3 to promote TLR innate response. Endogenous iGb3 production in TLR-activated macrophages could enhance CD1d phosphorylation at Tyr332 and facilitate CD1d to recruit and activate Pyk2. Pyk2 then associated with IKKβ and TBK1, and enhanced their phosphorylation, thus promoted the full activation of innate immune response. Our findings uncover a non-classical function of antigen presenting molecule CD1d in innate immunity, and provide new evidence on the close connection between cellular glycolipid metabolism and innate immunity.

P0597

Hypoxia/reoxygenation-induced HMGB1 translocation and release promotes islet proinflammatory cytokine production and early islet graft failure through TLRs signaling

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Our objective was to determine the role of High-mobility group box 1 (HMGB1) and the degree of activation of TLR-related signal transduction pathways in hypoxia/reoxygenation(H/R)-induced proinflammatory cytokine production and intra-islet graft inflammation. After islets are exposed to H/R, TLR2/4 expression and TLR-mediated signaling was up-regulated, and HMGB1 was translocated and released to the extracellular, Moreover, proinflammatory cytokine production (IL-1β and TNF-α) and islet injury were significantly increased, and these effects depend on TLR2/4 signaling pathways. Exogenous HMGB1 also induces islet inflammation and increases the phosphorylation of STAT3, p38 and IκBα in wild-type islets. TLR2 deficiency resulted in the inhibition of IL-1β production and STAT3/p38 phosphorylation after HMGB1 exposure. TLR4 deficiency resulted in the inhibition of TNF-

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DOI: 10.1002/eji.201970400

α production and IκBα phosphorylation after HMGB1 exposure. Pre-incubation of the STAT3, p38, or NF-κB inhibitors significantly inhibited HMGB1-induced IL-1β or TNF-α production in islets, but the effect of HMGB1 or H/R-induced islet injury was not counteracted by the inhibitors. HMGB1 inhibition by ethyl pyruvate or blockade by neutralizing antibodies significantly decreased the phosphorylation of STAT3, p38 and IκBα, the production of IL-1β and TNF-α, and the islet injury in wild-type islets after exposure to H/R and significantly improved early islet graft failure. Thus, our results suggest that HMGB1 released from H/R induced islets works in an autocrine manner to up-regulate STAT or p38 and augment IL-1β production via TLR2, and up-regulate NF-κB and augment TNF-α production via TLR4 in intra-islet, which are associated with H/R-induced islet injury and early graft failure.

P0598

Bap1 and Ubec3c reciprocally control IRF3 ubiqutination and regulate anti-viral immunity

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The activity of IRF3 has a key role in the innate immune response to viral infection, and dysregulation of IRF3 is associated with a variety of immune diseases. Ubiquitination is a dynamic multifaceted PTM, reciprocally regulated by the E3 ubiquitinase and deubiquitinating enzymes (DUBs). Although IRF3 ubiquitination and related E3 has been reported, its deubiquitination remains unknown. In this study, we screened potential DUBs in antiviral immunity with siRNA library of 103 DUBs family members, and BRCA1-associated protein 1 (BAP1) was picked out for robust and consistent regulatory effects. BAP1 significantly increased the secretion of IFN-β induced by viral infection, *and* inhibited influenza virus infection in the lung. Mechanically, BAP1 was proved to interact with IRF3, reduce its K48-linked ubiquitination and protect IRF3 from proteasome degradation in the nucleus. Lys77 was proved to be the main site for K48-linked polyubiquitination and degradation of IRF3, which was removed by BAP1. Moreover, E3 ligases targeting IRF3 were screened and UBE3C was found to specifically mediate the K48-linked polyubiquitination at Lys77. Reciprocal expression of Bap1 and Ubec3c governed IRF3 degradation and innate anti-viral immunity. Early after viral infection, Bap1 dominates and prevents IRF3 K48 ubiquitination to ensure effective and robust viral clearance; Later, Ubec3c expression governs IRF3 ubiquitinatian and degradation, preventing excessive innate immune responses and immunopathogenesis. Our work extended the regulatory network of IRF3, revealed a new insight into the tumor suppressor BAP1, and provided a potential target for intervening viral infection.

P0599

RNF144B inhibits LPS-induced inflammatory responses via binding TBK1

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Innate immune responses need to be precisely controlled to avoid prolonged inflammation and prevent unwanted damage to the host. Here we report that RNF144B responded dynamically to LPS stimulation and negatively regulated LPS-induced inflammation. We found that RNF144B interacted with the scaffold/dimerization domain (SDD) of TANK binding kinase 1 (TBK1) through the in between

DOI: 10.1002/eji.201970400

RING (IBR) domain to inhibit its phosphorylation and K-63 linked polyubiquitination, which led to TBK1 inactivation, IRF3 dephosphorylation, and interferon beta (IFN-β) reduction. RNF144B knockdown with siRNA increased IRF3 activation and IFN-β production in response to LPS stimulation. Our study identifies that RNF144B interaction with TBK1 is sufficient to inactivate TBK1 and delineates a previously unrecognized role for RNF144B in innate immune responses.

P0600

Cell wall mannoprotein of Candida albicans polarizes macrophages and affects proliferation and apoptosis through activation of the Akt signal pathway

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Objective: *Candida albicans* is a commensal fungus that associates with human hosts. Under normal circumstances this interaction does not produce any severe life-threatening disease, as macrophages of the innate immune system will result in its clearance. However, disorders may arise in immunosuppressed individuals.
Methods: To understand the bioactivity of *Candida albicans* cell wall polysaccharides, which represent an important component of its function, mannoprotein from this fungus was extracted, purified and analyzed. Mannoprotein with α-(1,2) and α-(1, 6) linkages was investigated with use of HPLC and NMR. Adding mannoprotein into macrophage, the expression level of macrophage markers was evaluated by flow cytometry and real-time PCR. The secretion level of cytokines was measured by ELISA and real-time PCR. The activation of Akt signal pathway and the alteration of cell cycle and apoptosis was confirmed by Western blot and flow cytometry..
Results: Co-incubation of mannoprotein with macrophages resulted in a mannoprotein with the potential to polarize macrophages to M1 and promote phagocytosis/microbial killing ability thus increasing the clearance of pathogens through Akt2. Moreover, mannoprotein within the cell wall promoted cell proliferation and inhibited apoptosis by activation of the Akt signaling pathway.
Conclusion: α-(1,6)(1,2)-mannoprotein, extracted from the cell wall of *Candida albicans*, demonstrates immune-enhancing effects by activation of the Akt signaling pathway. These findings provide important new insights into the biological effects of polysaccharides on macrophages. Such information can then serve as the foundation for the development of novel anti-fungal medications.

P0601

TcpC serves as an E3 ubiquitin ligase that promotes MyD88 hydrolysis in dendritic cells and macrophages

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Backgroud: Toll/interleukin-1 receptor domain-containing protein encoded by *E. coli* (TcpC) is an important virulence factor in the majority of uropathogenic *E. coli* (UPEC) strains and blocks the TLR signaling pathway by binding to MyD88, hereby inhibiting macrophage-mediated innate immunity. We found that TcpC secreting CFT073 UPEC or recombinant TcpC (rTcpC) treatment resulted in decreased concentrations of MyD88 in dendritic cells and macrophages.
Objectives: To clarify the mechanisms by which TcpC inhibits the expression of MyD88.
Methods: Protein and mRNA expressions of MyD88 in rTcpC treated dendritic cells and macrophages

DOI: 10.1002/eji.201970400

were measured by Western blot and real-time qRT-PCR respectively. Ubiquitination of MyD88 in rTcpC treated dendritic cells and macrophages was detected by co-immunoprecipitation. To identify the activity of E3 ubiquitin ligase of TcpC, an ubiquitination kit was used.
Results: rTcpC treatment dose dependently inhibits protein levels of MyD88 in both dendritic cells and macrophages, but does not affect its mRNA expression. Ubiquitination of MyD88 in rTcpC treated dendritic cells and macrophages was significantly enhanced compared with the control group. Ubiquitination kit tests demonstrated that rTcpC acted as the E3 ubiquitin ligase. This enhanced ubiquitination of MyD88 by rTcpC could be abrogated by the E3 ubiquitin ligase inhibitor Nutlin-3.
Conclusion: Our data showed for the first time that TcpC could serve as an E3 ubiquitin ligase that promotes hydrolysis of MyD88 via enhanced ubiquitination, which is a novel mechanism by which TcpC subverts the innate immunity.

P0602

DNA sensor IFI16 and its degradation modulate the type I interferon response against hepatitis B virus infection

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Background and aims: IFI16 was recently characterized as a DNA sensor targeting incoming viral genome. The role of IFI16 in innate immunity against hepatitis B virus (HBV) infection remains to be elucidated.
Methods: HepG2-based cell models of HBV infection and replication were used to determine the interaction of IFI16 and HBV DNA, the feature of intracellular IFI16, and the regulation of interferon-beta (IFN-β) response by IFI16. The peripheral blood mononuclear cells (PBMCs), sera, exosomes, and liver tissues of the patients with chronic hepatitis B (CHB) were analyzed to assess the role of IFI16 in the chronicity and interferon therapy.
Results: In the cell models, nuclear IFI16 interacted with HBV cccDNA, leading to a cytoplasmic migration followed by cleavage of the protein. The cytoplasmic IFI16 triggered an IFN-β response, while its degradation segment impaired the response. In CHB patients, the early changes of *IFI16* mRNA in PBMCs, but not serum IFI16 protein, were predictive to HBeAg seroconversion in IFN-α therapy. Although IFI16 was rarely detected in normal hepatocytes, IFI16 and its degradation were largely detectable in the PBMCs, exosomes, infiltrating inflammatory cells and neighboring hepatocytes in the liver.
Conclusion: IFI16 is not only a trigger of IFN-β response, but also an inhibitor due to its degradation after sensing of HBV DNA. Given that IFI16 and its degradation are largely detected in the chronic hepatitis B patients, this protein may significantly contribute to the modulation of IFN-β response and persistence of viral infection.

P0603

Porcine epidemic diarrhea virus infection activates proinflammatory responses via the NOD2 signaling pathway

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DOI: 10.1002/eji.201970400

NOD-like receptors (NLRs) play critical roles in innate immunity. NOD2, the first identified member in the NLR family, recognizes the peptidoglycan from bacteria and induces the production of proinflammatory. The functions of NOD2 in Crohn's disease and early onset sarcoidosis indicate that it is an essential factor to maintain intestinal homeostasis. However, its functions in intestinal viral infection still remain unclear. Porcine epidemic diarrhea virus (PEDV), a devastating enteric virus, causes up to 100% mortality in newborn piglets. To investigate the detailed functions of NOD2 during PEDV infection, intestinal porcine epithelial cell-jejunum 2 (IPEC-J2) cells were inoculated with PEDV. The results showed that PEDV infection upregulated NOD2 expression but not other NLR members. In addition, the NF-κB signaling pathway was activated, leading to the production of proinflammatory factors. To confirm these findings, the RIPK2, key factor connecting NOD2 and NF-κB signaling pathway, and NOD knock-down and knock-out IPEC-J2 cells were infected with PEDV. The results demonstrated that proinflammatory response was absent. A recent publication indicated that PEDV was able to infect pigs via the respiratory tract. We next checked if NOD2 was involved in respiratory infection of PEDV. To do so, porcine alveolar macrophages were isolated and infected with PEDV. The preliminary results suggested that NOD2 recognized PEDV in respiratory tract and facilitated downstream proinflammatory response. To our knowledge, this is the first piece of evidence elucidated the roles of NOD2 in PEDV infection and immune responses. These findings will facilitate the understanding of host responses to enteric viral infection.

P0604

TREM2/beta-catenin attenuates macrophage pyroptosis to promote bacterial eradication of Pseudomonas aeruginosa

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Triggering receptors expressed on myeloid cells 2 (TREM2) inhibits inflammatory responses, but promotes bacterial clearance during in vivo/vitro infections with *Pseudomonas aeruginosa* (PA). However, the underlying mechanism remains unclear. Our study demonstrated that deficiency/ knockdown of TREM2 significantly enhanced PA-induced macrophage pyroptosis, a novel form of pro-inflammatory programmed cell death. Blockage of pyroptosis by knocking out/down Caspase-1 or gasdermin D (GSDMD) enhanced macrophage-mediated PA clearance. And treatment with Caspase-1 inhibitor relieved TREM2 deficiency-mediated suppression of PA clearance. In addition, TREM2 enhanced beta-catenin stability via promoting its phosphorylation at S675. Moreover, silencing of beta-catenin enhanced pyroptosis and impaired PA clearance, while overexpression of beta-catenin inhibited pyroptosis and restored bacterial elimination in TREM2-silenced macrophages. Notably, in vitro studies suggested that TREM2/beta-catenin suppressed NLRP3 and NLRC4 inflammasome activation at priming and/or assembly stage. Collectively, our study provides a novel mechanism of TREM2-mediated antibacterial immunity and explores the relationship between pyroptosis and bacterial eradication.
Keywords: triggering receptors expressed on myeloid cells 2 (TREM2); pyroptosis; *Pseudomonas aeruginosa*; macrophage; bacterial killing

DOI: 10.1002/eji.201970400

P0605

Basal interferon signaling and therapeutic use of interferons in controlling peste des petits ruminants virus infection

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Peste des petits ruminants virus (PPRV) causes highly contagious diseases in domestic and particular wild small ruminants, leading to substantial economic loss. Since interferons (IFNs) serve as the important defense line against viral infection, we have investigated the roles of types I and III IFNs in PPRV infection *in vitro*. Upon PPRV infection, IFN-λ3 was strongly induced, while IFN-β and IFN-λ2 were moderately induced in transcriptional level in human embryonic kidney 293T (HEK293T) cells. Although the transcription of type I and III IFNs were triggered, the production of functional IFN products was not detected. Importantly, the replication of PPRV was strongly inhibited in HEK293T cells treated by the exogenous IFNs (IFN-α-2b and IFN-λ3). Consistently, these IFNs significantly activate a panel of IFN-stimulated genes (ISGs). The inhibition of JAK-STAT pathway by JAK I inhibitor can abrogate the anti-PPRV activity of IFNs. Thus, our study shall contribute to better understanding of the complex PPRV-host interactions and provide rationale for therapeutic development of IFN-based treatment against PPRV infection.

P0606

SRCR proteins as microorganism pattern recognition receptors

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The initial sensing of an invasive pathogen is one of the most critical events during an infection. This sensing or recognition is mediated by germline-encoded pattern recognition receptors (PRRs), which recognize conserved pathogen-associated molecular patterns (PAMPs) of microbes. Importantly, the efficiency and velocity at which a PRR can recognize an invasive pathogen can dictate the outcome of the infection. Since the pioneering discovery of Toll-like receptors by the Nobel laureate Jules Hoffmann, many other families of PRRs with different specificities have being described. Recent work has uncovered PRR features of several proteins that belong to the scavenger receptor cysteine-rich superfamily (SRCR-SF). Most SRCRs are plasma membrane integral proteins expressed at the surface of immune cells; however, a subset of these proteins consists of secreted receptors that are therefore in circulation. As such, they have exceptional features to intercept, recognize and neutralize invasive microbes and thus to contain infections. We have investigated the biological role and the microbe-binding capacity of the circulating SRCR proteins Spα and SSC4D in infections. Using conventional protein-bacteria or protein-parasite binding assays, we uncovered the capacity of SSC4D to selectively bind to different species of bacteria and parasites. Furthermore, we demonstrate that Spα, a well-established PRR for bacteria, is also able to differentially recognize different protozoan parasites. Our newly generated mice with disrupted genes for each of the proteins showed increased susceptibility to models of disease induced by the different classes of pathogen, opening the possibility of using these proteins as therapeutic agents for infectious diseases.

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DOI: 10.1002/eji.201970400

P0607

Synergistic activation of inflammatory cytokine genes and NLRP3 inflammasome in human macrophages by CXCL4 and TLR8 signaling

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Crosstalk between cytokines and Toll-like receptor (TLR) signaling modulates inflammatory gene expression, which is crucial for disease control or inflammatory disease pathogenesis. TLR8 is important for inflammatory diseases. However, it is less studied because mouse TLR8 lacks 5 amine acids that are necessary for its single-stranded RNA (ssRNA) ligand recognition. Chemokine (C-X-C motif) ligand 4 (CXCL4) is strongly correlated with inflammatory diseases, such as systemic sclerosis and rheumatoid arthritis while its receptor on myeloid cells is still unknown. Here, we found that co-stimulation with CXCL4 and TLR8 ligand ORN8L induced extremely high levels of inflammatory gene expression, including *IL6*, *IL12B* and *TNF* in human blood monocyte derived macrophages in vitro. In addition, NLRP3 mediated inflammasome and interleukin-1 β (IL-1β) processing were activated in human macrophages by CXCL4 and TLR8 signaling. Ongoing work is to decipher the receptor of CXCL4 on macrophages and synergy mechanism whereby CXCL4 licenses a primed signaling to augment TLR8-induced gene expression and NLRP3 inflammasome activation. Humanized transgenic mice expressing human TLR8 under the control of its own regulatory elements in a BAC construct will be used to investigate the crosstalk between CXCL4 and TLR8 in controlling autoimmune disease pathogenesis and to identify the contribution of NLRP3 inflammasome in CXCL4 and TLR8 signaling mediated inflammatory disease pathogenesis.

P0608

Multiple phagocytosis related factors underpin the pathogenesis of age-related macular degeneration

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A characteristic feature of early age-related macular degeneration (AMD) is the accumulation of debris in important locations within the posterior eye. Here, we evaluated whether peripheral blood monocyte function was impaired in various stages of AMD. AMD participants with either early stages or late disease were recruited, and their monocyte function compared with aged-matched healthy controls. Using a real-time tri-colour flow cytometry method, phagocytic function of peripheral blood monocyte subsets (non-classic, intermediate and classic) was found to be reduced in all three subsets of monocytes isolated from participants with earlier stage and late stage AMD. In addition, surface expression of phagocytosis related molecules including CD11b, CD11c, CD33 and CD34, was reduced. Finally, the phagocytic deficit could be restored *in vitro* by glatiramer acetate (Copaxone®) treatment, and the increments of improvement were correlated with size of drusen and area of reticular pseudodrusen (RPD). Our findings indicate, for the first time, that defective systemic phagocytosis is associated with both early and late stages of AMD, highlighting a potential role in the accumulation of debris that occurs in this disease. Assessing peripheral monocyte phagocytic function and phagocytosis related leukocyte surface biomarkers may provide additional insights into the aetiology of this disease and offer a novel therapeutic target.

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DOI: 10.1002/eji.201970400

P1192

TOB1 inhibits IRF3-directed antiviral responses by recruiting HDAC8 to specifically suppress IFN-β expression

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Innate immunity is the first line of host to defense against viral invasion and need to be precisely controlled. Viral infection induced type I interferons (IFNs) production play fundamental roles in innate immunity against virus and maintain immune homeostasis. However, the epigenetic regulatory mechanisms of type I IFNs production is unclear. The transducer of ErbB-2.1 (TOB1) is a member of the anti-proliferative family of BTG/TOB (B cell migration factor/erbB2). TOB1 plays crucial regulatory roles in T cell activation and cancer, via interacting with SMAD4 or SMAD2. However, its potential roles in innate immunity is unknown. In the present study, we found that the crystal structures of SMAD and IRF3 are very similar, and TOB1 could interact with IRF3. TOB1 expression could be markedly induced during viral infection in macrophages. TOB1 deficiency enhances both RNA and DNA viral-induced IFN-β production, and inhibits viral replication in macrophages. Mechanistically, TOB1 associates with IRF3, recruits HDAC8, and promotes HDAC8 binding to IFN-β promoter. Thus, TOB1 attenuates the acetylation of histone in the IFN-β promoter region, and thus inhibiting IFN-β transcription.
Therefore, we identified TOB1 as a negative regulator of IFN-β production and outlined a new feedback mechanism for the control of antiviral immune responses.

P1193

Toll-like receptor signaling cross-activates the autophagic pathway to restrict *Salmonella* Typhimurium growth in macrophages

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It has been long recognized that activation of Toll-like receptors (TLRs) induces autophagy to restrict intracellular bacterial growth. However, the mechanisms of TLR-induced autophagy are incompletely understood. *Salmonella (S)* Typhimurium is an intracellular pathogen that causes food poisoning and gastroenteritis in humans. Whether TLR activation contributes to *S.* Typhimurium-induced autophagy has not been investigated. Here we report that *S.* Typhimurium and TLRs shared a common pathway to induce autophagy in macrophages. We first showed that *S.* Typhimurium-induced autophagy in a RAW264.7 murine macrophage cell line was mediated by the AMP-activated protein kinase (AMPK) through activation of the TGF-b-activated kinase (TAK1), a kinase activated by multiple TLRs. AMPK activation led to increased phosphorylation of Unc-51-like autophagy activating kinase (ULK1) at S317 and S555. ULK1 phosphorylation at these two sites in *S.* Typhimurium-infected macrophages overrode the inhibitory effect of mTOR on ULK1 activity due to mTOR-mediated ULK1 phosphorylation at S757. Lipopolysaccharide (LPS), flagellin, and CpG oligodeoxynucleotide (ODN), which activate TLR4, TLR5, and TLR9, respectively, increased TAK1 and AMPK phosphorylation, and induced autophagy in RAW264.7 cells and in bone marrow-derived macrophages. However, LPS was unable to induce TAK1 and AMPK phosphorylation, and unable to induce autophagy in TLR4-deficient macrophages. TAK1 and AMPK-specific inhibitors blocked *S.* Typhimurium-induced autophagy and increased the bacterial growth in RAW264.7 cells. These observations collectively suggest that activation of the TAK1-AMPK axis through TLRs plays an important role in *S.* Typhimurium-induced

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DOI: 10.1002/eji.201970400

autophagy. Our study provides novel insights into how TLR signaling cross-activates the autophagic pathway to clear intracellular bacteria.

P1194

TRIM21 promotes innate immune response to RNA viral infection through lys27-linked polyubiquitination of MAVS

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Human innate immunity responds to viral infection by activating the production of interferons (IFNs) and proinflammatory cytokines. The mitochondrial adaptor molecule MAVS plays a critical role in innate immune response to viral infection. In this study, we show that TRIM21 interacts with MAVS to positively regulate innate immunity. Under viral infection, TRIM21 is upregulated through the IFN/JAK/STAT signaling pathway. Knockdown of TRIM21 dramatically impairs innate immune response to viral infection. Moreover, TRIM21 interacts with MAVS and catalyzes the K27-linked polyubiquitination of MAVS, thereby promoting the recruitment of TBK1 to MAVS. Specifically, the PRY-SPRY domain of TRIM21 is the key domain for its interaction with MAVS, while the RING domain of TRIM21 facilitates the polyubiquitination chains of MAVS. In addition, MAVS-mediated innate immune response is enhanced by both of the PRY-SPRY and RING domain of TRIM21. Mutation analyses on all lysine residues of MAVS further reveal that Lys325 of MAVS is catalyzed by TRIM21 for the K27-linked polyubiquitination. Overall, this study reveals a novel mechanism that TRIM21 promotes the K27-linked polyubiquitination of MAVS to positively regulate innate immune response, thereby inhibiting viral infection.

P1195

NFAT1 positively regulates antiviral innate immune response by preventing RIG-I degradation

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Type I interferons (IFNs) is the most profound effector against viral infections in innate immune responses. Investigations on the regulatory mechanisms underlying host type I IFN production are currently of great interest. Here, we found that viral infection significantly regulated NFAT1 expression at transcriptional and translational levels in mouse macrophages. NFAT1 positively regulates type I IFN production in the innate immune response against RNA virus infection by preventing RIG-I degradation. Upon VSV infection, NFAT1 regulates posttranslational modifications of RIG-I, and consequently prevents degradation of RIG-I, which enhances RIG-I/MAVS mediated type I IFNs production and promotes the antiviral innate immune response. Our results provide a new manner of NFAT1 in antiviral innate immunity, and reveal a new positive regulatory mechanism for type I IFN production in the antiviral innate immune response. This study may provide a potential target for intervention of inflammatory diseases.

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DOI: 10.1002/eji.201970400

P1196

The E3 ubiquitin ligase RNF39 differentially regulates DNA and RNA sensing pathways by targeting DDX3

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Innate cytosolic sensing of foreign nucleic acids is vital for the activation of innate immune responses in various physiological and pathological conditions. Cytosolic DNA is mainly recognized by cyclic GMP-AMP (cGAMP) synthase (cGAS), whereas pathogen-derived RNA in the cytosol is sensed by the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). Although STING- and MAVS-dependent pathways are distinct responsible for cGAS and RLR pathways respectively, there is a high degree of interconnection between the two pathways. Many molecules have been identified as regulators of the DNA or the RNA pathways that regulate cytokine production during the innate immune response. However, it remains unknown how the balance between the DNA and RNA pathways is maintained. In particular, no molecule has been identified that is able to oppositely regulate the DNA and RNA pathways.In the present study, we show that RNF39 differentially regulates DNA and RNA sensing pathways. RNF39 enhances cGAS-STING dependent signaling, but it suppresses RLR pathway, by targeting DDX3. RNF39 promotes K48-linked polyubiquitination of DDX3 at residue K51, K130 and K162, and facilitates the protein degradation of DDX3 in proteasome. Consistently, RNF39 deficiency inhibits DNA viral infection induced innate responses, while enhances anti-viral innate responses against RNA viruses. In addition, we demonstrate that DDX3 functions as a negative regulator of DNA pathway, which is opposite from its role in RNA pathway. Our results identify RNF39 as a unique regulatory molecule that balances DNA and RNA sensing pathway and thus facilitate the maintenance of immune homeostasis.

P1197

Crosstalk between IL-33/ST2 and TGF-β signaling promotes renal interstitial fibrosis

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Renal fibrosis comprises the inevitable consequence that occurs in virtually all types of chronic kidney diseases. Tubular epithelial-mesenchymal transition (EMT), along with activation of transforming growth factor-β (TGF-β) signaling, has been identified as the major avenue for the generation of matrix-producing cells in the progression of renal fibrosis. In this study, we show that deficiency of IL-33 or ST2 severely affected the activation of TGF-β signaling, thereby preventing the development of renal fibrosis in a unilateral ureteral obstruction animal model. In HK-2 renal epithelial cells, IL-33 induced TGF-β receptor I, Smad2, and Smad3 phosphorylation, promoting extracellular matrix (ECM) production and EMT. Moreover, interfering the expression of ST2 receptor or using sST2 effectively disturbed the activation of the Smad pathway and reduced EMT. In addition, TGF-β receptor I / II inhibitor or siRNA also effectively inhibited the activation of the Smad pathway and reduced ECM production in HK2 cells, suggesting that both ST2 and TGF-β receptors are required to promote IL-33-induced ECM synthesis. **Furthermore, we show for the first time that IL-33 promotes cooperation between ST2 and TGF-β receptors, which activates Smad2 and Smad3 downstream of TGF-β receptors to promote EMT, and that cooperation between ST2 and TGF-β receptors is essential**

DOI: 10.1002/eji.201970400

for the development of renal fibrosis. Therefore, targeting IL-33/ST2 signaling may constitute an effective therapeutic strategy for renal interstitial fibrosis.

P1198

Development of nano-inhibitors targeting Toll-like receptor signaling

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Pharmacological regulation of Toll-like receptor (TLR) responses is anticipated to be beneficial in treating many inflammatory diseases. However, there are currently very few compounds available to inhibit TLR signaling. The rapid advances in nano-science and technology that have contributed to the creation of diverse materials with various surface chemistry and properties may aid to the development of new types of TLR inhibitors. My research objective is to search novel nano-inhibitors for TLR signaling. The ultimate goal is to supply a potent anti-inflammatory “nano-drug” for effective pharmacological treatments of acute lung injury (ALI). In the past decade, we constructed a library of physiologically stable peptide-gold nanoparticle hybrids with tunable surface chemistry, serving as a “nano-drug” discovery platform. We employed THP-1 cell-based NF-kB and IRF reporter cells as a primary screening tool to discover nanoparticles with capability of inhibiting TLR signaling from the established nanoparticle library. We identified a unique nanoparticle that exhibited potent inhibitory activity on TLR4 signaling of both NF-kB and IRF3 pathways. Mechanistic studies revealed that such inhibitory activity was through high cellular uptake and blocking the endosomal acidification. Using a LPS-induced ALI mouse model, we further demonstrated that the nanoparticle significantly reduced proinflammatory cytokine production and neutrophil infiltration in the lung, suggesting that the lead TLR nano-inhibitor had potent inhibitory effects on TLR4 signaling in vivo. This work identifies a novel nanoparticle inhibitor for TLR4 signaling that may represent a novel class of anti-inflammatory therapeutics for ALI.

P1199

Hyperglycemia triggered S1P/S1PR3 signaling worsens liver ischemia/reperfusion injury by regulating M1/M2 polarization

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Hyperglycemia aggravates hepatic ischemia/reperfusion injury (IRI) but the underlying mechanism remains elusive. Sphingosine 1-phosphate (S1P)/S1P receptors (S1PRs) have been implicated in metabolic and inflammatory diseases. Here, we clarify whether and how S1P/S1PRs are involved in hyperglycemia-related liver IRI. S1P/S1PRs was analyzed in liver and Kupffer cells (KCs). We investigated the function/mechanisms by which S1P/S1PRs may influence hyperglycemia-related liver IRI. S1P levels were significantly higher in liver from patients and mice with diabetes. S1PR3, but not S1PR1 and S1PR2, was specifically activated in liver tissues and KCs under hyperglycemic conditions. S1PR3 antagonist CAY-10444 attenuated hyperglycemia-related liver IRI based on hepatic biochemistry, histology, and inflammatory responses. Diabetic liver expressed higher M1 markers [NO synthase 2 and phosphorylated signal transducer and activator of transcription (p-STAT)1], but lower M2 markers (arginase, mannose receptor C type 1, p-STAT1, and p-STAT6) at baseline and post-IR.

DOI: 10.1002/eji.201970400

Dual-immunofluorescence staining showed that hyperglycemia promoted M1 (CD11b/CD68) differentiation and inhibited M2 (CD11b/CD206) differentiation. Importantly, CAY10444 reversed hyperglycemia-modulated M1/M2 polarization. *In vitro*, high glucose (HG) medium triggered S1P/S1PR3 signaling, promoted M1 polarization, inhibited M2 polarization, and enhanced inflammatory responses compared with low glucose (LG) medium in bone marrow-derived macrophages (BMDMs). In contrast, S1PR3 knockdown significantly retrieved hyperglycemia-modulated M1/M2 polarization, and attenuated Toll-like-receptor-4-related inflammation. Administration of HG-cultured BMDMs worsened liver IRI, which was rescued by S1PR3 knockdown. This study reveals that hyperglycemia specifically triggers S1P/S1PR3 signaling and exacerbates liver IRI by facilitating M1 polarization and inhibiting M2 polarization, which may represent an effective therapeutic strategy for hyperglycemia-exacerbated liver IRI.

P1200

Myeloid cell expression of IRF5 enhances influenza-induced inflammation

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Recognition of Influenza A virus (IAV) by the innate immune system triggers the activation of pathways that restrict viral replication, activate innate immune cells, and induce and regulate adaptive immunity. However, excessive activation of innate immune responses can exaggerate disease. The pathways that promote excessive activation are incompletely understood, and there are limited experimental models to robustly investigate mechanisms that drive influenza-induced inflammation in humans. Interferon regulatory factor (IRF5) is a transcription factor that plays important roles in Toll-like receptor (TLR) pathways and subsequent induction of cytokines after viral sensing. Using a murine model of IAV infection, we identified a role for IRF5 in immune pathology without impacting virus replication. Reduced inflammatory cytokine production observed in lungs of *Irf5*$^{-/-}$ mice correlated with a specific reduction in cytokine-producing myeloid cell populations. In accordance with a role for myeloid cell-expressed IRF5 in orchestrating lung immune responses, using cytometry by time-of-flight (CyTOF) we identified that human lung IRF5 expression was highest in cells of the myeloid lineage. To investigate the role of IRF5 in mediating inflammatory responses to IAV, we used human induced pluripotent stem cells (hIPSCs) with biallelic mutations in *IRF5*, differentiated to dendritic cells and macrophages. Analogous to findings in mice, IRF5 deficiency in human myeloid cells corresponded with a reduction in virus-induced inflammatory cytokine production, with IRF5 acting downstream of TLR7 and RIG-I after viral sensing. Thus, IRF5 acts as a regulator for myeloid cell inflammatory cytokine production during IAV infection in mice and humans, and drives immune-mediated viral pathogenesis.

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DOI: 10.1002/eji.201970400

P1201

MyD88 inhibitor (ST2825) decreases the cytokine secretion in LPS-stimulated peripheral blood mononuclear cells

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Chronic inflammation is one of the main characteristics that lead to tissue damage in both autoinflammatory and autoimmune diseases. It is known that stimuli with mitogens lead to an elevated production of proinflammatory cytokines by immune system cells. New studies have focused on identifying new therapeutic targets that can inhibit the cytokine-mediated inflammatory process and therefore, reduce the damage. The aim of this work was to determine the stimuli effect of LPS and MyD88 inhibitor (ST2825) on the cytokine production in PBMC from healthy blood donors (HBD). PBMC were obtained from 10 HBD by density gradient separation (Histopaque®-1077), and were cultured in 24-well plates ($1x10^6$ PBMC/mL); stimulation was carried out with LPS (30 ng/mL) and ST2825 (30 µM) for 24 h. Cytokine quantitation was performed using the 27-Plex panel (Bio-Plex Pro Human Cytokine 27-Plex Immunoassay). Statistical analysis was performed using the GraphPad 5 software and a value of $p< 0.05$ was taken as significant. After stimulation with LPS, PBMC significantly secrete the proinflammatory cytokines IFN-γ, IL-12, IL-17A, IL-1β, IL-6, TNF-α, GM-CSF, IL-15 and VEGF. ST2825 showed an inhibition of IFN-γ ($p< 0.001$), IL-12 ($p< 0.05$), IL-6 ($p< 0.05$), IL-15 ($p< 0.05$) and VEGF ($p< 0.05$). Anti-inflammatory cytokines significantly elevated after stimulation with LPS were IL-1Ra, IL-4, IL-5, IL-13 and IL-10. It was observed that ST2825 is able to inhibit IL-1Ra ($p< 0.001$), IL-4 ($p< 0.05$), IL-5 ($p< 0.05$) and IL-13 ($p< 0.01$). In conclusion, ST2825 is a potent inhibitor of the production of proinflammatory and anti-inflammatory cytokines in LPS-stimulated PBMC.

P1202

TLR9 signaling in fibroblastic reticular cells regulates peritoneal immunity

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Fibroblastic reticular cells (FRCs), a subpopulation of stromal cells in lymphoid organs and fat-associated lymphoid clusters (FALCs) in adipose tissue, play immune-regulatory roles in the host response to infection and may be useful as a form of cell therapy in sepsis. Here, we found an unexpected major role of TLR9 in controlling peritoneal immune cell recruitment and FALC formation at baseline and after sepsis induced by cecal ligation and puncture (CLP). TLR9 regulated peritoneal immunity via suppression of chemokine production by FRCs. Interesting, activation of TLR9 signaling suppressed LPS-induced upregulation of chemokine expression in FRCs *in vitro,* suggesting a dominant role of TLR9 in the regulation of chemokine production by FRC. Adoptive transfer of *Tlr9* deficient FRCs more effectively decreased mortality, bacterial load, and systemic inflammation after CLP than wild type FRCs. Importantly, we found that activation of TLR9 signaling suppressed chemokine production by human adipose tissue-derived FRCs. Together, our results indicate that TLR9 plays critical roles in regulating peritoneal immunity via suppression of chemokine production by FRCs. These data form a knowledge basis upon which to design new therapeutic strategies to improve the therapeutic efficacy of FRC-based treatments for sepsis and immune dysregulation diseases.

DOI: 10.1002/eji.201970400

P1203

Role of GRK2 in the TLR signaling-mediated iNOS induction pathway in microglial cells

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G protein-coupled receptor kinase 2 (GRK2) has emerged as an integrative node in different signaling pathways. We previously showed that GRK2 is required for LPS-induced iNOS production in MG6 microglial cells, although it remains unclear how GRK2 regulates Toll-like receptor 4 (TLR4) signaling. In this study we investigated the role of GRK2 in signaling pathways via TLRs to induce iNOS expression. We found that LPS induces expression of proinflammatory mediators such as iNOS, IL-1β, IL-6, IP-10 and IRF7. GRK2 knockdown decreased the expression of these mediators without affecting NF-kB activation. Additionally, LPS stimulation induced IRF1 expression and nuclear translocation. Knockdown of IRF1 also decreased LPS-induced iNOS expression. GRK2 knockdown decreased LPS-induced IRF1 protein level and nuclear translocation without affecting IRF1 mRNA expression. LPS-induced STAT1 and STAT3 phosphorylation are required for iNOS expression. GRK2 knockdown decreased STAT1/3 phosphorylation and nuclear translocation. IRF1 knockdown decreased LPS-induced expression of interferon-β (IFN-β) that can activate JAK-STAT signaling pathway via type I IFN receptor. GRK2 knockdown also decreased LPS-induced IFN-β expression. Furthermore, GRK2 knockdown decreased exogenously supplied IFN-β-induced STAT1/3 phosphorylation. TLR3 and TLR9-dependent IFN-β expressions were also suppressed by GRK2 knockdown. Inhibition of kinase activity of GRK2 decreased LPS-induced STAT1/3 phosphorylation and iNOS expression without affecting IRF1 protein level and nuclear translocation. Our data demonstrated the differential regulatory mechanisms of GRK2 in the IRF1 and STAT1/3 activation, namely, kinase-independent IRF1 expression and kinase-activity-dependent STAT1/3 activation uncovering novel role of GRK2 in multiple TLR signaling.

P1786

The function of TRIM E3 ligase in innate immunity response

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Ubiquitination is one major protein post-translational modification (PTM) mechanism that regulates the degradation, intracellular location and function of proteins. It has been approved that ubiquitination is involved in many aspects of immune response, including innate and adaptive immune response, as well as autoimmune disorders. Here we reported an ubiquitin E3 ligase belonging to TRIM family, TRIM29, is induced by dsDNA stimulation specifically in bone marrow derived macrophage and dendritic cells, suggesting TRIM29 might play an important role regulating dsDNA-induced signaling pathway. TRIM29 deficiency elevates IFN-I and proinflammatory cytokine production upon viral DNA and cytosolic dsDNA stimulation. In vivo experiments show that TRIM29 deficient mice are more resistant to HSV-1 infection than WT controls, indicated by better survival rate and reduced viral load in organs. Mechanism studies suggest that STING-TBK1-IRF3 signaling pathway in TRIM29 KO cells is significantly enhanced and the degradation of STING was impaired. Furthermore, we identified that TRIM29 targets STING for K48-ubiquitination and degradation. Our study indicates that TRIM29 is a crucial negative regulator in immune response to DNA virus and cytosolic DNA, preventing potential damage caused by overcommitted immune responses.

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DOI: 10.1002/eji.201970400

P1787

ATP is the second key signal of gout flare besides MSU

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Forty hyperuricemia rats were prepared then followed ATP stimulation about 95% (38 rats) developed spontaneous arthritis. This type of arthritis is very different from the Coderre method in the following aspects: (1) The onset time of spontaneous arthritis is faster. The time of joint swelling in spontaneous arthritis peaked at 6±2 hours after ATP injection, while it took 2 to 3 days for arthritis caused by local injection of MSU to reach its peak. (2)The affected joints were different: The Coderre method joints swelling only at the MSU injection site. Spontaneous arthritis can involve multiple joints, in which 71% have one joint affected, 21% have two joints affected and 8% with three or more than three joints affected by redness and swelling. (3) Distinct histopathologic characteristic: In Coderre method, lymphocytes caused by local injection account for about 60%, with fewer neutrophils; In spontaneous arthritis, neutrophils account for more than 90% and lymphocytes are less. (4) Different sources of MSU: there is local MSU in spontaneous arthritis, which is associated with hyperuricemia; the local presence of MSU in Coderre´s arthritis was artificially injected. Our results suggested that ATP is the key second signal besides MSU to stimulate gout attack.
Appendix: Confocal observation of rat arthritis induced by ATP and MSU

P1788

Comparative transcriptome analysis of TLR8 signaling cells revealed the porcine TLR8 specific differentially expressed genes

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TLRs are the first discovered family of pattern recognition receptors (PRRs). They recognize pathogen associated molecular patterns (PAMPs) and initiate protective immune response. TLR8 as the main endolysosomal TLR, has recently regained attention especially for its structure and function. We previously found TLR8 exhibits species-specific activation by TLR7 specific agonist, Imiquimod (R837). Thus, we next initiated the identification of porcine TLR8 (pTLR8) specific downstream differentially expressed genes (DEGs) by parallel transcriptome analysis of porcine TLR8 and human TLR8 (hTLR8) signaling stable NF-kB reporter cells activated by TLR8 agonist Resiquimod (R848). It turned out that the two TLR8 NF-kB reporter cells can recapitulate the species-specific activity of pTLR8 and hTLR8, transcriptome analysis revealed a number of pTLR8 specific DEGs activated by R848, and some of these gene expressions were confirmed in porcine alveolar macrophages (PAMs) but not occurred in human cell types. Among the pTLR8 specific GO enriched item of cell recognition, 3 DEGs HAVCR2, CATSPERG and CNTN2 were validated to be upregulated by R848 stimulation in PAMs. HAVCR2 also known as Tim-3 has been recognized as an immune checkpoint together with PD-1, but Tim-3 in both mice and humans has also been shown to possess various functions in a context-dependent manner. The potential cooperation between porcine TLR8 and Tim-3 needs to be investigated in the future.

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DOI: 10.1002/eji.201970400

P1789

Salivary rAlb-34k2 protein of Aedes albopictus recognizes Escherichia Coli and inhibits its growth

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Mosquito saliva has been reported to include many immune active molecules, such as C-type lectin, peptidoglycan recognition protein and lysozyme, which participate in the maintenance of mosquito´s immune defense. 34k2 is a secreted protein which was found highly and specifically expressed in the saliva of *Aedes albopictus* mosquitoes. This study focused on the possible effects of recombinant salivary specific 34k2 protein (rAlb-3k42) of *A. albopictus* on recognizing *Escherichia coli, Staphylococcus aureus* and other bacteria, and its effects on the growth of *E. coli*. The results showed that rAlb-34k2 protein could bind to *E. coli*, *A. baumannii*, *S. aureus* and *P. aeruginosa*, but not to *β-hemolytic streptococcus* via bacterial binding assay. Moreover, the growth curves of *E. coli* which was treated with high concentrations of rAlb-34k2 protein (137ng/µL and 112ng/µL) were found smoothly gentle and its OD_{600} ranged from 0.063 to 0.294 and 0.097 to 0.349 within 24 hours, respectively, which was unrelated to Ca^{2+}. Conversely, the rAalb-CTL2 protein (a C-type lectin from recombinant saliva protein of *A. albopictus*) could promote the growth of *E. coli* in a manner of Ca^{2+} dependence. These data reveals that the rAlb-34k2 protein in the saliva of *A. albopictus* may act as a pattern recognition receptor of mosquitoes, which can recognize several kinds of bacteria and also inhibits the growth of *E. coli* in dose-dependent manner.
Keywords: *Aedes albopictus;* rAlb-34k2 protein; *E. coli;* pattern recognition receptor

P1790

The C Type lectin-like receptor OCILrP2 negatively regulates IL-6 in macrophage

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C-type lectin-like receptors (CTLRs) have gained increasing attention for their role in the fine tuning of both innate and adaptive immunity. OCILrP2 (Osteoclast inhibitory lectin related protein 2, also known as Clec2i and Clr-g) is a member of CTLRs and preferentially expressed in the spleen, liver, intestines, and activated immune cells. We have previously reported that the importance of OCILrP2 in T cell activation and DC cell maturation. However, its role in macrophage has not been fully identified. Here, we found that the expression level of OCILrP2 was significantly up-regulated during LPS stimulation of macrophages (RAW264.7 and peritoneal macrophage). And in the RAW264.7 silencing OCILrP2 significantly increased the expression levels of IL-6. Further, we observed the same result in mouse peritoneal macrophage cells silenced with OCILrP2 by siRNA. Interestingly, higher levels of IL-6 expression were maintained in OCILrP2-deficient cells at late phase of LPS response in macrophage. Taken together, our results have revealed that OCILrP2 plays an important role in the innate immunity and may negatively regulate IL-6 expression in macrophage.

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DOI: 10.1002/eji.201970400

P1791

Cancer-derived immunoglobulin G promotes LPS-induced proinflammatory cytokine production via binding to TLR4 in cervical cancer cells

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Numerous studies have shown that various cancer cells express immunoglobulin G (IgG). However, the function of cancer-derived IgG and the underlying mechanism remain unclear. In this study, we demonstrated that IgG expression was significantly altered after exposure to LPS in cervical cancer cells, suggesting that IgG was potentially involved in regulation of TLR4 signaling. Reduction of IgG attenuated LPS-induced proinflammatory cytokine production. The phosphorylation levels of NF-κB and MAPK were consistently suppressed by knockdown of IgG, which in turn impaired NF-κB nuclear translocation and the activity of NF-κB responsive element. Furthermore, we found that IgG was recruited to TLR4 in the cytoplasm after LPS stimulation, and IgG silencing inhibited LPS-initiated proinflammatory cytokine production through downregulating TLR4 expression. Similar results were obtained in a mouse model of endotoxemia and human tissues. Taken together, our findings demonstrate that IgG is a positive regulator of LPS-induced proinflammatory cytokine production by binding to TLR4 and enhancing its expression. TLR4 signaling plays a positive role in the development of many inflammation induced cancers such as cervical cancer. Our study strongly indicates that IgG may promote cervical cancer cell proliferation through enhancing TLR4 signaling. IgG may be a novel therapeutic target in treating inflammation mediated cancers.

P1792

Curcumin inhibits liver cancer by inhibiting DAMP molecule HSP70 and TLR4 signaling

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Curcumin has been revealed to inhibit liver cancer, however, no studies have reported that the mechanism of curcumin´s action on liver cancer is related to damage-associated molecular pattern (DAMP) molecules heat shock protein 70 (HSP70) and the toll-like receptor 4 (TLR4) signaling. This study aimed to investigate whether the activation of TLR4 signaling by HSP70 could be inhibited by curcumin, thus investigating the possible mechanism of curcumin in the inhibition of liver cancer. Western blotting was used to evaluate the expression of the HSP70 and TLR4 in HepG2 cells and ELISA was used to detect the concentration of HSP70 in cell culture medium. A thermal tolerance HepG2 (HepG2TT) cell model was established to simulate HSP70 accumulation in the microenvironment. A certain concentration of curcumin was co-cultured with HepG2 and HepG2TT cells to observe the changes of HSP70 and TLR4. Our results revealed that heat stress significantly increased the expression of extracellular HSP70 (eHSP70) and TLR4 ($P< 0.01$), but significantly reduced the expression of intracellular HSP70 ($P< 0.01$). Curcumin inhibited proliferation, invasion, and metastasis of HepG2 cells, caused cells to remain in the DNA S phase, promoted apoptosis, and significantly reduced intracellular HSP70, eHSP70 and TLR4 levels of HepG2TT cells. Following the

DOI: 10.1002/eji.201970400

removal of curcumin, eHSP70 increased again. In summary, our results demonstrated that the antitumor effect of curcumin was related to the inhibition HSP70-TLR4 signaling.

P1793

All-trans retinoic acid triggers cell surface hsp90 complex-mediated bacterial flagellin internalization in human monocytic THP-1 cells

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Heat shock protein 90 (HSP90) is a fundamental molecular chaperone that not only plays the key role in maintaining conformational integrity of various cellular proteins but also involves in the modulation of the immune response. Recent studies have identified membrane-bound HSP90 whose function is related to immune response but the underlying mechanism has remained unclear. In this study, we investigated the role of cell surface HSP90α complex induced by all-trans retinoic acid (ATRA), an active form of vitamin A, in response to bacterial flagellin. Western blot data were obtained by using cell surface cross-linking reagent BS3, and revealed that 1 μM ATRA induces formation of HSP90α complex on the cell surface of human monocyte THP-1. The HSP90α complex formation was dependent on the concentrations and incubation time of ATRA. In addition, internalization of bacterial flagellin was found to depend on the concentrations and incubation time of ATRA. Uptake of flagellin by ATRA-treated THP-1 cells was almost completely blocked by the treatment with cell surface HSP90α-specific inhibitor. Experiments with different types of endocytosis inhibitors demonstrated that bacterial flagellins are primarily taken up by clathrin-mediated endocytosis. In conclusion our results suggest that ATRA induces formation of cell surface HSP90α complex, which plays a critical role in bacterial flagellin internalization through clathrin-mediated endocytosis pathways.

P1794

Spred2 deficiency accelerates cancer growth, migration and invasion in HepG2 cells via activating ERK-MAPK pathway

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Activation of the Ras/Raf/ERK-MAPK pathway is involved in cancer development. However, the regulatory mechanism remains unclear. In the present study, we investigated whether Sprouty-related EVH1-domain-containing protein 2 (Spred2), a negative regulator of Ras/Raf/ERK-MAPK pathway, plays a role in cancer pathology. We first generated Spred2 knockout HepG2 cells (human hepatocellular carcinoma cell line) by CRISPR/Cas9 system. Spred2$^{-/-}$ cells showed enhanced ERK activation as compared with wild-type (WT) cells and demonstrated increased cell proliferation with enhanced expressions of cell-cycle markers, c-fos, CAD, and cyclinD1. STAT3 activation and anti-apoptotic markers such as bcl-2 and c-Myc were up-regulated in Spred2$^{-/-}$ cells. Interestingly, Spred2$^{-/-}$ cells were fibroblastic-like appearance and exhibited higher migration and invasion activities relative to the WT-cells. Of note, Spred2$^{-/-}$ cells showed decreased E-cadherin expression and in contrast, increased N-cadherin and Snail expression, thus demonstrating epithelial-mesenchymal transition (EMT) phenotype. Moreover, increased tumorigenicity was observed in Spred2$^{-/-}$ cells after implantation of the cells into nude mice. Spred2 deficiency may affect cancer stemness. We also found that Spred2$^{-/-}$ cells demonstrated higher ability of sphere formation. Expression of CD44 was up-regulated in Spred2$^{-/-}$ cells, as compared with that of WT cells. Up-regulated expression of stem cell markers including Nanog, KLF4, and c-Myc were reduced by over-expressing Spred2. Taken together,

DOI: 10.1002/eji.201970400

these results suggest that endogenous Spred2 down-regulates tumor cell growth, migration, invasion and possibly cancer stemness, thus controlling malignant potential of cancer cells.

P1795

Yellow fever virus modulates expression of key regulators of jak/stat signaling in human cell lines

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JAK/STAT signaling is activated in viral infections but pathway is highly regulated by suppressors of cytokine signaling (SOCS) and protein inhibitors of activated STATs (PIAS). Modulating expression of SOCS and PIAS as evasion mechanism has been described in some flaviviruses. In this study, we determined expression of SOCS and PIAS upon Yellow Fever Virus (YFV) infection and their effects on ISGs and viral titres using cell line model. HeLa cells were treated or not with IFN-b and were infected with YFV strains Asibi or YF-17D (MOI=1) or mock infected and we determined viral titres and mRNA expression at time points 3, 12, 24 and 48 hours-post-infection (hpi). Asibi and YF-17D evade IFN-b treatment as viral replication still progressed in IFN-b treated cells. At same MOI=1, YF-17D replicated more efficiently to yield significantly higher viral titres than Asibi strain. Expression of SOCS1 is kept around basal levels in YF-17D infected cells whiles Asibi downregulated SOCS1. SOCS5 was upregulated by both Asibi and YF-17D in early infection (3-12 hpi) but reduced to basal levels in late phases of infection 24 - 48 hpi. MxA and OAS1 (antiviral molecules), were upregulated throughout Asibi infection but remained basal till late phase in YF-17D infected cells. Expression of PIAS1 is upregulated early in Asibi-infected cells whiles both Asibi and YF-17D upregulated expression of PIAS4 in late phases of infection. Taken together, both Asibi and YF-17D evade host IFN response and reduced SOCS1 (antiproliferative molecule) in Asibi infected cells maintained cell viability for continued viral replication

P1796

CD44 alternative splicing isoforms and trafficking properties of antigen-specific T cells are regulated by TLRs engagement on T cells

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T cell trafficking is a highly regulated event in the immune response. Pathogen/pathogen receptors (PR) systems have a role in the control of trafficking of lymphocytes.
This study about the role of TLR2 in the regulation of trafficking of T cells, focused on its ability to reshuffle the role of CD44 and the alternative splicing of its pre-mRNA. Specifically, in the SJL mice, the regulation of alternative splicing of CD44 pre-mRNA by TLR2 depended on the CD62low/CD62high status and on the simultaneous activation *in vitro* via CD3+/CD28+, and led to over-representation of mRNAs specific for isoforms v8-v10 and, more prominently, v9-v10. mRNA specific for isoform v9-v10 was upregulated via activation of TLR2/TLR6 and TLR2/TLR1; mRNA specific for isoform v8-v10 was upregulated only by TLR2/TLR1 dimerization. Migration assays demonstrated that trafficking via TLR2 in T cells is CD44-dependent. In the C57/Bl6 mouse strain, only

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DOI: 10.1002/eji.201970400

isoform v8-v10-specific mRNA was upregulated, and the failure to produce isoform v9-v10-specific mRNA was dependent on the polymorphism of TLR2 between SJL and B6 mice. Finally, the stimulation of T cells by LPS or CpG (that engage the other TLRs expressed by activated T cells) modified alternative splicing of CD44 pre-mRNA, with a pattern of CD44 isoforms-specific mRNAs that varied according to each stimulus.
Thus, TLRs collectively represent a pathway through which pathogens or commensals of viral and bacterial origin modify trafficking of self- and allo-reactive antigen-activated T cells, by modulating the relative ratios of CD44 isoforms, leading to induction or inability to enter the CNS.

LB114

Sensing of mycobacterial arabinogalactan by galectin-9 leads to lung injury

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Mycobacterial arabinogalactan (MAG) is an essential cell wall component of *Mycobacterium tuberculosis* (*Mtb*), but its role in virulence and its host receptor remain unknown. Here, we show that chemically synthesized MAG induced matrix metalloproteinases (MMPs) to cause lung injury. Administration of MAG-specific aptamers eliminated *Mtb*-induced MMPs, and increased the survival of *Mtb*-infected mice or *M. marinum*-infected zebrafish. MAG interacted with galectin-9 with high affinity, and activated extracellular signal-regulated kinase (ERK) to induce the expression of MMPs. Moreover, deletion of galectin-9 or inhibition of MMPs alleviated MAG-induced lung injury. These results demonstrate that MAG is a novel *Mtb* virulence factor and galectin-9 is a novel host receptor for *Mtb* paving the way for the development of a novel type of effective immune modulators for tuberculosis (TB) therapy.

P2394

The essential role of stress granules in cGAS sensing of DNA

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cGAS (cyclic GMP-AMP synthase) is a major sensor of cytosolic DNA from invading pathogens and damaged cellular organelles. Activation of cGAS promotes the formation of membrane-less subcellular structures. Here, we found that cGAS bound G3BP1, a double stranded nucleic acid helicase involved in the formation of stress granules. Depletion of G3BP1 blocked subcellular cGAS condensation, diminished DNA-dependent cGAMP synthesis, and suppressed the interferon response to intracellular DNA and viral infection. We further demonstrated that cGAS directly bound PKR, which associated with subcellular cGAS deposits and promoted cGAS-dependent interferon responses. Together, these results illustrated that PKR-mediated stress granule formation augmented the DNA sensing function of cGAS, which suggests that there is a strong link between innate immune sensing and the cellular stress response to infection.

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DOI: 10.1002/eji.201970400

P2395

Dhx15 against enteric pathogen infection by mediating wnt signaling

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RNA Helicases contain more than 100 members including DEAH, DEAD and DExH box helicases. They are involved in various essential biological processes such as RNA splicing and editing, and immune responses toward viruses and tumor. However, there is a lack of genetic tools to study tissue or cell specific function of RNA helicases, as the complete deficient mice are normally lethal due to their central role in fundamental cellular processes. Previously we identified a RNA helicase Dhx15 as a potential viral RNA binder through pull down of Nod-like receptor Nlrp6 which is specifically expressed in intestine. Most recently we generate intestinal epithelial cell (IEC)- specific deficient mouse for Dhx15. Intriguingly, these mice are small in size and develop spontaneous ileitis. Though RNA sequencing of the ileal epithelial cells, we found significant reduction of wnt-targeted genes such as alpha defensins and Olfm4 which are critical for paneth cell and stem cell function in the intestine. Thought biochemical approaches, we found wnt3a is sufficient to induce Dhx15 cleavage, which promote its nucleic translocation, in turn to stabilize b-catenin to facilitate the transcription of wnt targets. Moreover, higher expressions of Dhx15, b-catenin, or HD5 are associated with worse prognosis of colorectal cancer (CRC), indicating the potential to targeting wnt signaling to treat CRC. These data for the first time identify an un-expected and indispensable role of a RNA helicase Dhx15 in intestinal development and anti-microbial response by mediating signaling of wnt.

P2396

FbaA protein of group A streptococcus initiates autophagy by binding integrin α5β1 receptor

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Group A streptococcus (GAS) is one of the most common pathogens, which can evade immune attack by invading epithelial or endothelial cells. However, GAS can be eliminated after 10 hours of invading cells in our research data. As we known, many pathogens can be eliminated by autophagy in host cells, which is currently considered to be the most effective way for non-phagocytic cells to defense pathogens . When we studied the relationship between GAS and autophagy, it was found that heat-inactivated GAS can induce autophagy, which suggested that the structural protein of GAS alone can initiate autophagy. Thus, we constructed two important surface protein mutant strains of GAS, and found that FbaA protein could be able to induce autophagy powerfully. But we also found that the autophagy induced by FbaA protein was not by binding to common pattern recognition receptor, such as Toll-like receptor. As FbaA was reported that is a main protein mediating cells invasion via binding to the receptor-integrin α5β1 of the host cell, we eventually confirmed that GAS activates intracellular autophagy signaling through its surface protein FbaA-FN-receptor-integrin α5β1 pattern. The Beclin-1 was activated through the mTOR-ULK1-Beclin-1 pathway, thereby enhancing the ability of Beclin-1/Vps34 complex to recruit Rab7, which ultimately promoted the formation of autophagosomes. These results suggest that integrin α5β1, a major family of non-phagocytic surface receptors, plays a protective role in innate immunity by initiating autophagy and eliminating invading intracellular pathogens.

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DOI: 10.1002/eji.201970400

P2397

CALML6 recruits CYLD to deubiquitinate TAK1 and confers protection against acute inflammation

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The nuclear factor-kappa b (NF-κB) signaling pathway plays crucial roles in innate immunity and inflammation. The aberrant activation of NF-κB signaling is implicated in diverse diseases and disorders. Here, we report that CALML6, a member of the EF-hand protein family, is a negative regulator of the NF-κB signaling pathway. CALML6 attenuated TNF-α-stimulated phosphorylation of proteins downstream of TGF-β-activated kinase 1 (TAK1) and inhibited TAK1-induced NF-κB activation. Further studies showed that CALML6 interacted with TAK1 and recruited the deubiquitylating enzyme cylindromatosis (CYLD) to repress the K63-linked polyubiquitination of TAK1. CALML6 transgenic mice had higher tolerances to lethal lipopolysaccharide (LPS) treatment and Vesicular Stomatitis Virus (VSV) infection in vivo. These findings suggest that CALML6 is a negative regulator of the NF-κB signaling pathway, which is important for maintaining the balance of the innate immune response.

P2398

Hyperglycemia triggered ATF6-CHOP signaling worsens liver ischemia/reperfusion Injury: immunomodulation by regulating β-catenin activity

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Hyperglycemia is an adverse factor of ischemia reperfusion injury (IRI) during liver transplantation or hepatectomy, but the underlying mechanism has not been elucidated. Herein, we addressed whether and how hyperglycemia triggered endoplasmic reticulum (ER) stress and affected liver IRI. Diabetic patients with benign liver condition undergoing hepatectomy were recruited in our study. Streptozotocin (STZ)-induced diabetic mice and control mice were subjected to hepatic IRI model. Macrophages were treated with low/high glucose (LG/HG) culture. In diabetic patients/mice, ER stress-related ATF6-CHOP pathway was specifically activated in liver. 4-phenylbutyrate (PBA), an inhibitor of ER stress, effectively attenuated hyperglycemia-related liver IRI based on biochemistry and histology, and decreased TLR4-related pro-inflammatory responses. Moreover, CHOP knockout (CHOP-KO) mice displayed CHOP-KO markedly reduced liver IRI and TLR4-related pro-inflammatory responses. Interestingly, β-catenin, a negative regulation factor of inflammation responses, was effectively inhibited accompanied by activation of ATF6-CHOP in diabetic mice, and almost restored in liver tissues from PBA-treated or CHOP-KO diabetic mice. Furthermore, β-catenin siRNA targeted Kupffer cells (KCs) abrogated CHOP KO-related protective effects on hyperglycemia-related liver IRI. In vitro, HG triggered ATF6-CHOP signaling, inhibited β-catenin expression, and resulted in TLR-inflammatory responses in bone marrow-derived macrophages (BMMs), which partly reserved in PBA-treated or CHOP-KO macrophage. Gain- and loss-of-function demonstrated that β-catenin play key roles in hyperglycemia triggered ATF6-CHOP signaling facilitated TLR4-related pro-inflammatory responses. This study demonstrates hyperglycemia triggers ATF6-CHOP signaling, inhibits β-catenin

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DOI: 10.1002/eji.201970400

activity, increases inflammatory responses, and exacerbates liver IRI, which might be provide a rationale for a novel therapeutic strategy for managing diabetic-related liver operation.

P2399

RNF170 acts as a negative regulator of TLR3-signaling by promoting TLR3 ubiquitination and degradation

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Toll-like receptor 3 (TLR3) as a type I transmembrane protein recognizes exogenous virus-derived double-strand RNA (dsRNA) and plays a critical role in antiviral innate immune responses. To avoid pathogenic inflammation, TLR3 signaling is subject to multilayer regulatory control mechanisms. Here, we identified that the E3 ubiquitin ligase RNF170 directly interacted with TLR3 protein by mass spectrometry analysis in mouse peritoneal macrophages upon dsRNA analog poly(I:C) stimulation. RNF170 selectively decreases TLR3-triggered signaling, leading to the inhibition of type I IFNs and proinflammatory cytokines in macrophage and mice. And mice with RNF170 knockout were less susceptible for EMCV infection in vivo. Mechanistically, RNF170 selectively mediated K48-linked ubiquitination on Lys766 of TIR domain of TLR3 and promoted TLR3 degradation through proteasome pathway. Our findings provide a feedback-negative way in terminating TLR3-triggered innate immune responses and preventing a protective response from causing injury to the host.

P2400

Nuclear CA-VI B epigenetically promotes IL-12 expression by interacting with PRMT5 in innate immune response

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Interleukin-12 (IL-12) plays a vital role for induction of protective immunity against intracellular bacterial infection. However, the regulation mechanisms of IL-12 expression are incompletely understood. Here we found that B type of carbonic anhydrase 6 (*Car6-b*, which encoded CA-VI B) is critical for host defense against *Listeria Monocytogenes* (LM) infection by epigenetically promoting IL-12 expression independent of its carbonic anhydrase activity. We found that *Car6-b* deficiency reduced IL-12 expression upon LM infection both *in vitro* and *in vivo*. As a result, loss of *Car6-b* impaired the clearance of LM in the mice, which caused much lower survival rate of *Car6*$^{-/-}$ mice compared to control groups after LM infection. Mechanistically, we observed that the nuclear CA-VI B interacted with protein arginine N-methyltransferase 5 (PRMT5) to selectively reduce symmetric dimethylation of histone H3 arginine 8 modification (H3R8me2s) at *Il12b* promoter, which increased the accessibility of chromatin and facilitated the transcription factor c-Rel to bind the promoter of *Il12b*. Our findings demonstrate a new function of *Car6-b* in regulating IL-12 expression through epigenetic mechanisms, which might provide the potential target for anti-inflammatory agents.

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DOI: 10.1002/eji.201970400

P2401

RNF2 suppresses interferon signaling by mediating K33-linked polyubiquitination of STAT1

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Interferon signaling pathway plays important roles in antiviral innate immune responses. STAT1 is the main transcription factor that initiates series of interferon-stimulated genes (ISGs) expression to restrict virus infection. Thus tight regulation of the transcriptional activity of STAT1 is necessary to protect host from virus infection as well as prevent pathogenesis of autoimmune diseases. Ubiquitination has been reported to be involved in the regulation of multiple signal transduction processes in antiviral responses, however, the relationship between ubiquitination and STAT1, especially regarding STAT1 transcriptional activity in the nucleus remains to be elucidated. Here we identified an E3 ubiquitin ligase RNF2, which is a central moiety of polycomb remodeling complex1 (PRC1), negatively regulated STAT1-mediated ISGs expression. Myeloid cell-specific RNF2-deficient macrophages exhibited much more enhanced antiviral responses, and myeloid cell-specific RNF2-deficient mice were more resistant to virus-induced death. We further demonstrated that RNF2 inhibited ISGs transcription mainly through K33-linked polyubiquitination on Lys379 of STAT1 in the nucleus, and by this modification, RNF2 promoted the disassociation of STAT1 from DNA and then indirectly promoted the dephosphorylation of STAT1. However, this blocking effect is beyond the chromatin remodeling function identified previously of RNF2. Our results demonstrate a new regulating pattern of ubiqutination in interferon signaling pathway, providing a new potential target for both infectious and over-reactive autoimmune diseases.

P2402

Effects of curcumin on intracellular and extracellular heat shock protein 70 in rat model of thrombosis

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Objective: Effects of curcumin on thrombosis model in rats and its relationship with damage-associated model molecule (DAMP) heat shock protein 70 (HSP70) were studied with turmeric extract as reference.
Methods: After 14 days of intragastric administration of curcumin and turmeric extract, thrombosis models were established in rats by physical method. Blood samples and vascular tissues of rats were obtained, and sham operation group and distilled water group as control group. Plasma HSP70 levels were detected by ELISA and the expression of HSP70 in vascular tissues of rats was measured by immunohistochemical staining.
Results: The plasma HSP70 level in thrombotic model group was significantly higher than that in sham-operated group (p=0.003). The plasma HSP70 level in curcumin low and high dose group was significantly lower than that in sham-operated group (p=0.005, p=0.002). Compared with sham-operated rats, the expression of HSP70 in vascular tissues of thrombotic model rats was increased. Curcumin could increase the expression of HSP70 in vascular tissues of thrombotic model rats, but curcuma extract had no such effect.
Conclusion: The anti-inflammatory effect of curcumin is related to the increase of intracellular HSP70

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DOI: 10.1002/eji.201970400

expression and the decrease of extracellular HSP70. The anti-inflammatory effect of turmeric extract may be through other mechanisms.

P2403

Ubiquitin-specific protease 15 negatively regulates IL-1β-induced and virus-induced NF-κB activation by targeting TRAF6 for deubiquitination

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Nuclear factor κB (NF-κB) is a key transcription factor in inflammation and immune responses. Ubiquitination of TRAF6 is essential in IL (interleukin)-1β-induced and Sendai virus (SeV)-induced NF-κB activation, yet the molecular mechanisms responsible for its negative regulation are still poorly understood. Here, we demonstrated that ubiquitin-specific protease 15 (USP15), a member of the deubiquitinases family, acts as a novel negative regulator in NF-κB signaling by targeting TRAF6 for deubiquitination in HEK293T cells. Overexpression of USP15 inhibited IL-1β-induced and SeV-induced activity of the NF-κB-dependent promoter, and downregulated the expression of phospho-p65 by using western blotting technique. And the mRNA level of proinflammatory cytokines, including IL-6, IL-8, and TNF-α, reduced markedly after USP15 overexpression. And knockdown of USP15 significantly increased IL-1β-induced cytokine expression. Furthermore, we found that USP15 specifically removed lysine 63-linked polyubiquitin chains attached to TRAF6 in a dependent manner. In addition, the residues of USP15 important for their role were also identified. Cysteine 269 and histidine 862 were identified as two critical residues of USP15 for TRAF6 deubiquitination activity. Taken together, our data reveal that USP15 acts as a negative regulator of IL-1β- and SeV-induced NF-κB signaling through deubiquitinating TRAF6.

P2404

Tyrosine phosphorylation negatively regulates TBK1 to alleviate innate antiviral responses

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Cytosolic nucleic acid sensing elicits interferon production for primary antiviral defense through cascades controlled by protein ubiquitination and Ser/Thr phosphorylation. Here we show that TBK1, a core kinase of antiviral pathways, is inhibited by tyrosine phosphorylation. The Src family kinases (SFKs) LCK, HCK and FGR directly phosphorylate TBK1 at Tyr354/394, to prevent TBK1 dimerization and activation. Accordingly, antiviral sensing and resistance was substantially enhanced in LCK/HCK/FGR triple knockout cells and ectopic expression of LCK/HCK/FGR dampened the antiviral defense in cells and zebrafish. Small molecule inhibitors of SFKs, which are conventional anti-tumor therapeutics, enhanced antiviral responses and protected zebrafish and mice from viral attack. Viral infection induced the expression of LCK/HCK/FGR through TBK1-mediated mobilization of IRF3, thus constituting a negative feedback loop. These findings unveil the negative regulation of TBK1 via tyrosine phosphorylation and the functional integration of SFKs into innate antiviral immunity.

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DOI: 10.1002/eji.201970400

P2405

E3 ubiquitin ligase RNF128 promotes innate antiviral immunity through K63-linked ubiquitination of TBK1

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TBK1 is essential for interferon-β (IFN-β) production and innate antiviral immunity, its activation must be tightly regulated to maintain immune homeostasis. TBK1 activation is regulated in a variety of ways, including protein ubiquitination and phosphorylation. It has been reported that several E3 ligases and deubiquitinating enzymes regulate TBK1 activation, however, their roles have not been confirmed in vivo. Especially, the E3 ubiquitin ligase responsible for K63-linked TBK1 polyubiquitination upon DNA-virus infection has not been identified. Here we demonstrated that E3 ubiquitin ligase RNF128 (also known as GRAIL) is an essential positive regulator of innate antiviral immunity to RNA virus and DNA virus. RNF128 knockdown or deficiency impaired IRF3 activation and IFN-β signaling. Consistently, RNF128 deficient mice were more susceptible than wild-type mice to infection with the RNA virus vesicular stomatitis virus (VSV) and the DNA virus herpes simplex virus type 1 (HSV-1). Mechanistically, RNF128 associated with TBK1 and promoted TBK1 kinase activity through conjugation of K63-linked ubiquitin chains. Thus, our study identified RNF128 as a new E3 ligase for K63-linked ubiquitination of TBK1 to RNA and DNA viruses.

P2406

Essential roles of S100A10 in Toll-like receptor signaling and immunity to infection

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Toll-like receptors (TLRs) are key pattern-recognition receptors that mediate innate immune responses to infection. However, uncontrolled TLR activation can lead to severe inflammatory disorders such as septic shock. The molecular mechanisms through which TLR responses are regulated are not fully understood. Here, we demonstrate an essential function of S100A10 in TLR signaling. S100A10 was constitutively expressed in macrophages but was significantly downregulated upon TLR activation. S100A10-deficient macrophages were hyper-responsive to TLR stimulation, and S100A10-deficient mice were more sensitive to endotoxin-induced lethal shock and *E.coli*-induced abdominal sepsis. Mechanistically, S100A10 regulated macrophages inflammatory responses by interfering with the appropriate recruitment and activation of the receptor-proximal signaling components, and eventually inhibited TLR-triggered downstream signaling. These findings expand our understanding of the TLR signaling, and establish S100A10 as an essential negative regulator of TLR function and potential therapeutic target for treating inflammatory diseases.

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DOI: 10.1002/eji.201970400

P2407

IKIP negatively regulates NF-κB activation and inflammation through inhibition of IKKα/β phosphorylation

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Nuclear factor kB (NF-kB), a key activator of inflammation, is essential for the initiation of host immune responses. However, excessive NF-κB activation can directly result in tissue damage, autoimmune diseases, and inflammation-associated carcinogenesis. Here, we demonstrated that the IKK-interacting protein (IKIP) negatively regulates NF-κB activation by inhibiting the phosphorylation of IKKα/β. IKIP Competed with NEMO for IKKα/β binding and inhibited the phosphorylation of IKKα/β. Upon stimulation, IKIP deficiency markedly enhanced the phosphorylation of IKKα/β and the production of NF-κB-responsive genes in macrophages. Moreover, IKIP deficient mice were more susceptible to LPS-induced septic shock and dextran sodium sulfate (DSS)-induced colitis. Our study confirms a previously unrecognized role for IKIP as a negative regulator of NF-κB signaling pathways and suggests an important role for IKIP in homeostatic control of innate immunity.

P2408

Incidence, risk factors and prognosis associated with brain metastases in newly diagnosed breast cancer: a population-based study

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Background: Breast cancer is a significant cause of mortality among patients. In previous studies, 5-15% of patients diagnosed with breast cancer have been found to have brain metastases. This study aimed to evaluate potential relationships between breast cancer and brain metastases, with an emphasis on the role of molecular subtypes.
Methods: Data were obtained from the Surveillance, Epidemiology, and End Results program (2010-2015). Among 379229 cases, 1562 patients with brain metastases were acquired. Multivariable logistic regression was performed to identify factors associated with the presence of brain metastases at diagnosis, and multivariable cox regression was performed to identify factors associated with all-cause mortality（ACM） and breast cancer-specific mortality (BCSM**).**
Results: Patients with the HR-negative HER2-positive (1.12%) and HR-positive HER2-negative (0.23%) subtypes displayed the highest and lowest incidence rates, respectively. The median survival was longest (20.0months) among patients with the HR-positive HER2-positive subtype and shortest (5.0months) among patients with the triple-negative subtype. Additionally, we found potential relationships between breast cancer with brain metastases and different clinical variables.
Conclusions: Based on our assessment of the incidence and prognosis of breast cancer patients with brain metastases at the time of diagnosis, we should establish a risk evaluation model with characteristics of breast cancer incidence in different countries, according to the results of risk assessment, targeted to focus on screening high-risk women. This will allow radiotherapy or novel targeted therapy to be administered earlier.

DOI: 10.1002/eji.201970400

P2409

The competitive function Sirtuin5 and Sirtuin2 on inflammation of during sepsis by binding and regulating of NF-κB p65

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Severe sepsis is a systemically mixed, pro- and anti-inflammatory response to an infectious organism and severe tissue injury, driven by a lack of normal immune homeostatic functions. Hyper-inflammation during acute phase and hypo-inflammation during immunosuppressive phase in macrophages/monocytes is the critical characters of septic response, in which Toll-like receptor (TLR)-triggered inflammatory response play a major function. Energy status and mitochondria metabolism during inflammation and sepsis progress needs to be further elusive. Here, we found that the NAD+ level was significantly related with of inflammatory cytokine production *in vivo* and *in vitro*. Screening the expression and function of NAD+ dependent enzyme, type III deacetylase Sirtuin family, Sirt3/5 had opposite expression pattern and function of Sirt2 on inflammation in macrophages. Sirt5 deficient mice were more resistant to LPS induced sepsis with decreased inflammation in both acute and suppressive phase. Sirt5 deficient macrophages showed decreased TLR4-induced signal and inflammatory cytokines production. Mechanism research found that Sirt5 competitively interacted with p65 of NF-κB with Sirt2 and block the deacetylation of p65 by Sirt2 in enzymatic activity independent way. Overexpression of SIRT5 in endotoxin tolerant macrophages could increase TLR4 induced response and ameliorate the hypo-inflammation status. Our results outline the novel function and mechanism of Sirtuin family sepsis, which indicates that targeting Sirtuin family at different phases of sepsis will be promising for therapy at precise medicine level.

P2410

E3 ligase Nedd4l promotes antiviral innate immunity by catalyzing K29-linked cysteine ubiquitination of TRAF3

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Ubiquitination of lysine residue is one of the most prevalent protein posttranslational modifications, playing important roles in diverse cellular and biological processes. However, little is known concerning about the physiological significance of non-lysine residue being ubiquitination site, such as cysteine (Cys, C). Nedd4l (neural precursor cell expressed developmentally down-regulated 4-like, also known as Nedd4-2) is a HECT E3 ubiquitin ligase of the Nedd4 family. Here we show that Nedd4l positively regulates antiviral immunity by catalyzing K29-linked cysteine ubiquitination of TRAF3. Deficiency of Nedd4l significantly impairs type I interferon and proinflammatory cytokine production in virus-infected macrophages. Nedd4l deficient mice are more susceptible to virus infection. Nedd4l deficiency inhibits virus-induced ubiquitination of TRAF3, the binding between TRAF3 and TBK1, and subsequent phosphorylation of TBK1 and IRF3. Nedd4l promotes PRR-triggered K63- and K48-linked ubiquitination of lysine residue in TRAF3 by catalyzing K29-linked ubiquitination of cysteine residue in TRAF3. Nedd4l directly interacts with TRAF3 and catalyzes K29-linked ubiquitination of Cys56 and Cys124, two cysteines located in zinc fingers of TRAF3. Mutation of Cys56 and Cys124 diminish

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DOI: 10.1002/eji.201970400

Nedd4l-catalyzed K29-linked ubiquitination, but robustly increase K63- and K48-linked self-ubiquitination of TRAF3 and TRAF3-mediated signaling, demonstrating that Nedd4l promotes antiviral immunity by catalyzing ubiquitination of zinc finger cysteines in TRAF3.

P2411

Ltn1 positively regulates antiviral responses by targeting STING protein for K63-linked ubiquitination

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Ltn1, a 60S ribosome-associated E3 ubiquitin ligase, is a major component of the quality control complex (RQC), which plays an essential role in protein homeostasis by targeting the aberrant nascent polypeptides for proteasomal degradation. The E3 ubiquitin ligase Ltn1 is likely involved in diverse biological processes. Their physiological functions in innate immunology, however, remain largely unknown. Here, we reported that the E3 ubiquitin ligase Ltn1 interacted with STING during HSV-1 infection. The expression of Ltn1 was increased after infected with HSV-1 in mouse peritoneal macrophages and human THP-1 monocytic cells. siRNA knockdown of Ltn1 expression reduced HSV-1-, cytoplasmic DNA- or cGAMP-induced production of type I interferons (IFNs) and facilitated HSV-1 replication. Phosphorylation of TBK1 and IRF3 after HSV-1 infection was also decreased in Ltn1 siRNA-transfected mouse peritoneal macrophages and THP-1 cells. Mechanistically, Ltn1 specifically catalyzed the K63-linked poly-ubiquitination of STING which promote the aggregation of STING. These results provide physiological evidence that links the Ltn1 with antiviral immune responses.

P2412

The metabolic regulator Lamtor5 suppresses inflammatory signaling via TFEB-dependent autophagic degradation of TLR4

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Full-fringe immune responses are essential for eliminating pathogens but must be tightly controlled to avoid sustained immune activation and potential tissue damage. Engagement of TLR4, a canonical pattern-recognition receptor, has been proposed to trigger the inflammatory responses with different magnitude and duration depending on its cellular compartmentalization. In the present study, we identified an unexpected role for Lamtor5, a newly identified component of the amino acid-sensing machinery, in modulating TLR4 signaling and controlling inflammation. Specifically, Lamtor5 associated with TLR4 via the LZ/TIR interaction and facilitated their co-localization at autolysosomes, preventing lysosomal tethering and activation of mTORC1 upon LPS stimulation and thereby de-repressing TFEB to promote autophagic degradation of TLR4. Loss of Lamtor5 was shown to be unable to trigger the TFEB-driven autolysosomal pathway and delay degradation of TLR4, leading to sustained inflammation and hence the increased mortality among Lamtor5 haploinsufficient mice during endotoxic shock. Intriguingly, we also found that nutrient deprivation, particularly leucine deletion, blunted inflammatory signaling and conferred protection for endotoxic mice. This effect however was largely abrogated upon Lamtor5 deletion. We thus propose a homeostatic function of

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DOI: 10.1002/eji.201970400

Lamtor5 that couples pathogenic insults and nutrient availability to shape the optimized inflammatory response, which may have implications for TLR4-associated inflammatory and metabolic disorders.

P2413

TAO3 acts as a direct upstream kinase for TBK1 and is essential in antiviral innate response

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Tank-binding kinase 1 (TBK1) functions as a central node in innate signaling initiated by multiple pattern recognition receptors for induction of type I interferons (IFN-I). However, upstream kinase for direct phosphorylation of TBK1 has not been identified. Here we report that the serine threonine kinase TAO3 directly phosphorylates TBK1 on Ser93 and promotes TBK1 autophosphorylation on Ser172, thus facilitating TBK1 activation. By using Tao3−/− cells and Tao3 conditional knockout mice, we demonstrate that TAO3 deficiency leads to significantly impaired TBK1 phosphorylation and activation as well as IFNβ production in vitro, and causes hindered antiviral innate response in vivo. Our study suggests that TAO3 is essential in TBK1 activation and antiviral innate response by acting as an upstream kinase for TBK1, and most likely TAO3 may participate in these pathological processes by activating TBK1.

P2414

NLRP2 negatively regulates antiviral immunity by interacting with TBK1

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Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are intracellular pattern recognition receptors (PRRs) that regulate a variety of inflammatory and host defense responses. Unlike the well-established NLRs, the roles of NLRP2 are controversial and poorly defined. Here, we report that NLRP2 acts as a negative regulator of TANK-binding kinase 1 (TBK1)-mediated type I interferon (IFN) signaling. Mechanistically, NLRP2 interacted directly with TBK1, and this binding disrupted the interaction of TBK1 and interferon regulatory factor 3 (IRF3), which interfered with TBK1-induced IRF3 phosphorylation. IFNs induce a series of proteins that have well-known antiviral or immune-regulatory functions, and tight control of the IFN signaling cascade is critical for limiting tissue damage and preventing autoimmunity. Our studies indicate that the NLRP2-TBK1 axis may serve as an additional signaling cascade to maintain immune homeostasis in response to viral infection.

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DOI: 10.1002/eji.201970400

P2415

CARD9 protects lung cancer development by suppressing the expansion of myeloid-derived suppressor cells and IDO production

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Caspase recruitment domain-containing protein 9 (CARD9) is an adaptor protein and highly expressed in myeloid cells. Our previous study demonstrates a critical protective effect of CARD9 in the development of colitis-associated colon cancer. Nevertheless, the effect of CARD9 in lung cancer is still unclear. Here, using a mouse lewis lung cancer model, we found the tumor burden of CARD9$^{-/-}$ mice was much heavier than that in wild-type (WT) mice. More myeloid-derived suppressor cells (MDSCs) was accumulated and less cytotoxicity T lymphocyte were found in tumor tissues of CARD9$^{-/-}$ mice, compared with WT mice. Depleting MDSCs using anti-Gr1 antibody can significantly decrease tumor burden in CARD9$^{-/-}$ mice. Furthermore, the non-canonical NF-κB pathway was activated in CARD9$^{-/-}$ mice-derived MDSCs. Deficiency of CARD9 enhanced expression of IDO in MDSCs via non-canonical NF-κB pathway. Moreover, correlations between *CARD9* expressions and MDSCs relative genes (*IDO*, *iNOS-2* and *ARG-1*) were further confirmed in tumor tissues from lung cancer patients. Taken together, we showed a CARD9-NF-κB-IDO pathway in MDSCs which can inhibit the suppressive function of MDSCs and protect lung cancer development.

P2416

Loss of RIG-I leads to a functional replacement with chMDA5-chLGP2 to sense RNA virus

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The ability to sense viral RNA has emerged as a critical component of innate immunity. Viral RNA is detected in the cytoplasm by the RIG-I-like receptors (RLRs; including RIG-I, MDA5 and LGP2), has been subjected to numerous microbial pathogens challenges and has undergone a dynamic evolution. RIG-I was only defected in several *teleosteans* and chicken. Previous studies demonstrated that chicken MDA5 (chMDA5) was believed to be responsible for avian influenza infection for compensating deficiency disadvantage. Our evolutionary analysis in this study was dissected that chMDA5 and chLGP2 had underwent positive selection, a potential trade-off to the loss of RIG-I. Moreover, chLGP2 could sense NDV, which is required for promoting chMDA5-mediated antiviral signaling, to successfully balance the defect of loss RIG-I in chicken. The positively selected amino acids sites in chMDA5 and chLGP2 endowed their diversity function. Together, our result suggested that loss of RIG-I leads to a functional replacement with MDA5, in co-operation with chLGP2 to conquer “arms-races” between host and virus.

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DOI: 10.1002/eji.201970400

P2417

Global identification of Tupaia lncRNAs reveals the regulation of HCV infection by a novel RNA

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Hepatitis C virus (HCV) evades the host response through a complex combination of processes that including signaling interference. The evasion strategies support persistent infection and the replication of HCV. Investigating the molecular mechanisms by which HCV regulates the host innate immune response is of crucial importance and may reveal targets for novel therapeutic strategies. Human and chimpanzee are the only known species susceptible to HCV infection. The restricted species tropism of HCV is the major limitation to HCV research and development of valid drugs and vaccines. The Chinese tree shrew (*Tupaia belangeri*), a close relative of primates, is emerging as a workable animal model for investigating the HCV infection. Although the genome and transcriptome data of the Chinese tree shrew were available, our current knowledge about the identity and function of lncRNAs in infected cells for the Chinese tree shrew is very limited. In this study, we compared the global RNA expression pattern of tree shrew primary liver-derived cells at three time points after HCV infection, and identified 3728 lncRNAs in normal conditions from RNA-seq. A total of 33 up-regulated and 17 down-regulated lncRNAs were detected in HCV infected cells. We focused on the role of lncRNA#2389 in the antiviral innate immune response. Using gain- or loss-of-function analyses, lncRNA 2389 is associated with type I IFN signaling and HCV replication. This study will help in the identification of novel cellular pathways and understanding the immune evasion strategies for chronic HCV infection.

P2418

USP29 maintains stability of housekeeping cGAS and promotes cellular antiviral responses and autoimmunity

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Cyclic GMP-AMP synthase (cGAS) is an essential sensor of cytosolic DNA and critically mediates innate immune responses and inflammation. The activity of cGAS is heavily and strictly regulated by various posttranslational modifications such as ubiquitination. However, the deubiquitination process of cGAS is not fully understood. Here, we report that ubiquitin-specific protease 29 (USP29) deubiquitinates housekeeping cGAS and promotes cellular innate immune responses against DNA virus infection as well as autoimmunity. USP29 constitutively interacts with cGAS and their association is potentiated after HSV-1 infection. Knockdown or knockout of USP29 severely impairs HSV-1-induced expression of type I interferons (IFNs) and proinflammatory cytokines and potentiates HSV-1 replication. Consistently, *Usp29*$^{-/-}$ mice produce decreased type I IFNs and proinflammatory cytokines after HSV-1 infection and exhibit increased susceptibility to lethal HSV-1 infection compared to wild-type littermates. Mechanistically, USP29 deconjugates K48-linked polyubiquitin chains from and stabilizes cGAS in uninfected cells or after HSV-1 infection. Reconstitution of cGAS into *Usp29*$^{-/-}$ cells fully rescues type I IFN induction and cellular antiviral responses after HSV-1 infection. In addition, knockout of USP29 in the *Trex1*$^{-/-}$ background rescued mice from lethality and alleviated the autoimmune symptoms. Our findings thus reveal a critical role of USP29 in cellular antiviral responses and autoimmunity and provide insight into the regulation of housekeeping cGAS.

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DOI: 10.1002/eji.201970400

Co-inhibitory Molecules and Receptors

O233

Tim-3 expression alleviates ROS-induced liver injury by regulating macrophage activation in NASH mice

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Tim-3, the well-known immune checkpoint, plays roles in regulation of both adaptive and innate immune cells including macrophages and greatly involves in chronic liver diseases. However, the precise roles of Tim-3 in nonalcoholic steatohepatitis (NASH) remain unstated. In the current study, we, for the first time, demonstrated the significantly increased expression of Tim-3 on all detected liver macrophage subsets in MCD mice, including F4/80$^+$CD11b$^+$, F4/80$^+$CD68$^+$ and F4/80$^+$CD169$^+$ macrophages. Remarkably, **Tim-3 knockout (KO) significantly accelerated MCD induced liver steatosis, displaying higher serum ALT, larger hepatic vacuolation, more liver lipid deposition and severer liver fibrosis.** Moreover, compared to wild type C57BL/6 mice, **Tim-3 KO MCD mice demonstrated enhanced expression of NOX2, NLRP3 and caspase-1 p20 together with increased generation of IL-1β and IL-18 in livers.** In vitro studies demonstrated that **Tim-3 negatively regulated production of reactive oxygen species (ROS) and related downstream pro-inflammatory cytokines IL-1β, IL-18 secretion in macrophages**. Exogenous administration of N-Acetyl-L-cysteine (NAC), a small molecular inhibitor of ROS, remarkably suppressed caspase-1 p20 expression and IL-1β, IL-18 production in livers of Tim-3 KO mice, thus significantly reduced the severity of steatohepatitis induced by MCD. In conclusion, Tim-3 is a promising protector in MCD induced steatohepatitis by controlling ROS and associated pro-inflammatory cytokine productions in macrophages.

O234

Quantitative interactomics in primary T cells provides a rationale for concomitant PD-1 and BTLA coinhibitor blockade in cancer immunotherapy

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Deciphering how TCR signals are modulated by coinhibitory receptors is of fundamental and clinical interest. A plethora of coinhibitory receptors is expressed on a T cell in variable levels, which cooperatively modulate T cell reactivity. Among coinhibitors, PD-1 and BTLA are evolutionary related, and coexpressed on human and mouse tumor-antigen specific CD8$^+$ T cells. Accordingly, BTLA can likely substitute for PD-1 in conditions where immune-checkpoint inhibitors target PD-1. **By using quantitative interactomics, we define the composition and dynamics of the PD-1 and BTLA coinhibitory signalosomes in primary effector T cells and at a T cell-antigen presenting cell interface.** We also solve the existing controversy regarding the role of the SHP-1 and SHP-2 protein-tyrosine phosphatases in mediating PD-1 coinhibition. **PD-1 predominantly recruits SHP-2, but when absent, it recruits SHP-1 and remains functional. In contrast, BTLA predominantly**

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DOI: 10.1002/eji.201970400

recruits SHP-1 and to a lower extent SHP-2. By separately analyzing the PD-1-SHP-1 and PD-1-SHP-2 complexes, we show that both dampen the TCR and CD28 signaling pathways equally. Therefore, our study illustrates how comparison of coinhibitory receptor signaling via quantitative interactomics in primary T cells unveils their extent of redundancy and provides a rationale for designing combinations of blocking antibodies in cancer immunotherapy based on undisputed modes of action.

O235

STAT3-mediated atypical IFN-gamma signaling modulates the PD-1 ligands in CD11b-positive subpopulation of MDS and AML cells

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Introduction: Upregulation of PD-L1 and PD-L2 on cancer cells is one of the unfavorable influences of IFN-γ, but how this potent anti-tumor cytokine regulates the PD-1 ligands in myeloid blasts is not clear. This study explores the role of STAT3, a pro-leukemogenic transcription factor, on IFN-γ-induced PD-L1 and PD-L2 expression in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). **Results:** CD11b$^+$ sub-population of AML and MDS cells displayed enhanced capacity to respond to IFN-γ and upregulate PD-L1 and PD-L2. Induction of myeloid blasts' maturation by all-trans retinoic acid (ATRA) or 1α,25-dihydroxyvitamin D3 (vitamin D) increased the CD11b$^+$ fraction that expressed PD-1 ligands. Intriguingly, STAT3 pathway was potently induced by IFN-γ and strengthened upon prolonged exposure. Genetic manipulation of STAT3 activity in the myeloid leukemia cells specifically modulated the expression of PD-L1. Interfering with the STAT1/STAT3 axis by a small-molecule inhibitor, stattic, also inhibited the expression of PD-L1.
Conclusion: The CD11b$^+$ AML and MDS cells represent a specific sub-population which can efficiently divert IFN-γ-mediated signals to facilitate immune resistance through STAT3 and PD-1 ligands.

LB041

Elevated expression of TIGIT on T cells associates with advanced disease and dysfunctional phenotype in oral squamous cell carcinoma patients

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The elevation of inhibitory immune checkpoint molecules within tumor microenvironment has a powerful effect on cancer progression. However, the representative immune checkpoint molecules might vary in different cancers. In our study, we firstly compared the expression levels of five common checkpoint molecules and found that the T-cell immunoreceptor with immunoglobulin and tyrosine-based inhibitory motif domains (TIGIT) was highly elevated on both CD4$^+$ and CD8$^+$ T cells from peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating lymphocytes (TILs) in patients with oral squamous cell carcinoma (OSCC) by flow cytometry. The elevation of TIGIT on CD4$^+$ T cells

DOI: 10.1002/eji.201970400

(18.1%) and CD8$^+$ T cells (34.9%) also correlated with higher T stage and nodal invasion in OSCC. To demonstrate the phenotype and function of TIGIT in OSCC patients, we revealed that TIGIT was preferentially expressed in activated CD4$^+$ and CD8$^+$ T cells in OSCC PBMCs and TILs. Furthermore, TIGIT$^+$CD4$^+$ and TIGIT$^+$CD8$^+$ T cells sorted from OSCC patients showed a dysfunctional phenotype (low cell proliferation and low secretion of IL-2, TNF-α and IFN-γ), and TIGIT$^+$CD4$^+$ T cells exhibited inhibitory function (high expression of Foxp3 and high amounts of IL-10). Importantly, TIGIT blockade could enhance the proliferation ability and effective cytokine production (IL-2, TNF-α and IFN-γ) for both CD4$^+$ and CD8$^+$ T cells *in vitro*. Results indicate that TIGIT plays an important role in the development of OSCC. TIGIT-expressing T cells exhibit a lower effector cytokine-releasing phenotype, which can be reversed by TIGIT blockade *in vitro*.

LB077

The molecular basis of the interaction between immune checkpoint CD226 and its ligand Necl-5

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As an indispensable part of the immune system, T lymphocytes and NK cells play important roles in responding to viral infection and malignancies by cell-mediated cytotoxicity. The functions of T lymphocytes and NK cells are regulated by various stimulatory or inhibitory receptors. Recently, a paired receptor family, which specifically recognizes the nectin and nectin-like (Necl) family members, was discovered. These emerging receptors are activating receptors CD226 and CRTAM, as well as inhibitory receptors TIGIT, CD96 and CD112R.
In our work, we reported the structures of human and mouse CD226, both of which present a unique side-by-side arrangement pattern of two tandem IgV domains. This arrangement mode is distinct from the conventional head-to-tail organizing mode, as shown in the structure nectin/Necl molecules, of all the known Ig-like superfamily members. The only exception we found is VCBP3, an Ig-like molecule in amphioxus that diverged early in phylogeny. Moreover, we systematically analyzed the binding mechanism between CD226 and Necl-5 by determining a hybrid complex structure of mouse CD226 bound to Necl-5, which revealed a conserved “double-lock-and-key” binding motif within the first domain of CD226. Intriguingly, we found that the second domain of CD226 both provides structural supports for the unique architecture of CD226 and forms direct interactions with CD155. This distinct binding model is unique in TIGIT-CD226-CD96 paired receptor family.
Taken together, our work might give a hint of the evolution of Ig-like molecules and provide molecular basis for the development of CD226-targeted antitumor immunotherapy.

P0022

Immune-checkpoint protein VISTA regulates the IL-23/IL-17 inflammatory axis in an IMQ induced psoriasis model

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V-domain Immunoglobulin Suppressor of T cell Activation (VISTA) is an inhibitory immune-checkpoint molecule that suppresses CD4$^+$ and CD8$^+$ T cell activation. *Vsir*$^{-/-}$ mice developed loss of peripheral tolerance and multi-organ chronic inflammatory phenotypes. Whether or not VISTA regulates innate immunity has not been demonstrated. Our current study shows that VISTA-blocking antibody enhanced anti-tumor T cell response, and synergized with a peptide vaccine and TLR7 agonist imiquimod as adjuvant to suppress tumor growth in the B16 melanoma model. To better define the

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DOI: 10.1002/eji.201970400

regulatory role of VISTA in inflammation, we employed a murine model of psoriasis induced by topical treatment of IMQ. Our data show that VISTA deficiency exacerbated the psoriasiform inflammation. Enhanced TLR7 signaling in *Vsir*$^{-/-}$ dendritic cells (DCs) led to the hyper-activation of Erk1/2 signaling and augmented the production of IL-23. IL-23, in turn, promoted the expression of IL-17A in both TCRgd^{+} T cells and CD4^{+} Th17 cells. Furthermore, VISTA regulates the peripheral homeostasis of CD27^{-} gd T cells, and their activation upon TCR-mediated or cytokine-mediated stimulation. IL-17A-producing CD27^{-} gd T cells were expanded in the Vsir$^{-/-}$ mice and amplified the inflammatory cascade. Together, these results indicate that VISTA is a critical regulator of the IL-23/IL-17-mediated inflammatory axis. Ongoing studies will define how IL-17 regulates tumor-reactive immunity in the context of VISTA blockade and TLR vaccine adjuvant.

P0023

The novel functional gene CPVL promotes the progression of the glioma by regulating the IFN-γ/Stat1 signaling pathway

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Background: Carboxypeptidase, vitellogenic-like (CPVL) is a serine carboxypeptidase of unknown function that was first characterized in human macrophages. The function of CPVL in various tumor diseases has not been reported to date, and the clinical significance and biologic role of CPVL in glioma remains unclear.
Methods: The expression of CPVL in normal glial cells, glioma cells, and five-paired glioma and adjacent noncancerous tissues was quantified using real-time polymerase chain reaction and western blotting. CPVL protein expression was analyzed in 179 archived, paraffin-embedded glioma samples using immunohistochemistry. The roles of CPVL in glioma cell apoptosis, cell proliferation, cell cycle, and tumorigenicity were explored *in vitro* and *in vivo*. Ingenuity Pathway Analysis (IPA) was used to determine the downstream target pathway affected by CPVL on glioma cell apoptosis. Rescue experiments were performed to verify the results of IPA.
Results: CPVL was significantly upregulated in glioma cells and tissues compared with normal cells and tissues. Immunohistochemical analysis revealed a significant correlation of CPVL expression with advanced clinical stage and poorer survival. Silencing of CPVL promoted glioma cell apoptosis, inhibited glioma cell proliferation and tumorigenicity *in vitro* and *in vivo*, and regulated the glioma cell cycle *in vitro*. CPVL silencing resulted in the activation of the IFN-γ/Stat1 signaling pathway, thereby inducing the apoptosis of glioma cells.
Conclusions: Our results suggest that **high CPVL expression is associated with poor overall survival in patients with glioma and that CPVL silencing activated the IFN-γ/Stat1 signaling pathway, resulting in the apoptosis of glioma cells.**

P0024

Discovery of a peptide inhibitor targeting human PD-L1 for cancer immunotherapy

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The human peroxiredoxin-5 (hPRDX5) was a member of the family of antioxidant enzymes, which could resist immunosuppression by promoting immune organs development, lymphocyte proliferation and up-regulation of the levels of serum cytokines. However, being a recombinant protein, the

DOI: 10.1002/eji.201970400

hPRDX5 have some issues including the high production cost and bad tissue penetration. Compared to macromolecular drugs, synthetic peptides have several advantages as drug candidates, including lower manufacturing costs, reduced immunogenicity, and better organ or tumor penetration. Herein in this work, in silico proteolysis and biological evaluation were used to automate the design of new peptides with anti-tumor activity. We found that **the most potent peptide could block the PD-1/PD-L1 interaction effectively with an IC_{50} of 0.646μM, and could restore the function of Jurkat T cells which had been suppressed by stimulated HCT116 cells. Moreover, it significantly slowed the growth of CT26 tumor in Balb/c mice relative to controls with no sign of bodyweight loss,** and showed extraordinary low acute toxicity in vivo. Peptides described here may provide novel low-molecular-weight drug candidates for cancer immunotherapy.

P0025

The signature of liver cancer in immune cells DNA methylation

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Background: The idea that changes to the host immune system are critical for cancer progression was proposed a century ago and recently regained experimental support.
Results: Herein, the hypothesis that hepatocellular carcinoma (HCC) leaves a molecular signature in the host peripheral immune system was tested by profiling DNA methylation in peripheral blood mononuclear cells (PBMC) and T cells from a discovery cohort (n = 69) of healthy controls, chronic hepatitis, and HCC using Illumina 450K platform and was validated
in two validation sets (n = 80 and n = 48) using pyrosequencing. Our result found that a CG site in the promoter of PD-1 is gradually demethylated in PBMC as HCC progresses, and the average methylation of all significantly hypomethylated PD-1 CGs was significantly correlated with HCC progression (Pearson cor R=-0.9, p< 1.7×10-321).
Conclusions: The study reveals a broad signature of HCC in PBMC and T cells DNA methylation which discriminates early HCC stage from chronic hepatitis B and C and healthy controls, intensifies with progression of HCC, and is highly enriched in immune function-related genes such as PD-1, a current cancer immunotherapy target. These data also support the feasibility of using these profiles for early detection of HCC.

P0026

Identification of APOBEC3 family in Tree shrew (Tupaia belangeri) and its anti-virus ability

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Many attempts to employ tree shrew (*Tupaia belangeri*) to build animal models for studying virus infections, even social stress and depression. For its unique characteristics: small body size, short reproductive cycle and life span, low-cost of maintenance, and a close relationship to primates. It is important to figure out the immune reference about animal model. In this study, we characterized tree shrew has five APOBEC3 family members. Naming the APOBEC3 genes, organized HxEx23-28PCx2-4C (x can be any amino acids), of tree shrew is just like CBX6-A3Z1a-A3Z2a-Z2b-A3Z2c-Z1b-A3Z2d-Z2e-A3Z3-CBX7 by the locus organization, naming as A3A/C/F/G/H for convenience. Expression spectrum shows that *tsAPOBEC3 mRNA* broadly express but highly express in immune tissues.
It refers that tsAPOBEC3s have anti-virus ability. We found the tsA3A/C are localized in nucleus and all tsAPOBEC3 are localized in cytoplasm. They could induce G to A and C to T hypermutations in viral genome. The tsA3A/C have strong ability of editing HBV DNA by 3D-PCR, even stronger than

DOI: 10.1002/eji.201970400

hA3G. They could restrict the HBV DNA, cccDNA, HBsAg and HBeAg level in Huh7. We subsequently identified tsAPOBEC3 proteins as active inhibitors of HIV-1 particle infectivity. They could edit the viral cDNA and reduced the infectivity ~10- to ~1000- fold. The tsAPOBEC3G protein could not be degraded by Vif and could disturb HIV-1 genome transport into nucleus and integrate into host genome.
Our works will provide more information about innate immune restriction factors in tree shrew and an essential guidance in building tree shrew virus infection model.

P0609

Comparison of the expression of PD-1 in neonatal cord blood and maternal peripheral blood T cells

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Objective: In order to effectively use stem cell therapy, we compare programmed cell death protein 1 (PD-1) in T cells of neonatal cord blood (CB) and maternal peripheral blood (PB).
Methods: 24 cases of healthy neonatal CB were collected as experimental group, and 20 cases of healthy maternal PB were used as control group. Flow cytometry was used to detect the mean fluorescence intensity (MFI) of $CD34^+$ and the expression of PD-1 on the surface of $CD4^+$ T cells before and after stimulation whole blood with phytohemagglutinin-L (PHA-L).
Results: The MFI of $CD34^+$ in CB was 23.98±1.43 and 8.74±2.09 in PB (P< 0.001). The expression level of PD-1 in $CD4^+$ T cells in fresh CB was 1.92±0.11%, which was lower than that in the control group (4.97±0.59%) (P< 0.001). After three days of PHA-L stimulation of CB, The level of PD-1 in $CD4^+$ T cells increased to 43.03±9.62%, which was significantly higher than that of the control group（3.97±1.77%）(P< 0.05).
Conclusion: CB contains a large number of hematopietic stem cells compare to PB. The immune tolerance function of newborns CB is lower than that of maternal PB, but the tolerance is strongly upregulated after activation in CB. This may explain the low incidence and severity of graft-versus-host disease（GVHD）when CB is used as a graft.
Keywords: neonatal cord blood; immune tolerance; $CD34^+$; PD-1

P0610

Upregulation of B7-H4 induced by the activation of insulin-like growth factor-1 receptor contributes to immunosuppressive microenvironment in EGFR-TKI-resistant lung cancer

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B7-H4 is a transmembrane protein and widely expressed in different types of cancers, and its expression correlates with poor clinical outcomes. Acquired resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs) in treatment of NSCLC patients is not rare scenario. Here, we for the first time demonstrate that B7-H4 expression is upregulated by IGF1 and this upregulation is primarily associated with IGF1R/MEK/ERK1/2 signalling. Prolonged treatment of lung cancer cells with EGFR-TKIs led to acquired resistance and IGF1R activation and B7-H4 upregulation *in vitro* and *vivo*. Knocking down B7-H4 by shRNA reversed T cell inhibition by EGFR-TKI-resistant lung cancer cells *in vitro* and increased tumor infiltrated $CD8^+$ T cells *in vivo*. This abrogation of B7-H4 expression further

DOI: 10.1002/eji.201970400

reduced erlotinib-resistant tumor growth and increased survival *in vivo*. Furthermore, immunohistochemical (IHC) studies of 108 NSCLC specimens showed significant positive correlation between B7-H4 and p-ERK1/2 levels. TCGA data analyses revealed that B7-H4 expression is correlated with exhaustion signature of $CD8^+$ T cells and negatively correlated with PD-1 and PD-L1 in NSCLC patients. **Therefore, our data reveal a novel regulation mechanism of B7-H4 expression by IGF1R/MEK/ERK1/2 pathway and involvement of B7-H4 in EGFR-TKI-resistant lung cancers. It suggests that B7-H4 might be a potential therapeutic target for tumors with hyperactivated IGF1R.**

P0611

B7-H3 promotes aerobic glycolysis and chemoresistance in colorectal cancer cells by regulating HK2

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Purpose: We studied the function of B7-H3 in regulating aerobic glycolysis and chemoresistance in colorectal cancer (CRC) and investigated whether blockade of B7-H3-HK2 axis could be a potential therapeutic approach for CRC treatment.
Experimental Design: The ability of B7-H3 in regulating aerobic glycolysis and chemoresistance was investigated in CRC cells via cell viability, clonogenicity and glycolysis analysis in vitro. The physiological relevance of B7-H3 in aerobic glycolysis and chemoresistance was further studied in the xenograft tumor models by intraperitoneal injection of oxaliplatin (L-OHP) and HK2 inhibitors. The correlation between B7-H3 and HK2 was examined in the CRC patients by immunohistochemistry (IHC) and pathological analyses.
Results: Overexpression of B7-H3 effectively increased the rate of glucose consumption and lactate production in CRC cells, whereas knockdown of B7-H3 had an opposite effect. Mechanistically, **we showed that B7-H3 increased glucose consumption and lactate production by promoting HK2 expression in CRC cells, and HK2 was a key mediator of B7-H3-induced CRC chemoresistance.** Downregulation of HK2 expression with siRNA or inhibition of HK2 in cells with its specific inhibitor reversed the B7-H3-induced increase in aerobic glycolysis and B7-H3-endowed chemoresistance of cancer cells. **Importantly, we verified a positive correlation between the expression of B7-H3 and HK2 in tumor tissues of CRC patients.**
Conclusions: Our findings indicate that B7-H3 is a novel regulator of glucose metabolism and chemoresistance by controlling HK2 expression in CRC cells, which could help utilize B7-H3 as a promising therapeutic target for CRC treatment.
Keywords: colorectal cancer; glycolysis; B7-H3; chemoresistance; HK2

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DOI: 10.1002/eji.201970400

P0612

The intracellular domain of LILRB4 mediates T cell suppression and infiltration of acute myeloid leukemia cells

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We recently demonstrated that the leukocyte Ig-like receptor 4 (LILRB4) expressed by monocytic acute myeloid leukemia (AML) cells mediates T cell inhibition and leukemia cell infiltration via its intracellular domain. The cytoplasmic tail of LILRB4 contains three immunoreceptor tyrosine-based inhibitory (ITIM) motifs; tyrosines at positions 360, 412, and 442 are phosphorylation sites. Here we analyzed how the ITIM motifs of LILRB4 mediate its function. Our *in vitro* and *in vivo* data show that Y_{412} and Y_{442}, but not Y_{360}, of LILRB4 are required in T cell inhibition, and all three ITIM motifs are needed for infiltration of AML cells. We constructed chimeric proteins containing the extracellular domain of LILRB4 and the intracellular domain of LILRB1 and vice versa. Based on T cell co-culture and cell migration assays, we found that the intracellular domain of LILRB4 but not that of LILRB1 mediates T cell suppression and migration of AMLs. Thus, these experiments defined the signaling roles of the ITIM motifs of LILRB4 in inhibition of T cell activity and migration of AMLs.

P0613

TIGIT/miR-206 pathway regulates biological function of hepatocellular carcinoma cells

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T-cell immunoglobulin and ITIM domain (TIGIT) is a new immune check point which plays a critical role in the various types of cancer, and miR-206 has a double edge role in regulating the biological functions of immune check points molecules including CTLA-4, PD-1, TIM-3, LAG-3 and multiple cancer cells. However, it is still unknown whether TIGIT is involved in the miR-206 mediated biological functions of hepatocellular carcinoma (HCC) cells. In this study, we found that the expression level of miR-206 was significantly lower in HCC tissues than that in adjacent non-cancerous tissues. Meanwhile, TIGIT was upregulated in HCC tissues and cell lines. Moreover, miR-206 downregulated TIGIT in HCC cells via directly binding to its mRNA 3′ UTR. Additionally, miR-206 suppressed HCC cell proliferation, migration and invasion while induced its apoptosis via TIGIT/miR-206 pathway. Similarly, silence of TIGIT also repressed HCC cell proliferation, migration and invasion while induced its apoptosis. Taken together, our results demonstrated that miR-206 inhibited the biological functions of HCC cells through targeting TIGIT, suggesting that the TIGIT/miR-206 pathway may be a novel target for the diagnosis and treatment of HCC.

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DOI: 10.1002/eji.201970400

P1204

BTLA contributes to *TP* immune escape associated with IFN-γ production in γδT cells

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Syphilis is still a serious problem in low-income and middle-income nations. So far, penicillin is still the only recommended treatment in clinics, but it is facing a growing threat of antibiotic-resistant syphilis strains. The main mechanism of *Treponema pallidum* (Tp) is associated with escaping from immune clearing and simultaneously inducing inflammation as well. γδT cell is the primary cell of the innate immune and skin mucosal immunity, while the roles of γδT cells in syphilis is undefined. B and T lymphocyte attenuator (BTLA) is an important molecule of immune checkpoints. However, impacts of BTLA on the function ofγδT cell in TP infected patients have not been clarified. In the current study, we assayed BTLA expression in γδT cells in TP infected patients and its changes in PBMCs stimulated by TPN47 protein by flow cytometry. The results showed that in syphilis patients $BTLA^+$γδT cells were significantly more than $BTLA^-$γδT cells, and IFN-γ level produced from $BTLA^+$γδT cells were significantly less than that from BTLA-γδT cells. Also, the plasma IFN-γ level is much higher in syphilis samples, which indicated a positive correlation with the percent of $BTLA^-$γδT cells but not with $BTLA^+$γδT cells. Using TPN47 protein stimulating PBMC, we also got the consistent results in vitro experiments. Collectively, we suggest that BTLA may contribute to Tp escaping from immune clearing associated with the production of IFN-γ in γδT cells.

P1205

Prognostic significance of B7-H4 expression in pancreatic cancer

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Pancreatic cancer is one of the common malignant tumors in the digestive system. Invasion and metastasis of pancreatic cancer is the leading causes of death in pancreatic cancer patients. Our previous research proved that B7-H4 might be involved in pancreatic cancer cell tumorigenesis. In this study, we intends to explore the expression characteristics of B7-H4 in the pathological progression of pancreatic cancer and the correlation with pancreatic cancer metastasis.
We found that the expression levels of B7-H4 in pancreatic cancer patients were significantly higher than in controls. B7-H4 was also highly expressed in 28 (65.1%) of the 43 liver metastases and 9 (60.0%) of the 15 matched primary tumors. The expression of B7-H4 in liver metastases was significantly higher than in the matched primary tumors. A significant difference in B7-H4 expression was observed between patients with late TNM stage and early TNM stage. The expression of B7-H4 was associated with distant metastasis and differentiation. In addition, Patients with high B7-H4 expression in their primary pancreatic cancer had higher risk of developing liver metastases. And the multivariate analysis identified that B7-H4 was an independent prognostic indicator. Using a microarray technique, we found that B7-H4 siRNA significantly up-regulated 57 miRNAs and down-regulated 14 miRNAs. GO and KEGG Pathway analysis of predicted miRNA targets showed that these genes were mainly involved in pathways in cancer, MAPK signaling pathway, and PI3K-Akt signaling pathway.
Thus, B7-H4 may promote pancreatic cancer progression and was promising to be a potential prognostic indicator of pancreatic cancer.

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DOI: 10.1002/eji.201970400

P1206

Hyperhomocysteinemia exacerbates IRI-induced acute kidney injury through mitochondrial damage pathway in mice

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Objective: The aim of this study was to investigate the effects of mitochondrial damage on the occurrence and development of acute renal injury in mice with hyperhomocysteinemia.
Methods: 60 male C57BL/6 mice with a body weight of 19-21g were selected and randomly divided into 4 groups: control group (n=15),HHcy group (n=15),AKI group（n=15）and HHcy+AKI group（n=15）. AKI and HHcy+AKI group were administrated with ischemia reperfusion injury（IRI）. All mice were sacrificed after 24 h of IRI.Renal biochemical function,renal pathological changes,renal injury markers,kidney mitochondrial function and structural changes were observed.
Results: The levels of serum creatinine,renal tubular injury score, $TUNEL^+$ cells, cleaved-caspase 3, γH2AX, cytochrome C, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-lα)，ATP synthase βand Mitochondrial DNA（mtDNA）copy number had no significant difference in control group and HHcy group(p>0.05).Compared with the AKI group, HHcy+AKI groups had significant higher levels of serum creatinine,renal tubular injury score,$TUNEL^+$ cells, cleaved-caspase 3,γH2AX and cytochrome C (p< 0.05).The levels of peroxisome proliferator-activated receptor-gamma coactivator-1 alpha(PGC-lα),ATP synthase βand Mitochondrial DNA（mtDNA）copy number in HHcy+AKI group were higher than AKI group (p< 0.05). Electron microscopy found tubular mitochondrial structural damage of HHcy+AKI group was more serious than AKI group.
Conclusion: Hyperhomocysteinemia aggravates IRI-induced acute kidney injury, which is probably mediated by exacerbating renal epithelial tubular cells apoptosis through promoting mitochondrial damage.

P1207

Ectopic B7-H4-Ig expression attenuates concanavalin A-induced hepatic injury

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Previous studies demonstrate that both membrane B7-H4 and B7-H4-Ig fusion protein could inhibit T-cell responses. In the present study, we explored the potential effect of B7-H4-Ig on liver injury in a hepatitis mouse model induced by concanavalin A (ConA). A B7-H4-Ig construct was introduced into animals by the hydrodynamic gene delivery approach. It was found that ectopic expression of B7-H4-Ig could inhibit ConA-induced elevation of serum levels of ALT and AST, suppress liver necrosis and even mortality of mice. Furthermore, we observed that pretreatment of B7-H4-Ig dramatically decreased serum levels and the expression of mRNA for IL-2, IFN-γ and IL-4, but increased IL-10 in ConA-treated mice. **Our results suggest that B7-H4-Ig may protect animals from liver injury induced by ConA, which could be associated with reduced serum levels for IL-2, IFN-γ and IL-4 as well as enhanced IL-10 production.**
This study is granted by Hainan key research and development programme (social development): **ZDYF2016167**.

DOI: 10.1002/eji.201970400

P1208

ILT5 inhibits macrophage-mediated bacterial killing and antigen presentation during sepsis

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Immunosuppression contributes to the mortality of sepsis. However, the underlying mechanism remains unclear. In the present study, we investigated the regulatory role of immunoglobulin(Ig)-like transcript 5 (ILT5) in sepsis. We first screened the expression of ILT family members, and found that ILT5 was dramatically up-regulated in the peripheral blood mononuclear cells (PBMCs) from sepsis patients vs healthy donors. Knockdown of ILT5 by siRNA increased bacterial killing and reactive oxygen species (ROS) production in THP-1 and RAW264.7 cells. Moreover, ILT5-expressing monocytes/macrophages exhibited lower expression of antigen-presenting molecules including MHC-II and CD80. In the *in vitro* co-culture system with monocytes/macrophages, blockage of ILT5 facilitated Th1 proliferation and differentiation of $CD4^+$T cells. Furthermore, *in vivo* experiment demonstrated that pretreatment with ILT5 blocking peptide improved the survival and pulmonary pathology of septic mice. Our study identified the regulatory role of ILT5 in bacterial killing and antigen presentation of macrophages, which contributed to $CD4^+$T cell proliferation and Th1 differentiation during sepsis. The expanded knowledge of ILT5 in the immune regulation holds the promise for clinical sepsis treatment.
Keywords: Sepsis; ILT5; Antigen presentation; Bacterial killing; Immunosuppression; Th1 differentiation.

P1209

The chloride channel CFTR dual-negatively regulates the activation and tumor immunity in γδ T cells

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γδ T cells have anti-tumor activities with high IFN-γ production in the infiltration of the tumor tissue,and become the strongest interest for cancer immunotherapy. Cystic fibrosis transmembrane conductance regulator (CFTR) is an important chloride channel and regulator in the apical membrane of epithelial cells. CFTR was reported to participate in the immune regulation and likely account for the risk of developing cancer. However, little is known about the effect of CFTR on γδ T cells tumor immunity. In this study, we would like to elucidate the regulatory mechanisms and indispensable functions of CFTR on γδ T cells in tumor immunity. We found that genetic deletion of CFTR displayed the increased TCR signaling and elevated IFN-γ release in peripheral γδ T cells, but not in $CD4^+$ T cells. The underlying mechanism of higher TCR signaling was that CFTR may participate in TCR signaling. Furthermore, CFTR was predominately expressed in immunogical synapes (IS) when γδ T cells was stimulated and CFTR deficiency may trigger more actin to IS region. Consistent with the data in mice, CFTR inhibition significantly increased the IFN-γ production in γδ T cells. Finally, $CFTR^{-/-}$ γδ T cells displayed higher cytolytic ability against the B16 melanoma and protected against B16 tumor growth. This study systematically defined the roles of CFTR in the activation of γδ T cells and its potential contribution to tumor immunity, which will enrich the γδ T cells biology and expand the sight for the application of CFTR in γδ T cells cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P1797

B7-H3 promotes multiple myeloma cell survival and proliferation by ROS-dependent activation of Src/STAT3 and c-Cbl-mediated degradation of SOCS3

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B7-H3 (CD276) is broadly over-expressed by multiple human cancers. It plays a vital role in tumor progression and has been accepted as one of the inhibitory B7 family checkpoint molecules. To identify the functions and underlying mechanisms of B7-H3 in multiple myeloma, we analyzed B7-H3 expression in myeloma patients and used siRNAs and overexpression plasmid of B7-H3 to investigate its roles and downstream signaling molecules in myeloma cell lines. The results showed that surface expression of B7-H3 was upregulated in myeloma samples and cell lines. Lower expression of B7-H3 in myeloma cells was associated with better progression-free survival. Myeloma cell survival, drug resistance, and tumor growth could be promoted by B7-H3. The molecular basis for these functional roles of B7-H3 involved the activation of JAK2/STAT3 via redox-mediated oxidation and activation of Src. We further identified a STAT3-promoting signaling pathway by which oxidant-mediated Src phosphorylation led to secondary activation of the E3 ubiquitin ligase c-Cbl. Activated c-Cbl subsequently caused specific proteasomal degradation of SOCS3, a negative regulator of JAK2/STAT3. These data indicate B7-H3's important role in the activation of ROS/Src/c-Cbl pathway in multiple myeloma which integrates redox regulation and sustained STAT3 activation at the level of degradation of STAT3 suppressor.

P1798

A CD300c-Fc fusion protein inhibits T cell immunity

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T cell responses are fine-tuned by co-stimulatory and co-inhibitory molecules. Among the T cell regulators, the B7 family members are of central importance. The recent success in targeting the B7 family molecules for the treatment of immune-related diseases has attracted intense interest in identifying additional B7-related molecules. In this study, we describe CD300c as a novel T cell co-inhibitory molecule that shares significant sequence homology with existing B7 family members. CD300c protein is expressed on professional antigen-presenting cells (APC), including B cells, monocytes, macrophages and dendritic cells (DCs). The putative CD300c counter-receptor is expressed on CD4 and CD8 T cells, and the expression levels are upregulated upon activation. Soluble human and mouse CD300c-Fc fusion proteins significantly inhibit the proliferation, activation, and cytokine production by CD4 and CD8 T cells *in vitro*. Administration of CD300c-Fc protein attenuates graft-versus-host disease (GVHD) and experimental autoimmune encephalomyelitis (EAE) in mice. Our results suggest that therapeutic interaction with the CD300c inhibitory pathway may represent a new strategy to modulate T cell-mediated immunity for the treatment of GVHD and autoimmune disease.

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DOI: 10.1002/eji.201970400

P1799

Identification of novel PD-L2 protein regulator by using whole genome CRISPR screening

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PD-L1 and PD-L2 are two members of the B7 family that can interact with PD-1. The interaction between PD-1 on T cells and PD-L1 on tumor cells inhibits activation, expansion and effector functions of antigen-specific CD8+ T cells and helps cancer cells evade immune destruction. However, the role of PD-L2 in cancer is particularly complex because of its contradictory effects on antitumor immunity. It is generally acknowledged that PD-L2 can inhibit both CD4+ and CD8+ T cell activation and T cell responses. PD-L2 is mostly expressed on antigen presenting cells, such as macrophages and dendritic cells. In view of the limited understanding of the function and regulation of PD-L2 expression, we set out to identify PD-L2 protein regulators through genetic screening.
We performed whole genome CRISPR-Cas9 screening in multiple cancer cell lines and selected PD-L2 high expression and low expression cell populations using fluorescence-activated cell sorting (FACS). For the sorting, cancer cell lines were pre-treated with IFN-γ and TNF-α simultaneously for 48h to stimulate the expression of PD-L2. By comparing the sgRNA enrichment between the high and low populations using MAGECK RRA method, we successfully identified some known regulators (like IFN-γ pathways genes) and some novel regulators. Our validation data further confirmed that a novel PD-L2 protein regulator can efficiently carry out its inhibitory function, and suggest potential new avenues to block this pathway to enhance tumor specific immunity.

P1800

Membrane translocation of mdm2 increases the immunogenicity of tubular epithelium via pd-l1 ubiquitination

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Increased immunogenicity of proximal tubular epithelium (PTE) contributes to donor acute kidney injury (AKI) induced allograft T cell mediated rejection. Formerly, we found MDM2, an E3 ubiquitin ligase, translocated from cytosol to basolateral membrane of tubules during AKI. Here, we explore the link between MDM2's translocation and tubular immunogenicity.
C57BL/6 mice were adopted to establish ischemic AKI, and divided into control, short-term or long-term warm ischemic groups. The expression and distribution of MDM2 and PD-L1 were analyzed by immunostaining. In vitro, mPTE was cocultured with T cell line (C10) to assess the immunogenicity of PTE and T cell activation during hypoxia. Actinomycin D and mutated MDM2 plasmid were utilized to interfere the subcellular transportation of MDM2 in vitro.
In normal, MDM2 is predominantly located in cytosol and nucleus. But, in warm-ischemic mice the distribution of MDM2 gradually shifts to basolateral membrane. Interestingly, PD-L1, a T cell co-inhibitory molecule, majorly presents on basolateral membrane of PTE, and co-localizes with MDM2. In long-term ischemic group the level of PD-L1 is weaken obviously. In vitro, we demonstrated that MDM2's trafficking attributes to PD-L1 ubiquitination and degradation during hypoxia. Coculture of mPTE with T cell line (C10) revealed that hypoxia increases the immunogenicity of mPTE indicating by enhanced CD40, MHC Ⅱ. Simultaneously, the T cells present activated status with CD40L upregulation and IL-2 secretion.
In summary, during ischemic AKI or hypoxia, the translocation of tubular MDM2 leads to PD-L1

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DOI: 10.1002/eji.201970400

ubiquitination and degradation which results in the weakened co-inhibitory signaling with T cell activation.

P1801

Distinct prognostic values of PD-L1 and PD-1 in lung adenocarcinoma and squamous cell carcinoma patients

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Although inhibitors targeting programmed cell death protein-1 (PD-1) and ligand (PD-L1) pathway have demonstrated similar clinical activities in the treatment of lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC), clinical trials have revealed different predictive roles for PD-L1 in ADC and SCC, implying that PD-L1/PD-1 pathway might have different functions in these malignancies. We performed a multi-dimensional analysis based on public database and two independent cohorts of lung cancer patients. Immunohistochemistry and multiplex immunofluorescence staining was used to detect immune factors. Kaplan-Meier method and multivariate analysis by COX were used to reveal the prognostic significance. We have discovered different expression and regulatory mechanisms of PD-L1 between SCC and ADC. PD-L1 significantly increased from mRNA to protein level in SCC group compared with ADC group. Meanwhile, PD-L1 on tumor cells (TC) showed positive correlation with amounts of $CD8^+$ tumor lymphocytic infiltration only in ADC but not made for SCC. More importantly, in survival analysis, the prognostic values of PD-L1 and PD-1 were distinct in two subtypes. For ADC patients, PD-L1 expression was an independent predictor for OS. On the contrary, PD-1 expression, especially, $CD8^+PD\text{-}1^+$ was predictors of poor prognosis in SCC patients. Overall, our study revealed PD-L1 in ADC and $CD8^+$PD-1 in SCC respectively representing the T cell function which is the crucial factor determining prognosis of patients. The distinct functionary biomarkers between two subtypes might provide potential avenues in guiding anti-PD-1/PD-L1 immunotherapy.

P2419

A low-molecular-weight compound exerts anticancer activity against breast and lung cancers by disrupting EGFR/Eps8 complex formation

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Eps8 is an important active kinase substrate of EGFR that directly binds to the juxtamembrane (JXM) domain of EGFR to form an EGFR/Eps8 complex. The EGFR/Eps8 complex is involved in regulating cancer progression and might be an idealtarget for antitumor therapy. In silico virtual screening was used to identify small-molecule EGFR/Eps8 complex inhibitors. The antitumor effects of the inhibitors were analyzed in cancer cells and xenograft models. In addition, the effect of the EE02 inhibitor on the protein expression of elements downstream of the EGFR/Eps8 complex was determined by western blotting and protein chip assays. In this study of nearly 390,000 compounds screened by virtual database screening, the top 29 compounds were identified as candidate small-molecule EGFR/Eps8 complex inhibitors and evaluated by using cell-based assays. The compound

DOI: 10.1002/eji.201970400

EE02 was identified as the best match to our selection criteria. **Further investigation demonstrated that EE02 directly bound to the JXM domain of EGFR and disrupted EGFR/Eps8 complex formation.** EE02 selectively suppressed growth and induced apoptosis in EGFR-positive and Eps8-positive breast cancer and NSCLC cells. More importantly, the PI3K/Akt/mTOR and MAPK/Erk pathways downstream of the EGFR/Eps8 complex were suppressed by EE02. In addition, the suppressive effect of EE02 on tumor growth in vivo was comparable to that of erlotinib at the same dose. We identified **EE02 as an EGFR/Eps8 complex inhibitor that demonstrated promising antitumor effects in breast cancer and NSCLC**. Our data suggest that **the EGFR/Eps8 complex offers a novel cancer drug target**.

P2420

3D-QSAR, molecular docking and molecular dynamics simulation of a novel thieno[3,4-d]pyrimidine inhibitor targeting human immune-odeficiency virus type 1 reverse transcriptase

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Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is one of the attractive drug targets for the treatment of AIDS. In this paper, 67 thieno[3,4-d]pyrimidine derivatives were selected as HIV-1 RT inhibitors, and they were subjected to 3D-QSAR studies by CoMFA, CoMSIA and T-CoMFA. The results show that CoMFA (n=8; q2=0.594; R2=0.974) and CoMSIA (n=7; q2=0.528; R2=0.965) have good stability and predictability. The study also found that the hydrogen bonding and van der Waals interactions of key residues such as Leu100, Lys101, Val106, Phe227 and Pro236 play a key role in ligand-receptor binding. Based on these results, Twelve new thieno[3,4-d]pyrimidines were designed and predicted for activity, and the results indicate that these compounds have good predictive activity. MD simulation analysis of 50 ns showed that compound 23j formed a total of four hydrogen bonds with the residues (Lys101, Lys104, Val106 and Thr318), and binds more closely to HIV-1RT than compound 23. Furthermore, the group at the position of the compound R1 and the compound horseshoe-like conformation are critical for the activity and stability of the inhibitor. These results provide powerful guidance for the discovery and design of a new generation of HIV-1 RT inhibitors.

P2421

3D-QSAR and docking studies on pyrimidine derivatives as CSF-1R inhibitors

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Background: Colony stimulating factor-1 receptor (CSF-1R) is associated with malignancy, invasiveness and poor prognosis of tumors, and pyrimidine derivatives are considered as a novel class of CSF-1R inhibitor.
Method: To explore the relationship between the structures of substituted pyrimidine derivatives and their inhibitory activities against CSF-1R, CoMFA and CoMSIA analyses, and molecular docking studies were performed on a dataset of forty-four compounds.
Results: We found in CoMFA model including steric and electrostatic fields for the training set, the cross-validated q2 value was 0.617 and the non-cross-validated r2 value was 0.983. While, the cross-validated q2 value was 0.637 and the non-cross-validated r2 value was 0.984 in CoMSIA Model which include steric, electrostatic and hydrophobic fields. 3D equipotential maps generated from CoMFA and CoMSIA along with the docking binding structures provided enough information about the structural requirements for better activity.

DOI: 10.1002/eji.201970400

Conclusion: The data generated from the present study helped us to predict the activity of new inhibitors and further design some novel and potent CSF-1R inhibitors.

P2422

Clophilin A inhibits human respiratory syncytial virus replication through interacting with viral protein M2-1

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Human respiratory syncytial virus (hRSV) is one of the most common pathogens causing upper respiratory tract infection in infants and immunocompromised individuals worldwide. Cyclophilin A (CypA) is the main member of the immunophilin superfamily that has peptidyl-prolyl cis-trans isomerase activity and it plays critical roles in several viruses. In our study, we found that RSV infection increased cyclophilin A (CypA) expression significantly in Hep2 cells, so we focused on CypA to survey its role in RSV replication. RSV N mRNA and protein level was increased in HEp-2 cells treated with siRNA targeting CypA or CypA inhibitor cyclosporin A (CsA) but decreased remarkably in cells with CypA overexpression.This results indicate that CypA inhibits RSV replication. Mechanistically, LC-MS/MS showed that CypA could interact with a variety of RSV proteins, including M2-1,phosphoprotein and nucleotide protein. M2-1 protein is a well known RSV transcription antiterminator protein and it plays an indispensable role in RSV transcription through preventing premature transcription termination and increasing the processivity of viral RNA dependent RNA polymerase (RdRP) complex. In our study, we verified whether there exists an interaction between CypA and M2-1 by immunoprecipitation (IP) assay and the result showed that M2-1 could be pulled down by CypA antibody and CsA could block their interaction. However, further research is required to determine whether their interaction is direct or indirect. Above all, these results suggest that CypA may play an antiviral role by interacting with viral protein M2-1 and act as a new potential antiviral drug target.

P2423

CDK5 upregulates PD-L1 expression via blocking the BIN1/c-MYC interactionin NSCLC

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Bridging integrator 1 (BIN1) has showed outstanding tumor-suppressive potential via inhibiting c-MYC-mediated tumorigenesis. However, a frequent phosphorylation of c-MYC at Ser-62 site could block the BIN1/c-MYC interaction and limits the tumor-suppressive effect of BIN1. Cyclin-dependent kinase 5 (CDK5), a generally dysregulated protein in various carcinomas,can mediate c-MYC phosphorylation at Ser-62 site. However, whether the existence of CDK5 could block the BIN1/c-MYC interaction remains unclear. In present study, we observed that CDK5 was highly expressed in H460 cells, and knockdown of CDK5 could restore the BIN1/c-MYC interaction. Meanwhile, low expression of CDK5 was observed in PC9 cells, and overexpression of CDK5 blocked the BIN1/c-MYC interaction. Consequently, the PD-L1 expression in H460 and PC9 cells could be upregulated by CDK5, while addition of CDK5 inhibitor Dinaciclib significantly downregulated PD-L1 expression. Furthermore, high expression of CDK5, along with low expression of BIN1, could predict poor postoperative prognosis of NSCLC patients. The patients with high expression of CDK5 and low expression of BIN1 showed similar prognosis, indicating that CDK5 could neutralize the tumor suppressing effect of BIN1 in clinical situation. Taken above, CDK5 upregulated the expression of PD-L1 via blocking the interaction of BIN1 and c-MYC, ultimately facilitated the progression of NSCLC.

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DOI: 10.1002/eji.201970400

P2424

M1-like macrophages induce PD-L1 expression in hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is a prototype of inflammation-related cancer, harboring M1-like and M2-like tumor-associated macrophages. M1-like macrophages are thought to be tumoricidal, but recent studies document its pro-tumor role. The programmed cell death-ligand (PD-L) 1 expressed in HCC cells is a critical checkpoint molecule to mediate immune escape of HCC. The PD-L1 expression in HCC cells is inducible. In the present study, we ask whether the M1-like macrophages induce the expression of PD-L1 in HCC cells. Double staining immunohistochemistry was used to detect CD68+HLA-DR+ M1-like macrophages and PD-L1 expression in HCC tissues. The infiltration of M1-like macrophages correlated with PD-L1 expression level in HCC cells. Besides, in vitro experiments show that M1 macrophages derived from THP-1 cell, RAW264.7 cell or murine bone marrow, induced expression of PD-L1 in HCC cells. Moreover, the data from siRNA assay and receptor blockade assay show that inflammatory cytokine IL-1β, IL-6 and TNF-α secreted by the M1-like macrophages accounted for the inducible PD-L1 expression. Furthermore, an enhanceosome containing transcription factor IRF1, p65 and STAT3 in the HCC cells mediated the inducible PD-L1 expression. All the results demonstrate that M1-like macrophages induced the expression of PD-L1 in HCC cells, supporting the pro-tumor role of M1-like macrophages.

P2425

Effects of metformin on the growth of human liver cancer transplanted subcutaneously in nude mice and the expression of inflammation-related proteins in tumor microenvironment

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The inhibitory effect of metformin on the growth of human liver cancer xenografts in nude mice was observed to investigate the molecular mechanism of metformin anti-liver cancer by improving the tumor microenvironment inflammation-related proteins TNF-α, MMP-9 and regulating EGF-NEDD9 pathway inhibit the expression of EMT-related proteins SLUG, TWSIT, β-catenin. BALB/c nude mice were used to prepare subcutaneous xenograft model of human liver cancer and randomly divided into model group and metformin group. The metformin group was administered with 6.25 mg/25 g body weight metformin for 7 days. The model group was given the same amount of normal saline for 21 days. The subcutaneous xenografts was removed and the volume and weight were measured to calculate the tumor inhibition rate. Western blot was used to detect the expression of TNF-α, MMP-9, EGF-NEDD9 and EMT-related proteins SLUG, TWSIT and β-catenin in Subcutaneous xenograft tissue. Compare with the model group, the volume and weight of the metformin group were significantly inhibited ($P< 0.001$). The results of Western blot showed that compared with the model group, the expression of TNF-α, MMP-9, EGF, NEDD9 and EMT-related proteins SLUG, TWSIT, β-catenin in the metformin group was decreased, and the difference was statistically significant ($P< 0.05$). Metformin significantly inhibit the growth of subcutaneous xenografts in human liver cancer nude mice and reduce the inflammatory state of tumor microenvironment by down-regulating the expression of TNF-α and MMP-9. Metformin can also reduce the expression of EMT-related proteins by EGF-NEDD9 pathway to reduce the invasiveness and metastasis of HCC.

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DOI: 10.1002/eji.201970400

P2426

CATT polymorphisms in MIF gene promoter is closely related to human pulmonary tuberculosis in a Southwestern China population

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Macrophage migration inhibitory factor（MIF) is deemed as an immunoregulatory and proinflammatory cytokine related to the progression of tuberculosis. To investigate whether Macrophage migration inhibitory factor gene *mif* CATT variants are associated with susceptibility to retreatment cases of TB and drug-resistant TB prevalence, genotyping of *MIF* −794 CATT polymorphism and quantifying of serum MIF were performed to associate *MIF*−794 CATT polymorphism with new patients and retreatment cases. Significant increases in *MIF* −794 CATT genotypes 7/8 and allele CATT 8 were observed in TB patients. Significant differences in the genotypic frequencies of *MIF* −794 CATT (5/X + 6/X vs. 7/7 + 7/8) were demonstrated upon comparing the total cases and the new cases of TB with the controls. Significant differences in the allelic frequencies of *MIF* −794 CATT (5 + 6 vs. 7 + 8) were observed in the total cases and new cases of TB. In conclusion, the *MIF* −794 CATT genotypes 7/8 and allele CATT 8 were highly associated with TB, no differences in the genotypic frequencies of the *MIF* −794 CATT (5/X + 6/X vs. 7/7 + 7/8) were observed between the new cases and retreatment cases.
Funding: This study was supported by grants from the National Natural Science Foundation of China (No.81560596, 81860644 and 31560051), the Yunnan Applied Basic Research Projects (No. 2017FE467-001 and 2014FA011).

P2427

The expression of B7-H3 on cancer-associated fibroblasts from renal cancer endowed anti-apoptotic activity

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Background: Renal cancer is one of the most common malignancies.B7-H3 has been described as an important tumor antigen in various human cancers. An abnormal high expression of B7-H3 molecules is often observed in tumor cells and tumor stromal cells in the tumor microenvironment. However, the impact of B7-H3 on cancer-associated fibroblasts (CAFs) from renal cancer remain unclear.
Methods: The expression of B7-H3 protein on CAFs was detected in renal cancer tissues. Then, the CAFs cells were stably transfected with shRNA to down-regulate the expression of B7-H3. The expression of apoptosis-related proteins, hepatocyte growth factor (HGF) protein and stromal cell-derived factor-1 (CXCL12) protein were detected by western blot. Finally, CAF-NC cells and CAFs-shRNA cells were co-cultured with A498 cells to assess the biological function of B7-H3 on changes of A498 cells.
Results: The obvious expression of B7-H3 on renal cancer CAFs were found. Over-expression of B7-H3 effectively increased the expression of HGF and CXCL12 in CAFs, whereas knockdown of B7-H3 had an opposite effect. Besides, B7-H3 expression has strong anti-apoptotic effect on CAFs. We also found that B7-H3^{high}-CAFs promotes the proliferation, invasion and migration of A498 cells in vitro and

DOI: 10.1002/eji.201970400

promotes the tumor formation of A498 in vivo.
Conclusion: Our findings indicate that B7-H3 is a crucial immune check point of renal cancer by controlling anti-apoptotic activity of cancer-associated fibroblasts cells, which could help utilize B7-H3 as a promising therapeutic target for renal cancer treatment.

P2428

B7H3 contributes to radioresistance in colorectal cancer via the KIF15/ERK pathway

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Neoadjuvant chemoradiotherapy (NCRT) has been widely applied in colorectal cancer (CRC) therapy, however, radioresistance has become a major obstacle. Here, we found that B7H3, an important costimulatory molecule, could effectively enhance the radioresistance. Furthermore, we showed that B7H3 enhanced CRC cells radioresistance of by modulating cell cycle progression and apoptosis. MRNA expression profiling revealed that the expression of KIF15 was significantly downregulated in B7H3-knockdown CRC cells. KIF15 siRNA or KIF15 inhibitor SB743921 could abolish the effect of B7H3 on CRC cells radioresistance. Luciferase assay confirmed that B7H3/KIF15 increased ERK signaling activity, which is associated with radioresistance. We also demonstrated that the expression of both B7H3 and KIF15 was significantly higher in CRC tissues than in tumor-adjacent tissues. Moreover, the KIF15 level was positively correlated with B7H3 level in CRC tissues. Overall, our findings provided an alternative mechanism by which CRC cells can acquire radioresistance via the B7H3-KIF15-ERK axis.
Keywords: colorectal cancer; radioresistance; B7-H3; KIF15; ERK signaling

P2429

Decreased siglec-9 expression on natural killer cell subset associated with persistent HBV replication

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Siglec-9 is an MHC-independent inhibitory receptor selectively expressed on CD56dim NK cells. Its role in infection diseases has not been investigated yet. Here, we studied the potential regulatory roles of NK Siglec-9 in the pathogenesis of chronic hepatitis B (CHB) infection. Flow cytometry evaluated the expression of Siglec-9 and other recep-tors on peripheral NK cells. Immunofluorescence staining was used to detect Siglec-9 ligands on liver biopsy tissues and cultured hepatocyte cell lines. Siglec-9 blocking assay was carried out and cytokine synthesis and CD107a degranulation was detected by flow cytometry. Compared to healthy donors, CHB patients had decreased Siglec-9+ NK cells, which reversely correlated with serum hepatitis B e antigen and HBV DNA titer. Siglec-9 expression on NK cells from patients achieving sustained virological response recovered to the level of normal donors. Neutralization of Siglec-9 restored cytokine synthesis and degranulation of NK cells from CHB patients. Immunofluorescence stain-ing showed increased expression of Siglec-9 ligands in liver biopsy tissues from CHB patients and in hepatocyte cell lines infected with HBV or stimulated with inflammatory cytokines (IL-6 or TGF-β). These findings identify Siglec-9 as a negative regulator for NK

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DOI: 10.1002/eji.201970400

cells contributing to HBV persistence and the intervention of Siglec-9 signaling might be of potentially translational significance.

Cytokines, Chemokines and Receptors

O257

PSMP/MSMP promotes hepatic fibrosis through CCR2

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Chemokines play a crucial role in the migration of leukocytes, which are involved in immune and inflammatory reactions with their receptors. PC3-secreted microprotein (PSMP) /microseminoprotein (MSMP) is a novel chemotactic cytokine discovered by a genome-wide bioinformatics analysis and PBMC chemoattractant screen. CCR2 was identified as a high-affinity receptor for PSMP. In this study, we found that PSMP was highly expressed in cirrhotic tissues from patients with multiple etiologies and then investigated the role of PSMP in liver fibrosis progression. PSMP deficiency (*Psmp*$^{-/-}$) resulted in a marked amelioration of hepatic injury and fibrosis in mice treated with carbon tetrachloride (CCl_4) or bile duct ligation. In CCl_4-induced hepatic injury, the infiltration of CCR2$^+$ monocytes and macrophages into the liver was significantly decreased in *Psmp*$^{-/-}$ mice. Consistent with the decreased levels of intrahepatic macrophages, proinflammatory cytokines were significantly reduced. Moreover, adeno-associated virus (AAV-8) vectors successfully overexpressing human PSMP in *Psmp*$^{-/-}$ mouse livers could reverse the attenuation of liver injury and fibrosis induced by CCl_4. However, this effect was not observed in double knockout PSMP and CCR2 mice. Treatment with a specific PSMP-neutralizing antibody, 3D5, significantly prevented liver injury and fibrosis induced by CCl_4 in a dose-dependent manner in mice. At the cellular level, recombinant PSMP protein directly promoted the activation of LX-2 cells and was blocked by CCR2 antagonist. Taken together, PSMP could enhance liver fibrosis through its receptor, CCR2, and was shown to be an attractive potential therapeutic target for liver fibrosis.

O258

Intracellular mature IL-37 suppresses tumor metastasis via inhibiting Rac1 activation

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IL-37, a newly found anti-inflammatory cytokine of the IL-1 family, has both extracellular and intracellular functions. Accumulating evidences indicate that it is also involved in tumor progression. However, the mechanism and its intracellular target are unclear. In this study, clinical data from 84 patients showed that loss or reduced expression of IL-37 in lung adenocarcinoma tissues was significantly associated with tumor metastasis. We further provided evidence that IL-37 inhibited effectively tumor metastasis in vitro and in vivo. Moreover, we uncovered a novel mechanism by which IL-37 suppressed tumor cell migration via its intracellular mature form (amino acids 46-218).

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DOI: 10.1002/eji.201970400

Intracellular mature form of IL-37, but not its extracellular form, markedly inhibited migration of multiple kinds of tumor cells through inhibiting Rac1 activation. Mechanistically, intracellular mature IL-37 directly bound to the CAAX motif in the C-terminal hypervariable region of Rac1, and then inhibited Rac1 membrane translocation and subsequent downstream signaling. Our research identifies intracellular mature IL-37 as a novel endogenous inhibitor of Rac1. Given the crucial roles of Rac1 in tumor angiogenesis and metastasis, intracellular mature IL-37 might serve as a potential strategy for the control of Rac1 activity and tumor progression.

O259

BAFF regulates follicular B cell homeostasis independent of intrinsic apoptosis

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The cytokine BAFF plays a non-redundant role in mature B-cell homeostasis and it has been implicated in autoimmune disorders and malignancies. Since its discovery, the pivotal role that BAFF plays in B cell biology has been linked to its ability to promote cell survival and to antagonise intrinsic apoptosis. In order to explore the role of apoptosis downstream of BAFF signalling, we have conditionally ablated intrinsic apoptosis in mature B lymphocytes in mice by deleting the ultimate apoptosis effectors Bak and Bax. $Bak^{-/-}$ $Bak^{D/D}$ follicular B cells are entirely resistant to apoptosis in vitro and have an increased lifespan in vivo. Surprisingly, apoptosis resistance does not lead to an unrestrained accumulation of B cells in vivo nor to the emergence of lymphoid malignancies. In conditional knockout animals, the numbers of follicular B-cells increase during the first weeks of life and plateau at ~2-fold normal levels at 10 weeks of age. This is accompanied by the decrease in size of follicular B cells, a phenomenon known as atrophy. We show that cellular atrophy results from the increased competition for BAFF and that it can be reversed upon exogenous BAFF administration. We are currently using transcriptomics to interrogate how BAFF regulates B-cell growth in vivo. Altogether, our results demonstrate that the role of BAFF in follicular B cell biology and homeostasis extends beyond blocking apoptosis.

O260

Spred2 deficiency exacerbated Concanavalin A-induced liver injury in mice through increased interferon γ production from T cells

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Background: Cytokines have been implicated in the progression of T cell-mediated liver injury, in which mitogen-activated protein kinase (MAPK) cascades may play a role. We focused on the functional role of Spred2, a negative regulator of MAPK that constitutively expressed in liver, in concanavalin A (ConA)-induced acute liver injury.
Methods: Wild-type (WT: C57BL/6J) and Spred2 KO mice were intravenously injected with ConA (10mg/kg). For some experiments, $CD4^+$ and $CD8^+$T cells were deleted by neutralizing antibody and ERK-MAPK pathway was blocked by a MEK/ERK inhibitor U0126, prior to Con A challenge.
Results: Spred2 deficiency exacerbated ConA-induced liver injury accompanied by increased plasma and hepatic level of interferon γ (IFNγ). Neutralization of IFNγ abolished ConA-induced liver injury in Spred2 KO mice with down-regulated hepatic STAT1 activation. ERK activation was enhanced in the Sperd2 KO livers, and U0126 alleviated liver injury with reduced IFNγ production. Upon ConA treatment, IFNγ-inducible chemokines CXCL9/10 were augmented in Spred2 KO livers. When stimulated with IFNγ, hepatocytes from Spred2 KO mice produced higher levels of CXCL9/10. FACS

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DOI: 10.1002/eji.201970400

analyses demonstrated the numbers of hepatic $CD4^+$T and $CD8^+$T cells in Spred2 KO liver were significantly increased. Depletion of either $CD4^+$T or $CD8^+$T cells ameliorated liver injury in Spred2 KO mice with decreased plasma level of IFNγ. In vitro studies defined splenic $CD4^+$T and $CD8^+$ T cells from Spred2 KO mice produced higher levels of IFNγ when stimulated with ConA or anti-CD3/CD28. **Conclusion:** Lacking Spred2 is deleterious in ConA-induced liver injury by enhanced IFNγ production from $CD4^+$T and $CD8^+$ T cells.

O261

KDM2B epigenetically promotes IL-6 production and inflammatory responses through Brg1-mediated chromatin remodeling

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IL-6 plays important and pleiotropic roles in infection and inflammatory diseases, and its production needs to be tightly regulated. However, the epigenetic mechanism underlying Il6 gene transcription remains to be fully elucidated. Here, we reported that deficiency of lysine-specific demethylase 2b (KDM2B), a demethylase of H3K36me2 and H3K4me3, preferentially inhibited innate IL-6 production triggered by Toll-like receptors (TLRs) in macrophages and dendritic cells, but not the production of TNF-α, IL-1 and IFN-β. KDM2B-deficient mice were more resistant to LPS- or poly(I:C)-triggered endotoxin shock and dextran-sulfate-sodium (DSS)-induced colitis with a less severe inflammatory pathogenesis phenotype and decreased IL-6 production in sera and tissue. KDM2B selectively bound the Il6 promoter, but did not alter the histone demethylation; instead, KDM2B interacted with Brg1, the core ATPase subunit of SWI/SNF chromatin remodeling complexes, to facilitate the chromatin accessibility of Il6 promoter. Furthermore, KDM2B directly recruited RNA Polymerase II to further initiate and promote Il6 transcription. Thus, our finding indentifies a novel non-classical function of KDM2B in the gene-specific transcription initiation and enhancement of Il6 independent of its demethylase activity, and adds a new mechanic insight into the specific epigenetic modification of inflammatory immune responses.

O262

Pro-inflammatory S100A8 induces PD-L1 expression in macrophages, mediating tumor immune escape

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S100A8 is a damage associated molecular pattern protein released by monocytes, playing a decisive role in the development of inflammation. Nonresolving inflammation is viewed as a driving force in tumorigenesis, and its role in tumor immune escape also attracted attentions. PD-1/PD-L1 axis is a critical determinant of physiological immune homeostasis, and anti-PD-1 or PD-L1 therapy has becoming the most exciting field of oncology. Multiple regulation mechanisms have been contributed to PD-L1 expression modulation including inflammatory mediators. Here we reported that S100A8 significantly induce PD-L1 expression in monocytes/macrophages, but not in tumor cells. S100A8 induce PD-L1 transcription through the TLR4 receptor and multiple crucial pathways of inflammation

DOI: 10.1002/eji.201970400

process. S100A8 modulate the histone modification of the PD-L1 promoter in monocytes/macrophages. S100A-pretreated macrophages had immunosuppressive function and attenuated the anti-tumor ability of cytotoxicity T cells both *in vitro* and *in vivo*. A highly positive correlation existed between S100A8 expression and PD-L1 expression in human cancer specimens. Our study uncovers a novel molecular mechanism for regulating PD-L1 transcription by an inflammatory mediator S100A8, and reveals the importance of comprehensive understanding the role of inflammation in tumorigenesis as well as in tumor immune escape.

O263

IL-37 levels correlate with better clinical and virological parameters in HIV infection

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Background: IL-37 is a member of the IL-1 family with potent anti-inflammatory and immune deviating effects. Little is known about the regulation and role of the cytokine and of its receptor in HIV infection.
Objectives: To understand how the cytokine and its receptor are regulated in this viral infection.
Methods: We measured circulating concentrations of IL-37 and the soluble form of its receptor, IL-1R8, in HIV-infected individuals, long-term non-progressors (LTNP) and healthy controls with commercial ELISA kits. The expression the cytokine receptor on immune cells was determined by flow cytometry. The transcripts for the cytokine isoforms (a-d) were determined in PBMC by RT-PCR using isoform-specific primers. The effect of recombinant human IL-37 (b) on HIV replication was determined in PHA blasts in *in vitro* assays.
Results: The cytokine concentrations were higher in LTNP and HIV-infected individuals on ART. No difference was found between treatment-naïve HIV-infected individuals and healthy controls. The expression of IL-1R8 was decreased on monocytes and NK cells making them hypo-responsive to the cytokine. Recombinant human IL-37 significantly decreased HIV replication in PHA blasts. Soluble IL-1R8 concentrations mirrored those of the cytokine, and attenuated anti-inflammatory effects of the cytokine. The transcripts for some active isoforms of the cytokine disappeared in HIV infected individuals. These results suggest a compromised functioning of the IL-37/IL-1R8 axis in the virus-infected individuals. Despite this, IL-137 concentrations correlated negatively with viral load and TNF-α concentrations, and positively with $CD4^+$ T cell counts.
Conclusions: IL-37/IL-1R8 axis plays a host beneficial role in HIV infection.

O264

Enhancers that regulate cytokine and chemokine gene expression in mast cells In response to antigenic stimulation of IgE receptors

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Mast cells are hematopoietic cells that abundant granules within the cytoplasm. These cells play critical roles in causing allergic inflammation and anaphylaxis. Activated mast cells undergo degranulation rapidly with the release inflammatory mediators including histamine, heparin, protease, and synthesize de novo cytokines and chemokines. However, genes that encode inflammatory mediators, cytokines and chemokines in mast cells have not been studied extensively. Especially, how

DOI: 10.1002/eji.201970400

enhancers in these genes respond to antigenic stimulation is not clear. In this study, we used next generation of sequencing was used to identify the putative enhancers in resting mast cells and mast cells that were stimulated with antigenic stimulation that cross-links IgE bound FceRI receptors (IgE cross-linking). We calculated the regulatory potential scores for the enhancers based on locations of the enhancers, the numbers of enhancers per gene and RNA expression levels of the genes in mast cells with or without IgE cross-linking. We found that the regulatory potential scores for enhancers in genes that were upregulated in response to IgE cross-linking increased, whereas the regulatory potential scores for enhancers in genes that were downregulated in response to IgE cross-linking decreased, and the regulatory potential scores for enhancers in genes that were not altered in response to IgE cross-linking remained unchanged. Our study supports that enhancers are the major target sites that are regulated by the positive and negative signals triggered by mast cell activation.

LB004

The association between genetic polymorphisms of interleukin 23 receptor gene and the risk of rheumatoid arthritis: an updated meta-analysis

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This meta-analysis was conducted to confirm whether interleukin-23 receptor (*IL-23R*) gene polymorphisms are associated with rheumatoid arthritis (RA) susceptibility. RevMan version 5.3 was used to calculate statistical data. Sixteen articles involving 11816 RA patients and 14268 healthy controls were included in this meta-analysis. A significant association was identified between the rs11209026 polymorphism and RA susceptibility in Caucasians (AA vs. GG: OR=1.78, 95% CI=1.02-3.10, P=0.04; AA vs. AG+GG: OR=2.56, 95% CI=1.07-6.12, P=0.03). Additionally, our result showed that the G allele of *IL23R*/rs10489629 had a significantly increased frequency in RA patients of Caucasians and Asians (A vs. G: OR=0.92, 95% CI=0.88-0.97, P=0.002; OR=0.62, 95% CI=0.44-0.87, P=0.006, respectively). Furthermore, the meta-analysis revealed a significant association between the rs1343151 polymorphism and RA susceptibility in Caucasians (C vs. T: OR=0.91, 95% CI=0.87-0.96, P=0.0004; CC vs. TT: OR=0.83, 95% CI=0.75-0.93, P=0.001; CC+CT vs. TT: OR=0.88, 95% CI=0.79-0.97, P=0.01; CC vs. CT+TT: OR=0.89, 95% CI=0.83-0.96, P=0.001).

LB005

IL-21 is a broad negative regulator of IgE class switch recombination in mouse and human B cells

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IgE antibodies may elicit potent allergic reactions and their production is tightly controlled. Thus, IgE-producing B cells are very rare in vivo under normal conditions. However, these cells can be readily generated in vitro, indicating extra extrinsic factors exist in vivo to restrain the generation of these cells. The tendency to generate IgE has been thought to reflect the balance between type 1 and type 2 cytokines, with the latter promoting IgE. Here we reevaluated this paradigm by a direct cellular analysis, demonstrating that IgE production was not limited to type 2 immune responses. IL-21 was found to be a critical negative regulator of IgE responses, whereas deficiency of IFN-γ, IL-6, and IL-10 had minimal effect. Follicular helper T cells were the primary source of IL-21 that inhibited IgE

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DOI: 10.1002/eji.201970400

responses by directly engaging the IL-21 receptor on B cells and triggering STAT3-dependent signaling. We reconciled previous discordant results between mouse and human B cells and revealed that the inhibition of IgE class switch recombination by IL-21 was attenuated by CD40 signaling, whereas IgG1 class switch recombination was potentiated by IL-21 in the context of limited IL-4. These findings establish key features of the extrinsic regulation of IgE production by cytokines.

LB042

NK cells are also the source of IL-9

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Interleukin-9 (IL-9), a cytokine secreted mainly by Th9 cells, is involved in a variety of physiological and pathological processes in the body. Activin A belongs to a multifunctional cytokine of transforming growth factor-β (TGF-β) superfamily, which can enhance Th9 cell differentiation. However, whether NK can secrete IL-9 remains unclear. In the present study, by intracellular cytokine staining, we found that not only $CD3^+CD4^+$Th cells, but also $CD3^-CD49b^+NKp46^+$NK cells of Balb/c mouse produced IL-9. Although activin A promoted the expression of IL-9 in $CD3^+CD4^+$Th cells, it inhibited the production of IL-9 in $CD3^-CD49b^+NKp46^+$NK cells of mouse. Furthermore, the enzyme-linked immunosorbent assay (ELISA) results showed that mouse NK cells could secrete mature IL-9 protein, and activin A inhibited IL-9 release by NK cells in a dose-dependent manner. Additionally, activin A inhibited IFN-γ production in NK cells of mouse, but promoted IL-2 production, and did not alter the production of IL-10. The inhibitory effects of activin A on IL-9 production by NK cells were attenuated in the presence of activin antagonist follistatin. These data demonstrated that although activin A up-regulates IL-9 expression in Th cells, it inhibits IL-9 production in NK cells, suggesting that biological effects of activin A on different cells are different.

LB078

The influence of interferon lambda (IFN-λ) on the viability and ability of canine mammary tumor cells to migrate *in vitro*

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Canine mammary tumor is the most common cancer in female dogs. Except for surgical removal of tumor, available therapies in veterinary are based on human chemo- and radiotherapy protocols. Immunotherapy is a new hope for effective anti-cancer therapy. Interferon lambdas are the last discovered interferon group. Their anti-viral properties are well-known, although their role in carcinogenesis is still unclear. In this work, the influence of IFN-λ1 and IFN-λ2 on the viability (MTT assay) and migration ability (wound healing assay and RT-qPCR) of P114, CMT-U27 and CMT-U309 canine mammary cancer cell lines were determined. The significant influence on cell proliferation was observed only in P114 cell line. Moreover, in P114 cell line IFN-λs promote wound healing compared to control conditions, probably because of the induction of matrix metalloproteinases *(MMPs)* expression (verify by qPCR). In CMT-U309 and CMT-U27 the IFN-λ had any influence on viability of cells. In CMT-U309 cell line the influence on cell migration and expression of *MMPs* and their inhibitors were not observed. in CMT-U27 cell line IFN-λ inhibit cells' ability to migration.

The research was founded by grant NCN no UMO-2016/23/D/NZ5/03276. Participation was funded by

DOI: 10.1002/eji.201970400

KNOW (Leading National Research Center) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

LB079

Expression of alternatively spliced IL-15 isoform modulates anti-viral immune responses in HSV-1 zosteriform mouse model

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We previously reported a Pedigree 191 (P191) of *N-ethyl-N-nitrosourea* (*ENU*) mutagenized mice that highly expressed an alternatively spliced IL-15 isoform with the deletion of first 16 amino acids of exon 7 (IL-15ΔE7). Expression of CD44 was suppressed within CD8+ T cells of P191 mice. Infection of P191 mice with herpes simplex virus-1 (HSV-1) via flank skin generated a prolonged and early re-emerged HSV-1 zosteriform rash compared to wild type (WT) C57BL/6 mice. Using K^b/$gB_{498-505}$ MHC Class I tetramer, we found that gB-specific CD8+ T cells were less expanded in draining lymph nodes (dLNs) and subsequently reduced CD8+ T cell infiltration to lesional skin in P191 compared to those in WT mice. While resident CD8α+DCs in dLNs were increased in number at 48 h and remained stable at 96 h in WT mice after HSV-1 infection, they were much reduced in P191 mice. Migration of Langerhans cells into dLNs was also decreased in P191 mice. The increases in the numbers of CD8α+DCs and skin-derived Langerhans cells were correlated with upregulated expression of IL-15 in dLNs of WT mice after HSV-1 infection. However, the reverse relationship between the cell numbers of CD8α+DCs and Langerhans cells and the increased expression of IL-15ΔE7 mRNA in dLN of P191 mice suggested a modulatory role for IL-15ΔE7 in T cell priming during early phase of HSV-1 infection. The molecular mechanisms by which the generation of HSV-1 specific CD8+ T cells is suppressed in P191 mice remain to be clarified.

P0027

Association between IL-1β polymorphisms and gastritis risk: a meta-analysis

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Objectives: *Helicobacter pylori* infection of human stomach regularly leads to chronic gastric inflammation. Cytokine gene interleukin-1β has been implicated in influencing the pathology of inflammation induced by *H. pylori* infection. Currently, several studies have been carried out to investigate the association of IL-1β-511 (rs16944) and IL-1β-31 (rs1143627) polymorphisms with gastritis risks; however, the results are inconsistent and inconclusive. To assess the effect of IL-1β polymorphisms on gastritis susceptibility, we conducted a meta-analysis.
Methods: Up to 15th Mar. 2016, 2,205 cases and 2,289 controls were collected from 12 published case-control studies. Summary odds ratios and corresponding 95% confidence intervals (CIs) for IL-1β-511 and IL-1β-31 polymorphisms and gastritis risk were estimated using fixed- or random-effects models when appropriate. Heterogeneity was assessed by chi-squared-based Q-statistic test, and the sources of heterogeneity were explored by subgroup analyses and logistic meta-regression analyses. Publication bias was evaluated by Begg's funnel plot and Egger's test. Sensitivity analyses were also performed.
Results: The results provided evidences that the **SNPs in IL-1β-31 might associate with the gastritis risks, especially in Caucasian population, while SNP in the IL-1β-511 might not.**
Conclusion: Our studies may perhaps supplement for the disease monitoring of gastritis in the future,

DOI: 10.1002/eji.201970400

and additional studies to determine the exact molecular mechanism might provide us with interventions to protect the susceptible subgroups.

P0028

IL-17A exacerbates cisplatin-based resistance of OVCA via upregulating the expression of ABCG2 and MDR1 through Gli1-mediated Hh signaling

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The major obstacle of chemotherapy for tumors, including ovarian cancer (OVCA), is drug resistance. However, the relevance of IL-17A with drug-resistance of OVCA has been poorly elaborated. In this study, we used 2 human OVCA cell lines to investigate the effects of IL-17A on cisplatin (CDDP or DDP)-based resistance in OVCA cells and the underlying mechanisms. Meanwhile, IL-17A-deficient mice and ID8 cells were used to verify the effects of IL-17A on OVCA chemo-resistance in vivo. Moreover, the relationship between IL-17A level and relevant indices were primarily assessed in ovarian specimens from 55 patients with OVCA. We found that rhIL-17A exacerbated DDP-based resistance of OVCA cells via up-regulating the expression of ABCG2 and MDR1 through Gli1-mediated Hh signal pathway. Animal experiment demonstrated that IL-17A significantly recede DDP-based treatment for ID8 tumor. Similar results were observed in preliminary clinical investigations. Our findings suggest that inhibiting IL-17A/IL-17RA-Gli1 signal may improve the resistance of OVCA to DDP.

P0029

1,25-dihydroxyvitamin D inhibits LPS induced HMGB1 secretion in macrophage via targeting Nrf2-HO-1-HMGB1 pathway

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Sepsis is common among critically ill patients and associated with considerable morbidity and Mortality. To date, the early cytokines, such as TNF-α and IL-1β, and the late cytokines high-mobility group box 1 (HMGB1) involved in sepsis have been identified. Recently, HMGB1 antibodies have been reported to inhibit endotoxin lethality in mice and inhibit lung inflammation. These findings suggest that HMGB1 may serve as a target to reduce mortality from sepsis. We have observed that 1,25-dihydroxyvitamin D (1,25(OH)2D3) treatment has a good effect and improves the survival rate in LPS-induced mouse sepsis model with blocking the secretion of HMGB1. 1,25(OH)2D3 attenuates LPS-induced HMGB1 secretion by blocking its translocation from nucleus to cytoplasm in macrophage. Heme oxygenase-1 (HO-1) is essential for HMBG1 nucleus translocation and blocks HMGB1 secretion, using SiHO-1 or HO-1 inhibitor attenuates 1,25(OH)2D3 inhibiting HMGB1 secretion. In addition, 1,25(OH)2D3 inhibits Nrf2 activation which control HO-1 expression via blocking its translocation from cytoplasm to nucleus upon LPS exposure. Together, we provide evidence in

DOI: 10.1002/eji.201970400

macrophage that 1,25(OH)2D3 inhibits Nrf2/HO-1 pathway to attenuate LPS-induced HMGB1 secretion.

P0030

The expression, purification of IL-21 recombinant protein and the function of inhibiting exosome secretion

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Background: Interleukin-21 (IL-21), a pleiotropy cytokine, plays an indispensable role in autoimmune, anaphylactic, inflammatory diseases and cancer. Increasing evidence has shown the potential therapeutic role of IL-21 in autoimmune diseases. However, the existing IL-21 protein cannot reach the higher requirements of well-modified spatial structure and intact physiological function to provide required protein activity.
Objectives: To construct a new IL-21 protein with complete bioactivity.
Methods: We successfully transferred overall-length IL-21 cDNA into HEK293F cells and innovatively cultured in suspension system. IL-21 protein was purified to homogeneity via sequential nickel affinity chromatography and ultrafiltration, with nondenaturing method to amend structural damage. Functional studies were performed by using $CD4^+$ T cells proliferation and differentiation induced by gradient concentration of recombinant IL-21, and its effects on PBMC released exosomes.
Results: The yield of recombinant IL-21 protein produced by HEK293F combined with suspension culture system was higher than HEK293T plus adherent culture system. Recombinant IL-21 demonstrated a function of prompting $CD4^+$ T cells proliferation with low concentration, while high concentration displayed reverse effects. In addition, we found that recombinant IL-21 suppressed exosome secretion significantly.
Conclusion: This study provides a full-length functional recombination human IL-21 protein, and demonstrates a new role that IL-21 inhibited exosome secretion which provides basis for fields of exosome research and cancer treatment.

P0031

Fucoidan inhibits CCL22 production through NF-κB pathway in M2 macrophages

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In tumor microenvironment, there are great amount of polarized M2 macrophages infiltration, which promote tumor progression via releasing multiple cytokines and chemokines. As a brown seaweed fucose-rich polysaccharide, fucoidan has antitumor activity and immune modulation through affecting tumor cells and lymphocytes. Here, we focused on the effect of fucoidan on macrophages especially M2 subtype, searching for internal connections between fucoidan and potential antitumor activity. Our results demonstrated that fucoidan down-regulated partial cytokines and chemokines, especially a M2-type chemokine CCL22 in M2 macrophages. Furthermore, fucoidan inhibited tumor cells migration and $CD4^+$ T lymphocytes, especially Treg cells, recruitment induced by M2 macrophages conditioned medium through suppression of CCL22. Mechanismly, fucoidan inhibited CCL22 via suppressing p65-NF-κB phosphorylation and nuclear translocation. In addition, p38-MAPK and PI3K-AKT also affected the expression of CCL22 through differential modulation of NF-κB transcriptional activity. Taken together, we reveal an interesting result that fucoidan can inhibit tumor cell migration and T lymphocyte subsets recruitment by suppressing CCL22 in M2 macrophages via NF-κB-dependent

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DOI: 10.1002/eji.201970400

transcription, which may be a novel and promising mechanism for tumor immunotherapy.
This work was supported by grants from the National Natural Science Foundation of China (No. 81772879) and National Key Research and Development Program of China (2018YFC1002803).

P0032

Association of gene polymorphism of CXCL10 and CXCL12 with tuberculosis susceptibility

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Objective: Explore the relationship of chemokines CXCL10-135G/A and CXCL12 -801G/A gene polymorphisms with susceptibility to tuberculosis.
Methods: CXCL10-135G/A and CXCL12-801G/A polymorphisms of 102 TB patients (case group) and 115 healthy controls (control group) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and analysis the relationship between the two polymorphisms and susceptibility to tuberculosis.
Results: The genotype analysis of CXCL10-135G/A and CXCL12 -801G/A in the case group and the control group accord with the law of Hardy-Weinberg equilibrium. The differences of genotype and allele distribution frequency of CXCL10-135G/A between the case group and the control group were statistically significant (all $P<0.05$); the frequency of G allele distribution in the case group was higher than that in the control group, and the frequency of A allele distribution was lower than that in the control group. The differences of genotype and allele distribution frequency of CXCL12-801G/A polymorphism between the case group and the control group were not statistically significant (all $P>0.05$).
Conclusion: Chemokine CXCL10-135G/A gene polymorphism was associated with susceptibility to pulmonary tuberculosis and CXCL12-801G/A gene polymorphism may not be associated with tuberculosis infection.

P0033

Low-dose IFN-γ induces tumor cell stemness in tumor microenvironment of non-small cell lung cancer

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Interferon-γ (IFN-γ) is conventionally recognized as an inflammatory cytokine that play a central role in antitumor immunity. Clinically, although has been used clinically to treat a variety of malignancies, low-level IFN-γ in the tumor microenvironment (TME) increases the risk of tumor metastasis during immunotherapy. Accumulating evidence has suggested that IFN-γ can induce cancer progression. The mechanisms underlying the controversial role of IFN-γ regulating tumor development remain unclear. Herein, we firstly revealed a dose-dependent effect of IFN-γ in inducing tumor stemness to accelerate cancer progression in patients with a variety of cancer types. Mechanically, low-level IFN-γ endowed cancer stem-like properties via the intercellular adhesion molecule-1 (ICAM1)-PI3K-Akt-Notch1 axis, whereas high-level IFN-γ activated the JAK1-STAT1-caspase pathway to induce apoptosis in non-small cell lung cancer (NSCLC). Inhibition of ICAM1 abrogated the stem-like properties of NSCLC cells induced by the low dose of IFN-γ both *in vitro* and *in vivo*. Our study first defines the role of low-level IFN-γ in conferring tumor stemness and clearly elucidate the distinct signaling pathways activated by IFN-γ in a dose-dependent manner, providing new insights into cancer treatment, particularly patients with low-level IFN-γ expression in the TME.

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DOI: 10.1002/eji.201970400

P0034

IL-37 isoform D downregulates proinflammatory cytokines expression in a Smad3-dependent manner

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IL-37 is a new member of IL-1 family and possesses five different isoforms (named as IL-37 a-e). IL-37b has been demonstrated as a physiological suppressor of immune responses. However, the function of other isoforms remains unknown. Here, we show that IL-37d possesses anti-inflammatory roles both *in vitro* and *in vivo*. Firstly, IL-37d is expressed in peripheral blood mononuclear cells (PBMCs) and umbilical cords-derived mesenchymal stem cells (UCMSCs). Secondly, IL-37d overexpression markedly inhibits IL-1β-induced IL-6 production in A549 cells. Consistently, bone marrow-derived macrophages (BMDMs) from IL-37d transgenic mice express low levels of pro-inflammatory cytokines (such as IL-6 and TNF-α) following LPS stimulation, compared with those from wild-type mice. Furthermore, IL-37d transgenic mice produce less pro-inflammatory cytokines, and show much less degree of LPS-induced endotoxemia *in vivo*. Mechanistically, IL-37d interacts with Smad3 and promotes nuclear translocation of pSmad3. SIS3 (a specific Smad3 inhibitor) treatment completely blocks the inhibitory effects of IL-37d. Thus, our data indicate that IL-37d is a functional cytokine that negatively regulates proinflammatory cytokines expression in a Smad3-dependent manner.

P0035

ZEB1/IL-8 axis recruits/polarizes TAMs to remodel the immune microenvironment in breast cancer

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The growth and development of tumor cells rely on a complex tumor microenvironment (TME). Among the various stromal cells, tumor-associated macrophages (TAMs) are the prominent components in TME. Accumulation of TAMs are associated with malignant progression and are thought to more closely resemble the M2-polarized phenotype. It has been demonstrated that ZEB1 functions as a transcription factor to promote tumor progression. However, the role for ZEB1 in the polarization and pro-tumor function of TAMs remains obscure. In this study, by using a spontaneous genetic model of breast cancer, we showed that **depletion of Zeb1 in the breast epithelium reduces TAMs recruitment and polarization in primary tumors.** Moreover, clodronate-induced depletion of macrophages *in vivo* attenuated the tumor development and progression promoted by Zeb1, demonstrating that Zeb1 regulates breast cancer growth through macrophages. Mechanistically, we performed RNA-sequencing and elucidated that **ectopic Zeb1 significantly upregulates IL-8 expression in MDA-MB-231 breast cancer cells.** Notably, addition of IL-8 neutralizing antibody abolished the recruitment and polarization of M2-TAMs induced by conditioned medium derived from Zeb1-overexpressing MDA-MB-231, demonstrating that **IL-8-dependent paracrine action is involved in the remodeling of tumor immune microenvironment (TIME) by Zeb1**. Furthermore, **IL-8-polarized M2-TAMs upregulate PD-L1 expression**, which occurs mainly on tumor-infiltrating immune cells rather than on tumor cells. Thus, **targeting both IL-8 and PD-L1 coordinately inhibits breast cancer aggressiveness and enhances the efficacy of TIME therapy.** Collectively, our study

DOI: 10.1002/eji.201970400

suggests a Zeb1-dependent mechanism that confers tumors with aggressive and lethal properties, such as fostering the function of TIME via TAMs recruitment and polarization.

P0036

The effects of β2GPI and anti-β2GPI antibody on the expression of pro-inflammatory cytokines and adhesion molecules in HUVEC

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Antiphospholipid syndrome (APS), an autoimmune disease, has been defined as a new risk factor for atherosclerosis (AS). Our previous studies have demonstrated that β2GPI, the main antigen in APS, and its autoantibody (anti-β2GPI) are tightly associated with foam cell formation and the secretion of tissue factor (TF) in monocytes and macrophages. However, the effects of β2GPI/anti-β2GPI with low density lipoprotein (oxLDL) complex on endothelial cell activation have yet to be investigated. In the present study, we focused on the effects and underlying mechanisms of the oxLDL/β2GPI/anti-β2GPI complex on HUVEC activation. Our results showed that the oxLDL/β2GPI/anti-β2GPI complex treatment significantly enhanced the pro-atherogenic activity of HUVEC, including the mRNA expression and protein secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), adhesion molecules (vWF, ICAM-1, VCAM-1), meanwhile significantly promoted their ability to recruit THP-1 cells; In addition, the expression of TLR4 and the phosphorylation of NF-κβ p65 and p38 were also upregulated in oxLDL/β2GPI/anti-β2GPI complex-treated HUVEC in compared to media treated ones. The effects of TLR4/p38/NF-κB pathway on oxLDL/β2GPI/anti-β2GPI complex-induced expression of pro-inflammatory cytokines and adhesion molecules in HUVEC and THP-1 adhesion were confirmed by the application of selective inhibitors. Taken together, our findings implicated that β2GPI and its autoantibody could promote the expression of pro-inflammatory cytokines and adhesion molecules in HUVEC by forming oxLDL/β2GPI/anti-β2GPI complex. TLR4/p38/NK-κB signal pathway was involved in this process.

P0037

Osteopontin exacerbates activation of hepatic stellate cells and liver fibrosis

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Backgrounds: Liver biopsy is the current reliable evaluation of liver fibrosis, while no specific sera applied in clinical diagnosis. Studies indicated that osteopontin (OPN) plays a pivotal role in numerous liver diseases. However, the molecular mechanism underlying the OPN in liver fibrosis is still obscure. In the present study, we investigated the potential of thrombin-cleaved OPN (Thr-OPN) in the pathogenesis of liver fibrosis.
Methods: We analyzed the correlation between Thr-OPN level and the severity of liver fibrosis and the involvement of Thr-OPN in liver fibrosis in patient samples, mouse models and LX-2 cells.
Results: Thr-OPN level was significantly higher in liver cirrhosis (LC) patients than that in chronic hepatitis B patients and was positively correlated to LC degree. The correlation between hepatic Thr-OPN levels and LC degree in mouse models was consistent with the above results. Neutralization of Thr-OPN alleviated LC in wild-type mice, whereas Thr-OPN could exacerbate liver fibrosis in OPN-deficient mice. Compared with full-length OPN (FL-OPN), Thr-OPN exhibited greater ability to promote hepatic stellate cell (HSC) activation, proliferation and migration via MAP kinase and NF-κB signaling pathways.

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DOI: 10.1002/eji.201970400

Conclusions: Thr-OPN, not FL-OPN, was critically involved in the pathogenesis of liver fibrosis via MAP kinase and NF-κB signaling pathway, thus representing a novel diagnosis biomarker and treatment target for liver cirrhosis.

P0038

Interleukin-27 induces expansion of myeloid-derived suppressor cells via increasing the numbers of granulocyte-monocyte progenitor

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Interleukin-27 (IL-27) is a pleiotropic cytokine that exhibits regulatory functions on multiple lineages of immune cells. Studies have revealed that IL-27 can enhance cancer immunity while inhibit autoimmunity, and has a potential to be used as a therapeutic for cancer and autoimmune diseases. Indeed, we recently found that systemic delivery of IL-27 using adeno-associated virus (AAV-IL-27) exhibited potent inhibition to the growth of tumors and development of autoimmune diseases in mouse models. However, we also found significant expansion of myeloid-derived suppressor cells (MDSCs) in AAV-IL-27-treated mice.

AAV-IL-27-induced MDSCs were mainly of the $CD11b^{+}Gr1^{+}Ly6C^{low}$ phenotype, whose expansion was IL-27R-dependent, but was inhibited by Stat1 signaling, since further expansion of MDSCs was observed in AAV-IL-27-treated Stat1-deficient mice.

Mixed bone marrow chimera experiments revealed that AAV-IL-27-induced expansion of MDSCs was myeloid lineage intrinsic. However, AAV-IL-27 treatment did not enhance the self-renewal capacity of MDSCs, but rather induced significant expansion of granulocyte-monocyte progenitor (GMP) cells in bone marrow, which are known to give rise to $CD11b^{+}Gr1^{+}$ myeloid cells.

Finally, we found that IL-27-induced MDSCs highly expressed signature genes such as S100A9, Arginase and IL-1b, and were metabolically active.

Thus, systemic delivery of IL-27 induces the expansion of MDSCs via increasing the numbers of GMPs in bone marrow, and targeting MDSCs may enhance IL-27-based immunotherapy of cancer and autoimmune diseases.

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DOI: 10.1002/eji.201970400

P0039

Inhibition of experimental corneal neovascularization by the tight junction protein Zonula Occludens 1

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Purpose: to explore the effects of the tight junction protein zonula occludens 1 (ZO-1) on experimental corneal neovascularization (CNV).
Methods: CNV models were established using NaOH. Anti-ZO-1 neutralizing antibody was topically applied to the burnt corneas of the mice in the ZO-1 antibody group after modeling. The mRNA and protein expression levels of ZO-1, VEGF, IL-1β, IL-6, IL-18, MCP-1, TNF-α, pPKC, and clusterin in burnt corneas were detected by RT-PCR and western blot analyses. The infiltration of neutrophils, macrophages and progenitor cells into corneas was examined by flow cytometry.
Results: Corneal mRNA and protein expression levels of VEGF, IL-1β, IL-6, IL-18, and MCP-1 were significantly up-regulated in the ZO-1 antibody group comparing to those in the control group. Intracorneal infiltration of neutrophils, macrophages and progenitor cells increased significantly in the ZO-1 antibody group in comparison with that in the control group. TNF-α expression level was elevated significantly in the 45 s alkali injury group comparing to that in the 15 s alkali injury group. However, the protein expression of pPKC and clusterin was decrease significantly in the 45 s injury group in comparison with that in the 15 s injury group.
Conclusion: Anti-ZO-1 neutralizing antibody-treated mice exhibited enhanced alkali-induced CNV through increased intracorneal infiltration of progenitor and inflammatory cells.

P0040

Interleukin-3 protects against secondary viral infections in sepsis

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Sepsis is a common and life-threatening syndrome associated to an acute systemic infection. Previously, we identified interleukin-3 (IL-3) as an important mediator in the development of acute inflammation during sepsis. However, its influence in the following immunosuppressive phase of sepsis remains unclear. In an experimental model of secondary viral pneumonia after the induction of septic immunosuppression by cecal ligation puncture (CLP), IL-3 promoted the recruitment of type 1 interferon producing circulating plasmacytoid dendritic cells (pDCs) into the lung parenchyma by stimulating pulmonary CD123+ (IL-3 receptor alpha chain) epithelial cells to secrete CXCL12, thus leading to a better protection against the virus. As well, local injection of recombinant IL-3 protected mice against primary pulmonary viral infection in naive mice. In a translational approach, the retrospective analysis of IL-3 plasma levels in septic patients (RAMMSES clinical study, n=60) revealed similarly a protective function of IL-3 against secondary viral pneumonia. Low plasma IL-3 levels was associated with increased HSV tracheal infection during sepsis. In addition, in bronchoalveolar lavage fluid low IL-3 levels was correlated with low pDC numbers and low CXCL12 levels from patients with different respiratory diseases. Altogether, our results indicate a protective function of IL-3 during viral infection, not only in the setting of septic immunosuppression.

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DOI: 10.1002/eji.201970400

P0041

Interleukin-34 contributes to Foxp3$^+$ treg suppressive function and inhibits allogeneic immune responses

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IL-34 is a recently discovered cytokine that binds to CSF-1R, CD138 and PTPz and is involved in differentiation and survival of macrophages. We discovered that IL-34 is expressed by rodent and human CD8$^+$ and CD4$^+$ Treg and that administration of an AAV-IL-34 induced long-term allograft tolerance in rat through M2 differentiation and subsequent Treg induction (Bezie, 2015).
To understand the function of IL-34 in immune responses, we generated IL-34$^{-/-}$ rats. These rats showed decreased CD8$^+$ and CD8$^+$CD45RC^{high} T cells in the spleen and CD8$^+$ and CD8$^+$Foxp3$^+$ T cells in the blood vs. WT animals. Interestingly, CSF-1 (the CSF-1R other ligand) serum level was significantly higher in IL-34$^{-/-}$ animals. In a model of wasting disease in *IL2rg*$^{-/-}$ rats injected with T cells, we show that IL-34-deficient CD4$^+$ Treg were not able to control the wasting disease compared to wt CD4$^+$ Treg, suggesting that IL-34 is essential for their suppressive function. Transcriptomic analysis of IL-34-deficient CD4$^+$ or CD8$^+$ Treg vs wt Tregs showed that only one gene and the same gene for both Treg subsets was altered in IL-34-deficient Tregs, suggesting an important role for this gene.
Finally, treatment with IL-34 and rapamycin at a suboptimal dose in a model of GVHD in NSG mice injected with hPBMCs reduced the incidence of aGVHD vs. rapamycin or IL-34 alone (median survival: 39, 28 and 14 respectively; n=4-8).
Altogether, our data demonstrate the implication of IL-34 in CD4$^+$ Treg function and the relevance of IL-34 as a regulatory cytokine in aGVHD.

P0042

EBI3, one of subunits shared among the IL-12 cytokine family, plays a critical role in up-regulation of MHC class I during DC maturation

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Epstein-Barr virus-induced gene 3 (EBI3) is one of subunits shared among the IL-12 cytokine family. Recently, we clarified the novel role for intracellular EBI3 in augmenting IL-23Ra protein expression through binding to IL-23Ra and calnexin in CD4$^+$ T cells under inflammatory conditions. Because calnexin is well known to be important for functional expression of MHC class I, we in the present study investigated the role of EBI3 in MHC class I up-regulation during DC maturation. Although expression of EBI3 was negligible in bone marrow-derived immature DCs, it was increased after activation with LPS. Cell surface protein expression of MHC class I was also enhanced on the activated WT DCs, but that on the activated EBI3-deficient DCs was only slightly increased. Consistent with the reduced expression of MHC class I, antigen-presenting activity of these EBI3-deficient DCs was also decreased. Moreover, forced expression of EBI3 in HEK293T cells augmented the endogenous protein expression of MHC class I in a dose-dependent manner by associating with it and calnexin. Thus, the present results suggest that EBI3 plays a critical role in augmenting MHC class I protein expression in DCs during their maturation. Taken together with our previous results on the role of EBI3 in IL-23Ra protein expression, these results further generalize the paradigm of intracellular EBI3 playing an important role in promoting the proper folding of target protein and augmenting its expression at the protein level by binding to calnexin and its target protein under inflammatory conditions, presumably through enhancing the chaperone activity.

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DOI: 10.1002/eji.201970400

P0043

Haplotypes of MIF gene polymorphisms (-794 $CATT_{5-8}$ and -173 G>C) and its soluble levels in basal cell carcinoma in western Mexican population

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Introduction: Basal cell carcinoma (BCC) is the dermatological neoplasm more prevalent worldwide. Despite its high incidence, there is little information about genetic factors that lead to the carcinogenesis of this neoplasms. In the last years, *MIF* has received particular attention, its expression has been linked to the development of different types of cancer. Specifically, in BCC controversial functions of MIF have been reported. Therefore, the aim of this study was to investigate the haplotypes of *MIF* gene polymorphisms (-794 $CATT_{5-8}$ and -173 G>C) and its soluble levels in BCC.
Methods: A total of 180 BCC patients and 180 individuals as a control group were recruited. Genotyping was performed by PCR and haplotypes were inferred. MIF soluble levels were measured with ELISA.
Results: It was found that the 5/5 genotype of the -794 $CATT_{5-8}$ *MIF* polymorphisms confers risk to BCC development (OR=3.7, p=0.04). Moreover, a significant association was found between the haplotype 7G and BCC (OR=1.99, p=0.02). Otherwise, the median of MIF soluble levels were different between BCC patients and control group (p=0.01).
Conclusion: our findings suggest that the 5/5 genotype of -794 $CATT_{5-8}$ *MIF* polymorphism and 7G haplotype is associated with the development of BCC in patients. Moreover, MIF levels are diminished in patients and could be a risk factor for the development of BCC. Nevertheless, further studies are needed due to the controversial results reported about MIF as a protection or risk factor in cancer no melanoma.

P0044

Stressed bone marrow environments accelerate hematopoietic recovery through cytokine production

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Hematopoietic stem and progenitor cells (HSPCs) maintain the turnover of mature blood cells in bone marrow (BM). While HSPCs divide at low rate during homeostasis, they are rapidly activated to proliferate in response to BM injury induced by anti-cancer drug 5-fluolouracil (5-FU). However, precise mechanisms of regulation of HSPC status by stressed microenvironments remain elusive. Our aim in this study is to elucidate the mechanism how HSPCs are activated in the stressed BM microenvironments. First, we conducted gene expression analyses of transplanted HSPCs. Lin^- c-kit^+ $Sca1^+$ (LSK) HSPC-enriched population was sorted from wild-type mice and transplanted into 5-FU-treated or non-treated control recipients. One day after transplantation, HSPCs were re-sorted from recipient BM, and RNA-seq analyses were performed to compare gene expression profiles between these 2 groups. We found that cell cycle-related genes were upregulated and cell death-related genes were downregulated in HSPCs that were transplanted into 5-FU treated recipient mice. We also conducted upstream analysis and found that several cytokine signals including GM-CSF were

DOI: 10.1002/eji.201970400

significantly activated in HSPCs transplanted into 5-FU-treated mice. Next, we performed dynamic live imaging of calvarial BM using two-photon excitation microscopy to analyze the location and the motility of transplanted HSPCs. The mean velocity of HSPCs in 5-FU treated recipients was significantly decreased compared to that in the control condition. Furthermore, a majority of HSPCs transplanted into 5-FU treated mice were persistently adhering to endothelial cells during time lapse imaging. These data suggested that transplanted HSPCs receive cytokine signals around perivascular areas in injured BM environments.

P0614

Human umbilical cord-derived mesenchymal stem cells inhibit hypoxia-induced neonatal porcine islet apoptosis via regulation of autophagy

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Background: Hypoxic damage greatly leads to the dysfunction of islet graft. Growing evidence suggests that mesenchymal stem cells (MSCs) are available to prevent β cells from various stress through regulating autophagy. In this study, we thus investigated whether human umbilical-cord derived mesenchymal stem cells (huc-MSCs) could ameliorate hypoxia-induced neonatal porcine islet cell clusters (NICCs) injury through modulation of autophagy and the underlying mechanism is investigated.
Methods: NICCs were cultured with huc-MSC-conditioned medium and native medium RPMI-1640 (Control) under hypoxic conditions (1%O_2) in vitro. 3-methyladenine (3-MA) and chloroquine (CQ) were used to examine the role of huc-MSC-CM in regulation of autophagy. Finally, several cytokines secreted by huc-MSCs were detected by ELISA, and the corresponding inhibitors were applied to determine which cytokine mediates the function of huc-MSC-CM. And the effects of huc-MSC-CM on NICCs viability and autophagy were examined by AO/PI staining with flow cytometry analysis, transmission electron microscope (TEM) and confocal fluorescence microscopy analysis.
Results: Compared to the control group, huc-MSC-CM treatment improved NICC viability, inhibited apoptosis, increased autophagy and up-regulated the levels of PI3K class III and pAkt, while the ratio of pmTOR/mTOR was reduced in NICCs. These changes could be reversed by CQ and 3-MA treatment. IL-6 was detected at high concentration in hu-MSC-CM. Furthermore, IL-6 pre-treatment played a similar role as huc-MSC-CM, and its effect could be reversed by Sarilumab.
Conclusion: Our results demonstrated that huc-MSC-CM could improve islet viability via elevating autophagy through PI3K/Akt/mTOR pathway under hypoxia. Additionally, IL-6 plays an important role in the function of huc-MSC-CM.

P0615

The novel cytokine CSBF/C10orf99 plays a protective role in psoriasis

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CSBF/C10orf99 is a novel potential cytokine identified with the strategy of immunogenomics in our lab. It is highly expressed in colon and skin. We have verified that CSBF/C10orf99 interacted with SUSD2 to inhibit colon cancer cell growth. Several studies show that CSBF/C10orf99 is upregulated in the lesion skin of psoriasis. Psoriasis is characterized by epidermal hyperplasia and dermal infiltration of inflammatory cells. We also found that CSBF/C10orf99 was dramatically upregulated in human and mouse psoriatic tissues and it was mainly expressed in the epidermis of lesion skin. IL-17A and

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DOI: 10.1002/eji.201970400

mCSBF/C10orf99 can significantly increase the expression of *Csbf/C10orf99* in primary keratinocytes. Administration of mCSBF/C10orf99 by intraperitoneal injection significantly ameliorates IMQ-induced psoriasiform skin with milder redness and scaling, reduced cell infiltration especially neutrophils and γδ T cells. To know its physiological function, we prepared *Csbf/C10orf99* knockout mice and found that *Csbf/C10orf99* deficiency had no obvious influence on the development of main organs and immune cells. The redness and scaling induced by IMQ is increased in *Csbf/C10orf99*$^{-/-}$ mice, with more cell infiltration including neutrophils and γδ T cells. Bone marrow transfer assay indicates that CSBF regulates psoriasis mainly through targeting non-hematopoietic cells, most likely keratinocytes. In summary, our results suggest that the novel potential cytokine CSBF/C10orf99 is increased in psoriasis, keratinocytes is the major source of CSBF/C10orf99, CSBF/C10orf99 can ameliorate the IMQ-induced psoriasiform skin inflammation.

P0616

IL-17 stimulates IL-6 to participate in the immune regulation of gastric cancer through NF-kappa B signaling pathway

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Introduction: Interleukin-17 (IL-17) is a pro-inflammatory cytokine and gastric cancer is the cumulative result of chronic gastritis. Studies have shown that IL-17 not only expressed significantly in gastric cancer cells, but also in serum and tumor tissues of patients with gastric cancer.However, the mechanism of L-17 on the occurrence and development of gastric cancer remains unclear. This study identified the role of IL-17 in gastric cancer and its possible mechanism of action.
Methods: The expression of IL-17 and IL-6 in serum of 60 patients with gastric cancer was from Wuwei Cancer Hospital in Gansu province and normal persons were detected by ELISA.The expression of IL-17, NF-kappa B, pNF-kappa B, IKK-beta and IL-6 in gastric cancer tissues and adjacent tissues were detected by immunohistochemistry and WB.
Results: Compared with healthy control group, the expression of IL-17 and IL-6 in serum of gastric cancer patients was up-regulated ($P < 0.05$); Compared with adjacent tissues, the expression of IL-17, NF-kappa B, pNF-kappa B, IKK-beta and IL-6 in gastric cancer tissues was increased ($P < 0.05$).
Conclusion: IL-17 can stimulate IL-6 to participate in the immune regulation of gastric cancer and promote the occurrence and development of gastric cancer through NF-kappa B signaling pathway. This may provide new insights into the pathogenesis of gastric cancer and provide a theoretical basis for IL-17 as a potential target for clinical treatment of gastric cancer.
Keywords: gastric cancer, immune factors, IL-17, NF-kappa B signaling pathway

P0617

The interleukin-2 were up-regulated in type 2 diabetic kidney disease

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We performed Gene Expression Omnibus (GEO) dataset parameters, disclose the the expression tendency of IL-2 in T2DKD. In GSE30122, IL-2 expression in renal tubular T2DKD and healthy was found to be significantly different (Figure 3F, P=0.0298); it was not found to be significantly different in other tissues (refer to Figure 1A-E, G and H; including adipose, artery, pancreas, peripheral blood mononuclear cells, renal tubular and renal glomeruli).
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis can find significant pathways in which DEGs participate. The mostly significant pathways for P-values from the KEGG pathway

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DOI: 10.1002/eji.201970400

database included ribosome, Huntington´s disease, oxidative phosphorylation, Parkinson´s disease, metabolic pathways, Alzheimer´s disease pathways in diabetic and others (Figure 2B). The STRING tool was used to get the PPI relationships of the DEGs. A total of 480 PPI relationships were gained with the combined score > 0.4. After the nodes of degree ≤ 5 were filtered out, we finally built the network with 355 nodes (Figure 2C).
Conclusion: In summary, our findings suggest that urine IL-2 expression down regulated in T2DKD patient. Therefore, this could aid the understanding of up regulated expression of IL-2 effects in T2DKD and reveal potential targets for therapeutic manipulation.

P0618

RNA-seq analysis reveals different gene expression of CXCL2 in ACPA-positive and negative rheumatoid arthritis

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Anti-citrullinated protein/peptide antibodies (ACPA) play important roles in the pathogenesis of rheumatoid arthritis (RA), and are associated with RA disease severity. It is suggested that ACPA-positive and negative RA belongs to different disease subsets with distinct differences of genetic variation and clinical outcomes. To compare the gene expression profile in ACPA-positive and negative RA, and to find novel candidate genes signatures which may serve as potential therapeutic targets, we performed a comprehensive transcriptome analysis of PBMC from ACPA-positive and negative RA patients by using the RNA-seq technique. We found that significant differences existed in the expression levels of both genes and gene isoforms between ACPA-positive and negative RA samples. Within these data, we analized chemokines expression and found that the expression of CXCL2 was significantly increased in ACPA-positive patients. The expression levels of CXCL2 in PBMC of RA were validated using real time PCR, as well as CXCL2 levels in serum and CD14+ monocytes supernatant using ELISA. Furthermore, we found that CXCL2 promoted the migration of CD14+ monocyte and increased osteoclast differentiation from ACPA-positive RA patients. We applied RAW264.7 macrophages to explore specific mechanisms, and found that CXCL2 stimulated osteoclastogenesis through ERK MAPK and NF-κB pathways. In conclusion, we identified novel pathways associated with ACPA-positive RA patients by RNA-seq and validated higher CXCL2 expression in ACPA-positive RA than anti-CCP negative RA. These results uncover a previously unappreciated role of CXCL2 during osteoclastogenesis in ACPA-positive RA, which suggests that CXCL2 blockade maybe a novel strategy for the treatment of RA.

P0620

IL-21 regulates macrophage polarization in rheumatoid arthritis

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Objectives: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by destructive arthritis. Macrophages are the main source of inflammatory mediators and play an important role in the pathogenesis of RA. Interleukin (IL)-21 is a cytokine involved in the regulation of various immune cells. This study was to investigate the effect of IL-21 on macrophage polarization in RA.
Methods: (1) Human monocytes were isolated from peripheral blood of patients with RA and differentiated into M1-like and M2-like macrophages by granulocyte-macrophage colony-stimulating

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DOI: 10.1002/eji.201970400

factor (GM-CSF), interferon-γ (IFN-γ) and LPS or M-CSF, IL-4 and IL-13, respectively. To determine the effect of IL-21 on macrophages polarization, Flow cytometry, RT-PCR and ELISA were used to detect macrophage phenotype, gene expression and cytokine secretion.
Results: IL-21 inhibited the expression of CD86 and CD80 and promoted the expression of CD163 during M1 polarization, and inhibited the expression of CD86 during M2 polarization. IL-21 inhibited the expression of TNF-α, IL-6, IL-1β, IL-8, and IL-12 during polarization to M1 macrophages and promoted IL-10, DC-SIGN, and Dectin-1 during polarization to M2 macrophages. IL-21 inhibited the secretion of TNF-α and IL-10 during the polarization to M1 macrophages; and promoted the secretion of IL-10 during the polarization to M2 macrophages.
Conclusion: IL-21 inhibits the polarization of peripheral blood monocytes derived macrophages to M1-like macrophages and promotes the polarization to M2-like macrophages in RA.
Key words: Rheumatoid arthritis, Interleukin-21, Macrophages, Polarization

P0622

IL-9 exerts anti-tumor effects in colon cancer and transforms the tumor microenvironment in vivo

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As a newly discovered cytokine, interleukin-9 (IL-9) was initially considered a T lymphocyte growth factor. IL-9 affects target cells by binding to a member of the γc-family of receptors, and is involved in inflammation, autoimmune diseases and other ailments. In recent years, mounting evidence reveals that IL-9 exerts anti-tumor effects, which has attracted considerable attention. Many previous studies were performed *in vivo* by establishing a mouse model of melanoma. Here, IL-9 protein and mRNA expression levels were both low in colon carcinoma tissue specimens, as assessed by immunohistochemistry and qRT-PCR. In addition, IL-9 expression in these samples was correlated with TNM staging, Ducks staging, lymph node metastasis and good prognosis, but not with gender, age, tumor size, tumor differentiation and hepatic metastasis. *In vivo*, by establishing a mouse subcutaneous xenograft model, we found that IL-9 overexpression inhibited tumor growth and resulted in longer survival time. Then, anti-tumor immune responses were increased by IL-9 as demonstrated by flow cytometry. Furthermore, IL-9 was shown to exert anti-tumor effects by regulating T cell function and killing tumor cells in the tumor microenvironment. Overall, this study revealed that IL-9 exerts robust anti-tumor effects in colon cancer and transforms the tumor microenvironment *in vivo*.
Keywords: Colon cancer, IL-9, anti-tumor effect, tumor microenvironment, cytokine

P0623

Relationship between the expression of autoimmune regulatory factors in different thymus tissues and myasthenia gravis

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Objective: To investigate the relationship between AIRE, FEZF2 and the pathogenesis of MG.
Method: The patients who underwent thymectomy and were pathologically diagnosed as thymoma after operation in the Department of Thoracic surgery of the first Hospital of Jilin University from

DOI: 10.1002/eji.201970400

January 2016 to December 2018 were collected. MG-T was thymoma complicated with MG; NMG-T was simple thymoma; HC was normal thymus tissue. The expression of AIRE, FEZF2 and their protein in thymus of MG-T and NMG-T was detected by immunofluorescence and immunohistochemical technique, respectively. RT-PCR detected the expression of *AIRE and FEZF2* mRNA in thymus.
Results: A total of 43 cases of thymus tissue were collected, including 17 cases in MG-T, 14 cases in NMG-T and 12 cases in HC. Immunofluorescence：almost all AIRE and FEZF2 were expressed in thymic medulla epithelial cells. The relative value of *AIRE* mRNA transcription in MG-T group was lower than that in HC and NMG-T group, P< 0.05. The relative value of *FEZF2* mRNA transcription in MG-T group was lower than that in HC group, P< 0.05. The percentage of AIRE and FEZF2 protein in MG-T group were significantly lower than those in NMG-T group, P< 0.05.
Conclusion: The expression of AIRE and FEZF2 was detected in thymic medulla epithelial cells. The expression of *AIRE* and *FEZF2* mRNA and protein in thymoma of MG-T group were lower. It is suggested that immunomodulatory factors AIRE and FEZF2 may be related to the pathogenesis of MG.
Keywords: Myasthenia gravis, AIRE, FEZF2, negative selection

P0624

The influences of electroacupuncture pretreatment on MCAO rat pallium microRNA-210 at different time

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Objectives: To observe the influences of electroacupuncture pretreatment on middle cerebral artery occultation (MCAO) rat neurological functions and MicroRNA-210 expression at different time.
Methods: SD rats were randomly divided into normal control group, model group, comfort acupuncture group, 5d electroacupuncture pretreatment group, 10d electroacupuncture pretreatment group and 15d electroacupuncture pretreatment group, 10 rats in each group. The MCAO model was prepared after pretreatment of electroacupuncture at different time. Longa standard was employed to evaluate neurological functions. Immunofluorescence was used to assess the proliferation of microvascular endothelial cells. Real-time fluorescence-based quantitative PCR was used to detect the expression of MicroRNA-210.
Results: (1) After electroacupuncture pretreatment, MCAO rat neurological function damage was alleviated, especially for 15d of electroacupuncture pretreatment group (P< 0.01). (2) The number of microvascular endothelial cell proliferation was significantly different (P< 0.05), the highest number of cell proliferation was observed for 15d electroacupuncture pretreatment group (P< 0.01). (3) Electroacupuncture pretreatment could inhibit MicroRNA-210 expression. This effect started showing up after 10d of electroacupuncture pretreatment.
Conclusions: Electroacupuncture pretreatment at different time can alleviate neurological function damage after cerebral ischemia. This may be related to regulating MicroRNA-210 expression to intervene in the proliferation of vascular endothelial cells, so as to promote local blood circulation after ischemia.
Keywords: MACO rats; electric needle; acupuncture; pretreatment; MicroRNA-210

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DOI: 10.1002/eji.201970400

P0625

Interleukin-33 deficiency exacerbated experimental autoimmune encephalomyelitis with an influence on immune cells and glia cells

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Interleukin (IL)-33, a member of the IL-1 cytokine family, is highly expressed in central nervous system (CNS), suggesting its potential role in CNS. Although some studies have focused on the role of IL-33 in multiple sclerosis (MS) / experimental autoimmune encephalomyelitis (EAE), an autoimmune disease in CNS, the exact role of IL-33 in MS/EAE remains unclear and controversial. Here, we used IL-33 knockout mice to clarify the role of endogenous IL-33 in EAE. We found that the clinical score in IL-33 knockout EAE mice was higher accompanied by more severe demyelination compared with the wild-type (WT) EAE mice. As for the main immune cells participating in EAE in IL-33 knockout mice, pathogenic effector T cells increased both in peripheral immune organs and CNS, while $CD4^+FOXP3^+$ regulatory T cells decreased in spleen and lymph nodes, Th2 cells and natural killer (NK) cells decreased in CNS. Additionally, the populations of microglia/macrophages and $CD11C^+CD11B^+$ dendritic cells (DCs) increased in CNS of IL-33 knockout mice with EAE, among which iNOS-producing microglia/macrophages increased. Moreover, resident astrocytes/microglia were more activated in IL-33 knockout mice with EAE. In vitro, after blocking the IL-33, the proliferation of primary astrocytes, the production of MCP-1/CCL2 and TNF-a by astrocytes, and the production of TNF-a by primary microglia stimulated by the homogenate of the peak stage of EAE were increased. Our results indicate that IL-33 plays a protective role in EAE and exerts extensive influences on multiple immune cells and neural cells involved in EAE.

P0626

In vitro experimental study on ASCs secretion of HGF from inbred line miniature pig of Wuzhishan to delay the progression of chronic renal disease fibrosis

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Chronic renal disease (CKD) is one of the most common urological diseases, and renal fibrosis is the common pathological feature of all the late stages of CKD. Delaying fibrosis and protecting residual renal function are the key to prevent and cure CKD.
Transforming growth factor beta 1 (TGF- β1) is an important factor of fibrogenesis. Hepatocyte growth factor (HGF) can specifically antagonize its fibrogenesis and delay the progression of fibrosis. However, the optimal time point of HGF/TGF- β 1 intervention and the mechanism of different intervention time points affect the progression of renal fibrosis have not been clarified.
In this study, adipose-derived mesenchymal stem cells (ASCs) from inbred Line miniature Pig of Wuzhishan were co-cultured with fibrotic kidney tissue in vitro. ELISA, IHC,WB and other methods were used to study the changes of HGF,TGF- β 1 content in renal tissue at different time points and its relationship with the progression of renal fibrosis. The results showed that there was an imbalance of HGF/TGF- β 1 ratio in renal tissue and the expression time of HGF was earlier than that of TGF- β 1. ADSCs could inhibit or delay the progression of CKD fibrosis by autocrine HGF.

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DOI: 10.1002/eji.201970400

P0627

A new heterodimeric cytokine consisting of IL-23p19 plays a role in promotion of differentiation into GM-CSF-producing CD4$^+$ T cells

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IL-23, a member of the IL-6/IL-12 heterodimeric cytokine family, is composed of a p19 subunit and the p40 subunit in common with IL-12. IL-23 is produced by activated macrophages and dendritic cells, and plays a critical role in the expansion and maintenance of pathogenic Th17 cells. In the present study, We initialy found the p19 is secreted from CD4$^+$ T cells after activation by T-cell receptor ligation. Therefore, We have further investigated the role of p19 produced by CD4$^+$ T cells in the development of experimental autoimmune encephalomyelitis (EAE) together with differentiation into effector CD4$^+$ T cells. CD4$^+$ T cell-specific p19-dificient mice showed significantly attenuated EAE with reduced frequency of GM-CSF$^+$CD4$^+$ T cells in the central nervous system. Simillar reduced frequency of GM-CSF$^+$CD4$^+$ T cells was observed in p19-dificient naive CD4$^+$ T cells after activation by T-cell receptor ligation in vitro. To investigate the molecular mechanism whereby p19 increases the frequency of GM-CSF$^+$CD4$^+$ T cells, Western blotting following immunoprecipitation revealed that p19 can associate with another molecule, tentatively called X, secreted from activated CD4$^+$ T cells. We then prepared purified recombinant protein of a single-chain fusion of p19 and X. This p19/X protein induced proliferation of cytokine-dependent cell line Ba/F3 expressing IL-12Rs, IL-23Rs, and IL-27Rs, and induced phosphorylation of STAT5. These results suggest that IL-23p19 can alternatively form a novel heterodimeric protein secreted from activated CD4$^+$ T cells, and contribute to the differentiation into GM-CSF-producing CD4$^+$ T cells in the development of EAE.

P0629

Homeostasisof CD4$^+$ central and effector memory cells in norm and in model of inflammation in vitro

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We hypothesized that the mechanism of memory T cell homeostasis can be modified in the inflammatory process. We developed a model in vitro: prior to cultivation an adhering fraction of PBMCs was activated with LPS to produce inflammatory cytokines.
The study included 5 healthy donors (average 45±4,7years). After isolation PBMCs or pure CD4$^+$45RO$^+$CCR7$^+$ (Tcm), CD4$^+$45RO$^+$CCR7$^-$(Tem) cells were stained with CFSE and cultivated with/without IL-7 during 7 days. Pure subsets of Tcm and Tem were isolated by magnetic separation. In culture of PBMCs, under IL-7 in the absence of MHC contact proportion of Tcm was enhanced while the number of Tem decreased compared to culture stimulated with IL-7. Also it was found an increase in number of proliferated to IL-7 Tcm in trans-wells versus PBMCs cultured with IL-7 in usual plate. Pretreatmentwith LPS promoted not significant increase in proliferation rate of all investigated T-cell subsets in all culture conditions. Major of pure Tcm maintained their phenotype under IL-7 in culture. Proliferation of pure Tcm didn't differ in the absence of MHC contact and/or in the presence of pretreatment with LPS. Culturing of pure Tem with and without IL-7 led to an increase of CD4$^+$45RO$^+$CCR7$^+$ cells. Trans-wells and pretreatment with LPS facilitated to enhance CCR7 expression on Tem. In all culture conditions Tem had a low proliferation rate. Thus, homeostasis of CD4$^+$Tcm is provided by IL-7, it doesn't change in the inflammatory conditions. However, under IL-7

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DOI: 10.1002/eji.201970400

Tem can covert to Tcm, and inflammation facilitate this process.
The study was supported by RSF(project№18-75-00068)

P0630

Proinflammatory cytokines in children with congenital cleft lip and palate

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Recently, the number of children with congenital cleft lip and palate, who have secondary immunodeficiencies, is progressively increasing throughout the world. In the postnatal adaptation of the immune system of newborns, one of the leading mechanisms is the activation of the cytokine system. The goal is to study the study of the levels of pro-inflammatory cytokines in children with cleft lip and palate. 46 children aged 0-14 months with congenital cleft lip and palate were examined. All sick children underwent a comprehensive examination by specialists. The control group consisted of 16 practically healthy children of similar age. We studied the levels of IL-2, IL-6, IL-18 and MCP-1 in serum by ELISA. It was revealed that the level of IL-18 was 3.5 times higher than the values of the control group (P < 0.01). Interleukin-18 stimulates the formation of various subpopulations of T-helper cells, acts as a polyclonal activator. The level of MCP-1 was 3.2 times higher than the control values (P < 0.01. MCP-1 is the most important factor of monocyte chemotaxis in the inflammatory focus. The level of IL-6 was also significantly increased compared with the control group (P < 0 , 01), while the level of IL-2 was below the control values. Noted changes in the state of cytokine status can be qualified as a secondary immunodeficiency state, requiring immunomodulatory measures

P1210

CXCL12 genotype, serum C-reactive protein levels, and incident coronary heart disease

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Background: Elevated concentrations of C-reactive protein (CRP) are associated with the long-term risk of coronary heart disease (CHD). Stromal cell-derived factor 1 (*CXCL12*) on chromosome 10q11 is associated with CHD, while the potential interaction between CRP and *CXCL12* genotype on CHD is unclear.
Objectives: To explore the associations of serum CRP levels with incident CHD, and to investigate the interaction between CRP, *CXCL12* genotype and incident CHD.
Methods: We included 3210 participants with measured serum CRP levels, of which 2979 participants with available genotyping data. We selected rs501120 on *CXCL12* reported to be associated with CHD at genome-wide significance in previous studies. We also tested the interaction between *CXCL12* rs501120 polymorphism and CRP on CHD.
Results: Serum CRP was significantly associated with a higher risk of incident CHD (Odds ratio [OR], 1.11; 95% coefficient interval [CI], 1.02-1.20). Significant interaction was observed between rs501120 and CRP on CHD (*P* for interaction= 0.006). The association between high CRP level and CHD was attenuated among individuals carrying the CT (OR, 1.01; 95% CI, 0.74-1.40) or CC (OR, 0.78; 95% CI, 0.53-1.16) alleles of rs501120, while among those carrying TT alleles of rs501120, the OR for CHD was significantly higher (1.46; 95% CI, 1.05-2.03).
Conclusion: Serum CRP was positively associated with incident CHD. Elevated concentrations of

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DOI: 10.1002/eji.201970400

CRP appeared to be associated with a higher risk of CHD only in homozygous carriers of the *CXCL12*-rs501120 T-allele. These results must be interpreted cautiously and need to be replicated in other populations with a larger sample size.

P1211

Molecular roles of CXCL8 and its receptors CXCR1 and CXCR2 in pathway from inflammatory to cancer in the patients with hepatocellular carcinoma

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Objective: To investigate the expression of CXCL8-CXCR1/2 axes in peripheral blood mononuclear cells (PBMCs), neutrophils and local cancer tissues of the patients with hepatocellular carcinoma (HCC) and their molecular roles in transformation from inflammation to cancer.
Methods: Forty-two typical patients were divided into two groups: the massive group (r>10cm) and the nodal group (r< 5cm). The levels of CXCL8, CXCR1, CXCR2 and HBV-DNA in PBMCs, PMNs and in local cancer tissues was detected by PCR and immunohistochemistry.
Results: The expression of CXCL8-CXCR1 were significantly increased in HCC. Among them, the mRNA levels of CXCL8, CXCR1 and CXCR2 in peripheral blood of patients were shown as follows: (massive-PBMCs: 0.80±0.30, massive-PMNs: 0.93±0.33; nodular-PBMCs: 0.89±0.28, nodular-PMNs: 1.04±0.29), (massive-PBMCs: 0.72±0.28, massive-PMNs: 0.90±0.26; nodular-PBMCs:0.88±0.18 nodular-PMNs: 1.05±0.17). SABC immunohistochemical staining showed that CXCL8 and CXCR1 were highly expressed in local tissues of HCC, and poor expression of CXCR2 in those of liver cancer tissues. CXCL8 and its receptors CXCR1/2 were diffusely expressed in the cytoplasm of hepatoma cells, inflammatory cells and vascular endothelial cells.
Conclusion: The high level of CXCL8-CXCR1 in peripheral blood and liver cancer tissues of HCC is positively correlated with concentration of ALT, GGT, GGT/ALT and HBV-DNA load, which play an important role in malignant hyperplasia and transformation from inflammation to cancer. However, the effect of CXCR2 may be relatively weaker.
Keywords: Hepatocellular carcinoma (HCC); CXCL8; CXCR1; CXCR2; Peripheral blood mononuclear cells (PBMCs); Neutrophils; Immunohistochemistry

P1213

Loss of A20 in BM-MSCs regulates the Th17/Treg balance in rheumatoid arthritis

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Mesenchymal stem cells (MSCs) are multi-potent cells that are self-renewable and possess the potential to differentiate into multiple lineages. Several studies demonstrated that MSCs could regulate an Th17/Treg balance and could be a potential therapeutic target for Rheumatoid Arthritis (RA). **A20** is highly expressed in many cell types after the stimulation of TNF-α, where it may inhibit pro-inflammatory cytokine secretion. However, the expression of A20 in BM-MSCs in RA is not fully

DOI: 10.1002/eji.201970400

understood. In our study, we found that A20 was decreased in RA patients' bone marrow MSCs (BM-MSCs), and with more IL-6 secretion, the balance of Th17/Treg was broken. In CIA mice, we found a moderate A20 decrease in mice MSCs as compared with those of control group in mRNA and protein levels. However, the IL-6 expression was increased. After umbilical cord MSCs treatment, A20 and IL-6 expressions were equal to the control group. **Thus, our study indicates that loss of A20 in MSCs regulates the Th17/Treg balance in RA and the regulatory role of A20 in pro-inflammatory IL-6 production could be a potential target for the transfer of MSCs in RA adoptive therapy.**

P1214

Molecular docking, QSAR and molecular dynamics simulation on phosphorus containing pyrimidines as CDK9 inhibitors

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The interaction modes between phosphorus containing pyrimidine cyclin dependent kinase 9(CDK9) inhibitors and the protein were studied using the combination of molecular docking and three-dimensional quantitative structure-activity relationships(3D-QSAR). The interaction mode obtained by molecular docking revealed that the molecule conformation, hydrophobic interaction and H-bond, as well as Cys106 played an important role in the binding of phosphorus containing pyrimidines and CDK9. Based on the result of molecular docking, 3D-QSAR models were established by comparative molecular field analysis(CoMFA), comparative molecular similarity indices analysis(CoMSIA) and Topomer CoMFA techniques. With the best CoMSIA-SEH model, the cross-validated value(Q2) was 0.557, the non-cross-validated value(R2) was 0.959. The predictive power of the CoMSIA-SEH model was determined from external test sets that were excluded during model development(r2=0.863). Ten new compounds were obtained based on the above modeling, and the results of molecular docking and molecular dynamics simulation suggested they could act as potential CDK9 inhibitors, especially compound 64d with more potent inhibitory activity proved by molecular dynamics simulation and binding free energy analysis. It was expected that these results could help establish the binding mechanism between phosphorus containing pyrimidines and CDK9, and provide a valuable reference for future anti-CDK9 drug design.

P1215

Long-term effects of high-voltage electric burn on serum PAP and sP-selectin in rats and intervention of Xuebijing and Ulinastatin

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Objective: To investigate the long-term effects of high voltage electric burn on serum PAF and sP-selectin in rats and the intervention effects of ulinastatin and Xuebijing.
Methods: SD rats were randomly divided into five groups: sham injury group, electric injury group, saline solution group, Xuebijing group and UTI group, with 70 rats in each group, total 350 rats. In addition to sham group and electric injury group, rats in other groups were given intraperitoneal injection of 2ml/kg normal saline, 10ml/kg Xuebijing and 2ml/Kg (2×10^4u/kg) UTI to intervene. The concentration of PAF and sP-selectin in rat serum was obtained and analyzed statistically.
Result: 1. The concentration of PAF in serum of rats in electro-injury group was significantly higher than that in sham-electric group and 15 minutes before electro-injury. The serum PAF level was

DOI: 10.1002/eji.201970400

decreased in Xuebijing group and UTI group after electric injury. 2. The concentration of sP-selectin in serum of rats in the electric injury group was significantly higher than that in the sham group and 15 minutes before the electric injury. The serum sP-selectin level was decreased in Xuebijing group and UTI group after electric injury.
Conclusion: 1. The levels of PAF and sP-selectin in serum of rats stimulated by high voltage electric burn increased significantly. 2. After high voltage electric burn, the concentration of PAF and sP-selectin in serum of rats was significantly decreased after the intervention of Xuebijing and UTI.
Keywords: High voltage electric burn, Platelet activating factor, Soluble platelet selectin, Xuebijing, Ulinastatin

P1216

Long-term effects of high-voltage electric burn on serum GM-CSF and PF-4 in rats and intervention of Xuebijing and Ulinastatin

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Objective: To study the long-term effects of high voltage electric burn on serum PF-4 and GM-CSF in rats and the intervention effects of Xuebijing and ulinastatin.
Methods: 350 SD rats were randomly divided into five groups: sham injury group, electric injury group, Xuebijing group and UTI group, with 70 rats in each group. High voltage electric burn model was made.In addition to the first two groups, the other groups were intraperitoneally injected with saline 2mL/kg, Xuebijing 10ml/kg and ulinastatin 2mL/kg.The levels of PF-4 and GM-CSF in serum of rats were detected.
Result: 1.The content of PF-4 in the electric injury group was significantly higher than that in the sham group.2. The content of GM-CSF in serum of rats in electric injury group was significantly higher than that in sham injury group. The content of GM-CSF in Xuebijing group and UTI group was significantly lower than that in electric injury group and saline group.
Conclusion:1. The concentration of PF-4 in serum of rats after high voltage electric burn is higher than that before injury. The secretion of PF-4 in serum of rats can be significantly inhibited by intraperitoneal injection of Xuebijing and UTI. 2. High voltage electric burn can increase the concentration of GM-CSF in rat serum. Xuebijing and UTI can inhibit the secretion of GM-CSF in rat serum.
Keywords: High voltage electric burn; Platelet factor4;Granulocyte-macrophage colony stimulating factor; Xuebijing; Ulinastatin

P1217

Long-term effects of high-voltage electric burn on serum GPIV,vWF and TSP in rats and intervention of Xuebijing and Ulinastatin

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Objective: To investigate the long-term effects of high voltage electric burn on serum GPIV and TSP in rats and the intervention effects of Xuebijing and ulinastatin.
Methods: 350 SD rats were randomly divided into sham group, electric injury group, saline group, Xuebijing group and UTI group, with 70 rats in each group. The left forelimb and right hind limb of rats were used as the "entrance" and "exit" of electric current to shock the rats. The rats were given

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DOI: 10.1002/eji.201970400

intraperitoneal injection of 2mL/kg saline, 10ml/kg Xuebijing and 2mL/kg UTI (2×10^4u/kg) according to the group and the time. The contents of GPIV and TSP in serum of rats were counted.
Results: 1. The serum GPIV level of rats in the electro-injury group was higher than that in the sham-electric group and before the electro-injury. GPIV in Xuebijing group and UTI group was lower than that in electric injury group and saline group. 2. The serum TSP level of rats at different time points after electric injury was higher than that of rats before electric injury and sham-electric group. Both Xuebijing and ulinastatin can reduce the serum TSP level after electric injury.

Conclusion: 1. High voltage electric burn can significantly increase the levels of GPIV and TSP in serum of rats. 2. Early intervention with Xuebijing injection and UTI after high voltage electric burn can significantly reduce the levels of GPIV and TSP in serum.**Keywords:** High voltage electric burn; Platelet membrane glycoprotein IV; Thrombin sensitive protein; Xuebijing; Ulinastatin

P1218

Long-term effects of high-voltage electric burn on serum GP-Ⅰα II α、GP-Ⅰb/IX/V in rats and intervention of Xuebijing and Ulinastatin

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Objective: To investigate the long-term effects of high voltage electric burn on serum GP-I alpha II alpha and GP-I b/IX/V in rats and the intervention effects of Xuebijing and UTI.
Methods: 1. The experimental animals were divided into five groups: sham-electric group, saline group, electric injury group, Xuebijing group and ulinastatin group. Each group contained 70 rats. 2. Model rat preparation: Electric burn model was established by 2 KV high voltage electrical link to the right hind limb and left forelimb of rats. 3. Drug administration: Intraperitoneal injection of saline 2mL/kg, 1% UTI (2×10^4u/kg) 2mL/kg and Xuebijing 10ml/kg were given to each group at each time. 4. Statistical methods were used to analyze the contents of GP-I alpha II alpha and GP-I b/IX/V in rat serum.
Result: 1. The contents of GP-I alpha II alpha and GP-I b/IX/V in serum of rats in electric injury group were lower than those in sham injury group and 15 minutes before electric injury, showing a downward trend. 2. The serum GP-I alpha II alpha and GP-I b/IX/V contents in Xuebijing group and UTI group decreased rapidly after electrification, and then increased with time.
Conclusion: 1. High voltage electric burn can lead to a sharp decrease in the concentration of GP-I b/IX/V and GP-I alpha II alpha in rat blood. 2. Early application of Xuebijing and ulinastatin after high voltage electric burn can reduce the decrease of GP-I b/IX/V in serum.
Keywords: High voltage electricity; Burn; Platelet membrane glycoprotein; Xuebijing; Ulinastatin

P1219

Long term effects of high voltage electrical burn on serum IL -6, IL -3, β-TG and the intervention of Xuebijing and Ulinastatin

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Objective: To investigate the long-term effects of high voltage electric burn on serum IL-6, IL-3 and beta-TG in rats and the intervention effects of Xuebijing and UTI.
Methods: 350 SD rats were randomly divided into sham electric group, electric injury group, saline group, Xuebijing group and ulinastatin group, 70 rats in each group. Each group of rats was divided

DOI: 10.1002/eji.201970400

into seven phases by random number table: 15 minutes before injury, 1 hour, 8 hours, 24 hours, 48 hours, 72 hours and 1 week after injury, with 10 rats in each phase. The rat model of high voltage electric burn was prepared according to the experimental requirements. The rats were given intraperitoneal injection of drugs according to group and time: 2ml/kg saline, 10ml/kg Xuebijing and 2×10^4u/kg ulinastatin. The concentrations of IL-6, IL-3 and beta-TG were calculated.
Result: 1. The serum levels of IL-6, IL-3 and beta-TG in the electro-injury group were significantly higher than those in the sham electro-injury group. The contents of IL-6, IL-3 and beta-TG in Xuebijing group and UTI group were significantly lower than those in saline group.
Conclusion: High voltage electric burn can cause significant increase of serum IL-6, IL-3 and beta-TG levels in rats. Xuebijing injection and UTI can inhibit the secretion of IL-6, IL-3 and beta-TG.
Keywords: High voltage electric burn; Interleukin-6; Interleukin-3; Beta-thromboglobulin; Ulinastatin; Xuebijing

P1220

Long-term effects ofhigh-voltage electric burn on serum PDGF and TPO in rats and intervention of Xuebijing and Ulinastatin

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Objective: To investigate the long-term effects of high voltage electric burn on serum PDGF and TPO in rats and the intervention effects of Xuebijing and ulinastatin.
Methods: 350 SD rats were randomly divided into five groups, 70 rats in each group: sham injury group, electric injury group, saline group, Xuebijing group and UTI group. According to the requirement, rats in each group were given intraperitoneal injection of saline 2mL/kg, Xuebijing 10ml/kg and 1% UTI (2×10^4u/kg) 2mL/kg at the same time. The contents of PDGF and TPO in serum of rats were detected by experimental techniques and statistical methods.
Result: 1. The serum PDGF content of rats at different time points after injury was higher than that of the sham injury group 15 minutes before the electric discharge. The serum PDGF levels in Xuebijing group and UTI group were significantly lower than those in electric injury group and saline group. 2. The content of TPO in serum of rats in electro-injury group was higher than that in sham-injury group and 15 minutes before electro-injury. The content of TPO in serum of rats in Xuebijing group and ulinastatin group was significantly lower than that in electro-injury group and saline group.
Conclusion: 1. High voltage electric burn can increase the concentration of PDGF and TPO in rat blood. 2. Early application of Xuebijing and UTI after high voltage electric injury can significantly reduce the content of PDGF and TPO in serum.
Keywords: High voltage electric burn; Platelet-derived growth factor; Thrombopoietin; Xuebijing; Ulinastatin

P1221

Interferon-λ2 maintaines intestinal epithelial barrier function to attenuate intestinal ischemia reperfusion injury in mice

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Purpose: INF-λ2 is involved in maintaining intestinal mucosal homeostasis and attenuating inflammatory responses. Intestinal ischemia/reperfusion (I/R) is a severe syndrome with disruption of the intestinal barrier function. The goal of the study is to investigate the role of IFN-λ2 in mouse

DOI: 10.1002/eji.201970400

models of intestinal I/R and intestinal barrier function.
Methods: Adult C57BL/6 mice were randomly divided into Sham operation, I/R and I/R+INF-λ2 groups. The mice were given injections of recombinant mouse INF-λ2 12 hours before the operation. Mice were sacrificed after I/R for 6 hours. The mucosal permeability was investigated and histology analyses were performed. Intestinal epithelial cells were isolated and the expression of tight junctions (TJs) and STAT1/pSTAT1 was assessed by Western blot analysis and Immunohistochemistry staining. A hypoxic caco-2 cell culture model was used to evaluate the role of IFN-λ2 in the development of TJs and epithelial permeability in vitro. Fludarabine was used to inhibit STAT1/pSTAT1 pathway.
Results: INF-λ2 significantly alleviated the increase of intestinal permeability and tissue damage caused by I/R. I/R induced the disrupted distribution of TJs while IFN-λ2 treatment attenuated that. The decreased expression of Claudin-1 in I/R mice was alleviated by treatment with INF-λ2. In Caco-2 cells, INF-λ2 treatment reversed the decrease in TER of monolayers exposed to hypoxic environments. The disrupted distribution of TJs was attenuated by IFN-λ2 administration. IFN-λ2 up-regulated the expression of Claudin-1via pSTAT1.
Conclusions: Interferon-λ2 could regulate epithelial barrier function through regulation of TJs. Interferon-λ2 might be potential means for treatment of this condition.

P1222

Pien Tze Huang suppresses the inflammation by regulating SPMs in hepatocellular carcinoma mice

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Inflammation is one of vital cancer-causing factors and participates in almost every stage of cancer progression. Pien Tze Huang (PZH), a well-known traditional Chinese formula, has anti-inflammatory effect and has been proved clinically effective in the treatment of hepatocellular carcinoma (HCC). Specialized pro-resolving mediators (SPMs) metabolized from polyunsaturated fatty acids (PUFA) have impact in regulating inflammation in some inflammation-related diseases including cancers. In this study, we aimed to detect the change of SPMs in HCC mice model after treating with PZH. HCC mice model with H22 tumor xenograft was established, the mice were then treated orally by different does (0.702g, 0.234g, 0.078g/kg/d) of PZH for two weeks. Then the pro-inflammatory cytokines and the D-series resolvins in serum of mice were tested by ELISA. The levels of serum endogenous SPMs including metabolic precursors of resolvins were detected by LC-TOF/MS. The results showed that PZH inhibited tumor growth and decreased the expression of IL-6, TNF-α, and IL-1β in serum of mice. Moreover, PZH could restore the disordered metabolism of SPMs, especially decreasing the levels of 17(S)-HDHA, 19(20)-EpDPE, resolvin D3, resolvin D5, resolvin D6 and increasing the level of resolvin D1. Consequently, these results indicated that regulating the serum endogenous SPMs might be one of the anti-tumor mechanisms of PZH.
Key words: Pien Tze Huang, hepatocellular carcinoma, inflammation, SPMs, resolvins
Acknowledge: This study was supported by National Science Foundation of China (No. 81873053).

DOI: 10.1002/eji.201970400

P1223

The effect of TNF-alpha and CAPE on apoptosis and metastasis in breast cancer cell lines

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Introduction and aim: Breast cancer is the most prevalent malignancy among women. TNF-alpha through messaging PI3K/AKT can lead to the activation of NF-KB Followed by that the increase of CXCR4 transcription occurs. Raising of CXCR4 expression is one of the factors that causing metastasis. Caffeic acid phenyl ester (CAPE) is an important active component of honey bee propolis with biological activities such as an anti-tumor. The purpose of this article, Determine the effects of antioxidant in the presence of cytokines secreted in the tumor environment.
Methods: MCF-7 and MDA-MB-231 Cells were maintained in 75 cm2 flasks in RPMI1640 supplemented with 10% FBS, Penicillin /Streptomycin (100 mg/ml). Cells seeded in 96-well plates then were treated with the different concentrations of CAPE alone and in keeping with cytokine TNF-a. After 24, 48 and 72 hours, the rate of apoptosis was studied with flow cytometry and the gene expression of the CXCR4 determined with using PCR-Real time method.
Results: CAPE has a dose and time-dependent manner of inhibiting cancer cell growth and induction of apoptosis in both of cell lines. CAPE revealed a significant reduction in CXCR4 expression at 24 and 48 hours in MCF-7 and MDA-MB-231 respectively. TNF-a leading to Reduces effects of CAPE and increased CXCR4 gene expression just in MCF-7.
Conclusion: CAPE is an effective component that was able to inhibit proliferation and apoptosis induction of cancer cells, but the combination with cytokines such as TNF-a is with different effects among various cell lines of breast cancer.

P1224

IL-37 suppresses hepatocellular carcinoma development by inhibiting tumor angiogenesis

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IL-37, a new member of IL-1 family, has been shown to play a critical role in both physiological and pathological conditions. However, the role of IL-37 in angiogenesis is still controversial. Recent studies demonstrated IL-37 served as a pro-angiogenetic factor through TGF-β signaling. However, other studies suggested that IL-37 inhibited angiogenesis during tumor development. In the current study, we found that tumor-derived IL-37 could inhibit angiogenesis in peritumoral area in murine hepatocellular carcinoma (HCC) model. Mechanism study revealed that recombinant IL-37 protein could directly promote migration, tube formation and VEGF, MMP2 expression in HUVEC cells. However, the supernatant from IL-37 expressing HCC cells showed an inhibition on the HUVEC cell migration and tube formation, suggesting its secondary role on angiogenesis through tumor cells could be dominant in the tumor microenvironment. Further studies showed that IL-37 could downregulate MMP2 expression in human HCC cells under hypoxia condition. These results suggested that IL-37 played opposite role on angiogenesis depending on its direct or indirect function. Overall, our data showed that IL-37 could inhibit HCC development through suppressing tumor angiogenesis.

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DOI: 10.1002/eji.201970400

P1225

A stabilized form of XCL1/lymphotactin used as an adjuvant recruits cross-presenting dendritic cells for efficient induction of antigen-specific CD8$^+$ T cell responses

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XCR1 is selectively expressed by cross-presenting dendritic cells (DCs), while its ligand XCL1/lymphotactin is produced by activated CD8$^+$ T cells and natural killer (NK) cells. XCL1 lacks one of the two disulfide bonds conserved in all other chemokines and has an unstable structure with a relatively weak chemotactic activity. Recent studies have shown that XCL1-antigen fusion proteins efficiently induce CD8$^+$ T cell responses by preferentially delivering the antigens to XCR1$^+$ DCs. However, XCL1 itself was found to be a poor adjuvant for induction of CD8$^+$ T cell responses. This may be due to its unstable structure and poor chemotactic activity. To test this hypothesis, we generated a stable form of murine XCL1 termed mXCL1-V21C/A59C by introducing a second disulfide bond. We confirmed that mXCL1-V21C/A59C had much more potent chemotactic and calcium mobilization activities than the wild type mXCL1 (mXCL1-WT). Upon intradermal injection, mXCL1-V21C/A59C but not mXCL1-WT efficiently induced accumulation of XCR1$^+$CD103$^+$ DCs, most of which also took up co-injected ovalbumin (OVA). XCR1$^+$CD103$^+$ DCs also migrated to the draining lymph nodes. Consistently, mXCL1-V21C/A59C strongly induced OVA-specific CD8$^+$ T cell responses. Mice immunized were also protected from E.G7-OVA tumor growth in both prophylactic and therapeutic protocols. Furthermore, memory CTL responses were induced in mice immunized with OVA and mXCL1-V21C/A59C. Collectively, our findings demonstrate for the first time that the stable form of XCL1 can be a useful adjuvant for cross-presenting DCs to induce antigen-specific CD8$^+$ T cell responses.

P1227

The role of ACKR3 in B cell lymphoma transmigration and correlation with IgM expression

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ACKR3 is an atypical chemokine receptor able to bind two different ligands: CXCL12 and CXCL11. Upon ligand binding ACKR3 does not activate G-protein-dependent signaling, instead its presumed role is to scavenge CXCL12 and CXCL11, which can lead to the formation of a chemokine gradient. The expression of canonical chemokine receptors is correlated with the pathogenicity of solid tumors and blood malignancies. The typical chemokine receptor CXCR4 as well as ACKR3 and CXCL12 are overexpressed in several human cancers where they contribute to tumor growth and metastasis. ACKR3 plays a key role in diffuse large B cell lymphoma (DLBCL) enabling the spreading of cells from local tumors through lymphatics. It was observed that the infiltration of brain and bone marrow by DLBCL is not present when ACKR3 was genetically ablated. The aim of this project is to investigate the role of the CXCL12/ACKR3/CXCR4 axis in DLBCL in trans endothelial migration experiments and in a localized tumor model. The migratory capacity will be tested depending on the ACKR3 expression levels. In order to evaluate the role of the atypical chemokine receptor in trans-endothelial migration, variants of ACKR3 have been engineered and re-expressed in otherwise ACKR3 null cells. The migratory capacity in vivo will be determined analyzing the ability of lymphoma cells to migrate to the

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DOI: 10.1002/eji.201970400

draining lymph node from the tumor mass in NOD/SCID mice. It was also observed that in DLBCL cells ACKR3 cell surface expression is correlated with the expression of the IgM B cell receptor (BCR).

P1228

Evolution of IL17A plasma rate during chemotherapy in Senegalese patients with cervical cancer

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Introduction: Cervical cancer (CC) is a serious public health problem and is associated with high cancer -related mortality in females worldwide. It is viral induced carcinoma, with a sexually transmitted High Risk HPV. The expression of interleukin 17A (IL-17A) can increase the migration and invasiveness of cervical cancer cell. We investigated the evolution of IL-17A in cervical cancer patients under chemotherapy.
Methods: Our study included 57 CC cases under chemotherapy and 59 healthy control. The IL-17A plasmatic rate were evaluated by ELISA on 3 samples collected before 3 chemotherapy spaced 21 days.
Results: Our result showed that, compared to healthy controls, the cervical cancer patients have significantly higher rate of IL-17A (p = 0.034). We also found that, a significant rate of IL-17A at the third sample compared to the first two samples (p= 0,047 and p = 0,043). Moreover, according to response to chemotherapy (no, partial or total response), the results showed that at 21 days after the second chemotherapy (P3), the IL-17A rate of patients with no response to chemotherapy is differed significantly (p = 0.048) to patients having partial and that having total response to chemotherapy. The same results have obtain at 21 days after the second chemotherapy.
Conclusion: Our results suggest that a high rate of IL-17A appears to be associated with a lack of regression.
Keywords: Cervical cancer, IL71A, chemotherapy

P1802

Interleukin-8 promote invasion and metastasis of esophageal squamous cell carcinouma by activating JAK2/STAT3 signaling pathway

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Elevated interleukin-8 (IL-8) expression promoting cancer metastasis has been reported in certain malignant tumors. However, studies investigating IL-8 in esophageal squamous cell carcinoma (ESCC) are rare. Here we assessed IL-8 levels in patients with ESCC and explored the relevant mechanism by modifying its expression in ESCC cell lines. Our results demonstrated that IL-8 expression was upregulated in patients with ESCC, both in carcinoma tissues and sera, as compared to controls. Suppression of IL-8 significantly downregulated (P< 0.01) the migration and invasion capacity of KYSE170 cells, while no significant differences in cell proliferation and apoptosis were observed. Whereas, overexpression of IL-8 in TE1 cells enhanced cell migration and invasion (P< 0.05). Furthermore, siRNA targeting of IL-8 significantly decreased the expression of CXCR2, p-JAK2, p-STAT3 and WASF3 in KYSE170 cells (P< 0.05), and overexpression of IL-8 in TE1 cells significantly

DOI: 10.1002/eji.201970400

elevated these molecules. Utilizing murine xenograft models of ESCC, we demonstrated that downregulating IL-8 and overexpression of IL-8 resulted in a significant reduction and enhancement of tumor growth kinetics, respectively. In conclusion, our results suggest that IL-8 is a cancer promoting factor in ESCC, and that inhibition of IL-8 suppress the phosphorylation of JAK2 and STAT3 via CXCR2, resulting in the decrease of WASF3, tumor invasion and metastasis. Thus, targeting IL-8 may represent a promising clinical strategy for patients diagnosed with ESCC.

P1803

Identification of adiponectin as a novel ligand for LAIR-1/CD305 negatively regulating of T cell activation

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LAIR-1(Leukocyte-associated immunoglobulin-like receptor 1, CD305) is a type I transmembrane inhibitory receptor with two ITIM motifs in its cytoplasmic tail expressed on majority of lymphocytes, NK cells, monocytes and dendritic cells. Collagen was once considered to be a natural and high affinity ligand of LAIR-1. As the highest level of adipokines in plasma, adiponectin (APN) performs its physiological function mainly to increase insulin sensitivity via its classical receptor AdipoR1/AdipoR2 and the following downstream AMPK and PPAR-α pathways. The inhibitory role of APN in immune response has been discussed recently and its mechanisms are still not clear. We found for the first time that APN could bind with LAIR-1 in vivo and in vitro via its globular domain rather than its collagenous domain. After their combination, APN could induce the activation of LAIR-1 by phosphorylating of SHP-2 and attenuate T cell activation. LAIR-2 (CD306), a putative soluble homolog of LAIR-1, antagonized the inhibitory potential of LAIR-1 induced by APN interaction. Consistent with these findings, we also found that APN increased in the plasma from the fever onset and remained higher level at the convalescent phase of hemorrhagic fever with renal syndrome (HFRS) patients. The sustained high level of APN in the convalescent phase was associated with disease severity. It is possible that LAIR-1/CD305 may serve as a bridge between immunosuppression and APN.

P1804

Role of IL-1β in regulating Leishmania infection

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Leishmaniasis is a vector-borne parasitic disease where *Leishmania* sp. invades various phagocytic cells, and resides within the harsh phagolysosomal environment. Macrophage activation functions as a crucial step for mounting protective immune responses against pathogens. Inflammasome activation is another protective response of macrophages against invading intracellular pathogens. Recently, inflammasome research for *Leishmaniasis* has received a huge impetus as inflammosome-mediated IL-1β production has been shown to provide resistance against pathogens by generating nitric oxide (NO). In fact, NOD-like receptor (NLR)- mediated caspase-1 activation is very important for inflammosome-induced IL-1β production. Therefore, it seems that early pro-inflammatory cytokines like IL-1β could be detrimental tointracellular parasites. We wanted to explore if IL-1β treatment has any effect on infection. Our data show that treatment with IL-1β increases parasitic burden. In corollary with in-vitro findings we noticed a similar trend in our in-vivo studies. With the treatment of IL-1β we noticed an increased expression of autophagic markers during *Leishmania* infection. As it is known

DOI: 10.1002/eji.201970400

that macrophages are highly plastic in nature, change their phenotypes in response external stimuli; we wanted to check if IL-1β treatment has any impact on macrophage polarization. Therefore, through our study we show thatIL-1β plays a crucial function in establishing *Leishmania* infection possibly modulating host metabolic circuit and setting pathogen favorable autophagic milieu.

P1805

Histamine in the regulation of IgE synthesis: on the role of H_3 and H_4 receptors*

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Through recent cloning of human histamine H_3 and H_4 receptors new studies have suggested that dual H_3 /H_4 receptor antagonists may possess a wide range of pharmacological activities.The goal of this investigation was to evaluate an impact of histamine on IgE response as well as the role of H_3 and H_4 receptors in its effects. In vitro cultivation of peripheral blood mononuclear cells (PBMC) of healthy donors and ragweed pollen sensitive patients have been assayed in the presence of several H_3/H_4 antagonists with different affinity and selective agonist for H_3 receptor subtype (R-alpha-methylhistamine -RMH). .The supernatants after 9-days culture of PBMC and DC from 10 healthy donors as well as 12 allergic subjects were assayed for total IgE by CAP FEIA method (Phadia). Concentration of cytokines were assessed by ELISA . It have been demonstrated that RMH at 10^{-5}M decreased IgE synthesis but low concentration (10^{-8}M) highly increased IgE synthesis up to 4-fold. IgE response in allergen-induced PBMC of ragweed sensitive patients responded to RMH less than healthy donor's cells. All studied H_3/H_4 antagonists dose- and affinity-dependently increased the synthesis of IgE and the main IgE-regulatory cytokines (IL-4, IL-10 and IFNg), by the exception of IL-13. This effect was more pronounce in allergen-induced culture. Thus, histamine H_3 and H_4 receptors are involved in IgE synthesis both in healthy donors and allergic patients as well as in synthesis of IgE-regulatory cytokines by PBMC.
*The publication has been prepared with the support of the "RUDN University Program 5-100"

P1806

Characterization of human T helper-like regulatory T cells and chemokine expression in oral squamous cell carcinoma

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Regulatory T-cells (Tregs) are a subset of CD4+T-cells that maintain immunological tolerance and regulate immune homeostasis. Tregs have been classified as regulatory T helper (Th)-like cells according to the expression of specific transcription factors, cytokines and chemokine receptors that mirror effector Th lineages. We have recently reported that tissue resident Tregs in patients with cancer are mainly Th2 type in comparison with tissue resident cells from heathy controls. However, it is not clear whether Tregs migrate in response to specific Th2-attractant chemokines or whether the environment supports the differentiation of Th2-like Tregs. In this study, we evaluated the phenotype of tissue resident Th-like Tregs and the expression of CCL1, CCL17, CCL18 and CCL22 in cancer tissues from patients with oral squamous cell carcinoma compared to healthy oral mucosa. Our results

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DOI: 10.1002/eji.201970400

demonstrated that patients with oral cancer exhibited higher percentages of Th2-like Tregs with high CCR8 expression. In addition, levels of CCL18 were increased in cancer samples in comparison with healthy mucosa. These results suggest that CCL18 may supports the migration of CCR8-expressing Th2-like Tregs to malignant areas in oral cancer.

P1807

IL-1β regulates the adhesion, proliferation, migration and promotes the ability of hPMSCs to modulate Th1/Tr1 balance via PDL1

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High level of IL-1β represents the salient characteristic of graft anti host diseases (GVHD). Mesenchymal stromal cells (MSCs) were regarded as a promising candidate for treating GVHD. However, the interaction between MSCs and proinflammatory cytokines during GVHD is still unclear. This study investigated the effect of IL-1β on the adhesion, migration and proliferation as well as hPMSCs ability to modulate balance between Th1 and Tr1. The results demonstrated that hPMSCs decreased the level of IL-1β and down-regulated the ratio of Th1/Tr1 *in vitro* and in the humanized xenogenic GVHD NOD/SCID model. In turn, the adhesion and proliferation of hPMSCs stimulated by IL-1β were inhibited, while the migration was promoted compared with non-treated hPMSCs. Moreover, IL-1β promoted hPMSCs ability to reduce the ratio of Th1/Tr1. Blockade of programmed death ligand 1 (PDL1) expressing on hPMSCs promoted the adhesion and proliferation of hPMSCs. Further studies showed that the expression of PDL1 increased by IL-1β was suppressed after administration of the JAK1/2 inhibitor or NF-κB inhibitor. Taken together, hPMSCs reduced the secretion of IL-1β from PBMC, in turn, IL-1β suppressed the adhesion and proliferation of hPMSCs and promote hPMSCs ability to modulate Th1/Tr1 balance by up-regulating the expression of PDL1 through JAK/STAT as well as NF-κB signal pathway. The results of this study will supplement the theoretical basis of hPMSCs treatment for GVHD.

P1808

Associations of urinary epidermal growth factor and monocyte chemotactic protein-1 with kidney involvement in patients with diabetic kidney disease

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Background: In diabetic kidney disease, it is important to find biomarkers for predicting the initiation and progression of the disease. Besides glomerular damage, kidney tubular injury and inflammation are also involved in the development of DKD. The current study investigated the associations of urinary epidermal growth factor (uEGF), monocyte chemotactic protein-1 (MCP-1) as well as uEGF/MCP-1 ratio with kidney involvement in patients at early and advanced stages of DKD.
Methods: The concentration of uEGF and uMCP-1 were measured in two Chinese population-based studies. The associations of uEGF, uMCP-1 and uEGF/MCP-1 with the occurrence of DKD were studied in a cross-sectional study (n=1811) of early stage of DKD and associations with kidney outcome were assessed in a longitudinal cohort (n=208) of advanced stage of DKD.

DOI: 10.1002/eji.201970400

Results: Positive correlations were found between uEGF/Cr and eGFR at sampling, and between uMCP-1/Cr and uACR. In the cross-sectional study, uEGF/Cr and uEGF/MCP-1 were negatively associated with the occurrence of DKD. In the longitudinal cohort, uEGF/MCP-1 ratio correlated more closely with the percentage change of eGFR slope, as compared with uEGF/Cr or uMCP-1/Cr alone. Composite endpoint was defined as end stage renal disease or 30% reduction of eGFR. These three markers were independently associated with composite endpoint after adjusting for potential confounders.
Conclusion: Urinary EGF/MCP-1 was negatively associated with the occurrence of DKD. Moreover, uEGF/MCP-1 had a better ability to predict the composite endpoint and correlated more closely with kidney function decline in advanced stage of DKD, as compared with uEGF/Cr or uMCP-1/Cr alone.

P1809

Dual role of interleukin-6 in LPS-induced attenuation of endurance in mice

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Systemic inflammation accompanies the majority of diseases and has a substantial influence on behavior and metabolism. Key proinflammatory cytokines, IL-1, TNF, and IL-6, are involved in inflammation-induced fatigue, however, the exact mechanisms of their action are not completely studied. We addressed the impact of IL-6 on behavior and physical performance during acute inflammation. We employed mice with inactivated IL-6 gene (IL-6 KO) and analyzed their response to LPS challenge, using the RotaRod performance test. In contrast to wild type (WT) mice, IL-6 KO animals were completely protected from inflammation-induced endurance loss after systemic (i.p.) LPS administration. Since IL-6 may affect both muscle metabolism and behavior we aimed to establish, which of these effects account for LPS-induced IL-6-mediated endurance loss. To answer this question, we tested WT and IL-6 KO mice using the RotaRod following local intramuscular (i.m.) or systemic (i.p.) administration of LPS. WT mice showed moderate reduction of endurance after i.m. LPS treatment. Strikingly, IL-6 KO mice, despite being fully protected from systemic LPS-induced endurance loss, demonstrated a significant reduction in performance on RotaRod following local induction of inflammation in the muscles. Altogether our data suggest the dual role of IL-6 in inflammation-induced fatigue and indicate that anti-IL-6 therapy may be beneficial for patients with systemic inflammatory response.
This work is supported by the Russian Science Foundation grant #19-75-30032.

P1810

The changes and significance of serum MIDKINE in Henoch-Schönlein purpura nephritis and Henoch-Schönlein purpura nephritis nephritis in children

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Objective: Through the detection of Midkine concentration in the serum of Henoch - Schonlein purpura and Henoch - Schonlein purpura nephritis patients,explore its clinical significance in the development of HSP and HSPN.
Methods: In the case group, 10 cases were HSP, 25 were HSPN, and 8 cases were reviewed after

DOI: 10.1002/eji.201970400

treatment.In which that control group were the 12 case in the same period.
Results: The case group was compared with the healthy control group on MK: (289.34±160.70pg/ml VS 100.03±56.75pg /ml), and the difference is statistically significant (P< 0.05).In the case group, the differences of MK concentration between the HSPN (nephrotic proteinuria) group, HSPN (non-nephrotic proteinuria) group and HSP group were still statistically significant:（449.91±141.91pg/ml VS 244.04±89.15pg/ml VS 175.94±46.30pg/ml）（P< 0.05）. The results of ROC subject curve showed that the AUC value of the MK prediction diagnosis HSP is 0.908 (95% CI, 0.828-0.988),the optimal value for the predicted diagnosis HSP based on the access index is 182.762pg/ml,the sensitivity is 81.4% and the specificity is 91.7%;the AUC value of MK prediction was 0.947(95% CI, 0.888-1.000), and the optimal value of the diagnosis of HSPN was 218.186pg/ml according to the prediction of the yiden index, and the sensitivity of the diagnosis of purpura nephritis was 84.0% and specificity 95.5%.
Conclusion: MK is involved in the development of HSP and HSPN which can provides a basis for early diagnosis of HSP and HSPN and the evaluation of HSPN renal damage degree and long-term prognosis.

P1811

A steroid alkaloid derivative 02F04 induced thymic stromal lymphopoietin production slowly and continuously through a novel Gq/11-ROCK-ERK1/2 signaling pathway in mouse keratinocytes

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Thymic stromal lymphopoietin (TSLP) plays critical roles in inducing and exacerbating allergic diseases. Chemical compounds that induce TSLP production can enhance sensitization to antigens and exacerbate allergic inflammation. Hence, identifying such chemicals will be important to prevent an increase in allergic disorders. In this study, for the first time, we found that a steroid alkaloid derivative 02F04, which has a unique skeletal structure compared with other TSLP-inducing chemicals, selectively induced TSLP production significantly in mouse keratinocytes, and the peak time of TSLP mRNA level at 48 h induced by 02F04 led to a slow and continuous TSLP production. As an analogue of the liver X receptor (LXR) endogenous ligand, 02F04 rapidly increased ABCA1 expression by regulating LXR. However, instead of being inhibited by LXR or other nuclear receptor antagonist, 02F04-induced TSLP production was markedly suppressed by inhibitors of ROCK, Gq/11 and ERK1/2, and by siRNA-mediated knockdown of Gq or G11. Treatment with 02F04 caused the formation of F-actin filaments surrounding the nucleus of PAM212 cells, which then disappeared following addition of ROCK or Gq/11 inhibitor. 02F04 induced phosphorylation level of ERK1/2 to a maximum at 24 h, but it was suppressed by both inhibitors of ROCK and Gq/11. Taken together, 02F04 induced TSLP production selectively, slowly and continuously by a novel Gq/11-ROCK-ERK signaling pathway rather than nuclear receptors. 02F04, with a unique skeletal structure on inducing TSLP production by a novel mechanism, can represent a potential new tool for investigating the role of TSLP in allergic diseases.

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DOI: 10.1002/eji.201970400

P1812

Covalent modification of clustered TiO2 nanotubular surfaces with pdgf-bb enhances osteogenic behaviours of bMSCs with a burst expression of osteocalcin

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A multitude of micro- and nano-surface structures have been developed to improve the clinical performance of endosseous titanium (Ti)implants. However, most of these surface structures only simulate the topographic elements on a micro- or nano-scale. Here, we describe a novel strategy to construct a bioinspired nano-micro hierarchical TiO2 clustered nanotubular surface structure using platelet derived growth factor-BB (PDGF-BB) covalently modified Ti implants, which highly mimic the structure of collagen in bone. This bio-activated hierarchical surface topography is referred to as NTPCP, nanotubular surface with PDGF-BB covalent modification via PhoA (11-hydroxyundecylphosphonic acid)/CDI (carbonyldiimidazole) chemistry. It provides a 3-dimentional spatial biomimetic structure for host cells, with negligible cytotoxicity and satisfactory bio-activity for significantly enhancing cell attachment and osteogenic functions (including proliferation, extracellular matrix synthesis, and mineralization) of human bone mesenchymal stem cells (bMSCs). Remarkably, we observed burst expression of osteocalcin (OCN), which mirrored prominent bone formation around the NTPCP implants in a rat model. Our study provides a promising strategy of surface modification to improve osseointegration of endosseous Ti implants. Considering the regulatory effects of OCN on glucose homeostasis, NTPCP may be applied in diabetic patients in the future.

P1813

Transcriptional blood signatures for active and amphotericin B treated visceral Leishmaniasis in India

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Visceral leishmaniasis (VL) elimination program aims to reduce the incidence rate in the Indian subcontinent to < 1/10,000 population/year. Ability to predict which asymptomatic individuals will progress to clinical VL would help in monitoring disease outbreaks. Here we examined whole blood transcriptional profiles associated with asymptomatic infection, active disease, and in treated cases. Two independent microarray experiments were performed, with analysis focussed primarily on differentially expressed genes (DEGs) concordant across both experiments. No DEGs were identified for IgG or Quantiferon positive asymptomatic groups compared to negative healthy endemic controls. In comparing concordant DEGs from active cases with all healthy controls, and with VL cases treated with different regimens of drug treatment, 6 major themes emerged: (i) enrichment of gene sets associated with erythrocyte function in active cases; (ii) strong enrichment of gene sets for cell cycle in comparing active cases with healthy controls; (iii) identification of *IFNG* encoding interferon-g as the major hub gene in concordant expression patterns across experiments comparing active cases with healthy controls or with treated cases; (iv) enrichment for interleukin signalling (IL-1/3/4/6/7/8) and a prominent role for CXCL10/9/11 and chemokine pathways in comparing active cases with treated cases; (v) the novel identification of Aryl Hydrocarbon Receptor signalling as a significant canonical pathway when comparing active cases with healthy controls or with treated cases; and (vi) global expression profiling support for more effective cure at day 30 post-treatment with a single dose of

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DOI: 10.1002/eji.201970400

liposomal encapsulated amphotericin B compared to multi-dose non-liposomal amphotericin B treatment over 30 days.

P1814

Diaminopimelic acid -mediated induction of acute liver injury in D-Galactosamine-sensitized mice: the detrimental role of IL-6

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Interleukin(IL)-6 is elevated in most hepatic diseases and its role in liver injury is controversial. In this study, **injection of diaminopimelic acid (DAP), a NOD1 receptor agonist, and D-galactosamin(D-GalN) was used to establish a novel murine fulminant hepatitis model**. We found that the frequency of hepatic NK, NKT and T cells and CD69 expression on these cells were not significantly affected by DAP/D-GalN injection. Interestingly, **serum IL-6 and hepatic neutrophils increased significantly after DAP/D-GalN administration. Moreover, neutralization of IL-6 could completely abolish the liver injury and the liver neutrophils accumulation significantly decreased after IL-6 neutralization. Conclusion:** In this study, we established a novel murine fulminant hepatitis model in which IL-6 played a dominant proinflammatory role, and the IL-6 induced liver neutrophils accumulation may participate in the mechanism of liver injury.

P1815

Bead-based multiplex immunoassay panels to measure mediators involved in immuno-oncology related research

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The global incidence of cancer is a growing cause of morbidity and mortality, with metastatic disease ultimately being responsible for approximately 90% of cancer-related deaths. Prior to this metastatic endpoint however, tumors must first induce angiogenesis and remodel the extracellular matrix in order to grow and disseminate via the endothelium away from the primary malignancy. Concurrently, tumors also must evade detection and clearance by the immune system through a process of immunoediting. All of these processes are orchestrated by a complex interplay of cytokines, growth factors, as well as adhesion and immune-checkpoint molecules within the tumor microenvironment. As our understanding of the biological systems which drive cancer progression have expanded so too has our development of novel therapeutic strategies.
In order to enable biomedical researchers to continue making these crucial and fundamental discoveries, we have developed a series of bead-based multiplex assay panels that simultaneously quantify multiple soluble mediators from one sample volume using commonly available flow cytometers. These panels use highly specific antibodies in order to ensure robust assay sensitivities, analytical accuracy, and reproducibility. In addition, our multiplex assay panels are validated with biological samples (serum, plasma, cell culture supernatant) and are economic, convenient tools to study the complicated biological mechanisms underlying the tumor microenvironment, extracellular matrix, and angiogenic processes which drive cancer progression.

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DOI: 10.1002/eji.201970400

P1818

Biological significance of CCR5/CCL5 axis in liver cancer

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Despite the improvement in liver cancer (LC) survival in recent decades, only 1 in 5 patient survives 5 years after diagnosis. Thus, there is an urgent need to find novel treatment options to improve patient survival rates. Chemokine CCL5 (RANTES) has been reported to facilitate tumor progression and metastasis in various cancers, including liver cancer. However, the function of CCR5/CCL5 interaction in liver cancer proliferation and migration is poorly understood. Hence, the present study was undertaken to investigate the expression and role of the CCR5/CCL5 axis interface in the progression and invasiveness of liver cancer. *In vitro* study of liver cancer cell lines SNU387, PLC/PRF 5, and SK-HEP-1 showed higher expression of CCR5. Flow cytometry, RT-PCR, western blot, immunofluorescence techniques were used to quantify the expression of chemokine CCR5 /CCL5 in LC cells. To determine the biological significance of CCR5 expressed by LC cell lines, a microarray of LC tissues stained for CCR5 /CCL5 were analyzed. Our results show significantly ($p < 0.001$) higher expression of CCR5 /CCL5 in cancerous tissues compared to normal liver tissues. Furthermore, in order to delineate the role of CCR5/CCL5 interaction in LC cell proliferation and migration, we pre-treated the LC cell lines with the Maraviroc (CCR5 antagonist) in the presence of CCL5. This data demonstrates both the biological and clinical significance of CCR5 /CCL5 axis, targeting this axis is a promising avenue for researchers to treat liver cancer.

P1819

Phospholipase D of Loxosceles laeta poison join to lipid raft of human skin fibroblasts, activates the PI3K/AKT and generate the production of chemokines

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Introduction: Phospholipase D (PLD) from *Loxoceles* spider venom, are responsible of dermonecrosis during human spider envenomation. On human skin fibroblasts, different recombinant isoforms of PLDs from *L. laeta*, induced IL-6, IL-8, CXCL1 and CCL2/MCP-1 production. However, the signaling pathway implicated in this process is unknown.
Aim: Evaluate the signaling pathway involved in chemokines production mediated by *Loxosceles*PLDs on skin fibroblast.
Methodology: Human skin fibroblasts HHF-1 cultures were incubated with recombinant PLDsfrom *L. laeta*(rLlPLD1 and rLlPLD2), and the mutant isoforms of rLlPLD1 (rLlPLD1-D259G and rLlPLD1-W256S), and the Akt activation were evaluated by western blot, using an anti-phospho-Akt1/PKBα (Ser473) monoclonal antibody. Additionally, PI3K participation was determined by Western Blot of phosphorylated Akt in presence or absence of PLDs plus wortmannin or LY294002. The PLDs-lipid raft binding was evaluated by immunofluorescence using kit Vybrant® Lipid Raft labeling kit. The phospholipase D activity of rPLDs was determinates by Amplex Red Sphingomyelinase Assay. The production of cytokines and chemokines was evaluated by ELISA.
Results: The recombinant PLDs from *L. laeta* activate Akt between 5-15 min in fibroblast cultures. This effect was decreased in cultures treated with rLlPLDs and wortmannin. Also, the substrate deficient rLlPLD1 mutants, showed a lower activation of PI3K/Akt pathway compared to native protein. Also, PLD binding to lipid rafts from fibroblasts plasmatic membrane seem to be involved in PI3K/Akt

DOI: 10.1002/eji.201970400

pathway activation.
Conclusion: PI3K/Akt signaling pathway is activated by rLIPLDs, involved binding to lipid rafts present in the plasma membrane of skin fibroblasts, suggesting a role of the latters in the chemokines expression during *Loxosceles* envenomation.

P1820

Interplay of lipidosome and inflammasome pathways drives crystalline silica induced sterile inflammation

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Silicosis is a lung inflammatory disease caused by chronic exposure to crystalline silica (CS). Leukotriene B_4 (LTB_4) plays an important role in neutrophilic inflammation that drives silicosis and promotes lung cancer. Here, we examined the interplay between the inflammasome (IL-1b) and lipidosome (LTB_4) pathways involved in CS-induced inflammation. Phagocytosis of CS particles is essential for the production of LTB_4 and IL-1b in mouse macrophages, mast cells and neutrophils. Mast cells play an important role in initiating the sterile inflammation by producing LTB_4 at the mucosal interphase. Studies with bafilomycin-A1 and NLRP3 deficient mice revealed that LTB_4 synthesis in the lipidosome is independent of inflammasome activation. In vivo studies in NLRP3$^{-/-}$, BLT1$^{-/-}$ and BLT1$^{-/-}$ NLRP3$^{-/-}$ mice showed that while the production of LTB_4 and IL-1b occurs independent of each other, there is synergy between these mediators in inducing sterile inflammation. Confocal microscopy studies showed that GTPases Rab5c, Rab35 Rab40c along with JNK1 are essential for lipidosome formation and LTB_4 production. BI-78D3, a JNK inhibitor abrogated CS-induced neutrophilic inflammation *in-vivo* in an air pouch model. Our results highlight the importance of lipidosome and inflammasome pathways in coordination of CS-induced sterile inflammation.

P1212

The effect of MT2A on P38 in cardiomyocytes under ischemia/reperfusion injury

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In the process of myocardial ischemia/reperfusion (MI / R), due to excessive production of ROS, oxidative stress is generated, which leads to the death of cardiomyocytes. Metallothionein 2A (MT2A) has a strong anti-oxidative stress function. However, its effects on MI/R injury are poorly understood. The purpose of this study was to investigate the protective effect of MT2A on cardiomyocytes injury induced by MI/R and its mechanism. We first established a model of myocardial hypoxia/reoxygenation in vitro to simulate the MI/R injury. Then we detected the p38 protein level in the myocardial hypoxia/reoxygenation model in vitro.The results showed that the MI/R can promote the active expression of p38 and lead to oxidative stress injury through the p38-MAPKs signaling pathway. Finally, we found that both endogenous overexpressed MT2A and exogenously added MT2A can inhibit the active expression of p38 during MI/R and play an anti-oxidative stress injury role through the p38-MAPKs signaling pathway. In summary, our study demonstrated that MT2A can inhibit the active expression of p38 and play an anti-oxidative stress role through the p38-MAPKs signaling pathway in the MI/R.

This work was supported by the National Natural Sciences Foundation of China

DOI: 10.1002/eji.201970400

(NO.81660064;81860075;81560055) and Hainan Province Natural Science Foundation (NO.20158338).

P2430

Cytokines expression in different kinds of *mycobacteria* infected cases

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Different kinds of *mycobacteria* infection lead to granulomatous disease, such as *M.tuberculosis*, *M. leprae*, *M. murinum*, *M. chelonae* and *M. haemophilus*. Here, we aimed to study cytokines expression in these different kinds of *mycobacteria* infected cases. Immunohistochemistry was performed in skin lesion of 10 leprosy, 6 patients with *M. murinum* and 10 healthy controls. We found that IL4, IL9, IL17 and FoxP3 increased in granuloma with infection of *M. leprae* and *M. murinum*. However, IL22 expression up-regulated in leprosy, and had no difference in *M. murinum* infected cases when compared with controls. It indicated that IL22 and Th22 may play important role when *mycobacteria* infection. To further explore the pathogenesis of different kinds of mycobacteria: cytokines expression will be further detected in skin of *M.tuberculosis*, *M. chelonae* and *M. haemophilus* infected cases by Immunohistochemistry; cytokines and chemokine will be investigated in serum by ELISA; proportion of Th1, Th2, Th17, Treg, Th9 and Th22 will be measured by flow cytomertry in different kinds of mycobacteria infected cases.

P2431

NEDD4L inhibit hypoxia-induced cardiomyocyte apoptosis by ubiquitination and degradation of IRS4

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Recent studies have shown that abnormal NEDD4L, an ubiquitin ligasecan, increase the morbidity and mortality of cardiovascular diseases. In this study, we validate the interaction between NEDD4L and IRS4 by point mutation and Co-IP. Also detected the ubiquitination level of IRS4 when NEDD4L was overexpressed to determine whether NEDD4L could mediate the ubiquitination of IRS4. Finally, we simulated the state of ischemia and hypoxia in myocardial cell in vivo and in vitro, respectively. The protein levels of NEDD4L and IRS4 as well as the apoptosis levels of myocardial cells were observed, so as to determine the relationship between NEDD4L-mediated ubiquitination degradation of IRS4 and apoptosis of myocardial cells.We found that NEDD4L binds directly to IRS4 in cells, and that PY(PPxY) and PPR motifs in IRS4 are important in the binding process. We also demonstrate that NEDD4L can mediate ubiquitination of IRS4. Subsequently, we observed that when myocardial cells were cultured under hypoxic conditions, intracellular NEDD4L mediated IRS4 ubiquitination degradation disorder, leading to IRS4 accumulation, accompanied by an increase in apoptosis level, and the phenomenon can be seen in the mouse MI model. These findings support that NEDD4L can mediate the ubiquitination degradation of IRS4 which this process may have the effect of anti-apoptosis and providing protection for myocardial cells after MI.

This work was supported by the Hainan Provincial Key Research and Development Program (ZDYF2018102), the National Science Foundations of China (81770487) .

DOI: 10.1002/eji.201970400

P2432

Discovery of vascular endothelial growth factor receptor tyrosine kinase inhibitors by quantitative structure-activity relationships, molecular dynamics simulation and free energy calculation

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Vascular endothelial growth factor (VEGF), along with its receptor tyrosine kinases VEGFR-2 or kinase insert domain receptor (KDR), are targets for development of novel anticancer agents. Accurately predicting the structural characteristics of the target and chemical features of ligands can greatly reduce the cost and shorten the cycle of designing selective KDR inhibitors with desired activity. In this study, a docking strategy and three dimensional holographic vector of atomic interaction field (3D-HoVAIF) were applied in QSAR analysis of KDR inhibitors. The optimal model was constructed by using stepwise regression combined with partial least squares regression (SMR-PLS). Integrating the results of QSAR analysis, ADMET, pharmacophore modeling and a reverse screening strategy, eight derivatives were identified as potential KDR inhibitors. Then molecular dynamics (MD) simulations and free energy calculations were employed to explore the detailed binding process, so as to compare the potential binding modes of inhibitors with different activities. By analyzing the key residues in the binding site, it was found that different KDR-ligand complexes had similar binding modes. The predicted binding affinities were highly correlated with the experimental biological activity. Free energy analysis indicated that van der Waals interactions provided the major driving force for the binding process. Furthermore, key residues, such as Leu840, Val848, Ala866, Lys868, Leu889, Val899, Thr916, Phe918, Cys919, Leu1035, Cys1045, Asp1046, and Phe1047 played a vital role in forming hydrogen bonds, salt bridges, and hydrophobic interactions with the conformation of KDR. The above results will help design more efficient KDR inhibitors.

P2433

Identification of potential CCR5 inhibitors through pharmacophore-based virtual screening, molecular dynamics simulation and binding free energy analysis

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CC chemokine receptor 5 (CCR5), a member of G protein-coupled receptors (GPCRs), plays a vital role in inflammatory responses to infection. Alterations in the expression of CCR5 have been correlated with disease progression in many types of cancers. The idea of using CCR5 as a target for therapeutic intervention has been demonstrated to prevent disease progression. To date, only a few compounds have been reported as CCR5 inhibitors. In this study, a series of CCR5 antagonists were used to construct pharmacophore models. Then the optimal model was utilized as a 3D query to identify novel chemical entities from structural databases. After refinement by molecular docking, drug-likeness analysis, molecular dynamics simulations (MDS) and binding free energy analysis, three potential inhibitors (25, 29 and 45) were identified. MD simulations suggested that the screened compounds retained the important common binding mode known for CCR5 inhibitors (maraviroc and nifeviroc), which occupied the bottom of a pocket and stabilized the conformation of CCR5. During the binding process, van der Waals interactions provided the substantial driving force. The most favorable contributions were from Tyr37, Trp86, Tyr89, Tyr108, Phe109, Phe112, Gln194, Thr195, Ile198, Trp248, Tyr251, Leu255, Thr259, Met279, Glu283 and Met287. The above results suggest that the hybrid strategy would provide a basis for rational drug design.

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DOI: 10.1002/eji.201970400

P2434

Study on the possibility of antagonizing tumor necrosis factor receptor 1 by small molecule in hedysari radix based on molecular docking

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Objective: Tumor necrosis factor (TNF) mainly binds to TNFR1(tumor necrosis factor receptor type 1) and plays an anti-inflammatory role by mediating downstream NF-κB pathway.Small molecule inhibitors derived from Chinese herbal extracts have the advantage of multiple targets.The aim of this study was to explore the small molecular compound of hedysari radix antagonizing TNFR1 through molecular docking.
Methods: The structures of small molecular compound of hedysari radix were downloaded from the chemical composition compound library of traditional Chinese medicine, and those structures were optimized. The three-dimensional structure of the inflammatory target TNFR1 (PDB ID:1TNR) was identified. After hydro-treating and an-hydrating, the combine pocket residues were identified according to the literature. According to the defined target structure and combine pocket, the composition compound library was docked with the target flexibly, and the scores were obtained. Based on the results of molecular docking, the top nine molecular compounds in ranking score were selected as candidate components. On this basis, the drug-likeness was evaluated, that is, small molecular compounds must meet the standards such as number of hydrogen-bonded receptors, number of hydrogen-bonded donors and etc. Finally, the binding mode was analyzed according to pharmacokinetic parameters and complex structure of composition-target docking.
Results: Identify the residue set of TNFR1 drug-binding pocket includes Glu109, Lys35, Ala62 and etc. Five small molecular compounds were screened by comprehensive methods including docking scoring.
Conclusion: As the active ingredients of hedysari radix, Quercetin, isoglycyrrhizin, naringin, pili isoflavone and glycyrrhizin may play an anti-inflammatory role by antagonizing TNFR1.

P2435

Cytokins enhance central memory T cell antitumor immunotherapy

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Background: Memory T lymphocytes exhibit a rapid and strong immune response to restimulation of tumor-related antigens, and perform the functions of immune surveillance and target cell killing. Cytokines play a prominent role in manipulate immune cells include T cells. A number of cytokine have been developed to elicit. In this study, we used various of cytokines to explore more efficiently cytokines combination in shaping naïve T cells into central memory T cells.
Methods: Monocytes were isolated from PBMC with healthy donors, and they were sorted by immunomagnetic cell separation then stimulated with anti-CD3/anti-CD8 beads for 72 hours. After stimulation, cells were seeds in 24-plate in the present of cytokines combination groups: (1) IL-2; (2)IL-7+IL-15; (3)IL-7+IL-15+IL-21 ; (4)IL-7+IL-15+IL-21+IL-23 for 21 days. Then cells were examined for proliferation, activation, phenotypic characteristics, and cytotoxic capacity.
Results: We found naïve T cell treated with combination cytokines formulation(IL-7, IL-15, IL-21 and IL-23) expressed high level of CD45RA-CCR7+, a memory marker and indicator of $CD4^+$ /$CD8^+$Tcm

DOI: 10.1002/eji.201970400

compared with other groups[(1) IL-2; (2)IL-7+IL-15; (3)IL-7+IL-15+IL-21], with the percentage of 52.9±3.6% and 40.0±3.6% respectively. Further more, T cells treated with combination cytokines formulation secreted a significantly higher amount of IFN-γ, GrB and Perforin than other groups. **Conclusions:** Our investigations demonstrate that mixed of IL-7, IL-15, IL-21 and IL-23 is a optimal cytokines combination that can efficiently induce naïve T cell into central memory T cell for cancer immunotherapy.

P2436

Lung cancer-derived extracellular vesicles induced myotube atrophy and adipocyte lipolysis via the extracellular IL-6-mediated STAT3 pathway

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Cancer-associated cachexia (CAC) constitutes a metabolic dysfunction characterized by systemic inflammation and body weight loss. Muscle atrophy and adipose tissue lipolysis might explain weight loss in CAC. Specific functions of numerous hormones and cytokines derived from tumours can provoke cachexia. Extracellular vesicles (EVs) can be involved in intercellular communication. However, whether EVs participate in this process has not been investigated thoroughly. Using Lewis lung carcinoma (LLC) cell cultures, we tested whether LLC-derived EVs induced C2C12 myotube atrophy and 3T3-L1 adipocyte lipolysis. EVs derived from LLC cells and serum from patients with lung cancer, non-lung cancer controls, tumour-bearing mice, and non-tumour-bearing control mice were isolated and characterized biochemically and biophysically. LLC cell-derived EVs induced dose-dependent effects of atrophy in C2C12 myotubes and lipolysis in 3T3-L1 adipocytes. Mechanistically, EVs directly fused with target C2C12 myotubes and 3T3-L1 adipocytes, and transferred interleukin-6 (IL-6) activate the STAT3 signalling pathway in C2C12 myotubes and 3T3-L1 adipocytes. Neutralization of extracellular IL-6 prevented the atrophy and lipolysis effects of EVs. Inhibiting the STAT3 signalling pathway also prevented the atrophy and lipolysis effects of EVs. PKH67-labelled (PKH 67 is a lipid dye that can be used to label extracellular vesicles) LLC-EVs were readily internalized into myotubes and adipocytes. Our data showed that LLC cell-derived EVs induced myotube atrophy and adipocyte lipolysis via the extracellular IL-6-mediated STAT3 pathway in target cells. These findings represent a potentially novel basis for further research in this field towards identifying targets and developing strategies for maintaining weight in CAC.

P2437

The contribution and genomic methylation characteristics of SETD4 in mouse bone marrow mesenchymal stem cells

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Bone marrow mesenchymal stem cells (BMSCs) are low immunogenic cells that have attracted much attention in cell transplantation therapy. However, epigenetic modification is the key mechanism affecting the biological function of BMSCs. SETD4 is a histone methyltransferase and is currently lacking in the biological role in BMSCs. In this study, we used CRISPR/Cas9 technology to obtain *SEDT4* knockout mice, and WT mice were served as controls, then we analyzed the differences in the

DOI: 10.1002/eji.201970400

proliferation and paracrine angiogenic growth factors of BMSCs, and we used Reduced Representation Bisulfite Sequencing (RRBS) to analyze the genomic methylation in the cells, and finally the possible involvement of SETD4 in cell function and signaling pathways was bioinformatics analyzed. The results showed that knockdown of *SETD4* significantly promoted the proliferation of BMSCs and inhibited apoptosis, which was characterized by enhanced cell activity and increased expression of PCNA, Cyclin A2, Cyclin E, CDK2 and CDK6 and decreased the expression of P16 and P21. Interestingly, *SETD4*deficiency impaired the ability of BMSCs to secrete VEGF and TGF-b1. The RRBS results showed that the genomic CpG islands and differential methylation regions (DMR) were significantly characterized by SETD4 deficiency, and the DMR involved 34 signaling pathways and 35 cellular functions. The results suggest for the first time that*SETD4* has a unique role in the biology in BMSCs.
This work was supported by the Hainan Provincial Key Research and Development Program(ZDYF2018102), the National Science Foundations of China (81670254), Science and Technology Project of Guangdong Province (2016A020214016).

P2438

The expression of peroxisome proliferator-activated receptor-α in rats with alcoholic liver injury

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Sixty SD rats were divided randomly into five groups. The observe groups rats were lavaged with distillate spirit for 4 , 8, 12 and 16 weeks respectively to establish the alcoholic liver injury animal models. The animals were sacrificed at each time point for study. The semi-quantitative reverse transcription polymerase (RT-PCR) was used for determining the expression of the PPAR-α mRNA and nuclear factor-κB (NF-κB) mRNA. The level of the blood-serum cytokine (TNF-α, IL-6) were determined with RIA, and the liver histopathology variations were observed under the light microscope. The expression of the PPAR-α mRNA in the observe groups rats decreased at the time point of 4, 8, 12 and 16 weeks. Along with the increase of lavage time, the expression decreased more conspicuously compared to those in control group, especially at 12 and 16 weeks (P< 0.05, 0.01 respectively). The expression of NF-κB (P65 subunit) mRNA and the level of the blood-serum cytokine (TNF-α, IL-6) increased evidently at each time point compared to those in control group (P< 0.01 respectively). Along with the increase of time, more and more fatty degeneration and inflammation appeared in livers. The expression of the PPAR-α in alcoholic liver injury decreased. There was certain inverse correlation among the expression of PPAR-α mRNA and the expression of NF-κB (P65 subunit), the level of TNF-α and IL-6, the liver histopathology variation. The study data suggested that the PPAR-α might play significant role in alcoholic liver injury.

This study was supported by Hainan natural science foundation 819MS122

DOI: 10.1002/eji.201970400

P2439

Regulation of TNFSF15 on vascular endothelial growth factor receptor

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Vascular endothelial growth factor receptor (VEGFR) is expressed on the surface of vascular and lymphatic endothelial cells. When combined with vascular endothelial growth factor, it can promote endothelial cell proliferation and enhance vascular permeability. Tumor necrosis factor superfamily member 15 (TNFSF15) secreted by vascular endothelial cells can inhibit the expression of vascular endothelial growth factor (VEGF), which can stimulate the production of microRNAs such as microRNA-20a and microRNA-31, targeting 30-UTR of TNFSF15, thereby down-regulating TNFSF15. TNFSF15 can also regulate the expression of three kinds of VEGFR and produce different physiological effects. TNFSF15 can promote Akt inactivation-dependent degradation of VEGFR1 in vascular endothelial cells, and alleviate the inhibition of Jmjd6 on the production of soluble VEGFR1 (sVEGFR1), thereby exerting anti-angiogenesis effect. When TNFSF15 acts with VEGFR2, its death receptor 3 (DR3) and phosphatase-1 (SHP-1) containing SH2 domain can compose protein complexes with VEGFR2, induce dephosphorylation of VEGFR2, inactivate the signal of VEGF-VEGFR2 and inhibit vascular permeability associated with VEGF; TNFSF15 can also promote the expression of VEGFR3 in lymphatic endothelial cells, thereby enhancing lymphangiogenesis.
Keywords: Tumor necrosis factor superfamily member 15; Vascular endothelial growth factor receptor; Regulation

P2440

IL-13 and IL-13Rα1 are overexpressed in extranodal natural killer/T cell lymphoma and mediate tumor cell proliferation

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Extranodal NK/T cell lymphoma (NKTCL) is a rare but aggressive subtype of non-Hodgkin lymphoma. Multiagent chemotherapy and involved-field radiotherapy are used to treat this disease, but the prognosis remains poor. Interleukin 13 and its receptors (IL-13Rs) are correlated with the pathogenesis and progression of various malignances. However, their roles in NKTCL have not been evaluated. In this study, we examined the roles of IL-13 and IL-13Rs in NKTCL and the underlying mechanisms. We found significantly higher serum IL-13 levels ($p< 0.001$) and IL-13Rα1 expression in tumor tissues (36 of 40, $p< 0.001$) in patients with NKTCL than those in control cohort. IL-13 secretion was observed in tumor tissues (30 of 40, $p< 0.001$) and several cell lines of NKTCL. However, we did not observe significant associations between clinical characteristics and the expression levels of IL-13 or IL-13Rs. In vitro, IL-13 activated Stat6 and promoted cell proliferation in a dose-dependent manner. In addition, blocking IL-13 exerted a negative effect on tumor cell growth. We conclude that IL-13 functions as an autocrine growth factor in NKTCL and contributes to its pathogenesis. Blocking IL-13 is thus a potential therapeutic approach of NKTCL.

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DOI: 10.1002/eji.201970400

P2441

Combined use of interferon alpha-1b, interleukin-2 and thalidomide to reverse minimal residual disease in acute myeloid leukemia

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Objective: To explore the effect of ITI regimen (interferon alpha-1b, interleukin-2 and thalidomide) on minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) who were in hematologic remission but MRD-positive.
Methods: Eighteen patients with AML admitted from July 2016 to June 2018, who were in hematologic remission but MRD-positive were treated with different doses of ITI regimen. Monitoring the MRD level.
Results: Among 18 patients who received a conventional dose of ITI regimen, MRD levels turned unmeasurable in seven patients, and MRD levels significantly decreased in three patients, hematologic recurrence occurred in five and three had a MRD increase. Three patients with MRD increase received a higher dose of ITI regimen, MRD levels turned unmeasurable in two patients, and one patients MRD level decreased. The response rate was 57.1% and 81.8% in those who presented a MRD≥1.0% and < 1.0%, respectively.
Conclusion: The ITI regimen can reduce the MRD level of patient with AML who are in hematologic remission but MRD-positive. Improved effect showed through a higher dose administration and a lower burden of MRD level before treatment.
Keyword: myeloid leukemia; interferon; interleukin-2; thalidomide; minimal residual disease

P2442

pORF5 plasmid protein of *chlamydia trachomatis* regulates the secretion of IL-1β through activation of p38 MAPK and NALP3 signaling pathways

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Objective: To investigate the molecular mechanism of 1L-1β production induced by pORF5 plasmid protein of *Chlamydia trachomatis* (Ct) in THP-1 cells.
Methods: pGEX-6p/pORF5 prokaryotic expression vector was transformed to *E.coli* to express GST-pORF5 fusion protein, the fusion protein was then cleaved to get pORF5 protein without GST tag. pORF5 protein was used to stimulate THP-1 cells at different concentrations and different time points, and IL-1β was detected by ELISA; After treated with NALP3 siRNA, ASC siRNA or Caspase-1 inhibitor, THP-1 cells were subsequently stimulated with pORF5 (24 µg/mL) for 24 h, then the level of IL-1β, mRNA expression of IL-1β and NALP3 inflammasome were detected by ELISA and Real-time PCR, Caspase-1 activity and p38 phosphorylation were determined by western blot.
Results: The pORF5 plasmid protein induced THP-1 cells to produce IL-1β in dose- and time-dependent manners, and IL-1β production reached its peak at concentration of 24µg/mL for 24 h. pORF5 protein increased mRNA expression of NALP3 inflammasome, and the levels of IL-1β were reduced by 37.7%, 71.3% and 40.1% respectively after treated with NALP3 siRNA, ASC siRNA and Caspase-1 inhibitor; p38 inhibitor reduced pORF5-induced IL-1β production and mRNA expression of NALP3 inflammasome significantly (P< 0.01), but there were no significant differences in p38 phosphorylation after inhibition the activation of NALP3 inflammasome (P>0.05). **Conclusion** pORF5

DOI: 10.1002/eji.201970400

plasmid protein induced the production of IL-1β relied on the NALP3 and p38MAPK signaling pathways.
Key words: *Chlamydia trachomatis*; pORF5 plasmid protein; NALP3 inflammasome; p38 MAPK; IL-1β

P2443

HMGB1: a immune-regulatory protein

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High mobility group box 1 (HMGB1) presents in almost all somatic cells as a component of the cell nucleus and is necessary for transcription regulation during cell development. Recent studies indicate that extracellular HMGB1, coming from necrotic cells or activated immune cells, triggers inflammatory response whereas intracellular HMGB1 controls the balance between autophagy and apoptosis. In addition, reduced HMGB1 also presents a potent function in tissue regeneration. HMGB1 shows a challenge that can be used for therapeutic improvement while preventing its pathological role.
It is interesting that different HMGB1 could act seems to be different functions. Fully reduced HMGB1 accelerates tissue regeneration through CXCL12/CXCR4 axis whereas disulfide HMGB1 promotes the inflammatory response by TLR2/TLR4/RAGE receptors. The accumulated cytoplasmic HMGB1 promotes autophagy.The cytoplasmic HMGB1 directly binds to autophagy protein Beclin-1 through the intramolecular disulfde bridge (C23/45) and promotes Bcl-2 dissociating from Beclin-1, which is required for Atg5/Atg7-dependent and -independent autophagy.The nucleus HMGB1 reduction promotes apoptosis. The HMGB1/p53 complex within the nucleus keeps cells stable. Once HMGB1 separated from complex, the cytoplasmic p53 protein mitochondrial translocation can induce cells apoptosis through inhibiting anti-apoptotic proteins BCL-X_L and Bcl-2, coupling with the pro-apoptotic proteins PUMA/Bax, which mediates mitochondrial pathway.
HMGB1 has been an effective therapeutic target for infectious and inflammatory diseases. Compared with the specific antibody of HMGB1, inhibitors(such as glycyrrhizin, ethyl pyruvate et al.) of HMGB1 secretion from immune cells get more attention in this research field.

P2444

Hypoxia regulates angeogenic-osteogenic coupling process *via* up-regulating IL-6 and IL-8 in human osteoblastic cells through hypoxia-inducible factor-1α pathway

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Inappropriate angiogenesis and osteogenesis are considered as the crucial factors of osteoporotic fracture. Hypoxia is a primary driving force for regulating the angiogenic-osteogenic coupling process. Our recent results indicated that hypoxia could improve angiogenesis as well as differentiation and activity of osteoblastic cells *via* up-regulating VEGF through HIF-1α pathway. Here we demonstrated that in human osteoblastic MG-63, U2-OS and Saos-2 cells, besides VEGF, the other two pro-angiogenic factors IL-6 and IL-8 were alsoboth up-regulated by hypoxia and $CoCl_2$. Results of mMechanism studies indicated overexpression of HIF-1α markedly increased the levels of IL-6 and IL-8 in osteoblastic cells. Furthermore, a luciferase reporter assay was performed using the reporter vector containing the IL-6 or IL-8 promoter sequence to illustrate observably increased activity of hypoxia-induced IL-6 and IL-8 promoter caused by overexpression of HIF-1α observably increased

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DOI: 10.1002/eji.201970400

hypoxia-induced IL-6 and IL-8 promoter activity. Additionally, chromatin immune-precipitation analysis showed hypoxia increased the DNA binding ability of HIF-1α to IL-6 or IL-8 promoter. Analysis *in vitro* by MTT test and Boyden chamber assay showed exogenous IL-6 and IL-8 (a relatively short period of treatment with recombinant IL-6 or IL-8 equivalent to the autocrine levels) could significantly promote the proliferation of human osteoblastic, endothelial and monocytic cells, as well as the migration of human endothelial cells. Taken together, these results indicate that IL-6 and IL-8 in osteoblastic cells may also contribute to the angiogenic-osteogenic coupling process *via* HIF-1α pathway. Besides VEGF, IL-6- or IL-8-targeted adjunctive therapy maybe a new strategy to improve the treatment of osteoporosis.

P2445

MiR-410-3p suppresses cytokine release from fibroblast-like synoviocytes by regulating NF-κB signaling in rheumatoid arthritis

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Background: MiR-410-3p acts as an oncogene or tumor suppressor in some malignancies. However, its role in rheumatoid arthritis (RA) is unknown. The study was conducted to investigate the effect of miR-410-3p on the pathogenesis of RA.
Methods: Real-time RT-PCR was used to determine the mRNA levels of miRNA-410-3p in synovial tissues and fibroblast-like synoviocytes (FLSs). An ELISA assay was performed to examine the production levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and matrix metalloproteinase (MMP)-9. Western blotting was conducted to determine the protein levels of IκB-α, p-IκBα, p65 and p-p65. Nuclear factor (NF)-κB activation and nuclear translocation assays were performed to confirm the activation of NF-κB.
Results: We found that the expression level of miR-410-3p was downregulated in synovial tissues and FLSs from RA. Overexpression of miR-410-3p significantly reduced the secretion of TNF-α, IL-1β, IL-6 and MMP-9 in HFLS-RA; whereas miR-410-3p inhibition increased the expression levels of these cytokines. Furthermore, miR-410-3p suppresses the activation of NF-κB signaling pathway. Moreover, NF-κB inhibitor restored the elevation of TNF-α, IL-1β, IL-6 and MMP-9 induced by miR-410-3p inhibition.
Conclusion: Our results demonstrate that miR-410-3p acts an inflammatory suppressor in the pathogenesis of RA by regulating the NF-κB signaling pathway. These data suggest a novel function of miR-410-3p and provide insight into the complex mechanisms involved in RA.
Keywords: Rheumatoid arthritis; miR-410-3p; fibroblast-like synoviocytes; cytokines; NF-κB signaling

P2446

HCMV-encoded UL128 enhances TNF-a and IL-6 expression and promotes PBMC proliferation through the MAPK/ERK pathway in vitro

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Cytomegalovirus (CMV) infection enhances expression of several cytokines, such as tumor necrosis factor-a (TNF-a), interleukin-6 (IL-6), granulocyte macrophage colony-stimulating factor (GM-CSF),

DOI: 10.1002/eji.201970400

and IL-8, to the benefit of virus replication and dissemination. However, the stimulus for certain cytokine production remains unclear. CMV encodes a series of proteins that alter and/or mimic functions of leukocyte migration, activation, and cytokine responses. Our study revealed that human CMV (HCMV)-encoded UL128 protein, which contains signal peptides and has similar amino acid sequences to the CC chemokine, recruits monocytes as human CC chemokine (microphage inflammatory protein 1a). Using RNA interference technology, we constructed an HCMV (UL128 + /UL128 -)-infected tissue cell (MRC-5) and peripheral blood mononuclear cell (PBMC) coculture system. We measured 6 cytokine levels (IL-2, IL-4, IL-6, IL-10, TNF-a, and interferon-c [IFN-c]) in the supernatant, and found significantly elevated IL-6 and elevated TNF-a levels in the HCMV UL128 + -infected
group. Conversely, we observed decreased levels in the UL128-knockout supernatant. PBMCs presented with UL128 (50 ng/mL) demonstrated better cell viability than the UL128-absent group. Finally, the MAPK/ERK pathway was found to be involved in UL128 induction of cell proliferation. Selective induction of cytokine expression indicates that HCMV-encoded UL128 is a potent inducer of several inflammatory mediators.

P2447

Downregulation of ENDOCAN in myeloid leukemia cells inhibits proliferation and promotes apoptosis by suppressing nuclear factor-kB activity

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Objective: ENDOCAN has been reported to be associated with poor prognosis. However, the functional role of ENDOCAN in the development of leukemia remains to be fully elucidated.
Methods: In the present study, the expression levels of ENDOCAN were detected in THP-1, U937, HL-60 and K562 cells, and it was found that ENDOCAN was increased in U937 and K562 cells, compared with the other two cell lines. Subsequently, ENDOCAN was knocked down in U937 and K562 cells via lentiviral infection. The effects of Endocan expression silencing on the proliferation, cell cycle and apoptosis of U937 and K562 cells were detected by CCK-8, Western blot and flow cytometry. Western blot was used to detect apoptosis related proteins. Western blot was used to detect NF-κB、P65、p-P65、NF-κB inhibitor alpha (IκBα)、p-IκBα、nuclear P65. The DNA binding activity of NF-κB was determined using the EMSA.
Results: ENDOCAN knockdown induced G0/G1-phase cell cycle arrest in myeloid leukemia cells with a decreased expression of cyclin D1. Cell apoptosis was increased in response to ENDOCAN silencing, which was accompanied by the downregulation of B-cell lymphoma (BCL2) and the upregulation of BCL2-associated X protein, cleaved caspases 3 and 9, and cleaved poly (ADP-ribose) polymerase. It was demonstrated that the knockdown of ENDOCAN inhibited nuclear factor-κB (NF-κB) activity, as evidenced by the increased expression of NF-κB inhibitor α (IκBα), decreased expression of phosphorylated (p-)IκBα, p-P65 and nuclear P65, and reduced NF-κB DNA-binding activity.
Conclusion: ENDOCAN may serve as a potential therapeutic target in the treatment of leukemia.

DOI: 10.1002/eji.201970400

P2448

Expression of interleukin 34 in patients with inflammatory bowel disease

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Objective: To detect the expression of interleukin 34 (IL-34) in inflammatory bowel disease (IBD) and investigate the correlation between serum IL-34 and other estabilished serum markers and disease severity.
Methods: A total of 48 patients with UC were chosen, including 28 patients with moderate to severe UC, 11 patients with mild UC, 9 patients in remission, and 21 patients with CD, including 9 patients with moderate to severe CD, 6 patients with mild CD, 6 patients in remission. Blood samples from 29 healthy volunteers were served as serum control. The colonic tissues from patients with colon cancer were served as tissue control. Immunohistochemistry and ELISA was used to detect the expression of IL-34 in colon and serum seperately. The data of Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were collected from patients with IBD.
Results: The expression of IL-34 in colonic mucosa and serum in active UC and CD group was significantly different from the control group and remission group respectively ($P< 0.05$). There was no significant difference between the remission group and the control group ($p>0.05$). The serum levels of IL-34 in moderate to severe UC group and mild UC group were significantly different, While the same to the CD group ($P< 0.05$). What's more, The levels of serum Il-34 were positively correlated with disease activity, ESR and CRP.
Conclusion: IL-34 is significantly increased in colonic tissue and serum of patients with IBD, suggesting that IL-34 may be important in the genesis and development of IBD.

P2449

Evaluation of the value of serum SAA, CRP and PCT in the diagnosis and differential diagnosis of aseptic inflammation

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Objective: To evaluation the diagnostic value of the serum amyloid A (SAA), C-reactive protein (CRP) and procalcitonin (PCT) in aseptic inflammatory.
Methods Ninety-one aseptic inflammation patients included acute myocardial infarction (AMI), acute pancreatitis (AP) and acute trauma (AT) enrolled in this study, infection diseases and healthy people as control. The levels of serum SAA, CRP and PCT were analyzed.
Results: Compared with the healthy control, the levels of serum SAA, CRP and PCT in patients with infectious diseases and aseptic inflammatory were significantly increased (p = 0.000 / 0.000 / 0.000), but the serum SAA,CRP and PCT in aseptic inflammation were obviously lower than that of infection control (p = 0.000/ 0.000/ 0.000); the levels of serum SAA and CRP in the AMI were significantly lower than those in the AP and AT (p = 0.000 / 0.000 / 0.000 / 0.005), there was not significant difference in the levels of serum PCT among the groups (p = 0.642); Serum SAA was positively correlated with CRP; AUC of SAA, CRP and PCT for diagnosis of infection and non-infection were 0.759, 0.747 and 0.871, cutoff values were 97.3 mg/L,42.15 mg/L and 0.64 ng/L; the AUC of SAA combined with PCT for diagnosis of infection and non-infection was 0.882, and the AUC of SAA,CRP and PCT combined detection for diagnosis of infection and non-infection was 0.884.

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DOI: 10.1002/eji.201970400

Conclusion: Serum SAA, CRP and PCT combined analysis in the differential diagnosis of infections and aseptic inflammatory play a certain role.

P2450

Reversal effect of adenovirus-mediated human interleukin-24 transfection on the cisplatin resistance ofA549/DDP lung cancer cells

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Interleukin-24 (IL-24) is a tumor-suppressor gene that has been documented in human melanoma cells. IL-24 has marked antitumor activities on various types of human cancer, but its underlying mechanism remains unclear. In the present, we investigated the effects of human IL-24 (hIL-24) on the chemotherapy resistance of lung cancer cells. The cisplatin (DDP)-resistant lung carcinoma cell line A549/DDP was subjected to adenovirus-mediated transfection with the human IL-24 gene (Ad-hIL-24). The growth-inhibitory and apoptotic effects of Ad-hIL-24 on A549/DDP cells were observed, and the expression levels of AKT, phosphorylated-AKT (p-AKT) and P-glycoprotein (P-gp) were detected. Ad-hIL-24 significantly decreased the levels of p-AKT and P-gp, and effectively inhibited A549/DDP cell growth. Furthermore, A549/DDP cells exhibited a significantly increased rate of apoptosis, as well as G2/M-phase arrest, following transfection with Ad-hIL-24, and these effects were increased in cells treated with Ad-IL-24 combined with DDP when compared with those treated with Ad-hIL-24 or DDP alone. These results suggest that hIL-24 can reverse the DDP resistance of lung cancer cells, and that the associated mechanism involves the induction of apoptosis and G2/M-phase arrest through the phosphoinositide3-kinase (PI3K)/AKT signaling pathway, as well as a decrease in drug resistance through P-gp expression (T*his work was supported in part by the National Natural Science Foundation of China (81872226, 81402368, 81402265, 81502346), Guangdong Provincial Science and Technology Project Foundation (2017A020215136), China Postdoctoral Science Foundation funded project (2016M592580)*.
Keywords: Interleukin-24, Lung cancer, drug resistance, AKT, P-gp

P2451

Identification of factors that specifically inhibit TNF-activated NF-κB pathway in THP-1 cells

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Chronic inflammation such as rheumatoid arthritis seriously affects the health of patients. TNFα induces the expression of inflammatory factors and is a key molecule for promoting chronic inflammation. At present, monoclonal antibodies against TNFα are increasingly used to treat patients with RA and IBD who are refractory to general drugs. However, these drugs have two major problems, no significant effect on too many patients and treatment costs are very high. Identification of factors that specifically regulate TNFα signaling may provide new targets for the development of drugs that interfere with the development of chronic inflammatory diseases. Our results indicate that both TNFα and LPS activate the NF-κB pathway in THP-1, but unlike LPS, TNFα induces a weak effect on IL-8 and other factors, reaching a peak at 60 min. In addition, TNFα-induced NF-κB pathway was abruptly attenuated at 30-60 min, while LPS-activated NF-κB pathway was still at a high level, indicating that

DOI: 10.1002/eji.201970400

TNFα induces a certain factor that specifically negatively regulating the activation of the NF-κB pathway in the early stage of stimulation in THP-1 cells.

P2452

Construction, expression and characterization of rSEA-EGF and in vitro evaluation of its antitumor activity against nasopharyngeal cancer

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Staphylococcal enterotoxin A is well known as a superantigen and able to be used for cancer immunotherapy. In this study, recombinant Staphylococcal enterotoxin A was genetically conjugated to epidermal growth factor to produce a chimeric protein recombinant Staphylococcal enterotoxin A-epidermal growth factor expressed in Escherichia coli. The recombinant Staphylococcal enterotoxin A-epidermal growth factor protein was purified using Strep-Tactin affinity chromatography and Endotoxin Removal Resin and identified by sodium dodecyl sulfate-polyacrylamide gel electropheresis and liquid chromatography-tandem mass spectrometry analysis. Furthermore, in vitro experiments showed purified recombinant Staphylococcal enterotoxin A-epidermal growth factor could successfully bind to the human nasopharyngeal carcinoma cell line CNE2, significantly promote the proliferation of human peripheral blood mononuclear cells, and enhance the secretion of several cytokines that have broad antitumor activities, such as interferon-g, tumor necrosis factor-a, and interleukin-2 . Importantly, recombinant Staphylococcal enterotoxin A-epidermal growth factor significantly inhibited proliferation of CNE2 cells and promoted apoptosis in CNE2 cells when cocultured with peripheral blood mononuclear cells. Finally, both the binding of recombinant Staphylococcal enterotoxin A-epidermal growth factor and the toxicity of recombinant Staphylococcal enterotoxin A-epidermal growth factor-activated peripheral blood mononuclear cells were demonstrated as specific and only effective on high epidermal growth factor receptor expressing cell lines. In all, our work suggests that recombinant Staphylococcal enterotoxin A-epidermal growth factor serves as a promising novel immunotherapeutic agent. More in vivo and in vitro studies are needed to verify its antitumor potency, as well as investigate the underlying mechanisms in cancer immunotherapy.

P2453

PBMC activation via the ERK and STAT signaling pathways enhances the anti-tumor activity of Staphylococcal enterotoxin A

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Staphylococcal enterotoxin A (SEA) is well known as a superantigen and is highly potent in activating T lymphocytes. And it has been used clinically as an immunomodifier in the treatment of a number of tumors for years. However, the mechanism of its action remains largely unclear. In this study, SEA was found to significantly inhibit the proliferation and induce the death of human lung carcinoma A549 cells when co-cultured with human peripheral blood mononuclear cells (PBMCs). SEA could also induce the proliferation of human PBMCs and stimulate human PBMCs to release a wide range of cytokines that have broad anti-tumor activities such as IFN-c, TNF-a, IL-2. Furthermore, SEA was

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DOI: 10.1002/eji.201970400

found in PBMCs to induce a rapid and long-lasting phosphorylation of extracellular signal-regulated kinases (ERKs) which was significantly inhibited by MEK/ERK pathway inhibitors U0126 and PD0325901, and a late onset of phosphorylation of signal transducers and activators of transcription (STATs) which was significantly inhibited by a pan-JAK inhibitor Pyridone 6 (P6). Unexpectedly constitutive ERK or STATs phosphorylation was also significantly inhibited by P6 or U0126 in a dose-dependent manner, respectively. Summing up, our data reveal SEA may function as a novel protein drug used for cancer immunotherapy via inducing activation of PBMCs, immune cell crosstalk-dependent activation of ERK and STATs, and production of tumor-suppressive cytokines.

P2454

Active immunization against FGF-2 modulates systemic antitumor immune responses and synergizes with a HPV16 E7 vaccine in TC-1 tumor-burden mice

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Background: The clinical efficacy of tumor vaccines is significantly limited by systemic and local immunosuppressive mechanisms developed with tumor growth. FGF-2 is tightly involved in the development of immunosuppressive tumor microenvironment.
Objective: This study aimed to investigate the effects of active immunization against FGF-2 on antitumor immune responses.
Methods: A set of thirteen synthesized peptides, covering all the possible linear B cell epitopes of mouse FGF-2, and recombinant FGF-2 protein were respectively conjugated to virus like particles (VLPs) of HBcAg. The VLPs were immunized in a TC-1 tumor model alone or in combination with a HPV16 E7-specific vaccine.
Results: Preventive immunization with VLPs produced high tittered FGF-2 specific antibody and inhibited tumor growth. Peptide 12, which locates in heparin binding site, or protein-conjugated VLPs represented the most significant effects on suppressing tumor growth. It was found that the levels of IFN-γ-expressing splenocytes and serum IFN-γwere significantly elevated; further, the immune effector cells $CD8^+$ IFN-γ^+ CTL and $CD4^+$ IFN-γ^+ Th1 cells were increased significantly, whereas immunosuppressive cells $CD4^+CD25^+FOXP3^+$ Treg and Gr-1^+CD11b^+ MDSC were decreased in the immunized mice. In addition, VLPs immunization significantly suppressed tumor vascularization and promoted tumor cell apoptosis. In therapeutic immunization study, VLPs still demonstrated inhibitory effects on the growth of established tumors, and the combined immunization presented significant synergic effects on suppressing tumor growth and boosting anti-tumor immunity.
Conclusion: Active immunization against FGF-2 modulated anti-tumor immune responses and synergized with a tumor vaccine, providing a promising tumor vaccine strategy.

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DOI: 10.1002/eji.201970400

P2455

Recombinant virus-like particles presenting IL-33 successfully modify the tumor microenvironment and facilitate antitumor immunity in a model of breast cancer

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Recently, interleukin (IL)-33 has been closely associated with a variety of clinical cancers. IL-33 presents both protumorigenic, and less frequently, antitumorigenic functions depending on disease conditions. IL-33 signaling appears to be a possible target for the treatment of applicable tumor diseases. This study aimed to develop an effective approach to intervene in IL-33 functioning in tumors and reveal the immunotherapeutic potential of anti-IL-33 active immunization. Recombinant truncated hepatitis B virus core antigen (HBcAg), presenting mature IL-33 molecules on the surface of virus-like particles (VLPs), was prepared and used to immunize BALB/c mice in a model of murine 4T1 breast cancer. The immunization was performed through either a preventive or therapeutic strategy in two separate studies. Anti-IL-33 immunization with VLPs elicited a persistent and highly titrated specific antibody response and significantly suppressed orthotopic tumor growth and lung metastasis in both studies. The underlying mechanisms might include promoting tumor-specific Th1/CTL cellular responses and the expression of the effector molecule IFN-γ, suppressing Th2 responses, and significantly reducing the infiltration of immunosuppressive Treg cells and MDSCs into tumor tissues in the immunized mice. In conclusion, anti-IL-33 active immunization employing recombinant VLPs as an antigen delivery platform effectively modified the tumor microenvironment and promoted antitumor immunity, indicating the potential of this approach as a new and promising immunotherapeutic strategy for the treatment of cancers where IL-33 plays a definite protumorigenic role.

P2456

The effects of anti-cytokine active immunization in combination with induction of tumor cell immunogenic death on mouse tumor growth

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Background: Our previous studies demonstrated that anti-cytokine active immunization successfully modified tumor microenvironment and promoted antitumor immune responses. Recently, it was reported that immunogenic cell death (ICD) of tumor cells could release “find me” and “eat me” signals and present multiple tumor antigens, indicating that ICD induction may represent a possible immunotherapy strategy getting over tumor heterogeneity.
Objective: This study is sought to investigate the therapeutic potentials of combining anti-cytokine immunization with ICD induction.
Methods: Recombinant HBcAg virus-like particles (VLPs) presenting either mature IL-33 molecule or antigenic epitopes of TGF-β1, FGF-2, IL-4, or IL-13 were prepared, and then immunized in a mouse

DOI: 10.1002/eji.201970400

model of breast cancer. The mice also received ICD-induced tumor cells or intratumor delivery of chemotherapeutic drugs.
Results: Mitoxantrone, Doxorubicin , oxaliplatin, and bortezomib presented a dose-dependent tumor-killing effects in 4T1 tumor burden mice. Mitoxantrone and Doxorubicin *in vitro* induced obvious tumor cell apoptosis and expression of key ICD mediators such as CRT, ATP, and HMGB1. Immunization with ICD tumor cells significantly suppressed subsequently inoculated homologous tumor. Anti-cytokine active immunization in combination with administration of ICD cells or intratumor injection of the drugs more significantly suppressed orthotopic tumor growth and lung metastasis than single immunotherapy, evidenced by more significant tumor suppression, longer survival period, reduced Tregs and MDSCs levels, and increased Th1 and CTLs responses.
Conclusion: The results indicated that combining the modification of tumor immunosuppressive microenvironment through anti-cytokine active immunization, with the induction of antitumor cellular immunity through ICD induction, might be a promising tumor vaccine strategy.

P2457

Clinical significance of serum type III interferons in patients with gastric cancer

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IFN-α and IFN-β are currently used in the treatment of several neoplasms. Type III interferon (IFN) is a newly established IFN that shows roles different from those of type I and II IFNs. However, the effect of type III IFN on the prognosis of patients with gastric cancer (GC) is controversial. This study used LEGENDplex bead-based immunoassays to analyze the serum IFN-α, IFN-β, IFN-γ, IFN-λ1, and IFN-λ2/3 levels in 84 patients with GC and 25 healthy volunteers, and used flow cytometry to test the IFN concentrations, aimed to investigate the effects of these IFNs levels on the survival of patients with GC. Compared with the healthy controls, the patients with GC had significantly decreased serum IFN-α, IFN-γ, and IFN-λ1 levels, but significantly increased serum IFN-λ2/3 level. And patients with GC showed significant correlations of serum IFN-α, IFN-β, IFN-γ, and IFN-λ2/3 levels with clinical stages and lymph node metastasis. In addition, serum IFN-λ2/3 levels were significantly correlated with distant metastasis. Serum IFN-λ1 levels significantly correlated with tumor location, histopathology, and lymph node involvement. Furthermore, serum IFN-β and IFN-γ levels were also significantly correlated with Lauren classification. The univariate analysis revealed that high IFN-λ2/3 level, tumor sizes of >5 cm, and higher clinical stage were significantly associated with poor prognosis. This study demonstrates the relationship between serum IFN level and progression of GC. Elevated levels of serum IFN-λ2/3 were associated with poor prognosis and may serve as a novel biomarker for assessing GC progression and predicting non-metastatic GC outcomes.

P2458

Changes in cytokines and chemokines in imflammtion cells after nickel treatment may be caused by methylation of RNA

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Objective: The study is to investigate the biological effects caused by nickel treatment.
Methods: In vitro, cytokines and chemokines (IL-1α, IL-1β, IFN-g, IL-6, IL-18, TNF-α, IFN-β, CXCL1,

DOI: 10.1002/eji.201970400

GSDMD, MIKL) were detected in nickel-treatment cultured cells at different time points. Cells were incubated with different concentrations of nickel for 24 h, and the total RNA methylation M1A was detected. In vivo, nickel exposure was used to make a mouse dermatitis model. Blood from the abdominal aorta and skin tissue homogenate was collected.The inflammation panel with item IL-23, IL-1α, IFN-γ, TNF-α, MCP-1, IL-12p70, IL-1β, IL-10, IL-6, IL-27, IL-17A, IFN-β, GM-CSF was detected by flow cytometry, and the level of CXCL1 in serum and skin homogenate supernatant was detected by ELISA. The total RNA methylation M1A in inflammation cells was detected.
Results: Compared with the control group, changes in cytokines and chemokines in vitro cultured cells generally occurred 24 h after nickel treatment. The total RNA methylation M1A of the cultured cells was decreased in a dose-dependent manner with different concentrations of nickel. The levels of IL-6 and MCP-1 in serum the model group were lower than control ($P < 0.05$), but the CXCL1 in serum and skin homogenate supernatant was higher than control ($P < 0.05$).
Conclusion: Nickel treatment caused changes in cytokines, chemokines, methylation of RNA. It suggests that changes in cytokines and chemokines caused by nickel treatment may be due to methylation of RNA.

P2459

CCL2 rs1024610 polymorphism can affect susceptibility to hepatitis C virus

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Background: An effective immune response to hepatitis C virus (HCV) infection requires efficient recruitment and activation of inflammatory cells to the liver, the site of infection. Chemokines are critically involved in this process, since they exert both chemotactic and immunoregulatory actions. The most prominent chemokines in HCV infection are the CC and CXC families, and they are highly expressed and associated with the progression of HCV infection. The SNP rs1024610 has been demonstrated to determine the serum concentration of CCL2 belonging to the CC family.
Aims: To determine the association between CCL2 rs1024610 polymorphism and hepatitis C virus susceptibility.
Methods: We collected blood samples from 250 HCV-infected patients and 299 blood donors as control subjects. Genotyping of CCL2 rs1024610 polymorphism was performed using TaqMan probes method. Comparisons between HCV-infected patients and control subjects were assessed using χ^2 test (or Fisher's exact test).
Results The genotype frequencies of AA, AT and TT were 83.2%, 16.4% and 0.4% in the patient group, and 89.3%, 9.7% and 1.0% in the control group, respectively. Three different genotypes of rs1024610 between HCV-infected patients and the control group were significant (P=0.048). Moreover, P values of AA vs. AT and (AT+TT) vs. AA models were 0.020(OR=0.551; 95% CI=0.331-0.917), and 0.037(OR=1.685; 95% CI=1.028-2.762), respectively.
Conclusion: Our result indicates that CCL2 rs1024610 polymorphism can affect susceptibility to HCV and the AA of rs1024610 may be a protective genotype against development of HCV.

DOI: 10.1002/eji.201970400

P2460

IL-36β promotes CD8⁺ T cell activation and antitumor immune responses by activating mTORC1

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Cytokine-amplified functional $CD8^+$ T cells ensure effective eradication of tumors. Interleukin 36α (IL-36α), IL-36β, and IL-36γ share the same receptor complex, composed of the IL-36 receptor (IL-36R) and IL-1RAcP. Recently, we revealed that IL-36γ greatly promoted $CD8^+$ T cell activation, contributing to antitumor immune responses. However, the underlying mechanism of IL-36-mediated $CD8^+$ T cell activation remains understood. In the current study, we proved that IL-36β had the same effect on $CD8^+$ T cell as IL-36γ, and uncovered that IL-36β significantly activated mammalian target of rapamycin complex 1 (mTORC1) of $CD8^+$ T cells. When mTORC1 was inhibited by rapamycin, IL-36β-stimulated $CD8^+$ T cell activation and expansion was drastically downregulated. Further, we elucidated that IL-36β-mediated mTORC1 activation was dependent on the pathway of phosphatidylinositol 3 kinase (PI3K)/Akt, IκB kinase (IKK) and myeloid differentiation factor 88 (MyD88). Inhibition of PI3K or IKK by inhibitor, or deficiency of MyD88 respectively suppressed mTORC1 signal, causing arrest of $CD8^+$ T cell activation. Additionally, it was validated that IL-36β significantly promoted mTORC1 activation and antitumor function of $CD8^+$ tumor-infiltrating lymphocytes (TILs) *in vivo*, resulting in inhibition of tumor growth and prolongation of survival of tumor-bearing mice. Taken together, we substantiated that IL-36β could promote $CD8^+$ T cell activation through activating mTORC1 dependent on PI3K/Akt, IKK and MyD88 pathways, leading to enhancement of antitumor immune responses, which laid the foundations for applying IL-36β into tumor immunotherapy.

P2461

MTB Driven B cells producing IL-35 and secreting high level of IL-10 in the patients with active pulmonary tuberculosis

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Regulatory B (Breg) cell have critical roles as negative regulators of immunity, mainly due to it secret high level of interleukin 10 (IL-10). Recently, a new subset of Breg cells also identified as a key source of IL-35, which is an immunosuppressive cytokine and conventionally thought to be secreted by regulatory T (Treg) cells. Our previous study showed that the level of IL-35 in serum elevated in the patients with active tuberculosis (ATB). However, none of the studies reported that IL-35 is secreted by B cells in ATB patients. In current study, we found that the mRNA expression of the both subunits (*p35* and *Ebi3*) of IL-35 in purified B cells in peripheral blood samples increased in ATB patients. *Mycobacterium tuberculosis* (MTB) lysate stimulation assay also demonstrated higher levels of IL-35 within purified B cells from healthy control group (HC). Flow cytometry analysis further showed that the

DOI: 10.1002/eji.201970400

IL-35-producing B cells from ATB patients possessed stronger ablility of producing IL-10. Moreover, by using immunohistochemistry and immunofluorescence staining, we found a subset of B cells infiltrated into the tuberculous granuloma of ATB patients also could express IL-35. Taken together, IL-35-producing B cells involved in the MTB infection and may play regulatory effect during the infectious process by producing more IL-10.

P2462

Eradication of CAF-induced metastasis by targeting LincRNA HOTAIR in breast cancer

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The communication between carcinoma associated fibroblasts (CAFs) and cancer cells facilitate tumor metastasis. While the underlying the epigenetic mechanism of CAFs feed the cancer cells and the molecular mediators involved in these processes is poorly understood. Here we reported that TGF-β1 was top one highest level of cytokine secreted by CAFs as revealed by cytokine antibody array, which is a crucial mediator of the interaction between stromal and cancer cells. The activation of HOTAIR in cancer cells was required for CAF induced EMT. Most importantly, SMAD2/3/4 directly bound the promoter site of HOTAIR, located between nucleotides -386 and -398, -440 and -452, suggesting that HOTAIR was a directly transcriptional target of SMAD2/3/4. And a small compound AC1NOD4Q (ADQ) targeting HOTAIR was identified by high-throughput molecular docking-based virtual screening of the PubChem library. By binding to the specific 36G46A micro-domain of HOTAIR, ADQ efficiently abrogate the HOTAIR/EZH2 interaction and consequently inhibit tumor metastasis through Wnt/β-catenin pathway in vitro and in orthotopic breast cancer models. Our findings demonstrated that CAFs promoted the metastatic activity of breast cancer cells by activating the transcription of HOTAIR via TGF-β1 secretion, supporting the pursuit of the TGF-β1/HOTAIR axis as a target in breast cancer treatment. Our study also discover the lead compound for targeted lincRNA therapy and potentially pave the way for exploiting ADQ as a scaffold for more effective small molecule drugs.

P2463

The clinical significance of Mda-7/IL-24 and C-myb expression in tumor tissues of patients with diffuse large B cell lymphoma

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Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin´s lymphoma in adults. Mda-7/IL-24 had been identified as a differentiation inducer of B phenotype lymphoma cells. Previous studies have revealed that knockdown of C-myb also leads to the terminal differentiation of B cell lymphoma. The aim of the present study was to investigate the association between the expression of Mda-7/IL-24 and C-myb, and their prognostic significance for DLBCL patients. The tumor tissues were collected from 72 cases of DLBCL patients and detected with reverse transcription-quantitative polymerase chain reaction, western blotting and immunohistochemistry assays. The results showed that, the expression of Mda-7/IL-24 mRNA and protein was lower while the expression of C-myb was higher in DLBCL tissues, compared with the specimens of normal lymph node tissues. Furthermore, C-myb expression was negatively correlated with Mda-7/IL-24 expression at mRNA and protein levels in DLBCL tissues. The expression of Mda-7/IL-24 and C-myb in DLBCL tissues was associated with some clinicopathological parameters such as clinical stage, infiltration in bone marrow, Ki67 expression level in the tumor tissues and overall survival rates. These results indicated that low expression of Mda-7/IL-24, along with high expression of C-myb, are predictor for poor prognosis of

DOI: 10.1002/eji.201970400

DLBCL patients, suggesting that Mda-7/IL-24 and C-myb may be potential targets for clinical treatment of DLBCL.

P2464

Novel increased resistin induced by TLR4 deficiency inhibits lipopolysaccharide-induced IL-1β and TNF-αexpression in C57BL/6J mice

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As well known, the activation of Toll-like receptor 4(TLR4) with lipopolysaccharide(LPS) increases many inflammatory cytokines and TLR4 knockout inhibits their expressions. Resistin is widely recognized as a kind of adipokine and preinflammatory factor which can lead to insulin resistance and associates with the chronic inflammation of vascular system. But the effect of TLR4 deficiency on resistin expression and its clinical significances on inflammatory reaction is not clear yet. In this paper we reported that resistin expression in spleen and serum in TLR4-/- mice was significantly and consistently higher than wild-type(WT) mice by the tests of qPCR, WB and ELISA ($P< 0.05$). This expression pattern was significantly attenuated post the intraperitoneal treatment of 5 mg/kg LPS for 24 hours($P< 0.05$). But TNF-αand IL-1βdidn't increase and had no obvious reaction on LPS treatment in TLR4-/- mice. By comparing with WT C57BL/6J mice, the significant increase of RORγt, FoxP3, Tbet and GATA3 mRNA in spleen lymphocyte, and significant decrease with the stimulation of 5 mg/kg LPS for 24 hours were found in TLR4-/- mice by qPCR($P< 0.05$). 25 ug/kg recombinant human resistin treatment for 12 hours obviously deceased LPS induced IL-1β and TNF-α expression in WT C57BL/6J mice($P< 0.05$). It implied that TLR4 deficiency increases resistin expression, while resistin inhibits LPS-induced IL-1βand TNF-α expression in C57BL/6J mice.

P2465

Elevated Interleukin-35 in colorectal cancer activated JAK1-STAT1/3 signal pathway in human $CD4^+$T cell

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IL-35 was reported to be produced from a wide range of cell types, especially by regulatory T cells, and to exert immunosuppression via downstream STATx signaling pathway. In this study, we demonstrated that IL-35 expression elevated both in serum and tumor in patients with colorectal cancer. Increased IL-35 expressions in tumor adjacent tissues were associated with tumor metastasis significantly and IL-35 mainly expressed in CD4+T cell in human colorectal cancer and adjacent tissues. To determine the roles of IL-35 on immune response, we purified CD4+ CD25-T and

DOI: 10.1002/eji.201970400

incubated with IL-35. Results showed IL-35 inhibited the CD4+CD25- T effector proliferation *in vitro*, and the suppression could be restored using IL-35 neutralizing antibodies. To determine the activation of IL-35, we incubated purified CD4+T cell and CD19+B cell with IL-35 for a period of time, observed that IL-35 activated the STAT1 and STAT3 phosphorylation in CD4+T cell and not in B cell as reported. IL-35 induced positive feedback promoted more IL-35 production to involve in tumor immunity. We observed that Tregs from colorectal cancer were more capable of inducing EBI3 and P35 expression than healthy individuals. To illustrate whether IL-35 directly participated IL-35 production, we constructed EBI3 promoter luciferase reporter plasmid. The EBI3 promoter activity was higher after IL-35 stimulation compared with mock plasmid. Thus, our study indicates high IL-35 level in colorectal cancer promoted its production via STAT1 and STAT3 to suppress the T cell proliferation in the tumor immunity.

P2466

STAT3/p53 pathway activation disrupts IFN-β-induced dormancy in tumor-repopulating cells

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Dynamic interaction with the immune system profoundly regulates tumor cell dormancy. How immunological cues trigger cancer cell-intrinsic signaling pathways for their entering dormancy remains an enigma. Here, we show that IFN-β treatment induces tumor-repopulating cells (TRC) to enter dormancy through an indolamine 2,3-dioxygenase (IDO)-kynurenine (Kyn)-aryl hydrocarbon receptor (AhR)-p27 dependent pathway. Blocking this metabolic circuitry does not relieve dormancy but leads to apoptosis of dormant TRCs in murine and human melanoma models. Specifically, blocking AhR re-directs IFN-β signaling to STAT3 phosphorylation through both tyrosine and serine sites, which facilitates STAT3 nuclear translocation and subsequent binding to p53 promoter in the nucleus. Upregulation of p53 in turn disrupts pentose phosphate pathway, leading to excessive ROS production and dormant TRC death. More importantly, high expression of IFN-β is correlated with tumor cell dormancy in melanoma patients. Revelation of this previously unrecognized mechanism for controlling TRC dormancy by IFN-β provides deeper insights into cancer-immune interaction and new cancer immunotherapeutic modalities.

P2467

Hippo kinase NDR2 inhibits IL-17 signaling by promoting Smurf1-mediated MEKK2 ubiquitination and degradation

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Background: Serine/threonine kinase 38/38L (Stk38/38L), also known as nuclear Dbf2p-related kinase 1/2 (NDR1/2), belong to NDR/LATS kinase family of serine-threonine kinases protein. NDR/LATS kinase family are conserved from yeast to man, but their roles in inflammation remains largely unknown.

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DOI: 10.1002/eji.201970400

Results: We find that knockdown of NDR2 significantly increases IL-17-induced IL-6, CXCL2 and CCL20 expression in Hela and HT-29 cells. Knockdown of NDR2 markedly enhances IL-17-induced MAPK and NF-κB activation. NDR2 interacts with E3 ubiquitin protein ligase Smurf1, promotes Smurf1-mediated K48-linked ubiquitination of MEKK2 and inhibits expression of MEKK2. Consistently, knockdown of Smurf1 increases IL-17-induced IL-6, CXCL2 and CCL20 expression. On the other hand, overexpression of MEKK2 increases IL-17-induced IL-6 expression. These results suggest that NDR2 may play important roles in IL-17-associated inflammation by promoting Smurf1-mediated MEKK2 ubiquitination and degradation.
Conclusion: The present study identifies NDR2 and Smurf1 as novel negative regulators and MEKK2 as a novel positive regulator of IL-17-induced inflammatory cytokine and chemokine production. This study brings new insight into the mechanism by which IL-17 signaling is regulated, and also provides new targets for the therapy of inflammatory diseases.
Keywords: NDR2; IL-17; Inflammation; MEKK; Smurf1

P2468

DDX5 regulates IL-36-induced REG3A expression to control skin wound healing

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RNA helicase DDX5 plays an important role in RNA splicing and regulates the proliferation and migration of keratinocytes. However, whether DDX5 could regulate IL-36-induced REG3A expression in keratinocytes to promote wound healing remains unknown. To address this, we first generated *DDX5*$^{-/-}$ keratinocytes by CRISP/Cas9. The deficiency of DDX5 in keratinocytes reduced the expression of REG3A induced by IL-36γ. *In vitro* keratinocyte scratch assay showed that IL-36γ failed to induce the proliferation and migration of *DDX5*$^{-/-}$ keratinocytes while the administration of REG3A could restore this event. Consistently, the specific ablation of DDX5 in murine keratinocyte delayed wound healing in the skin of mice. Mechanistically, DDX5 regulated IL-36γ-induced REG3A pre-mRNA splicing to generate different REG3A mRNA variants. The deficiency of DDX5 in keratinocytes led to decreased expression of *REG3A* mRNA variant 1&3 but increased expression of REG3A mRNA variant 2, thus resulting in decreased production of REG3A. Altogether, our data demonstrate that **DDX5 is involved in IL-36γ-regulated REG3A expression. Impaired DDX5 production leads to decreased REG3A expression and delayed wound healing.** Our findings reveal a new mechanism of the regulation of REG3A by DDX5, and provide a potential therapeutic target for treatment of diabetic wounds.

P2469

Tumor-derived exosomes induce fibroblasts differentiation and establish an immunosuppressive microenvironment

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The effect of tumor-derived exosomes on fibroblasts play a critical role in facilitating cancer progression. Recent studies have demonstrated that tumor-derived exosomes promote tumor progression. However, little is known regarding the role of exosomes in fibroblasts activation. To investigate whether lung tumor cell-secreted exosomes could mediate normal fibroblasts polarized to tumor-associated fibroblasts (TAFs) and further contribute to tumor growth, we isolated exosomes from Lewis lung carcinoma (LLC) cells by ultracentrifugation, followed by stimulating immortalized mouse embryonic fibroblasts (iMEF). We found that LLC cell-derived exosomes could be phagocytized

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by fibroblasts. A cytokine array was used to identify the production of inflammatory cytokines in exosome-induced fibroblasts, we found that exosomes induced high expression of CCL1 while reduced expression of CCL2 in fibroblasts, thus leading to the differentiation from T cells to regulatory T cells. This processes established an immunosuppressive microenvironment thus promoting cancer progression. Current results indicated that LLC-derived exosomes could mediate the intercellular communication between tumor cells and fibroblasts, which has a crucial effect on the microenvironment and may provide potential therapeutic target in the treatment of lung cancer.

P2470

Molecular identification and antiviral function of the guanylate-binding protein (GBP) genes in the Chinese tree shrew (*Tupaia belangeri chinesis*)

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Following viral detection and interferons (IFNs) production, several hundreds of IFN-stimulated genes (ISGs) are subsequently induced to act as direct antiviral effectors or regulators of the IFN signaling. The guanylate-binding protein (GBP) family belongs to IFN-inducible GTPases defending the host against a diverse group of invading pathogens such as parasites, bacteria and viruses. The Chinese tree shrew (*Tupaia belangeri chinese*) has been increasingly used as an alternative experimental animal to primates in studying viral infectious diseases. Hitherto, the tree shrew GBP family has not been characterized. In this study, we identified five tree shrew *GBP* genes (*tGBP1*, *tGBP2*, *tGBP4*, *tGBP5* and *tGBP7*) and characterized their antiviral activities. All these *tGBPs* were ubiquitously expressed in heart, spleen, intestines, kidney, liver, lung and brain tissues of the tree shrew. IFN-γ treatment of tree shrew primary renal cells (TSPRCs) significantly induced the mRNA expression of *tGBPs*. Infections with Newcastle disease virus (NDV), encephalomyocarditis virus (EMCV) and type 1 herpes simplex virus (HSV-1) enhanced *tGBPs* mRNA expression in TSPRCs, but had no effect on the localization of tGBP proteins in the cytoplasm. tGBP1, but not the other four tGBPs, showed antiviral activity against vesicular stomatitis virus (VSV) and HSV-1 infections in TSPRCs. Mechanistically, tGBP1 inhibited VSV replication via suppressed the viral transcription. Taken together, this study provided the first-hand information of the GBP family members in the Chinese tree shrew, which might assist the development of tree shrew animal model for infectious diseases.

Complement and Receptors

O177

The bona fide structure of IgM pentamer and its binding mode with AIM/CD5L molecule

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Soluble IgM forms a predominantly pentameric complex that also contains a small polypeptide J chain. While the IgM pentamer has various immune functions to defend against foreign pathogens, it also behaves as a carrier of the circulating apoptosis inhibitor of macrophage (AIM, also called CD5L). The binding to IgM pentamer protects AIM from renal excretion, leading to high serum AIM levels. When AIM dissociates from IgM, it becomes functionally active and facilitates the repair of a variety of diseases including acute kidney injury and hepatocellular carcinoma. Since the precise manner of

DOI: 10.1002/eji.201970400

AIM-IgM pentamer binding remains unknown, we examined the structure using a latest single-particle negative-stain electron microscopy. Surprisingly, the IgM-Fc pentamer shapes one-piece-missing hexagon containing one large gap, which is markedly different from the textbook prediction of a symmetric pentagon model. A single AIM molecule stably fits into the gap, cross-bridging two IgM-Fc through a disulfide bond and a charge-based interaction. We also found that the association of AIM with IgM pentamer not only inactivates AIM but also progresses inflammatory immune-complex formation in certain autoimmune diseases such as IgA nephropathy. Thus, our current findings of the *bona fide* structure of the AIM/IgM pentamer complex provides an important insight into the releasing mechanism of AIM from IgM, which could be the basis for the development of new therapeutic strategies against multiple diseases, via the increase of active AIM as well as the disruption of immune-complex formation.

O178

N-terminal region of Plasmodium falciparum circumsporozoite protein mediates immune evasion by hijacking a complement inhibitor

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RTS,S is the only licensed vaccine for malaria, but with limited efficacy. In the present study we have looked for possibilities, how the efficacy of this *Plasmodium falciparum* circumsporozoite protein (CSP) -based vaccine could be improved. It is known that pathogens have developed mechanisms to escape immune attack, especially the complement system. Hijacking inhibitors of complement from the blood circulation is the pathogens' common evasion tactic to inhibit complement activity. Here, we report hijacking of complement C4b binding protein (C4bp), the regulator of the antibody-mediated classical pathway of complement activation, by the sporozoite stage of the *P. falciparum* parasite. Four consecutive peptides from the N-terminal region upstream of the NANP repeats of *P. falciparum* CSP bound to C4bp in membrane-bound peptide arrays (PepSpots). A biotinylated peptide covering the same area on the N-terminal CSP (N-termCSP) region pulled down C4bp from human serum. A recombinantly expressed N-termCSP also bound to C4bp. The recombinant protein inhibited binding of C4bp to the biotinylated peptide. C4bp also bound to the surface of live sporozoites. In conclusion, the binding site of the complement inhibitor C4bp to the sporozoite surface was mapped to the N-terminal region of CSP that is missing from the RTS,S malaria vaccine. The inability of the vaccine-mediated immunity to neutralise this important immune evasion region of CSP may explain the limited efficacy of the RTS,S vaccine. Incorporating this functional N-terminal domain of CSP into the vaccine formulation could increase its efficacy.

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DOI: 10.1002/eji.201970400

O179

Complement C5a receptors, C5aR1 and C5aR2, mediate opposing pathologies in a mouse model of melanoma

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The canonical complement C5a receptor (C5aR1) has well described roles in tumorigenesis, but the contribution of the second receptor, C5aR2, is unclear. The present study demonstrates that B16.F0 melanoma cells express mRNA for both *C5aR1* and *C5aR2,* albeit at much lower levels than the murine macrophage cell line, J774. Although B16.F0 cells signal through ERK and p38 mitogen-activated protein kinases in response to C5a, the anaphylatoxin had no impact on melanoma cell proliferation or migration *in vitro*. In accord with previous reports in other murine tumour models, B16.F0 melanoma development and growth was significantly reduced in C5aR1$^{-/-}$ mice or wild-type mice treated with the C5aR1 antagonist, PMX53. In contrast, tumour growth was significantly enhanced in C5aR2$^{-/-}$ mice. Analysis of tumor-infiltrating leukocyte populations showed no significant differences between wild-type and C5aR2-/- mice. Conversely, percentages of myeloid derived suppressor cells (MDSC), macrophages and regulatory T lymphocytes (Tregs) were lower in tumors from C5aR1-/- mice, whereas total (CD3^{+}) T lymphocytes and CD4^{+} subsets were higher. Analysis of cytokine/chemokine levels showed plasma IFNγ was higher and tumor CCL2 lower in the absence of C5aR1.
The results suggest that C5aR1 signalling supports melanoma growth by promoting infiltration of immunosuppressive leukocyte populations into the tumor microenvironment, whereas C5aR2 has a more restricted, but beneficial role in limiting tumor growth. Overall, these data support the potential of C5aR1-inhibitory therapies for melanoma.

O180

Interplay of leucocyte membrane complement regulatory protein MCP with cytokines ,CIC and C3 in the pathophysiology of rheumatoid arthritis

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Earlier studies suggested disease related modulation of leukocyte CR1, CD59 and DAF in RA suggesting their disease modulating role. Here, we report the modulation of leukocyte membrane cofactor protein(MCP),a complement regulatoy protein and its relations with the cytokine profiles and path-physiology of RA. One hundred healthy volunteers and 100 DMARD and Corticosteroid naive patients diagnosed at AIIMS hospital with active RA were enrolled for retrospective and prospective studies(0, 3 and 6 months,n-38;12 months,n=17 of treatment). The levels of MCP transcript,cell surface proteins, cytokine transcripts (TNF-α, IL-6, IL-17A, IL-18, IFN-γ,IL-10 ,TGF-β) were determined in total leukocytes, PBMCs and Neutrophils from the study subjects. The CIC, complement proteins and CRP were determined in serum.Flowcytometery,RTPCR,Nephelometry,ELISA,Spectrophotometry were used as relevant. In patients, the level of surface MCP expression declined significantly in the order of lymphocytes (p = 0.023) > monocytes (p = 0.036). The expression of PBMC-MCP transcripts were significantly lower (p=0.02). Levels of MCP transcript increased in early responder and correlated negatively with DAS28, CIC and C3 Significant decreases in the levels of IFNg and IL10 transcripts with increases in other cytokines were observed.IL17A and Immune complexes down regulated the expression of MCP, IFNγ upregulated the same in culture. To conclude, we found a significant negative correlation and negative

DOI: 10.1002/eji.201970400

modulation of MCP with increasing disease activity and vice versa. Correlation and culture studies with cytokines suggest a concerted impact of MCP and cytokines in determining the pathophysiology and prognosis of RA.Upregulation of MCP may serve as a therapeutic strategy.

P0045

Complement deposition in renal histopathology of patients with diabetic nephropathy

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Aims: The potential role of complement system in diabetic nephropathy (DN) is increasingly reported. The current study aimed to investigate the deposition in renal histopathology of C1q and C3c, as well as their association with clinical and pathological parameters in DN patients.
Methods: Renal biopsy specimens from 161 DN patients were investigated using direct immunofluorescence, light and electron microscopy. For direct immunofluorescence, the staining of C1q and C3c on fresh frozen renal tissues was performed immediately after renal biopsy. Complement deposition was defined as the presence of C1q or C3c for at least 1+ in a 0~4+ scale. The association between complements deposition and clinico-pathological data was analyzed.
Results: In direct immunofluorescence microscopy, C1q and C3c could be detected in specimens of 44/161 (27.3%) and 89/161 (55.3%) patients. For clinical data, patients with C1q deposition had significantly higher levels of urinary protein (7.25±4.20 g/24h vs. 4.97±3.76 g/24h, $P< 0.01$) and significantly lower estimated glomerular filtration rate (eGFR) (34.16±25.21 mL/min/1.73m^2 vs. 51.17±31.56 mL/min/1.73m^2, $P< 0.01$). Patients with C3c deposition had significantly lower eGFR than those without (40.09±27.97 mL/min/1.73m^2 vs. 54.48±32.49 mL/min/1.73m^2, $P< 0.01$). For renal histopathology, patients with C1q deposition had significantly higher interstitial fibrosis and tubular atrophy (IFTA) score, higher interstitial inflammation score, higher vascular lesions score (all P values< 0.05). Patients with C3c deposition had significantly higher IFTA score, higher proportion of global sclerosis ($P< 0.01$, $P< 0.01$).
Conclusions: Complement deposition of C1q and C3c in renal histopathology was associated with more severe kidney damage in diabetic nephropathy.

P0046

Role of glycosylation in the interaction between uromodulin and lectin pathway initiated molecules

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Background: Uromodulin (UMOD) is a glycoprotein rich of carbohydrate contents. Initiated molecules of lectin pathway, including MBL, ficolins and collectin, can recognizing carbohydrates. We hypothesized that UMOD could bind with initiated molecules of lectin pathway and influence complement activation.
Methods: Hemolytic assay was used to explore the effect of UMOD on lectin pathway activation. Binding affinity of uromodulin and de-glycosylated uromodulin (dUMOD) with MBL, collectin11 and ficolins were probed by ELISA. Glycosylation in acute kidney injury (AKI) was detected with MALDI-TOF MS and lectin array chip.
Results: Hemolytic assay showed that UMOD inhibited activation of complement lectin pathway in dose dependent manner. Uromodulin could bind to MBL, collectin 11 and ficolins. The binding of UMOD with ficolins was stronger than MBL and collectin 11. The binding strength decreased between

DOI: 10.1002/eji.201970400

dUMOD and CL-11/ficolins, but increased between dUMOD and MBL, compared with normal UMOD. Both MS and lectin array analysis detected more amount of high mannose and core fucosylation in patients with AKI when compared with normal controls.
Conclusion: UMOD could bind to lectin pathway initiated molecules, and inhibited the complement activation and the interaction was changed by abnormal glycan structure of UMOD. Similar abnormal glycosylation of UMOD could be observed in patients with acute kidney injury, indicating an important role of UMOD glycosylation during acute kidney injury.

P0047

Complement activation in patients with diabetic nephropathy

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Aim: The current study aimed to investigate the levels of various complement components in plasma and urine of diabetic nephropathy patients, and their correlation with clinicopathological parameters was studied.
Methods: Sixty-eight biopsy-proven diabetic nephropathy patients with plasma samples were recruited, among whom, urine samples were available in fifty patients. Seven complement components including C1q, MBL, Bb, C4d, C3a, C5a, soluble C5b-9 were measured by enzyme linked immunosorbent assay. Associations between levels of complement components and clinicopathological parameters were investigated in diabetic nephropathy patients.
Results: In diabetic nephropathy patients, the plasma levels of C1q, MBL, Bb, C4d, C3a, C5a and sC5b-9 were significantly higher than those in diabetic patients without renal involvement. The urinary levels of MBL, Bb, C4d, C3a, C5a and sC5b-9 were significantly higher than diabetic patients without renal involvement, while no significant difference was found in urinary C1q levels between these two groups. In patients with diabetic nephropathy, the urinary levels of C3a and C5a significantly correlated with serum creatinine, urinary protein and estimated glomerular filtration rate. The urinary sC5b-9 significantly correlated with urinary protein and estimated glomerular filtration rate. Besides, urinary levels of MBL, Bb and C4d significantly correlated with urinary protein. Urinary levels of C3a, C4d and Bb significantly correlated with the classification of glomerular lesions of DN.
Conclusion: In diabetic nephropathy patients, the complement system is activated; among the three complement pathways, activation of lectin pathway and alternative pathway was associated with renal damage.
Keyword: diabetic nephropathy; complement

P0631

Complement C5a/C5aR pathway potentiates the pathogenesis of gastric cancer by down-regulating p21 expression

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Although the complement C5a/C5aR pathway is suggested to play a critical role in tumor pathogenesis, the underlying mechanism has yet to be fully elucidated. In the present study, we found that in patients with gastric cancer in different clinical stages (from stage Ⅰ to stage Ⅳ, both C5aR and p-PI3K/AKT levels were significantly higher in tumoral tissues than in adjacent non-tumoral tissues. In contrast, p21/p-p21 levels were significantly lower in tumoral tissues than in adjacent non-tumoral tissues. In vitro recombinant C5a administration remarkably promoted p-PI3K/p-AKT expression, but

DOI: 10.1002/eji.201970400

inhibited p21/p-p21 expression. Blockage of C5a/C5aR signaling with a C5aR antagonist reversed the C5a-induced inhibitory effect on p21/p-p21 expression. C5a administration to cells pre-treated with a PI3K inhibitor also prevented this inhibitory effect, suggesting the involvement of the PI3K/AKT signaling pathway in C5a/C5aR-mediated suppression of p21/p-p21 expression. In vivo C5aR antagonist treatment caused significant reduction in tumor growth in mice, accompanied by a remarkable elevation in p21/p-p21 expression and reduction in p-PI3K/AKT activation. These results indicate that the C5a/C5aR pathway promotes gastric cancer pathogenesis by suppressing p21/p-p21 expression via activation of PI3K/AKT signaling.

P0632

C1q A08 is a half-cryptic epitope of anti-C1qA08 antibodies in lupus nephritis and important for the activation of classical complement pathway

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All C1q A08 antibodies isolated from ten lupus nephritis patients could not bind C1q coating on the microtitre plate and the anti-C1q autoantibodies were not able to bind to resin coupled with C1q A08 peptide. One C1q A08 mAb, 32-4, binds to six amino acids of the N-terminal, while another C1q A08 mAb, 17-9, binds to eight or ten amino acids of the C-teminal. The third and fourth C1q A08 mAb, 1A12 and 4B11, who binds to the whole sequence of C1qA08. 32-4 mAb binds to C1q coating on microtitre plate, while 17-9 mAb, 1A12 mAb and 4B11 mAb could not. However, different result was shown by biolayer interference method that is, 17-9 mAb, 1A12 mAb and 4B11 mAb could bind to C1q but 32-4 mAb could not. 1A12 mAb and 4B11 mAb were able to prevent the activation of classical complement pathway, while 32-4 and 17-9 mAb could not.
C1q A08 is a half-cryptic epitope of C1q, only part of the six amino acids is cryptic. When C1q was coated on microtiter plates, the conformation of C1q changes and several C-terminal amino acids turn cryptic which results in that most A08 antibodies can not bind to C1q except 32-4 mAb. A08 plays an important role in activation of classical complement pathway and some anti-A08 antibodies such as 1A12 mAb and 4B11 mAb are able to prevent this process.

P0633

Sialic acid on uromodulin mediated the interaction between uromodulin and complement factor H

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Uromodulin is an important protective protein during renal injury. Previous studies indicated that uromodulin acted as a molecular recognizing molecule. It can bind with complement factor H (cFH), and enhance the function of cFH as a co-factor of factor I to degrade C3b. As a highly glycosylated protein, sialic acids (SAs) are the outermost properties of the glycan chains on uromodulin. Thus we hypothesized that the interaction between uromodulin and cFH was mediated by SAs.
By using sheep erythrocytes hemolysis assay, we found that binding of uromodulin to cFH reduced the hemolysis from ~60% (0μg/ml uromodulin) to ~25% (50ug/ml uromodulin). Then, we removed SAs from uromodulin by using neuraminidase, and by using ELISA binding assay and co-factor activity assay, we found that, compared to untreated uromodulin, uromodulin lost SAs showed significantly

DOI: 10.1002/eji.201970400

reduced binding to cFH, and loss of the ability to enhance the C3b degradation. Finally, by using lectin-ELISA we detected α-2,3 SAs levels of urinary uromodulin from 36 CKD patients and 17 healthy controls, and we found that there was a significant decrease in SAs from CKD patients ($p< 0.05$). We concluded that the SAs property mediated the interaction of uromodulin and cFH, and affected uromodulin's function in inhibiting complement alternative pathway

P0634

Whole-exome sequencing detects mutations in pediatric patients with atypical hemolytic uremic syndrome in Taiwan

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Although atypical hemolytic uremic syndrome (aHUS) is a genetic disorder, molecular defects are detected in only sixty percent of patients. We aim to dissect the genetic background by whole exome sequence and the clinical characteristics of pediatric patients with aHUS. Ten patients (6 male and 4 female) with mean age 5.2±5.0 years were enrolled. The age at onset ranged from 2 days to 11 years. Eighteen different mutations (17 missense, 2 nonsense, and 11 novel) on 7 complement and 3 coagulation genes were detected in all patients. The majority of mutation was heterozygous and S1191L on *CFH* were the recurrent mutation. Sixty percent of patients had multiple genetic mutations. Nine mutations were associated with genes known to be implicated in aHUS (*CFH, CFI, CD46, CFHR5, and DGKE*), while 4 and 5 mutations were detected on complement- (*C8B, C9, and MASP1*) and coagulation-associated (*VWF* and *CD36*) genes, respectively. *CD36* may be a candidate gene act as disease modifier for aHUS through the contribution of thrombosis by impairing the interaction with TSP-1 and ADAMTS 13 shown in simulation model. Genetic defects on both complement and coagulation pathways play pathogenic roles on aHUS. *CD36* may be a novel candidate gene act as disease modifier of aHUS.

P0635

Covalent binding of complement C3 to MHC-II mediates trogocytosis between conventional dendritic cells and MZ B cells in the spleen

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Major Histocompatibility Complex class II (MHC-II) molecules in antigen presentation to CD4+ T cells and adaptive immunity have been well characterised. Complement component 3 (C3) is the central component of the complement system and essential for protective functions as part of the innate immune system. Here, we describe a new function for both molecules, namely the covalent binding of activated C3 to MHC-II on the surface of conventional dendritic cells (cDCs) in the spleen, mediating their interaction with marginal zone (MZ) B cells.
Surface expression of MHC-II in antigen presenting cells is tightly controlled by MARCH1-mediated ubiquitination. Using mice lacking this post-translational modification, and HLA-deficient human PBMCs, we observed that surface expression of MHC-II in cDCs correlates with the deposition of extracellular C3. This is due to the covalent binding of activated C3dg/C3d to the α-chain of MHC-II. Mice harbouring C3 surface-coated cDCs, observed reduced numbers of cDCs and “abnormal” MZ B cells that displayed several DC surface markers. These markers were not synthesised by the MZ B

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DOI: 10.1002/eji.201970400

cells themselves. Rather, co-culturing experiments demonstrated transfer of membrane fractions from C3-coated cDCs to B cells, a phenomenon known as *trogocytosis*. Blocking or deleting complement receptor (CR2) in splenic B cells reduced trogocytosis.
Our results reveal a fundamental new role for MHC-II and C3 in splenic cDCs, in which surface MHC-II serves as an acceptor for covalent C3 binding. The resulting MHC-II-C3 complexes in turn act as recognition ligands for MZ B cells, mediating cell-cell interactions and directional extraction of membrane proteins.

P1229

The interaction between C-reactive protein and C1q in lupus nephritis

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Systemic lupus erythematosus (SLE) is one of the most common chronic autoimmune diseases in China characterized by the production of various autoantibodies in serum and the systemic involvement of multiple organs including the kidney. Lupus nephritis (LN), a common and serious complication of SLE, is the leading cause of the secondary glomerulonephritis in China, which seriously affects the prognosis of patients with SLE. The exact immunological pathogenesis of SLE and LN is still unclear with us. Howere, it was speculated that the renal damage was attributed to the complement activation caused by the disorder of apoptotic cell clearance, which in turn formed circulating immune complex deposited in the kidney or an in situ immune complex of the kidney. Previous studies showed that complement C1q molecules played a key role in the clearance of apoptotic cells as well as immune complexes. It was also closely related to the occurrence of lupus nephritis. C-reactive protein (CRP) is a highly conserved non-specific inflammatory marker with most of its pro-inflammatory effects exerted by the form of mCRP. In the first part of this study, we identified the major recognition sites for C1q and mCRP were C1qA08 and aa.35-47 through ELISA and SPR. apoptotic cells.For the second part, the effect of aa.35-47 on the biological functions of C1q and A08 was further investigated. As the main recognition site for C1q, A08 is predicted to be the critical sequence for regulating macrophage phagocytic cells.

P1230

Tumor associated macrophages protect pancreatic cancer from complement dependent cytotoxicity by up-regulation of complement regulator CD59 via IL-6R/STAT3 signaling pathway

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Cooperation between tumor-associated macrophages (TAMs) and pancreatic cancer cells plays crucial roles to promote varieties of malignant behaviors of pancreatic cancer. CD59 is a GPI-anchored membrane protein that prevents complement activation by inhibiting the formation of the membrane attack complex, which may protect cancer cells from complement-dependent cytotoxicity (CDC). The interactions between CD59, TAMs and pancreatic cancer has not been reported. A tissue microarray of pancreatic cancer patients was constructed to evaluate the interrelationship of CD59 and TAMs and their survival impacts. The roles of TAMs to up-regulate the expression of CD59 in

DOI: 10.1002/eji.201970400

pancreatic cancer cells were demonstrated by real-time PCR, western blot and immunofluorescence staining. That CD59 protected pancreatic cancer cells from CDC was demonstrated in vitro. To explore the potential mechanisms, RNA sequencing of pancreatic cancer cells with or without co-culture of TAMs were performed and the results showed that IL-6R/STAT3 signaling pathway may participate in the regulation, which was further demonstrated by target-siRNA transfection, antibody neutralization and STAT3 inhibitors. Our data showed that the infiltration of TAMs and the expression of CD59 of pancreatic cancer paralleled and higher infiltration of TAMs and higher expression of CD59 predicted worse survival of pancreatic cancer patients. TAMs could protect pancreatic cancer from CDC by up-regulation of complementary regulator CD59 via IL-6R/STAT3 signaling pathway. These findings uncovered the novel mechanisms between TAMs and CD59, contributing to provide a new promising target for immunotherapy of pancreatic cancer.

P1231

Coupling mannose binding protein (MBL) genotyping to recurrent infections in childhood and treatment with vitamin D (VD)

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The innate and adoptive immunity are responssible for defence, control of apoptosis, antitumor protection, inflammation, tissues repair and remodelling. Dendritic cells (DC) express a variety of C-type lectins (macrophage mannose receptor, dectin-1 and several asialo-glycoprotein receptors), and Tool like receptors which presumably mediate the recognition of foreign antigens and regulate important adhesion processes. MBL and MBL cation-independent C1q/collectin receptor(s) are markedly upregulated in immature dendritic cells. MBL is characterized by being a soluble pattern recognition receptor (PRR) to recognize conserved microbial regions containing e.g. mannose, glycosamine, glucose or fucose termed pathogen associated molecular pattern (PAMP) and subsequently activates the complement system through MBL associated serine proteases (MASP-1, MASP-2 and MASP-3). The vitamin D has substantial effect on DCs in this study the effects of vitamin D is tested on various inflammation and autoimmune-like responses particularly on MBL expresion and its functional potential in complement activation. In our study, children with recurrent infectious diseases, chronic recurrent viral infections, chronic inflammatory diseases, metabolic and endocrine diseases, anemia in chronic disease, are tested for mutation/polimorfism in MBL gene (gene seqwensing) /expresion and functional studies of MBL. We measurement of serum C-reactive Protein (CRP), serum vitamin D3, MBL levels, WBC, T, NK, B cells in vitamin D treated patients. This study shown that despite the genetic lack of MBL, the treatment of D-vitamin improves the functionality of Complement, and give a better understanding and perhaps a connection between MBL correlated to chronic diseases and an effectively treatment with vitamin D

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DOI: 10.1002/eji.201970400

P1821

Genome-wide analysis of DNA methylation and serum complement component 4 in a Chinese population

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Background: Complement component 4 (C4) plays a key role in the physiological activities of the complement system, and more recent studies showed a pro-tumor effect of complement.
Objectives: We aimed to investigate genome-wide methylation in relation to serum C4 in a Chinese population.
Methods: We measured the methylation levels at > 485,000 CpG sites (CpGs) in DNA from leukocytes using the HumanMethylation450 array and conducted a genome-wide meta-analysis of DNA methylation and serum C4 in a total of 832 Chinese participants. We further evaluated the associations of C4-related CpGs with the expression of corresponding genes (< 50kbp), tumor biomarkers, and incident cancer during 8 years of follow-up.
Results: We identified 6 CpGs whose methylation levels were associated with serum C4 concentration at a genome-wide significant level (*FDR*< 0.05), and all of them annotated to cancer-related genes. Of these C4-related CpGs, cg21722170 (in the body of *TNXA* and *TNXB*) showed significant correlations with the expression of corresponding genes (including *C4A*, *RDBP*, *SKIV2L*, *STK19*) and was associated with the risk of incident cancer; cg27449793 (in the TSS200 of *CYP21A2*) showed significant correlations with the expression of corresponding genes (including *STK19* and *TNXA*); and cg08650890 (in the body of *TNXB*) was significantly associated with alpha-fetoprotein levels, which is a biomarker of multiple malignant tumors.
Conclusions: We identified novel DNA methylation loci associated with serum C4 that could serve as candidate loci for future researches, including 2 loci that involved in tumor biomarker or incident cancer.

P1822

The imbalance in the complement system and its possible physiological mechanisms in patients with lung cancer

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The clinical and experimental evidences for complement-cancer relationships are solid, whereas an epidemiological study reporting the imbalance of complement system in patients is still lacking.Using publicly available databases, we jointly compared the levels of complement components in plasma and lung cancer tissues. Using paired co-cultures of human normal QSG-7701 hepatocytes with lung cancer cells (A549, LTEP-α-2 or NCI-H1703) or human normal bronchial epithelial (HBE) cells, we examined the effects of lung cancer cells on complement synthesis and secretion in QSG-7701 hepatocytes. An integrated analysis of transcriptome and proteome datasets from 43 previous studies revealed lower mRNA and protein levels of 25 complement and complement-related components in lung cancer tissues than those in normal control tissues, while the levels of complement protein in plasma of patients with lung cancer were the opposite. The iTRAQ proteome study identified

DOI: 10.1002/eji.201970400

decreased and increased levels of 31 and 2 complement and complement-related proteins, respectively, in lung cancer tissues. Paired co-cultures of QSG-7701 hepatocytes with A549, LTEP-α-2, NCI-H1703 or HBE cells indicated that lung cancer cells increased complement synthesis and secretion in QSG-7701 cells compared to HBE cells.The possible mechanism of the imbalance may be associated not only with the decreased complement levels in lung cancer tissues but also the concurrent lung cancer tissue-induced increase in hepatocyte complement synthesis and plasma secretion in patients. And the imbalance should be accompanied by a suppression of complement-dependent immunity in lung cancer tissues coupled with a burden of complement immunity in the circulation of patients.

P1823

Hypoxia promotes C5a-associated inflammation in rheumatoid arthritis

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Hypoxia-inducible miRNAs (hi-miR) are identified in hypoxia-associated physiological and pathological processes. Hypoxia plays a pivotal role in the pathogenesis of several inflammatory diseases, particularly rheumatoid arthritis (RA). However, the roles and underlying mechanisms of hi-miR in autoimmune diseases remain poorly understood. Considering that synovial dendritic cells (DCs) and C5a-associated inflammation are critical for RA pathogenesis, here we investigated what hi-miR regulated C5a-mediated inflammatory responsiveness of DCs. **Hypoxia upregulated the expression of C5aR1 on DCs from human monocytes, consequently promoting C5a-mediated inflammatory responsiveness of DCs**. miR microarrays have identified several hypoxia-upregulated miRNAs, some of which are reported to be involved in the pathogenic process of RA. Blocking their expression by miRNA inhibitors, three of them are responsible for the increased expression of C5aR1 by hypoxia, among which the abundance of miR-x is highest. The onset and severity of RA models are markedly attenuated by blockade of miR-x by antgomir or in miR-$x^{-/-}$ mice. So, **miR-x is a hypoxia-regulated RA-associated miRNA**. RNA-seq and miRNA pulldown assays indicate **miR-x enhances the C5aR1 expression via promoting the transcription of the *C5aR1* gene and stabilizing the C5aR1 mRNA**. So, our study identifies hypoxia-inducible miR-x as an important mediator for DC and C5a-associated inflammatory pathogenesis in autoimmune diseases.

P2471

Polymorphism of mannan-binding lectin-associated serine protease 2 genes (MASP-2) in Zhangjiakou population

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Mannan-binding lectin-associated serine protease 2 (MASP-2) plays a critical role in the lectin pathway of complement. Polymorphisms of MASP-2 gene are closely associated with MASP-2 levels, as well as the risk for inflammatory disorders and infections. In this study, DNA sequencing and polymerase chain reaction (PCR) were used to investigate single nucleotide polymorphisms (SNPs) and frequency in the whole 11 exons fragments of MASP-2 genes among 301 subjects in the Zhangjiakou area, China. The results showed that 7 already reported SNP were observed, which all mutation type were substitutions, include 3 SNP were synonymous: rs201972294 (p.A169A mutation, 1/301, 0.33%), rs12142107 (p.A297A mutation, 51/301, 16.94%) and rs1782455 (p.S493S mutation, 297/301, 98.67%), other 4 SNP were missense: rs2273343 (p.H155R mutation, 23/301, 7.64%),

DOI: 10.1002/eji.201970400

rs12711521 (p.D371Y mutation, 263/301, 87.38%), rs2273346 (p.V377A mutation, 110/301, 36.54%) and rs144471433 (p.Q577H mutation, 26/301, 8.64%). Didn't find any to be homozygous for the mutant allele rs201972294; Wild type, heterozygous and homozygous were exist simultaneously at other 6 SNP. From the exon region, all mutations we found mainly exist in the exon 4, 7, 9 and 11. In the Zhangjiakou area, a total of seven SNPs were detected, did not find unknown mutations. The influence of these mutations on MASP-2 function and the relationship between them and different disease are still not sure.

P2472

Study on the subsets of monocytes in preeclampsia

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Objective: To study the changes of monocyt subsets in preeclampsia.
Methods: 26 women with preeclampsia in Shengjing hospital of China medical university, were selected from February to October in 2017. 23 normal pregnant women were selected over the same period as the control group. The subsets of monocytes (classical mononuclear cells $CD14^{++}CD16^{-}$, intermediate monocytes $CD14^{++}CD16^{+}$, and non-classical monocyte $CD14^{+}CD16^{++}$ were detected by flowcytometry. the expression and change of Integrin alpha 2(CD49b) and intercellular adhesion molecule -1 ICAM-1(CD54) on them was checked . The experimental results were analyzed by SPSS software package.
Results: 1. The percentage of peripheral blood monocytes in patients with pre-eclampsia 6.92±2.3% was increased compared with normal pregnant women 5.62±1.7%, t=2.207, P< 0.05; The classical mononuclear cells $CD14^{++}CD16^{-}$ level in patients with preeclampsia 42±15.4% was decreased significantly compared with those in normal pregnancy women63.7±12.8%（t=5.269, P< 0.01）; there was no significant difference in the intermediate monocytes $CD14^{++}CD16^{+}$ level in patients with preeclampsia 9.0±9.0% and normal pregnant women 10.2±10.2%,t=-1.014, P>0.05,; The non-classical monocyte $CD14^{+}CD16^{++}$ in patients with preeclampsia was 45.0± 16.1%, increased significantly compared with those in normal pregnancy women26.2± 12.5%, t = 4.516, P < 0.01; There was no significant difference in the expression of CD54 and CD49 in the monocyte subsets in preeclamptic patients and normal pregnant women.
Conclusion: The level of mononuclear cells in peripheral blood of preeclampsia patients was increased, and changes in monocyte subset may be involved in the pathgenesis of preeclampsia

P2473

Stundy of molecular mechanisms on cluster of differentiation 59 GPI by LAT in promoting Jurkat cell signal transduction

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Cluster of differentiation 59 (CD59) is a glyco-sylphosphatidylinositol-anchored protein. Cross-linking of CD59 with specific monoclonal antibodies can cause a series of intracellular signal transduction

DOI: 10.1002/eji.201970400

events. However, the under-lying molecular mechanisms are poorly understood. Linker for activation of T-cells (LAT) is a crucial adaptor protein in T-cell signaling, and its phosphorylation and palmitoylation are essential for its localization and function. In a previous study by the present authors, it was demonstrated that CD59 may be responsible for LAT palmitoylation, there by regulating T-cell signal transduction. The present study detected the co-localization of LAT and CD59 in lipid rafts by transfecting Jurkat cells with lentivirus vectors carrying the LAT-enhanced green fluorescent protein fusion protein. In addition, LAT and CD59 were shown to have a synergistic effect on the prolif- eration of Jurkat cells. The results also indicated that CD59 may transfer the palmitate group from phosphatidylinositol to LAT to form LAT palmitate, which then localizes to lipid rafts to regulate T-cell activation. The results of the present study provided novel insights into the role of CD59 in T-cell signal transduction.

P2474

Associations of erythrocyte complement receptor type 1 and single nucleotide polymorphisms of its gene with hepatocellular carcinoma

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In this study, we investigated the association of erythrocyte complement receptor type 1(CR1) and single nucleotide polymorphisms(SNP) of its gene with hepatocellular carcinoma(HCC). The CR1 on erythrocytes from 98 healthy persons and 102 HCC patients was measured by flow cytometer, and genotyping with five tag SNPs from CR1 gene was done by the GenomeLab™ SNPstream® 12-plex/48-plex genotyping system. The CR1 levels between HCC group and control group were compared with the CHISS 2005 software. The SPSS V 13.0 software and Haploview V4.1 software was used for the inter-group distributional differences comparison of genotypes, alleles and halotypes of SNPs between SLE group and control group, respectively. The odds ratios (OR) and 95% confidence intervals (CI) for carriers vs. non-carriers of genotypes, alleles or halotypes were calculated. The geometric mean fluorescence intensity ratio of CR1 (CR1-GMFIR) in HCC patients was lower than that in controls(P< 0.01). There were significant associations between SNP rs4844600G>A and HCC(P< 0.01). When compared with noncarriers, the risk of developing HCC of carrier of CR1-rs4844600G>A/GG genotype increased by 2.458 folds (95%CI: 1.357-4.451) and that of carrier of G allele increased by 1.945 folds (95% CI:1.183-3.199) . While that of carrier of GA genotype decreased by 0.404 folds (95%CI: 0.218-0.746). The other 4 SNPs, rs17048010T>C, rs3818361C>T, rs11118167T>C and rs9429945C>T, and rs11118167-rs3818361-rs17048010/TCT, TTC, CCT and TTT haplotypes were not associatied with HCC(P>0.05). In conclusion, the CR1 level decreases in patients with HCC. SNP rs4844600G>A of CR1 gene is associated with HCC.

DOI: 10.1002/eji.201970400

P2475

Circulating heme oxygenase-1 and complement activation in transplant-associated thrombotic microangiopathy

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Objective: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe complication in patients following hematopoietic stem cell transplantation (HSCT), which is associated with endothelial injury. However, the pathogenesis of TA-TMA is still unclear. In this study, we explore whether heme oxygenase-1 (HO-1) is deficiency in the plasma of TA-TMA patients.
Methods: Fifteen patients diagnosed as TA-TMA at the First Affiliated Hospital of Soochow University from June 2011 through December 2016 were enrolled in our study. We also enrolled 75 matched participants, including 15 patients with III-IV grade graft-versus-host disease (GVHD), 15 patients with severe infections after HSCT, 15 patients with no complications after HSCT, 15 healthy subjects, which were negative controls, and 15 patients with myocardial infarction (MI), which were positive controls. Plasma HO-1 concentrations in patients with various diseases were detected by ELISA. HUVECs incubated with TA-TMA plasma was as a vitro model. We explored the level of decay-accelerating factor (DAF) and complement in HUVECs incubated with TA-TMA plasma.
Results: Firstly, plasma levels of HO-1 in these 15 TA-TMA patients were significantly lower than the controls after HSCT. Of course HO-1 in MI patients were lower than healthy controls.
Secondly, cell-surface DAF level was decreased in TA-TMA plasma treated cells. Moreover, C3 deposition was increased in the HUVECs incubated with the TA-TMA plasma.
Conclusions: Comparing with other complications, plasma level of HO-1 in TA-TMA patients is deficiency. Perhaps, it may be used for diagnosed. DAF level was decreased in TA-TMA plasma treated cells. It may reveal a new pathway of TA-TMA.

Inflammasome and Immunity

O060

The UAF1 deubiquitinase complexes promote NLRP3 expression and enhance NLRP3 inflammasome activation

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NLRP3 inflammasome is a multimolecular complex, which comprises NOD like receptor NLRP3, an adapter protein ASC and an effector pro-caspase-1, and play fundamental roles in inflammation. Aberrant NLRP3 inflammasome activation is involved in many types of diseases, such as infectious diseases, gout, type 2 diabetes, atherosclerosis, Alzheimer's disease and cancer. Therefore, NLRP3 inflammasome activation should be tightly regulated. Ubiquitination is a key post-translational modification in regulating NLRP3 inflammasome activation. However, the roles and molecular mechanisms of K48 deubiquitination of NLRP3 in NLRP3 inflammasome activation are unclear. In the present study, we show that UAF1 facilitates NLRP3 inflammasome activation by recruiting the deubiquitnase USP1, USP12 and USP46. UAF1/USP1 complex interacts with NLRP3, removes its

DOI: 10.1002/eji.201970400

K48-linked polyubiquitination, and stabilizes NLRP3 protein. UAF1/USP12 and UAF1/USP46 complexes interact with p65, inhibits its ubiquitination and degradation, and then promotes NF-κB activation. Concordanatly, UAF1 deficiency inhibited NLRP3 inflammasome activation and ameliorated NLRP3-dependent inflammation in vivo. Thus, the UAF1 deubiquitinase complexes are indispensable for NLRP3 expression and license subsequent NLRP3 inflammasome activation. Our results uncover a novel mechanism of NLRP3 activation and suggests a new approach for modulating NLRP3-dependent immunopathologies.
Keywords: NLRP3, inflammasome, inflammation, ubiquitination, UAF1

O061

A novel pharmacological role of ticagrelor: controlling NLRP3 inflammasome activation independent of the $P2Y_{12}$ signaling pathway

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Ticagrelor was the first reversibly-binding oral $P2Y_{12}$ receptor antagonist to inhibit platelet activation in patients with coronary artery disease, which was preferred over numerous antiplatelet agents and recommended as first-line antiplatelet treatment by clinical guidelines, whereas other pharmacological functions of ticagrelor remain poorly understood. The NLRP3 inflammasome plays a protective role in the innate immunity system, while its excessive activation also contributes to the pathogenesis of complex diseases such as atherosclerosis and acute myocardial infarction. It is unclear whether ticagrelor inhibits the NLRP3 inflammasome activation in addition to its classical antiplatelet properties. Herein, we revealed that ticagrelor showed potent and rapid inhibitory effect on the NLRP3-inflammasome-mediated IL-1β secretion in macrophages. Interestingly, **our study demonstrated that ticagrelor controlled activation of the NLRP3 inflammasome via blocking the formation of ASC specks independent of the $P2Y_{12}$ signaling pathway.** Furthermore, experiments on lipopolysaccharide-induced sepsis and alum-induced peritonitis in mice both confirmed that ticagrelor significantly mitigated the severity of systemic inflammation beyond $P2Y_{12}$ receptor antagonism. Most importantly, oral treatment with ticagrelor (90 mg twice daily) for 3 days rapidly and strongly regulated NLRP3 inflammasome activation in peripheral blood mononuclear cells from patients with coronary artery disease. **Taken together, our study discovers a novel function of ticagrelor in addition to its classical antiplatelet properties, which suggest that ticagrelor may serve as a potential therapeutic agent for NLRP3-associated syndromes.**

DOI: 10.1002/eji.201970400

O062

Galectin-3 promotes non-canonical inflammasome activation through LPS glycans recognition

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Pyroptosis is a typical inflammatory form of immune cell death coordinated by inflammatory caspases. The various stimuli have been well elucidated in canonical inflammasome signaling pathway via caspase-1, the mechanism in non-canonical signaling pathway mediated by caspase-4/5/11 is still poorly understood. Lipopolysaccharides (LPS) have been shown to induce non-canonical inflammasome activation by binding to caspase-4/5/11 through its lipid A moiety intracellularly and causing oligomerization and activation. Galectin-3, belonging to a beta-galactoside-binding protein family, can bind to LPSs from different Gram-negative bacteria, which contain beta-galactoside-containing polysaccharide chains. Galectin-3 is known to self-associate upon binding to multivalent glycans through its non-lectin domain. We hypothesized that galectin-3 could cross-link LPS and consequently amplify LPS-induced caspase-4/5/11 oligomerization and activation. Cytoplasmic LPS induced less pyroptosis in galectin-3$^{-/-}$ BMDM compared to wild-type cells. The immunofluorescence staining indicated that LPS colocalized with galectin-3 and caspase-11 in J774 cells. Co-immunoprecipitation also confirmed the interaction between galectin-3 and caspase-11 after LPS intracellular delivery. Further, reconstitution of inflammasome system in HEK293 cells also demonstrated that galectin-3 potentiates caspase-4 complex formation. In cell-free system, we showed that galectin-3 facilitates the assembly of LPS-induced caspase-4 oligomerization and activation. To link its biological relevance, outer membrane vesicles (OMVs) were applied. *E. coli* OMVs induced less pyroptosis and IL-1 responses in galectin-3$^{-/-}$ THP-1 macrophages. In vivo study, galectin-3$^{-/-}$ mice displayed lower lethal sepsis after OMVs injection. In conclusion, galectin-3 is a novel host factor in the induction of a higher degree of caspase-4/5/11 oligomerization and activation through LPS binding, resulting in more intense pyroptosis.

O063

A zebrafish (danio rerio) model for inflammasome signaling in mycobacterial infection

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Every year over one million people die of tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis* infection. In addition, over 10 million people become infected each year, with an increasing percentage of them by multi drug resistant bacterial strains. Morbidity and mortality related to tuberculosis result from the lack of a functional vaccine as well as the requirement for extensive treatment. *M. tuberculosis* manipulates host immunity to establish itself inside the granulomas, which are the hallmark feature of tuberculosis. Granulomas are formed of necrotic aggragates of immune cells and bacteria and are known to help bacteria escape clearance. One of the pathways manipulated by mycobacteria is the inflammasome pathway. Inflammasomes are involved in early bacterial clearance and recruitment of adaptive immunity. The zebrafish (*Danio rerio*) / *Mycobacterium marinum* infection model has been shown to be suitable for studying the different phases of human tuberculosis as well as inflammasome structure and function. As the zebrafish larvae are protected solely by innate immunity, we can use the larvae to study the innate arm of immunity in the absence of the adaptive arm. To study the defense mechanisms that protect against mycobacterial infection, we have developed a knockout zebrafish model devoid of inflammasome activation. We will

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DOI: 10.1002/eji.201970400

use this model to determine the significance and the role of inflammasome signaling in mycobacterial infection and granuloma formation. Our knockout zebrafish model will shed light on the role of inflammasome signaling in early phases of mycobacterial infection and offer a platform for studying host protective immunity.

O064

Short-chain fatty acids regulate Toll-like receptor-mediated inflammatory response by human primary macrophage

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Short-chain fatty acids (SCFAs), namely acetate, propionate and butyrate, are metabolites derived from commensal bacterial fermentation of non-digestible polysaccharides in the gut lumen. SCFAs reach the circulation and may play an important role in the prevention of inflammatory diseases affecting the cellular response of immune cells in the periphery including the blood and the bone marrow compartments. Within the immune cells, macrophages play a critical role in initiating inflammation and maintaining homeostasis and
are one of the main drivers of several inflammatory diseases, e.g. inflammatory bowel diseases (IBD), rheumatoid arthritis (RA). Here we aim to decipher how SCFAs modulate human macrophages and how SCFAs can change responses towards immune stimulants.
To address the modulatory effects that SCFAs could exert on the inflammatory response mediated by macrophages, we treated in vitro human monocyte-derived macrophages from healthy donors with TLR ligands in the presence of SCFAs. Our results show that administration of SCFAs together with LPS can considerably influence the response of primary human macrophages to innate immune receptor stimulation. Differential effects of SCFAs were observed on NLRP3 inflammasome-dependent and -independent cytokines.
Taken together, we demonstrate that SCFAs considerably regulate the TLR-mediated inflammatory responses of human primary macrophages towards immune stimulants. Our results confirm recent studies that illustrated an important role for dietary fibers consumption in various immune responses and cell types. Our results provide new insights into the underlying mechanisms by how metabolites influence the development of inflammatory diseases.

LB043

Sequential regulation by tristetraprolin, CD38 and Sirt1 promotes resolution of inflammation and bacterial clearance

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The resolution phase of acute inflammation is essential for tissue homeostasis, yet the underlying mechanisms remain unclear. We demonstrate here that resolution of inflammation involves interactions between CD38 and tristetraprolin (TTP). During the onset of acute inflammation, CD38

DOI: 10.1002/eji.201970400

levels were increased, leading to the production of Ca^{2+}-signaling messengers, nicotinic acid adenine dinucleotide phosphate (NAADP), ADP ribose (ADPR) and cyclic ADPR (cADPR) from $NAD(P)^+$. To initiate the onset of resolution, TTP expression was increased by the second messengers, NAADP and cADPR, which downregulated CD38 expression. The activation of TTP by Sirt1-dependent deacetylation, in response to reduced NAD^+ levels, suppressed the acute inflammatory response and decreased Rheb expression, inhibited mTORC1 and induced autophagolysomes for bacterial clearance. TTP may represent a mechanistic target of anti-inflammatory agents, such as carbon monoxide. TTP mediates crosstalk between acute inflammation and autophagic clearance of bacteria from damaged tissue in the resolution of inflammation during sepsis.

P0049

Targeted cancer immunotherapy using engineered oncolytic *Salmonella typhimurium*

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Attenuated Salmonella typhimurium show tumor-targeting capacities and significantly suppress tumor growth in mice. The levels of IL-1β and TNF-α were markedly increased in tumors colonized by ΔppGpp Salmonella. The transcript levels of the core molecules of inflammasome signaling, IPAF, NLRP3 and P2X7, were significantly elevated only in Salmonella-treated tumors. ATP released from damaged cancer cells was also identified as a mechanism of NLRP3 activation. Inhibiting IL-1β production in Salmonella-treated mice restored tumor growth, whereas tumor growth was suppressed for longer by local administration of recombinant IL-1β or TNF-α in conjunction with Salmonella therapy. Based on this finding, we developed novel strategy of enhanced cancer immunotherapy using an engineered strain of attenuated S. typhimurium secreting Vibrio vulnificus flagellin B (FlaB) or cytolysin in tumor tissues. Engineered Salmonella with payloads significantly suppressed tumor growth and metastasis in mouse models and prolonged survival, along with increased immune cell infiltration in the tumor microenvironment. These targeted therapies with modification of tumor immune microenvironment could be used as novel cancer immunotherapeutics.

P0050

C5aR2 contributes to NLRP3 inflammasome activation and HMGB1 release via promoting PKR expression in macrophages

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The NLR family pyrin domain-containing 3 (NLRP3) inflammasome, a multimeric protein complex that mediates the maturation of interleukin (IL) -1β and IL-18 as well as the release of high mobility group box 1 (HMGB1), contributs to several inflammatory diseases, including sepsis, gout and type 2 diabetes. The active complement fragment C5a and its receptor C5aR1 or C5aR2 are well studied as their interaction could orchestrate the inflammatory responses in many diseases. Although C5a-C5a receptor interaction in NLRP3 associated diseases has been suggested, little is known about the details of C5a-C5a receptor crosstalk with the NLRP3 inflammasome in macrophages. In this study, we demonstrated that C5aR2 deficiency restricts the activation of NLRP3 inflammasome and the release of HMGB1 in vitro and in vivo. Mechanistically, C5aR2 promotes the NLRP3 activation via amplifying double-stranded RNA-dependent protein kinase (PKR) expression, which is an important NLRP3 activating factor. Further, elevation of PKR expression by C5a-C5aR2 interaction depends on mitogen-activated protein (MAP) kinase kinase (MEK)/ extracellular signal-regulated kinases (ERK)

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DOI: 10.1002/eji.201970400

pathway and type I interferon (IFN) signaling. Thus, our study reveals that C5aR2 contributes to NLRP3 inflammasome activation and HMGB1 release in macrophages.

P0051

Caspse-11- GSDMD pathway is required for serum ferritin secretion in sepsis

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Ferritin is the major iron storage molecule of vertebrates, which can be detected in serum under numerous conditions, including inflammatory, neurodegenerative, and malignant diseases. Given this character, serum ferritin is frequently used as a biomarker in clinical settings. How the ferritin secreted to the serum has attracted much attention. Although some studies have found ferritin was mediated via the endoplasmic reticulum (ER)-Golgi secretion pathway or secretory lysosomes trafficking pathway under normal conditions, the secretion pathway of ferritin under pathological conditions, especially in sepsis is not very clear. In this report, we adopt a murine sepsis model to study the secretion pathway of ferritin in sepsis. We demonstrated caspase-11-GSDMD pathway and associated pyroptosis is required for secretion of ferritin in vitro and in vivo in sepsis. Moreover, our work connects pyroptosis to serum ferritin secretion and suggests a passive release process of ferritin, enhancing our understanding of the mechanism of ferritin secretion.

P0052

Vitamin K inhibits inflammation by targeting the NLRP3 inflammasome

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Vitamin K is a group of structurally similar, fat-soluble vitamins, which has an anti-inflammatory activity, but the underlying mechanisms of its anti-inflammatory action remain unclear. The NLRP3 inflammasome is a multiple protein complex, dysregulation of which has been linked to various human inflammatory diseases, such as type 2 diabetes (T2D), atherosclerosis and gout. Here, we show that vitamin K is a selective, potent inhibitor for NLRP3 inflammasome and it specifically blocks the interaction between NLRP3 and ASC, thereby inhibiting NLRP3 inflammasome assembly. Moreover, vitamin K treatment attenuated the severity of inflammation in mouse models of peritonitis. Our results thus demonstrate that vitamin K exerts anti-inflammatory activity via inhibiting NLRP3 inflammation activation and indicate that vitamin K deficiency may be linked with NLRP3-associated diseases in vivo.

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DOI: 10.1002/eji.201970400

P0053

CD1d1 intrinsic signaling in macrophages controls NLRP3-inflammasome expression during inflammation

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Dysregulation of immune responses in the gut often associates with inflammatory bowel diseases (IBD) for imposing problems to human health. Mouse CD1d1, an ortholog of human CD1D mainly participating in lipid-antigen presentation to NKT cells, is able to generate intrinsic signals upon stimulation. We here showed mice with macrophage-specific *CD1d1* deficiency ($Lym^{CD1d1-/-}$) acquire resistance to dextran sodium sulfate (DSS)-induced colitis due to the absence of CD1d1-dependent transcriptional inhibition of NLRP3 inflammasome components. DSS-induced NLRP3-inflammasome hyperactivation accounts for gut epithelial proliferation and intestine-blood-barrier integrity. Mechanically, occupancy by the natural-ligand iGb3, CD1d1 responds with intracellular Ser^{330} dephosphorylation, by thus to cause reduction of the PRDX1-associated AKT-STAT1 phosphorylation and subsequent NF-κB activation, eventually leading to transcriptional down-regulation of *Nlrp3* and its immediate substrates *Il1b* and *Il18* in macrophages. Therefore, the counterbalancing role of CD1d1 in macrophages determines severity of DSS-colitis. These findings propose new intervention strategies for treating IBD and other inflammatory disorders.

P0054

Regulation of JAK/STAT signal pathway by miR-21 in the pathogenesis of juvenile idiopathic arthritis

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Background: To analyze the expression of miR-21 and its relationship with JAK/STAT signal pathway in Juvenile idiopathic arthritis (JIA).
Methods: Total RNA was extracted from PBMCs in active JIA patients (systemic juvenile idiopathic arthritis (sJIA), n=20/ polyarticular juvenile idiopathic arthritis (pJIA), n=13)) and healthy controls (n=20) (HC). The relative expressions of miR-21, STAT3 and SOCS3 in PBMCs were measured by real-time polymerase chain reaction (PCR) and their expressions were measured by Western blotting and Dual-Luciferase Reported Assay. Inducing the differentiation of RASF derived osteoclasts and identifying factors that can impact on their differential ability.
Results: The expression of miR-21 was significantly lower in JIA patients than in healthy controls ($P< 0.05$). The level of STAT3 increased in PBMCs of JIA group compared with control group($P< 0.05$). Furthermore, the expression levels of miR-21 in sJIA and pJIA group had a negative correlation with STAT3 ($r=-0.5854/r=-0.6134$, $P< 0.05$)). The expression of STAT3 changed little after the stimulation of IL-6 or not in rheumatoid arthritis fibroblast-like synovial cells (RASFs) with transfection of LV3-miR-21. The expression of p-STAT3 decreased after the stimulation of IL-6 in RASFs transfected by LV3-miR-21($P< 0.05$). M-GSF was used for inducing the RASFs to osteoclasts. According to the manifestation of TRAP staining, the amounts of osteoclasts in group miR-21 mimics were significantly lower than the group NC ($p< 0.05$)
Conclusions: We proved that miR-21 might affect the JAK/STAT signal pathway by suppressing the

DOI: 10.1002/eji.201970400

expression of STAT3 and phosphorylation of STAT3. miR-21 could inhibit the production of osteoclasts induced from RASFs by M-GSF.

P0055

Epigallocatechin Gallate suppresses Interleukin-1β Secretion by inhibition of NLRP3 inflammasome

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Epigallocatechin Gallate (EGCG), which is the main polyphenol component of green tea, has been shown to have the ability to inhibit oxidation and inflammation. However, the exact mechanism of anti-inflammatory is still not clear.
In this study, we report that EGCG inhibits caspase-1 activation and IL-1β secretion by suppressing NLRP3 inflammasome activation in mouse bone marrow-derived macrophages. EGCG inhibits NLRP3 inflammasome activation by interfering with mitochondrial spatial arrangement, blocks NLRP3-mediated ASC speckle formation and reduces mitochondrial impairment. Reactive oxygen species, autophagy, or acetylated a-tubulin was ruled out as the mechanism by which EGCG inhibits the inflammasome. Importantly, in vivo data show that EGCG attenuated IL-1b secretion and prevented high-fat diet-induced insulin resistance in wide-type C57BL/6 mice.In addition, our findings indicate that EGCG reduces pyroptosis.
EGCG can be used as an inhibitor of common NLRP3 inflammasome activation and may become a potential therapeutic drug for NLRP3-driven diseases in the future.

P0056

Oridonin is a covalent NLRP3 inhibitor with strong anti-inflammasome activity

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Oridonin (Ori) is the major active ingredient of the traditional Chinese medicinal herb Rabdosia rubescens and has anti-inflammatory activity, but the target of Ori remains unknown. NLRP3 is a central component of NLRP3 inflammasome and has been involved in a wide variety of chronic inflammation-driven human diseases. Here we show that Ori is a specific and covalent inhibitor for NLRP3 inflammasome. Ori forms a covalent bond with the cysteine 279 of NLRP3 in NACHT domain to block the interaction between NLRP3 and NEK7, thereby inhibiting NLRP3 inflammasome assembly and activation. Importantly, Ori has both preventive or therapeutic effects on mouse models of peritonitis, gouty arthritis and type 2 diabetes, via inhibition of NLRP3 activation. Our results thus identify NLRP3 as the direct target of Ori for mediating Ori's anti-inflammatory activity. Ori could serve as a lead for developing new therapeutics against NLRP3-driven diseases.

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DOI: 10.1002/eji.201970400

P0057

Effect of oridonin-mediated hallmark changes on inflammatory pathways in human pancreatic cancer (BxPC-3) cells

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Oridonin, which has been extracted from Rabdosiarubescens, is a tetracyclic diterpenoid compound. Structuraland pharmacological studies indicate that oridonin is one of the most important therapeutic compounds found in Rabdosia rubescens, due to its heat-clearing, detoxifying,anti-inflammatory, anti-nociceptive, and anti-tumorigenic properties. Pancreatic cancer is a common cancer of the digestive system and is a leading cause of morbidity and mortality in both developed and developing countries. Pancreatic cancer is characterized by a covert anatomical structure, aggressive biology, resistance to conventional therapeutic agents, and no early detectable biomarkers, resulting in a 5-year survival rate of only 5% among patients . Therefore, searching for new strategies for the prevention and treatment of pancreatic cancer is essential. To investigate the effect of oridonin on nuclear transcription factors and to study the relationship between biological behavior and inflammatory factors in human pancreatic cancer (BxPC-3) cells. In this report, we demonstrate that oridonin is able to inhibit the growth of human pancreatic cancer (BxPC-3) cells and change the expression of cancer hallmark-related proteins. Inflammatory factors such as interleukin-1β (IL-1β), IL-6, IL-33 were decreased in BxPC-3 cells treated with oridonin, and oridonin also downregulated nuclear transcription factor pathways such as IL-6/STATE3 and IL-1β/IL-33/NF-κB. We propose that oridonin can reduce growth, metastasis, and angiogenesis in BxPC-3 cells by regulating the nuclear transcription factor mediated expression of related inflammatory factors.

P0058

Irgm1 suppresses LPS-induced endotoxemia through inhibition of NLRP3 Inflammasome signaling pathway

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Immune-related GTPase M (Irgm1) plays a crucial role in resolving immune responses during infection. The molecular mechanism underlying the immunoregulatory function of Irgm1 is not fully understood. NLRP3 inflammasome triggered by a variety of stimuli are actively involved in immune responses during both physiological and pathological conditions. We hypothesized that Irgm1 may suppress innate immune responses through regulating NLRP3-Caspase-1-IL-1β signal cascades. To test that hypothesis, we injected LPS to either wide-type (WT) mice or Irgm1-knockout (Irgm1$^{-/-}$) mice. We showed that Irgm1$^{-/-}$ mice are more vulnerable to LPS induce inflammation as indicated by their lower survival rate and hyper-inflammatory responses in particular higher level of IL-1β. In keeping with that, macrophage isolated from Irgm1$^{-/-}$ mice also produce more pro-inflammatory cytokines including IL-1β. Interestingly, we observed that the expression of NLRP3 inflammasome related protein such as NLRP3, ASC and cleaved caspase-1 are also up-regulated in the Irgm1$^{-/-}$ macrophage. Inhibiting NLRP3 inflammasome significantly enhanced the survival of Irgm1$^{-/-}$ mice and normalized the up-regulation of IL-1β observed in Irgm$^{-/-}$ macrophages, suggesting that increased NLRP3 mediated inflammation is responsible for the vulnerability of Irgm1$^{-/-}$ mice to LPS. In addition, Irgm$^{-/-}$ macrophages displayed impaired autophagy flux, which was associated with the suppression of NLRP3 inflammasome degradation. Taken together, these data suggest that Irgm1 negatively

DOI: 10.1002/eji.201970400

regulates NLRP3 inflammasome activation and IL-1β secretion by autophagy, which may be involved in the resolution of immune responses.

P0059

Shikonin alleviates DSS-induced ulcerative colitis via inhibiting NLRP3 inflammsome activation

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Shikonin is a plant-derived lipophilic naphatoguinone that exhibits diverse pharmacological properties, has been widely used in several pharmaceutical and cosmetic preparations, as well as in food colorants. Shikonin has been reported antipyretic and anti-inflammatory properties, but the mechanism of inhibition has not been determined. The NLRP3 (NLR family, pyrin domain-containing 3 protein) inflammasome is a cytosolic multiprotein complex that plays a critical role in the innate immune response and pathogenesis of multiple inflammatory disorders. Here we found that shikonin inhibited NLRP3 inflammasome assembly via reduction of ROS and inhibited ASC oligomerization and ASC speck information ,which disrupted the interaction between ASC and NLRP3.And shikonin also suppressed caspase-1 activation in mouse macrophages and suppressed the activating of isolated caspase-1,demonstrating that it may directly targets caspase-1. Furthermore shikonin also reduced the levels of the IL-1βand IL-18,however the production of TNF-α, an inflammasome-independent cytokine, was not affected. This effect was further confirmed in vivo using mouse models of DSS-induced ulcerative colitis (UC).These results suggest the therapeutic value of shikonin by targeting NLRP3-driven inflammatory diseases.

P0060

SpiC protein is SifA-dependently expressed and negatively regulate Salmonella Enteritidis proliferation in RAW264.7 macrophage cells

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Salmonella Enteritidis (SE) is an important zoonotic pathogen, which could bring an important public health problem worldwide. SE secretes effector proteins encoded by *Salmonella* pathogenicity island 2 (SPI-2) to manipulate host cells to facilitate its replication and proliferation for survival and dissemination. SpiC protein as one of main effectors of SPI-2, which was not completely known for its real-time expression and exact function. In this study, the *Salmonella* strain which expressed SpiC-GFP fusion protein was successfully constructed. And further, SpiC protein was found to express specially in the infected RAW264.7 cells and SifA-dependently. SpiC could negatively regulate SE replication in RAW264.7 cells, intracellular replication ability of the *spiC* mutant was significantly stronger than that of the wild-type strain within 24 hours post infection in RAW264.7 cells. The *spiC* mutant which was released from RAW264.7 cells into cell supernatant was significantly fewer, compared to that of wild type *Salmonella* Enteritidis, its escape ability from macrophage cells decreased strongly. The *spiC* mutant significantly decreased the level of cytokine IFN-γ and increased the level of cytokine IL-10 in cell supernatant. Eukaryotic expressed SpiC was found to stimulate NF-kB activation in reporter cell lines as well as when it was during *Salmonella i*nfection, and SpiC is also efficient for IkBa phosphorylation and degradation in infected cells. Taken together, this study demonstrated special intracellular expression of SpiC protein, intracellular hyperproliferation ability of the *spiC* mutant in RAW264.7 cells and involvement of potential cytokine signal pathway as proinflammatory factor for possible regulation mechanism of SpiC protein.

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DOI: 10.1002/eji.201970400

P0061

Gasdermin D Drives the Non-exosomal Secretion of Galectin-3

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As a critical component of the innate immune system, inflammasome governs caspase-1 auto-activation, IL-1β maturation, and gasdermin D (GSDMD)-mediated pyroptosis. Inflammasome activation also drives the release of additional proteins, whose secretion mechanism and function remain elusive. Herein, our secretome analysis identified galectin-3 as an effector protein downstream of inflammasome, which has been demonstrated desensitizing insulin signaling. Mechanistically, the non-pyroptotic caspase-1-GSDMD axis drove non-exosomal secretion of galectin-3 through plasma membrane pores. *In vivo*, galectin-3 was essential for insulin resistance induction in mice harboring the hyperactive *Nlrp3*^{A350V} allele. Moreover, decreased circulating galectin-3 in obese *Nlrp3*$^{-/-}$ mice correlated with improved insulin sensitivity, which was aggravated by infusion of recombinant galectin-3. Therefore, our study identified galectin-3 as an effector of inflammasome activation. These findings potentiate galectin-3 as target to interfere with insulin resistance and other diseases related with inflammasome.

P0062

Role of Syk in atherosclerosis and IL-1 production

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Objective: Atherosclerosis is one of the complications in rheumatic diseases and is mediated by chronic inflammatory processes along with altered lipid metabolism. CANTOS trial showed that anti-IL-1β monoclonal antibody reduced the cardiovascular events. This study aims to examine the role of the spleen tyrosine kinase (Syk) in the pathogenesis of atherosclerosis in mice. The other aim is to investigate the role of Syk in the production of IL-1β.
Methods: First, we generated with Syk$^{flox/flox}$Rosa26CreER$^{(T2)+/+}$LDLR$^{-/-}$ mice. The Syk was knocked out by tamoxifen. Syk$^{del/del}$ mice received a high-fat diet for 16 weeks. The atherosclerotic lesions in aortic sinus and aorta were measured. Next, Syk$^{del/del}$Rosa26CreER$^{(T2)+/+}$ mice were injected intraperitoneally with cholesterol crystals which induced IL-1 dependent inflammation. After 6 h, the absolute number of monocytes in peritoneal lavage was measured with FCM. Third, LPS primed BMDM from Syk$^{del/del}$Rosa26CreER$^{(T2)+/+}$ mice were incubated with cholesterol crystals. The IL-1β in the supernatants was measured by ELISA.
Results: The cumulative area of atherosclerosis in Syk$^{del/del}$ was significantly lower than that in Syk$^{+/+}$ in both aortic sinus and aorta [$p < 0.05$]. The absolute number of monocytes in peritoneal lavage and the IL-1β production of BMDM from Syk$^{del/del}$ mice was significantly higher than that from Syk$^{+/+}$ mice [$p < 0.05$].
Conclusions: The deletion of Syk gene suppressed atherosclerosis in mice. On the contrary, Syk deletion augmented the cholesterol crystal-induced acute inflammation in vivo or IL-1β β production in vitro. Reduced atherosclerosis in Syk deleted mice is attributed to other mechanisms than the cholesterol - IL-1β pathway.

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DOI: 10.1002/eji.201970400

P0636

Homotypic CARD-CARD interaction is critical for NLRP1 inflammasome activation

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Cytosolic inflammasomes are supermolecular complexes in response to intracellular pathogens and danger signals. However, the mechanism of homotypic caspase recruitment domain (CARD) interaction between the inflammasome adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) and NLRP1 in the NLRP1 inflammasome is largely unknown. In this study, a crystal structure with death domain fold of the ASC^{CARD} fused to maltose-binding protein was determined. According to this structure, we find that the CARDs interaction of between ASC and NLRP1 is mediated by three conserved interaction types of death domain superfamily (type I, II, III). The functional relevance of these important surfaces is verified in the NLRP1 inflammasome in vitro. Moreover, $NLRP1^{CARD}$ and ASC^{CARD} can polymerize to form the filamentous supermolecular complexes. This finding suggests a novel mechanism of homotypic CARDs interactions for the NLRP1 inflammasome activation and gives insights into the inflammasome-mediated signal amplification mechanism.

P0637

Crystal structure of casapse11 CARD reveals insight into non-canonical inflammasome caspase activation

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The murine non-canonical inflammasome caspase-11 could respond to intracellular LPS and lead to pyroptosis. Caspase-11 contains CARD and caspase domains at its N-terminal and C-terminal. Caspase domain contains two subunits, p20 and p10. However the activation mechanism of caspase-11 remains unknown to date. LPS produced by gram-negative bacterial as a ligand of intercellular caspase11 that initiates non-canonical inflammasome signaling pathway and lead to pyroptosis in vitro and in vivo. Here, we find the CARD of caspase11 undergoes aggregation via a tetramer formation, which is supported by crystal structure. The hydrophobic interface among the "tetramer" in crystal model is mediated by a long helix in CARD-domain. It leads to our hypothesis that after ligand engagement in an unknown mechanism, the CARD undergoes oligomerization, which results in activation of caspase-11. Then caspase-11 cleaves into caspase domain, and initiates pyroptosis. Point mutants on CARD long helix destroy the hydrophobic interface can abolish the aggregation of CARD and fail to induce pyroptosis. Furthermore, we found the first 10 amino acids and the linker between CARD and caspase domain are required for CARD oligomerization which is in a sequence independent manner. These findings demonstrate a critical role of the tetramer formation, as observed in the CARD crystal structure, in caspase11 activation during Gram-negative bacterial infection.

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DOI: 10.1002/eji.201970400

P0638

Metformin improves ulcerative colitis by regulating NLRP3 inflammasome activation

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The NLRP3 inflammasome is a complex of multiple proteins in the cytoplasm that plays an important role in immune defense and innate immune responses. However, its excessive activation or abnormality is associated with many diseases such as type 2 diabetes, Alzheimer's disease and Parkinson's disease, ulcerative colitis, Therefore, targeted regulation of excessive activation of NLRP3 inflammasome is a very important strategy for the treatment of related diseases. In this study we mainly investigated whether the anti-inflammatory effects of metformin improve ulcerative colitis by regulating the activation of NLRP3 inflammasome. Our study found that metformin can specifically inhibit the activation of NLRP3 inflammasome and reduce the maturation of caspase-1 and IL-1β. Further mechanism studies have found that metformin mainly mediates ASC polymerization and mediates ROS production, and can also pass The lysosomal pathway regulates the degradation of NLRP3 ubiquitination, thereby ameliorating its condition in a mouse model of ulcerative colitis. In conclusion, this study not only elaborated the mechanism of metformin inhibiting NLRP3 inflammasome, but also provided a very safe treatment strategy for the treatment of ulcerative colitis.

P0639

Ceramide production induced by a high-fat diet promotes hepatocellular carcinoma development in a murine model

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Occult HBV infection (OBI) was associated with an increased risk of hepatocellular carcinoma (HCC). Hepatocytes steatosis might be an important cofactor for OBI progression into HCC. We fed wild-type mice and Alb1HBV mice with a high-fat diet and normal diet for 13 weeks. The results showed that the tumor occurred earlier in Alb1HBV mice and developed significantly more hepatic tumor nodules than those in the wild-type mice after they were all fed with a high-fat diet in the same periods ($p< 0.01$). Further analysis in liver tissues showed that the ceramide levels in the Alb1HBV mice liver were significantly higher than that in the wild-type mice ($p< 0.05$). After being treated with Myriocin to inhibit *de novo* synthesis of ceramides, the liver tumor burden in Alb1HBV mice were significantly reduced ($p< 0.01$). Further analysis indicated that ceramides activated NLRP3 inflammasome in hepatic $CD11b^{dim}$ $F4/80^{+}$ cells, triggered liver inflammation for HCC promotion ($p< 0.05$).
Conclusion: After occult HBV infection, a high-fat diet could be an important cofactor to promote HCC development , which is probably by enhancing the production of ceramides. Inhibiting ceramides production might be helpful for intervening the OBI progression into HCC.
Keywords: Occult HBV infection, High-fat diet, Ceramides, Chronic inflammation, Hepatocellular carcinoma

DOI: 10.1002/eji.201970400

P0640

The ubiquitin E3 ligase TRIM26 positively regulates TLR-mediated Inflammatory responses

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Toll-like receptors (TLRs) play a pivotal role in defense against invading pathogens through the detection of pathogen-associated molecular patterns (PRRs). TLRs-mediated signaling needs to be effectively terminated to avoid excessive immune responses and harmful damages to the host. However, the precise mechanisms involved in the regulation of TLR signaling remain unclear. TRIM26 is an E3 ubiquitin ligase of the TRIM family of proteins. Previously, we found TRIM26 negatively regulates interferon-β production and antiviral response through degrading nuclear IRF3, while the function of TRIM26 in TLRs-induced inflammatory responses is unknown. To study the function of TRIM26 in TLRs-mediated signaling, we prepared macrophages from WT and TRIM26 $^{-/-}$ mice, then stimulated with various TLR ligand including LPS, poly(I:C) and R848, and the expression of inflammation-related cytokines were measured by ELISA and quantitative RT-PCR. We found that expression of TNF-α and IL-6 was significantly decreased in the TRIM26-deficient macrophages. Consistently, LPS-induced phosphorylation of P65 and IKKα/β was dramatically decreased in TRIM26-deficient macrophages. Moreover, TRIM26$^{-/-}$ mice showed a decreased sensitivity to LPS-induced inflammatory death compared to WT mice. Our study provides a novel mechanism of TRIM26-mediated regulation of Inflammatory responses and identifies new therapeutic targets for inflammatory diseases.
Keywords: TRIM26**;** Inflammatory responses; Toll-like receptor

P0641

Dynamic changes of proteasome and protective effect of bortezomib, a proteasome inhibitor, in mice with acute pancreatitis

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The proteasome is involved in the activation of NF-κB and can regulate the progression of inflammatory diseases. However, the role of proteasome in acute pancreatitis (AP) has not been demonstrated. In this study, we first observed that the protein level and activity of proteasome 20S were increased significantly in pancreatic injury tissues after caerulein-induced mild acute pancreatitis (MAP) induction, which was in consistent with the expression of the NF-κB nucleoprotein and positively correlated with the severity of AP. Then, bortezomib, a classical proteasome inhibitor, was used to intervene the progression of MAP in mice. The results showed that bortezomib administration reduced the serum amylase and lipase levels and mitigated histopathological manifestation of pancreatic injury in mice. Meanwhile, bortezomib decreased the expression of NF-κB p65 nucleoprotein as well as total proteasome 20S protein, and inhibited the activity of 20S in pancreatic tissues. In addition, we found that bortezomib could protect pancreatic acinar cell against necrosis and mitigate the severity of AP in a severe acute pancreatitis model induced by sodium taurocholate hydrate. Taken together, our study for the first time confirmed that the proteasome participated in the pathogenesis of AP and its inhibitor bortezomib could protect against AP in mice.

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DOI: 10.1002/eji.201970400

P0642

CLICs-dependent chloride efflux is an essential and proximal upstream event for NLRP3 inflammasome activation

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NLRP3 inflammasome plays an important role in innate immunity and diverse inflammatory diseases. Many different stimuli including both pathogen-associated molecular patterns(PAMPs) and danger-associated molecular patterns(DAMPs) induce potassium efflux, which causes mitochondrial damage and reactive oxygen species(ROS) production to mediate NLRP3 inflammasome activation. However, the underlying signaling events remain unclear. Here we report the identification of chloride intracellular channels(CLICs) as downstream of the potassium efflux-mitochondrial ROS axis to regulate NLRP3 activation. CLICs mediate NLRP3 inflammasome activation in vitro and in vivo. NLRP3-activating stimuli induces potassium efflux and mitochondrial ROS production, which facilitates the plasma membrane translocation of CLICs. CLICs on the cell membrane induce chloride efflux to promote NEK7-NLRP3 interaction and inflammasome assembly. Thus, our results demonstrate that CLICs-dependent chloride efflux is an essential signaling event upstream of NLRP3 inflammasome activation.

P0643

LPS promoted metastasis via NF-κB/Snail/HK3 signaling pathway related glycolysis acceleration in colorectal cancer

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Colorectal cancer (CRC) is leading cause of death related to cancer in global. Lipopolysaccharide (LPS) has significantly higher concentration in CRC tissue than in normal tissue. However, the effects of LPS on inflammasomes activation in tumor cells and metastasis are unclarified.
The degree of inflammasomes activation in 8 CRC cell lines was different by detecting the protein expression of Caspase-1, cleaved Caspase-1 (P20) and IL-1β-P17. LPS treatment promoted inflammasomes activation, and potentiated migration and invasion, which could be reversed by specific inhibitor for caspase-1 (Ac-YVAD-CHO). Knockdown of p65NF-kB not only reduced P20 and IL-1β-P17 expression, impaired LPS-induced upregulation of caspase-1 enzyme activity, but also suppressed LPS-induced motility. Nuclear Snail expression also was increased after LPS stimulation. LPS didn't increase motility of CRC cells after knockdown of Snail. LPS promoted cells motility in a glucose-nourished environment but not in a glucose-starved condition. Glucose and lactate concentration assays suggested that LPS promoted glucose consumption and lactate production, which could be reversed by Snail knockdown. Hence, we detected the expression of key rate-limiting enzymes in glycolysis, and HK3 was the only upregulated enzyme after LPS treatment. LPS no longer enhanced cell motility and glycolysis after HK3 knockdown. Co-IP and ChIP showed that p65NF-κB formed a protein complex with Snail, then bound to the promoter region of HK3, inducing the expression of HK3.
In conclusion, LPS directly active inflammasomes in cancer cells and promote metastasis through NF-κB/Snail/HK3 signaling pathway-related glycolysis acceleration in CRC.

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DOI: 10.1002/eji.201970400

P0644

The expression and immune function of NOD2 in Myd88-knockout mice by mycobacteria infection

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Background: With an increasing incidence in recent years, the tuberculosis is the second leading infectious disease cause deaths in adults. Ningxia is one of the provinces with higher number of tuberculosis cases in China. We have previous uncovered a NOD (nucleotide-binding oligomerization domain receptor) -mediated signaling transduction between the alveolar epithelial cells and macrophages using an air-liquid interface (ALI) co-culture cell model. However, it is not clear about immunoregulatory mechanism underpinning the interaction between Toll-like receptor (TLR) signaling and NOD signaling in lung of mice by M. tuberculosis infection.
Objective: To investigate the expression and immune function of NOD2 signal in MyD88-/- mice by mycobacteria infection.
Methods: Pulmonary infection of MyD88-/- mice and wild-type C57BL/6 mice were induced by intratracheal instillation with BCG, PBS as negative control. After 24h infection, we detect the expression of NOD2 mRNA and protein in lung of mice by real-time PCR and Western-blotting; serum IL-6 levels were detected by ELISA.
Results: The expression of NOD2 mRNA and protein were significantly increased in BCG infection group, compared with PBS group. NOD2 protein expression of MyD88-/- mice were higher than wild-type mice. The serum levels of IL-6 MyD88-/- mice and wild type mice with BCG infection were higher than PBS group, the difference was statistically significant.
Conclusions: BCG can activate the NOD2 signaling pathways in MyD88 function loss of mouse lung tissue .
Keywords: Mycobacterium tuberculosis; BCG; NOD2; MyD88; IL-6

P0645

Interleukin-18 exacerbates skin inflammation and affects microabscesses and scale formation in an imiquimod-induced mouse model of psoriasis

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As a potent pro-inflammatory cytokine of the interleukin (IL)-1 family, IL-18 was elevated in early active and progressive plaque-type psoriatic lesions and that serum or plasma levels of IL-18 correlated with the Psoriasis Area and Severity Index. Although results from previous studies have established that IL-18 may aggravate psoriatic inflammation, the mechanisms of this process remain unknown. In this study, IL-18 knock out (KO) mice and Wild-type (WT) mice were used to investigate the effects of IL-18 within a mouse model of psoriasis. We found that IMQ-induced IL-18KO mice showed significantly milder acanthosis and dermal cell infiltration compared with WT mice($P< 0.01$). IMQ-induced IL-18 KO mice manifested larger areas of Munro's microabscesses and scales ($P< 0.01$) as comparing with WT mice. In skin lesions of IL-18 KO mice, the expressions of IL-1B, IL-4 and IL-27 were all significantly upregulated but IL-17 was decreased. Histologically, strong positive signals of Ly6g were observed within the epidermis of IL-18 KO mice but expressions of CXCL1 were decreased. **IL-18 may exacerbate prominent inflammation and influence pathological features in IMQ-induced mouse model of psoriasis.** IL-18 may upregulate pro-inflammatory cytokines and reduce protective

DOI: 10.1002/eji.201970400

cytokines, thus aggravating psoriatic inflammation. In addition, **IL-18 may be involved in the formation of Munro's microabscesses and scales.**

P0646

Inhibition of NLRP3 inflammasome by MCC950 effectively attenuates disease activity through suppressing monocyte pyroptosis in systemic lupus erythematosus

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Background: Pyroptosis is a lytic mode of programmed cell death which can be mediated by inflammatory caspase-1/GSDMD pathway. The activation of NLRP3 inflammasome contributes to the pathogenesis and development of immune and inflammatory diseases including systemic lupus erythematosus (SLE). In this study, we aim to investigate the effects of MCC950, a selective small-molecule inhibitor of NLRP3 inflammasome, on pyroptosis and its effects on disease activity in MRL/lpr mice.
Methods: In vitro, human peripheral blood monocytes (PBMCs) or THP-1 cell line were incubated with MCC950, followed by stimulated with LPS/ATP or LPS/Nigericin. And then, the percentage of caspase-1+PI+ cells, the release of LDH and the levels of IL-1β and IL-18 were examined. In vivo, MRL/lpr mice were randomly subdivided into saline-treated and MCC950-treated groups. Proteinuria, serum autoantibodies, kidneys histopathology, IgG deposition and the expression levels of GSDMD-N domain were detected.
Results: In vitro, LPS/ATP or LPS/Nigericin induced pyroptosis of human PBMCs or THP-1 cells could be evidently inhibited by MCC950. In MRL/lpr mice, MCC950 treatment significantly decreased the proteinuria, circulating autoantibody levels, deposition of immune complex and the expression of GSDMD-N domain in the kidneys.
Conclusion: Overall, MCC950 treatment ameliorated lupus manifestations in MRL/lpr mice by blocking NLRP3 inflammasome-mediated pyroptosis. These findings emphasize the vital role of NLRP3 inflammasome-mediated pyroptosis in SLE pathogenesis and reveal that MCC950 might be a potential therapeutic drug for SLE treatment.

P0647

Expression of inflammasomes -NLRP1, NLRP3 and AIM2 in different pathologic classification of lupus nephritis

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Background: Lupus nephritis（LN）is an immune-complex mediated nephritis which contributes to the morbidity and mortality of patients with SLE. The pathogenesis of LN is complex, including activation of inflammasome.
Objectives: Our aim at this study is to find whether inflammasome is provoked in renal pathology of LN patients and to confirm the activation of inflammasome in different class of LN renal tissues and its

DOI: 10.1002/eji.201970400

association with disease activity.
Methods: Patients with renal biopsy-proven CKD in Xiangya Hospital of Central South University from January 2015 to August 2018(n=86) were enrolled in this study. They were divided into minimal lesion nephritis（MLN）group (n=17), IgA nephropathy（IgAN）group (n=17) and lupus nephritis（LN）group (n=52) accordingly. Clinical and pathological records of patients were retrospectively analyzed. The histologic classes of LN according to the ISN/RSP were defined. Immunofluorescence of NLRP1, NLRP3 and AIM2 in human renal tissue were examined to show the different expression of inflammasomes in LN patients.
Results: The expressions of NLRP3, NLRP1 and AIM2 were significantly increased in renal tissues of LN. AIM2 expressed in glomerular cells of LN class II, NLRP1 expressed in tubular cells of LN class II and class IV, NLRP3 expressed in tubular cells of LN class IV. Additionally, NLRP3 expression had a positive correlation with AI score in patients with LN.
Conclusions: Activated Inflammasomes of NLRP3, NLRP1 and AIM2 are involved in the pathogenesis of LN. NLRP3 has positive correlation with AI score of LN patients.

P0648

Syk and JNK promotes IL-1β production via downregulation of Nek7 upon Staphylococcus aureus infection

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Staphylococcus aureus (S. aureus), a gram positive pathogen, causes pneumonia, sepsis and other serious infectious diseases to lead high mortality. It was previously reported that NLRP3 inflammasome is involved in production of IL-1β during the *S. aureus* infection. However, the underlying regulatory molecular mechanism is not clearly understood. In the present study, the roles of Syk, JNK and Nek7 in NLRP3 inflammasome activation were investigated upon *S. aureus* infection in vitro. J774A.1 cells were pretreated with or without Syk/JNK inhibitors for 1h or transfected with siRNA for Syk, JNK or Nek7 for 48h, subsequently infected with *S. aureus* for 24h, the protein levels of Syk, JNK, Nek7 and NLRP3 inflammasome-associated downstream molecules in cells were determined. The results showed that the protein levels of phospho(p)-Syk, p-JNK, p20, IL-1β in cells were increased and the protein level of Nek7 was reduced after *S. aureus* infection. Whereas, the Syk and JNK inhibitors significantly reduced the expression of p20, mature IL-1β and increased the expression of Nek7 during *S. aureus* infection. Futhermore, the Syk-siRNA and JNK-siRNA showed the similar result. Moreover, Nek7-siRNA decreased the protein level of p20 and mature IL-1β after *S. aureus* infection. Therefore, it was demonstrated that Syk/JNK promotes IL-1β production by downregulation of Nek7 upon *S. aureus* infection.
This work was supported by the National Natural Science Foundation of China (81772252）

P0649

Effect of Piper longum extract on intestinal flora in rats with liver cirrhosis

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Piper longum is a traditional Mongolian medicine. To study the preventive effect and mechanism of piper longum on liver cirrhosis though testing intestinal flora changes in rats. 60 SD rats were randomly divided into blank control group, cirrhosis model group, high, medium and low dose groups

DOI: 10.1002/eji.201970400

of Piper extract. Except blank control group, rats in other groups were induced with phenobarbital sodium solution for 1 week. 60% of CCl4 was injected subcutaneously at multiple points of rats.The results of intestinal flora sequencing showed that the appropriate dose of Piper jasminoides extract could play an anti-fibrotic role by promoting the growth of beneficial bacteria in rat intestinal tract and preventing the destruction of intestinal mucosal barrier. The volume of each injection was 0.3ml/100g, two times a week, and the dosage was adjusted according to the change of body weight. After 14 weeks of continuous intragastric administration, 1 ml/100g volume was administered to the stomach. The changes of body weight, mental state and colour of feces were observed during the experiment. Medication was ended at the end of 14 weeks. The rats were sacrificed at week 15 and liver tissue was removed. At the same time, intestinal flora of rats was collected and stored -80℃. The liver pathological changes were observed by HE staining and Masson staining. Piper longum can delay liver fibrosis and regulate the growth of beneficial bacteria in intestine to maintain the balance of intestinal microecology.

P0650

Local hyperexpression of inflammasome genes in psoriatic plaque

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Psoriasis is a chronic, autoimmune, inflammatory skin disease. An important role in the development of psoriatic inflammation is activation of inflammasome. Inflammasome is a multiprotein complex which main function is recognizing stress molecules and processing of pro-inflammatory cytokines IL-1β and IL-18. In the this study we investigated expression of TLR9 gene, components of the inflammasome complex (NLRP1, NLRP3, CASP1, CASP5) and effector molecules IL-1β and IL-18 in biopsies of healthy and psoriatic skin in 35 patients with severe psoriasis. We used the PCR-RT method.
It was found that in the affected skin compared with healthy expression of the TLR9 gene was significantly increased by 4.1 fold. We showed that NLRP3 gene expression did not differ in the samples of healthy and affected skin in patients with psoriasis. At the same time expression of the NLRP1 gene in affected keratinocytes was higher in 6 fold than in healthy skin samples. We founded that expression of CASP5 was increased in 250 fold in psoriatic plaque. Expression of the IL-18 gene in the psoriatic plaque was increased 10.1 fold. NLRP1 has the ability to recruit caspase-5, which enhances processing of IL-18, which may explain the increase in its expression IL-18. The study of molecular mechanisms of development inflammation in psoriatic lesions can help identify new targets for the effective therapy.

P1233

NLRP3 inflammsome is involved in hypoxia induced inflammatory response in islet transplantation

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Introduction: A large loss of islet cells occurred soon after transplantation, mainly mediated by hypoxia. Activation of inflammasomes in ischemic condition is identified in various tissues. Here we investigated whether hypoxia activates the inflammasome in beta cells and the role of inflammsome plays in hypoxic stress.
Methods: NICC(porcine islet) were transplanted to NSG mouse kidney capsule. NICC and MIN6 cells were exposed to hypoxia(1% O_2) for indicated time. NICC cell death was measured by AO/PI staining.

DOI: 10.1002/eji.201970400

Cytokine releasing was detected by ELISA. Protein level of inflammsome component was measured by Western blot. NLRP3 and ASC were knocked down using siRNA.
Results: NLRP3 Inflammasome is expressed in islets 6 weeks after transplantation. In both NICC and MIN6, hypoxia(1% O_2) time dependently increased NLRP3 and ASC protein level associated with up-regulation of NF-κB signaling. In NICC, hypoxia directly increased protein level of cleaved caspase-1 and mature form of IL-1β and IL-18 and decreased precursor form of them, while in MIN6, it worked only in the presence of a small dose of LPS(10ng/ml). Knockdown of NLRP3 and ASC markedly reduced mature form of IL-1β and IL-18 and decreased HMGB1 releasing after exposure to hypoxia. Moreover, it partially protected against hypoxia-induced cell death. Finally, pretreated with ROS scavenger NAC significantly decreased protein level of cleaved caspase-1 and mature form of IL-1β and IL-18 and NICC cell death.
Conclusions: Our data indicate for the first time that inflammasome is involved in hypoxia-induced beta cell death and HMGB1 releasing in islet transplantation.

P1234

The effect of protein energy wasting and calcium-phosphorus metabolism by injecting L-carnitine before dialysis in patients with maintenance hemodialysis

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Analyze the difference of blood calcium,blood phosphorus,iPTH and PEW on poor HD tolerance patients who were injected l-carnitine before versus after HD,in order to provide the basis for MHD and improve patients´ quality of life.
Methods: Tewnty-five patients of the observation group were injected l-carnitine before HD 15 minutes, and 25 patients of the control group were injected l-carnitine after HD.L-carnitine dosage is 1g, a total of 3 months. Analyze the difference of blood calcium, blood phosphorus, iPTH,dialysis adequacy (urea clearance index Kt/V), serum creatinine, prealbumin, albumin, hemoglobin, CRP, cholesterol, lean body mass, high-density lipoprotein cholesterol (hdl-c), low-density lipoprotein cholesterol (ldl-c), and triglycerides of the two groups,compared the two groups before and after treatment
Results: After three months, the PEW related index comparison showed that prealbumin, albumin,and lean body mass significantly increased, CRP, low-density lipoprotein cholesterol (ldl-c) and triglycerides decreased in observation group,the difference was statistically significant ($P< 0.05$).There was only triglycerides decreased in control group,the rest of the various indexes had no statistical significance ($P > 0.05$).Calcium and phosphorus metabolism dialysis adequacy index comparison showed that blood calcium and Kt/V significantly increased, blood phosphorus, iPTH and serum creatinine decreased in control group,the difference was statistically significant ($P< 0.05$).
Conclusion: Maintenance hemodialysis patients with supplementary l-carnitine before HD can effectively improve the PEW, calcium phosphorus metabolism disorder and hyperparathyroidism, and it also can improve the dialysis adequacy,dialysis tolerance of HD, and the quality of life.

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DOI: 10.1002/eji.201970400

P1235

Thymosin β4 alleviates renal fibrosis and tubular cell apoptosis through TGF-β pathway inhibition in UUO rat models

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This study aimed to examine the effects of Tβ4 on the renal apoptosis and the expression of transforming growth factor (TGF-β), E-cadherin, and α-smooth muscle actin (α-SMA) in CRTIF rat models.Two weeks after UUO, there were no differences in blood urea nitrogen and creatinine among the four groups (P>0.05). Compared to the UUO group, Tβ4 treatment decreased the 24-h proteinuria (P< 0.001), reduced the area of pathological change (P< 0.01), and these decreases were more obvious in the UUO+high-dose Tβ4 group. The level of E-cadherin protein was lower in the UUO group than the Tβ4 groups, and high-dose Tβ4 treatment further increase E-cadherin expression. Cell apoptosis in the renal interstitial tissue was improved by Tβ4 treatment. Moreover, the decrease of apoptosis was more obvious in the UUO+ high-dose Tβ4 group. In vitro experiments of tubular epithelial cells revealed thatα-SMA mRNA and protein levels decreased, E-cadherin mRNA and protein levels increased by Tβ4 treatment, and similarly, these changes were more significant in the UUO+high-dose Tβ4 group. The apoptosis of In vitro tubular epithelial cells was improved by Tβ4 treatment compared with pure TGF-β stimulation, and equally, the decrease of apoptosis was more obvious in the TGF-β+high-dose Tβ4 group. Tβ4 treatment might alleviate the renal fibrosis and apoptosis of tubular epithelial cells through TGF-β pathway inhibition in UUO rats with CRTIF.

P1236

Association of white blood cell count and makers of central obesity, visceral obesity in hemodialysis patients

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To investigate the association between white blood cell (WBC) count and makers of central obesity, visceral obesity in maintenance hemodialysis (MHD) patients in China.
Method: We conducted a multicenter, cross-sectional study in the hemodialysis centers of 11 hospitals in Guizhou Province, southwest China from June 1, 2017 to September 30, 2017. Visceral adiposity index (VAI), lipid accumulation product (LAP) , hypertriglyceridemic waist phenotype (HW phenotype) were used to evaluate visceral obesity. Linear regression models were used to evaluate the association between WBC and BMI, waist circumference, waist-to-height ratio, waist-to-hip ratio.
Results: We involved 582 participants with valid data in our study at last. White blood cell (WBC) counts were associated with BMI(r=0.122, P< 0.001), waist circumference(r=0.12, P< 0.001), waist-to-height ratio （r=0.123, P< 0.001）but not associated with waist-to-hip ratio(r=-0.01, P=0.82) in MHD patients after adjust for age and sex. Relationships between white blood cell count and markers of visceral obesity(VAI、LAP) seemed to be stronger(VAI r=0.19, P< 0.001;LAP r=0.2, < 0.001). Participants in quartile 4 showed a 2.83-fold higher risk of HW phenotype (95% CI 1.67, 4.8, P< 0.001) compared with those in quartile1 in the unadjusted model. The relationship between WBC count and HW phenotype remained statistical significance（OR 2.46, 95% CI 1.13-5.36, P< 0.001）after adjusted for sex, age, high blood pressure and diabetes.
Conclusions: WBC count is positively related to makers of central and visceral obesity in MHD patients. Moreover, this relationship is more distinguishable with visceral adiposity than central adiposity.

DOI: 10.1002/eji.201970400

P1237

Dialysis with continuous renal replacement therapy in the treatment of acute kidney injury after cardiac surgery

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To compare the efficacy and infection characteristics of automated peritoneal dialysis (APD) with continuous renal replacement therapy (CRRT) in the treatment of acute kidney injury (AKI) after cardiac surgery.
Methods We retrospectively analyzed 197 patients with AKI after cardiac surgery, who were divided into APD group (95 cases) and CRRT group (102 cases) according to treatment methods, and evaluated the renal functions, heart rate (HR), mean arterial pressure (MAP), left ventricular ejection fraction (LVEF), cardiac output (CO), serum biochemical indexes (K^+,Na^+,$HCO3^-$), infection rates and infection characteristics of two groups before and 3, 7 days after treatment.
Results The renal functions and serum biochemical indexes of both groups were improved after treatment, and the $HCO3^-$ of APD group was significantly higher than CRRT group. At 7 days after treatment, the HR and MAP of APD group were significantly lower than CRRT group, whereas the LVEF and CO were obviously higher than CRRT group. The infection rate of APD group was 9.47%, and lower than CRRT group (14.71%). The dosages of vancomycin, teicoplanin, meropenem and imipenem used in APD group were all lower than those in CRRT group.
Conclusion Both APD and CRRT can improve renal function and correct electrolyte imbalances, but APD has advantages in improving heart function and reducing infection rate.

P1238

The value of neutrophil and lymphocyte ratio in the evaluation of inflammation status in patients with peritoneal dialysis

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Objective: To investigate the relationship between neutrophil lymphocyte ratio (NLR) and inflammation in patients with continuous ambulatory peritoneal dialysis(CAPD) in order to provide a simple method to evaluate inflammation status in CAPD patients.
Methods: 86 CAPD patients (male 48 patients;female 38 patients) with stable clinical conditions treated in People's Hospital of GuiZhou Province were recruited and divided into two groups according to the median value of NLR for this study. T
Results: Compared with control group, Lymphocyte count, NLR and hsCRP in CAPD patients were significantly higher (3.46±0.68×109/L vs 1.46±0.48×109/L, 1.16±0.47 and 2.15±1.36 mg/L), respectively, , and were , 3.65±1.58 and 14.57±10.26 mg/L, respectively, ;the three values in CAPD patients than in control group,and the values of NLR and hsCRP were also significantly higher in CAPD patients than in the control group patients ($P<0.05$). ALB was 42.64±7.68 g/L in control group patients, and was 32.56±6.78g/L in CAPD patients, significantly lower in CAPD patients than in the control group patients. ($P<0.05$). ALB and triglyceride were 30.66 ± 5.69 g/L and 1.56 ± 0.47 mmol/L, respectively, in CPAD patients with NLR ≥3.5, significantly lower than those in patients with NLR$<$3.5 ($P<0.05$); hsCRP was 18.61±10.80 mg/L in CAPD patients with NLR≥3.5, significantly higher than that in patients with NLR$<$3.5 ($P<0.05$). NLR was negatively correlated with ALB (r=-0.327, P=0.012)

DOI: 10.1002/eji.201970400

and positively correlated with hsCRP (r=0.367, P =0.007)in CAPD patients.
Conclusion: NLR may be used as a marker of systemic inflammation in CAPD patients.

P1239

The expression of HMGN1/TLR4 and association with diabetic kidney disease explored in mouse models

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To determine the renal expression of high-mobility group nucleosome binding protein 1 (HMGN1) and toll-like receptor (TLR4) in diabetic kidney disease (DKD) mouse models.In vivo: 1. Compared with control groups, the blood glucose, kidney index, UACR and mesangial index were significantly increased [(8.57±0.70) vs (24.28±1.944 mmol/L, (1.44±0.02) vs (1.70±0.09) mg/g, (97.69±10.68) vs (697.67±168.78)μg/mg, $P < 0.05$]; characteristic lesions are shown with mesangial expansion, renal tubules with different degrees of injury and tubulointerstitial infiltration with inflammatory cells; the expression of HMGN1 and TLR4 in kidney tissue was up-regulated in T1DN groups ($P< 0.05$); a significant positive correlation was showed among the expression of HMGN1, TLR4 and mesangial index (r=0.337, 0532, 0.369, 0.366, 0.574, 0.826, 0.359, 0.3175, $P< 0.05$); no significant changes were observed in the insulin treatment group ($P>0.05$). 2. In T2DN group, both biochemical and pathological indicators suggested the features of diabetic nephropathy in T2DN group, HMGN1 expression in renal tissue was increased at 6 weeks of age and reduced at 8 weeks of age in the kidney tissue, TLR4 expression maintained high level along with aging, when compared with WT group ($P< 0.05$). Moverover, the depressed level of HMGN1 was negatively correlated with TLR4 expression, UACR, mesangial index, respectively (r=-0.450, -0.515, -0.622, -0.295, $P< 0.05$). In vitro: The decreased expression of HMGN1 and increased expression of TLR4 were detected in high glucose group when compared with control group ($P< 0.05$).

P1240

Inhibition of phosphorylated IGF-1R alleviates diabetic tubulopathy through interventing the F-actin reorganization

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Diabetic kidney disease (DKD) is known as the major threat because it is a major cause of end-stage renal disease (ESRD) and a leading cause of motility. Changes in glomerular structure are undoubtedly major features of DKD. However, emerging evidence suggests a main role for the diabetic tubulopathy in causing renal tissue injury and decline of kidney function in DKD, this insight offers more new therapeutic opportunities. Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-1 receptor (IGF-1R) overexpression play the main role in DKD histopathology. To regulate its biological function, IGF-1 interacts with its receptor. Then IGF-1R is phosphorylated and the signal is sent to downstream. In this report, to examine the effect of the inhibition of phosphorylated IGF-1R on diabetic tubulopathy and its mechanism, we used IGF-1R inhibitor treatment to streptozocin (STZ)-induced diabetic mice. Our results demonstrated that IGF-1R inhibitor reduced tubular stenosis, swelling of tubule, drop of epithelial cells and tubular atrophy in DKD. IGF-1R inhibitor treatment significantly increased the total number of proximal tubular epithelium in the renal cortex and outer stripe of the medulla of both kidneys {N [(PTE cells, Cortex+OSM)]}. Furthermore, IGF-1R inhibitor intervented reorganization of F-actin and overexpression of vinculin induced by high glucose in vivo

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DOI: 10.1002/eji.201970400

and in vitro. Our study suggests that inhibition of phosphorylated IGF-1R may be a promising approach for the prevention and treatment of diabetic tubulopathy.

P1241

IGF-1/IGF-1R blockade ameliorates diabetic kidney disease through normalizing Snail1 expression in a mouse model

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This study investigated the role of insulin-like growth factor-1/insulin-like growth factor-1 receptor (IGF-1/IGF-1R) in the genesis and progression of diabetic kidney disease (DKD) in a streptozocin (STZ)-induced mouse diabetes model. We show elevated IGF-1 expression in the DKD kidneys after 16 weeks of diabetic onset. Intraperitoneal administration of IGF-1R inhibitor (GSK4529) from weeks 8 to 16 post-diabetes induction ameliorated urinary albumin excretion and kidney histological changes due to diabetes, including amelioration of glomerulomegaly, inflammatory infiltration, and tubulointerstitial fibrosis. The GSK4529 treatment also attenuated alterations in renal tubular expressions of E-cad1 and matrix protein fibronectin. Moreover, renal fibrosis in DKD (without treatment) was associated with Snail1 overexpression that was effectively prevented by IGF-1R inhibition. Further experiments in cultured renal epithelial cells (NRK-52E) showed that IGF-1 silencing reproduced in vivo effects of IGF-1R inhibition with markedly attenuated Snail1 expression and nearly normalization of the Ecad1 and fibronectin expression pattern. Further Snail1 silencing prevented high-glucose induced changes without affecting IGF-1 expression, consistent with Snail1 acting downstream to IGF-1. The antifibrotic effects were also shown with benazepril or insulin treatment but to a much lesser degree. In summary, in STZ-induced diabetic mice, activation of IGF-1 in diabetic kidneys induces fibrogenesis through Snail1 upregulation. The diabetic related histological and functional changes, as well as fibrogenesis, can be attenuated by IGF-1/IGF-1R inhibition.

P1242

The role of NLRP3 inflammasome in 5-fluorouracil resistance of oral squamous cell carcinoma

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5-Fluorouracil (5-FU) is a widely used drug for the therapy of cancer. However, the chemoresistance of tumor cells to 5-FU usually limits its clinical effectiveness. In this study, we explored the role of NLRP3 inflammasome in 5-FU resistance of oral squamous cell carcinoma (OSCC). We found that expression and activation of NLRP3 inflammasome was clearly increased in OSCC tissues of patients who received 5-FU-based chemotherapy. Multivariate Cox regression analysis revealed that this high expression was significantly correlated with tumor stage and differentiation, and was associated with poor prognosis. Moreover, 5-FU treatment increased expression and activation of NLRP3 inflammasome in OSCC cells in a cell culture system and xenograft mouse model. Silencing of NLRP3 expression significantly inhibited OSCC cell proliferation and enhanced 5-FU-induced apoptosis of OSCC cells. Further investigation showed that intracellular ROS induced by 5-FU promoted the expression and activation of NLRP3 inflammasome and increased the production of interleukin (IL)-1β, which then mediated the chemoresistance. With the carcinogen-induced OSCC model, we found less and later tumor incidence in Nlrp3−/− and Caspase1−/− mice than wild-type mice. And greater decrease of tumor area was observed in the gene deficient mice treated with 5-FU. Our findings suggest that NLRP3 inflammasome promoted 5-FU resistance of OSCC both in vitro and

DOI: 10.1002/eji.201970400

in vivo, and targeting the ROS/NLRP3 inflammasome/IL-1β signaling pathway may help 5-FU-based adjuvant chemotherapy of OSCC.

P1243

Evaluation of a burkholderia pseudomallei invaded THP-1 cell model and primary investigation on mechanism of its infection to host cells

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The growth curves of BP and BT were drawn to probe their proliferation rule.
Transmission electron microscopy was used to observe the ultra-structure pathological changes of the number of intracellular bacteria and the infected host cells for the reaction of the bacteria and host cells. Plate counting was tested for bacterial colonies at different hours after invasion to analyze the intracellular growth of bacteria. The cytokines in the supernatant of the infected cells and patients'serum samples were detected to evaluate the reactivity of host cells and the effect of PEG3350 on the bacteria-affected cells and intracellular survival. The inflammatory responses of infected cells to BP and BT strains were contrasted and analyzed. Thereby the stabilityof this model was evaluated. The model of BP-infected THP-1 cells is constructed successfully in this study. The mechanism of BP infecting the host cells is obtained primarily, which founding a precondition for the interaction between this pathogenic bacteria and host cells in the further study.
Funding: Project supported by key science and technology program of hainan province ZDXM2014143
Funding: Project supported by hainan natural science foundation 817398

P1244

BPI-ANCA in chronic obstructive pulmonary disease with pulmonary Pseudomonas aeruginosa colonization: a potentially novel indicator for poor prognosis

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Objective: The aim of the current study was to evaluate the clinical value of serum antineutrophil cytoplasm autoantibodies (ANCA) directed against bactericidal/permeability-increasing protein (BPI) detection in chronic obstructive pulmonary disease (COPD) patients with pulmonary Pseudomonas aeruginosa (P.aeruginosa)colonization.
Materials and methods: A total of 126 patients with COPD complicated with lung P.aeruginosa colonization between June 2014 and August 2016 were enrolled. Serum BPI-ANCA was detected by enzyme-linked immunosorbent assay and patients were divided into BPI-ANCA(+) group and BPI-ANCA (-) group. Lung function test, chronic obstructive pulmonary disease assessment test (CAT) and 6-minute walking test (6MWT) were conducted for each patient during the period of clinical stabilization and FEV1%pred, FEV1/FVC(%), CAT score, and 6 min walking distance (6 MWD)were compared between two groups at entry and after median follow-up of 6, 12 and 18 months.
Results: Positive rate of BPI-ANCA was 46.8%; FEV1% pred and FEV1/FVC(%)were lower in BPI-ANCA(+) group than BPI-ANCA(-) group and healthy controls at entry; after a follow-up period of 6, 12, and 18 months, FEV1%pred, FEV1/FVC(%), and 6MWD decreased lower in BPI-ANCA(+) patients compared to BPI-ANCA(-) group, while CAT scores as well as number of hospital readmission

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DOI: 10.1002/eji.201970400

and hospitalization days in acute exacerbation period were higher with difference statistically significant (P< 0.05).
Conclusion: Positive serum BPI-ANCA was a poor prognostic factor for COPD patients with pulmonary P.aeruginosa colonization, which can be used as an indicator of patient condition monitoring.

P1245

CD157 confers host resistance to Mycobacterium tuberculosis via TLR2-CD157-PKCzeta-induced ROS production

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Recruitment of monocyte to the infection site is critical for host resistance against Mycobacterium tuberculosis (Mtb). CD157 has a crucial role in neutrophil and monocyte transendothelial migration and adhesion, but its role in tuberculosis is unclear. Here, we show that both mRNA and protein levels of Cd157 are significantly increased during Mtb infection. Deficiency of Cd157 impaired host response to Mtb infection by increasing bacterial burden and inflammation in lung in murine TB model. In vitro experiments show that the bactericidal ability was compromised in Cd157 KO macrophages, which was due to impaired Mtb-induced ROS production. We further reveal that CD157 interacts with TLR2 and PKCzeta and facilitates Mtb-induced ROS production, which resulted in enhancing Mtb killing. For the clinic aspect, we observe that the expression of CD157 decreases after effective anti-TB chemotherapy. CD157 is specific increased in pleural fluids in tuberculous pleurisy patients compared to pneumonia and lung cancer. Interestingly, the levels of soluble CD157 correlate with human peripheral monocyte derived macrophage bactericidal activity. Exogeneous application of soluble CD157 could compensate for macrophage bactericidal ability and restore ROS production. In conclusion, we have identified a novel protective immune function of CD157 during Mtb infection via TLR2-dependent ROS production. Application of sCD157 might be an effective strategy for host directed therapy against TB in those with insufficient CD157 production.

P1246

Beta 3 adrenoceptor signaling pathway inhibites inflammasome activity in macrophages

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Background: Inflammatory bowel diseases (IBD) are a worldwide health-care problem with a continuously increasing incidence, caused by an impaired or inappropriate inflammatory response toward intestinal bacteria that is mainly headed by the recruitment of inflammatory macrophages. Among environmental factors, stress, *via* the secretion of cathecholamines, has been shown to

DOI: 10.1002/eji.201970400

activate catecholaminergic receptors. a-adrenoreceptors boost inflammation/oxidative stress while b2-adrenergic (b2-AR) signaling suppresses immunity. We demonstrated that b3-AR, is also expressed and functional in human macrophages. Thus, the investigation of b3-AR pathway could be of interest for the treatment of IBD.
Methods: the effects of β3AR stimulation on inflammasome activation are elusive and the underlying mechanisms need to be explored
Results: However,. In this study, we found that ICI 215,001 (10^{-8} M-10^{-5} M) prevented the cleavage of caspase-1 and the activation of NLRP3 inflammasome in THP-1 derived macrophages, reduced the release of pro-inflammatory cytokines and interleukin-1β (IL-1β) in vitro. Blockade of adenosine 3′,5′cyclic monophosphate (cAMP)/protein kinase A (PKA) signaling pathway showed inhibition to the anti-inflammatory effects of ICI 215,001. These results imply that the stimulation of β3AR signal pathway shows anti-inflammatory effect via inhibiting NLRP3 inflammasome.
Conclusion: These preliminary results suggest that the use of b3-agonists may be of interest for the pharmacological managementof IBD.

P1247

Investigation of VEGF, FGF and PDGF factors in patients with brain tumor in Iran

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Background: Gliomas and meningiomas are the two most common types of human brain tumors. Hypoxic tumor cells secrete angiogenic cytokines, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) that stimulate neovascular formation. We measured these serum levels in patients with meningioma and glial cell tumors.
Materials and methods: 90 samples on patients diagnosed with meningioma/glioma were collected. All demographic and clinical data were registered. Serum levels of VEGF, PDGF and FGF were measured by ELISA methods. The intraoperative bleeding and tumor volume were recorded.
Results: Ninety patients were enrolled in this study, 30 in each group. Patients VEGF level with cranial tumor, glioma/meningioma had increased. VEGF level was highest among larger tumors, grade tumors, and in glioblastoma multiform. The highest VEGF levels were seen in parasagittal meningioma ($p \leq 0.05$). PDGF was slightly raised in glial cell tumors, which was significantly higher in meningioma ($p \leq 0.05$). Higher PDGF correlated with increased intraoperative bleeding, especially in meningioma cases. Oligodendroglial tumors expressed higher PDGF levels in contrast to other glial tumors ($p \leq 0.05$). FGF level was not statistically significant.
Conclusion: We observe increased serum levels of VEGF and PDGF in brain patient´s tumor. A different role for PDGF was found in the pathogenesis of meningioma.
Key Words: Angiogenic factors, Meningioma, Glioma

P1248

Caspase-1 initiates apoptosis in the absence of gasdermin D

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Caspase-1 activated in inflammasomes triggers a programmed necrosis called pyroptosis, which is mediated by gasdermin D (GSDMD). However, GSDMD-deficient cells are still susceptible to caspase-1-mediated cell death. Therefore, here, we investigated the mechanism of caspase-1-initiated cell

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DOI: 10.1002/eji.201970400

death in GSDMD-deficient cells. Inflammasome stimuli induced apoptosis accompanied by caspase-3 activation in GSDMD-deficient macrophages, which largely relied on caspase-1. Chemical dimerization of caspase-1 induced pyroptosis in GSDMD-sufficient cells, but apoptosis in GSDMD-deficient cells. We found that caspase-1-induced apoptosis involves the Bid-caspase-9-caspase-3 axis, which can be followed by GSDME-dependent secondary necrosis/pyroptosis. However, Bid ablation did not completely abolish the cell death, suggesting the existence of an additional mechanism. Furthermore, cortical neurons and mast cells exhibited little or low GSDMD expression and underwent apoptosis after oxygen glucose deprivation and nigericin stimulation, respectively, in a caspase-1- and Bid-dependent manner. This study clarifies the molecular mechanism and biological roles of caspase-1-induced apoptosis in GSDMD-low/null cell types.

P1824

A20 degraded MyD88 by facilitating Nrdp1 interacting with E2 ubiquitin, and reduced activation of TBK1 by inhibiting the binding between Nrdp1 and TBK1

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A20 can alleviate inflammation by negatively regulating the pathways (such as TLRs, IL-1βR, TNFR, BCR, TCR, NOD2, IL-17R) through its ubiquitin editing effect. Recent studies have shown that A20 can inhibit the polyubiquitination of TRAF6 through its de-ubiquitination editing effect, and then inhibits the activation of NF-kappa B and aggravates inflammatory damage. Nrdp1 (neuregulin receptor degradation protein1) has been shown to bind with MyD88 to promote ubiquitination and degradation of MyD88, and then inhibits the activation of the key downstream signaling molecule NF-κB. However, the regulatory mechanism of the Nrdp1 polyubiquitination is unclear.. Both Nrdp1 and TRAF6 are the E3 ubiquitin ligases of RING family and share the similar structure. Therefore we hypothesized that A20 may promote Nrdp1 polyubiquitination by interacting with Nrdp1, and then degrade MyD88 and facilitate the inactivation of NF-κB, to reduce the inflammatory injury. We constructed A20- and Nrdp1-cells using CRISPR/Cas9 and detected the interaction between A20 and Nrdp1 by co-immunoprecipitation after LPS stimulation. We have shown that A20 can suppress the activation of NF-κB and release of inflammatory factors by facilitating Nrdp1 interacting with the E2 ubiquitin and then degrading MyD88, which depends on the ZF4 in the C-terminal domain of A20. In addition, we found that A20 can inhibit the binding between Nrdp1 and TBK1, reduced activation of TBK1 mediated by K63 linked polyubiquitination, thereby reduced the secretion of IFN-β. These findings suggest a new mechanism of A20 acting in the inflammatory response and antiviral signal regulating.

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DOI: 10.1002/eji.201970400

P1825

The expression and tissue distribution of T cell immunoglobulin and mucin-domain-1 (TIM-1) in Hepatitis B Virus (HBV)-associated hepatocellular carcinoma

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T cell immunoglobulin and mucin-domain-1 (TIM-1) has been illustrated to regulate the progress of various kinds of carcinomas but its role in the pathogenesis of hepatitis B virus (HBV)-associated hepatocellular carcinoma (HBV-HCC) is unclear. Here the expression and tissue distribution of TIM-1 in **fifty-two** cases of HBV-HCC were determined by immunofluorescence staining. Results showed that all of these HBV-HCC samples are positive for TIM-1. TIM-1 expression was found in tumor cells, tissue infiltrating lymphocytes, and it restricts on cell membrane and in the cytoplasm. Interestingly, double immunofluorescence staining shows that TIM-1 co-expresses with $CD3^+$T cells, $CD68^+$ macrophages, $CD11c^+$ dendritic cells, $CD16^+$ NK Cells, $CD31^+$ Endothelial cells and $CK\text{-}18^+$ epithelial cells. Our results clearly showed the characteristic expression and anatomical distribution of TIM-1 in HBV-HCC, and a clear understanding of their functional roles may further elucidate the pathogenesis of this carcinoma and potentially contribute to the development of novel immunotherapeutic strategies.

P1826

NLRP3 inflammasome expression in peripheral blood monocytes of patients with systemic juvenile idiopathic arthritis

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Objective: We assessed the expression of NLRP3 inflammasome and IL-1β and IL-18 in PBMCs in patients with SJIA at three time points, and to explore the role of NLRP3 inflammasome in the pathogenesis of SJIA.
Methods: A total of 38 SJIA patients and 28 controls were recruited in the study and the PBMCs were collected at three time points. The mRNA of NLRP3 were measured in cell lysates by qRT-PCR. The protein of NLRP3 in supernatant and cell lysate were evaluated by Western Blot assay. The concentration of IL-1β and IL-18 in the supernatant were measured by ELISA. Data were analyzed using student's t test.
Results: Compared with control group, the protein expression of NLRP3 in cell lysate was lower; the mRNA and protein levels of IL-1β in cell lysates and the concentration of IL-1β and IL-18 in supernatant were higher in SJIA (0W) group (all $P< 0.05$). Compared with SJIA (0W) group, the protein expression of NLRP3 in cell lysate was higher; the protein expression of IL-1β and the concentration of IL-1β and IL-18 in supernatant were significantly decreased in SJIA (2W) group ($P< 0.05$). Compared with SJIA (0W) group, the concentration of IL-18 in supernatant of PBMCs in SJIA (8W) group decreased significantly ($P< 0.05$). Other protein was no significantly difference (all $P>0.05$) .
Conclusion: The NLRP3 inflammasome may be involved in the pathogenesis of SJIA. The level of IL-1β and IL-18 in peripheral blood may be as monitoring indicator of disease activity.

DOI: 10.1002/eji.201970400

P1827

Oral delivery of FSD-C10, a novel Rho kinase inhibitor, shows effectiveness in treating experimental autoimmune encephalomyelitis

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Background: FSD-C10 has a potential therapeutic effect on experimental autoimmune encephalomyelitis(EAE) mice, but the oral effect and mechanism of FSD-C10 were not studied.
Objective: To explore the therapeutic effect and mechanism of FSD-C10 on EAE mice.
Material and methods: EAE mice were randomly divided into model group and FSD-C10 group. The mice in FSD-C10 group were orally administrated with FSD-C10 from the 3rd day after immunization to the 27th day. The mice in model group were treated with same dosage of saline. The clinical score and body mass were recorded every other day. At day 28 after immunization, the mice were sacrificed and spinal cords were obtained for HE staining and myelin staining. The cytokine of splenocyte were analyzed by ELISA.
Results: Compared with the model group, FSD-C10 decreased the clinical scores of EAE, inhibited the inflammatory cell infiltration and demyelination in the spinal cord, decreased the expressions of IL-1β、IL-6、TNF-α, and increased expression of IL-10.
Conclusion: Oral treatment with FSD-C10 attenuates the behavioral and pathological changes in the EAE mice, and its mechanism may be related to the Immune regulation. (Shanxi International Science and Technology cooperation project 201703D421016, NNSF of China 81703978, 81102552 and 81473577, Shanxi Scholarship Council project 2017-129 and 2017-19, Scientific and technological innovation team of integrated Chinese and western medicine for the prevention and treatment on nervous system diseases of Shanxi university of Chinese medicine 2018TD-012, Shanxi Key Laboratory project SXIDL-2018-04, Shanxi Key project 201803D31209.).

P1828

HDAC10 inhibits NLRP3 inflammasome activation by promoting NLRP3 degradation

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Post-translational modifications (PTMs) play a crucial role in NLRP3 inflammasome activation. However, whether NLRP3 has any acetylation or deacetylation is not known. Here, we aim to investigate whether acetylation and deacetylation have any effect on NLRP3 inflammasome activation. Our investigation showed that histone acetyltransferases (HATs) especially Tip60 could induce acetylation of NLRP3 and increase its protein stability. After treatment with trichostatin A, a specific deacetylase inhibitor, acetylation of NLRP3 was significantly increased, which indicated that NLRP3

DOI: 10.1002/eji.201970400

could be deacetylated by HDAC family members. After screening of interacted histone deacetylases with NLRP3, we identified histone deacetylase 10 (HDAC10) as a potential interacted protein with NLRP3. Co-immunoprecipitation assay further confirmed the interaction between HDAC10 and NLRP3. After construction of truncated mutants of these two proteins, we demonstrated HDAC10 directly bound with NLRP3 via its DAC domain. Further assay showed that HDAC10 could specifically induce deacetylation of NLRP3 and dramatically decrease the protein level of NLRP3 in a dose-dependent manner. In vitro experiments showed that knockdown of HDAC10 robustly increased the protein level of NLRP3 and its downstream signaling, with no influence on its mRNA level, which indicated a post-translational modification of NLRP3 by HDAC10. CHX assay further verified HDAC10 could reduce the stability of NLRP3 protein. In vivo study showed HDAC10 deficiency promoted caspase-1 activation and IL-1β secretion in Alum-induced peritonitis. In conclusion, HDAC10 could directly interact with NLRP3, induce its deacetylation and further inhibit NLRP3 inflammasome activation, which indicated a potential therapeutic strategy for NLRP3 inflammasome-related diseases.

P1829

High salt diet accelerates the progress of murine lupus through dendritic cells via P38MAPK and the STAT1 signaling pathway

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In recent years, autoimmune diseases have seen an increased worldwide incidence and prevalence. Genetic predisposition and environmental factors have been identified as contributors for systemic lupus erythematosus (SLE). Recently, changes in modern dietary habits have been suggested to play a role in the increased incidence of SLE. Given the importance of dendritic cells to the pathogenesis in SLE, we herein explored the influence of excessive dietary sodium chloride on dendritic cells in a murine systemic lupus erythematosus model in which bone marrow derived dendritic cells (BMDC) incubated with activated lymphocyte-derived DNA (ALD-DNA) were transferred to C57BL/6 mouse recipients. We observed that high dietary salt intake not only markedly aggravated the lupus disease progression but was also accompanied by elevated activation of dendritic cells. Sodium chloride treatment also stimulated the vitro maturation, activation and antigen presenting capacity of dendritic cells and pretreatment of the BMDCs with sodium chloride also exacerbated BMDC-ALD-DNA induced lupus. These mice produced greater levels of autoantibodies, pro-inflammatory cytokines, greater splenomegaly and lymphadenopathy and enhanced pathogenic renal lesions. The P38MAPK-STAT1 pathway played an important role in several parameters of sodium chloride-induced enhancement of dendritic cell immune activities. Taken together, our results demonstrate that high salt dietary intake promotes immune activation of dendritic cells through the P38MAPK-STAT1 signaling pathway and aggravates the features of systemic lupus erythematosus. Thus, changes in diet may not only provide a novel strategy for prevention or amelioration of disease manifestations, but which may also facilitate treatment of patients with lupus and other autoimmune diseases.

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DOI: 10.1002/eji.201970400

P1830

Echinococcus granulosus cyst fluid is recognized by receptors on DCs to inhibits TLR4 response via c-Raf phosphorylation

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Echinococcus granulosus can escape the inflammatory response in the host and survive for a long period. Dendritic cells (DCs) play a major role in host immune responses. However, the DCs receptors and intracellular pathways targeted by *Echinococcus granulosus* are poorly understood. In this study, we found that *Echinococcus granulosus* cyst fluid (EgCF) interfered with the LPS-induced expression of colony-stimulating factors CD80 and CD86, as well as the activation of NFκB p65 and p38 MAPK signaling pathways in vitro. Furthermore, we showed that EgCF-induced c-Raf phosphorylation was critical for down-regulating the phosphorylation of NFκB p65 and the secretion of IL-12 and TNF-α. Finally, we demonstrated that glycosylated molecules in EgCF could trigger c-Raf phosphorylation by binding to multiple receptors including mannose receptor (MR) and macrophage galactose C-type lectin (MGL) on BMDCs. Together, our data indicated that c-Raf phosphorylation induced by glycomolecules in EgCF binding to C-type lectin played an important role in the interference with TLR4-mediated signaling pathways.

P1831

Hyperglycemia aggravates acute liver injury by promoting liver-resident macrophage NLRP3 inflammasome activation via inhibiting AMPK/mTOR-mediated autophagy induction

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Although the detrimental effects of diabetes mellitus/hyperglycemia have been observed in many liver disease models, the function and mechanism of hyperglycemia regulating liver-resident macrophages (Kupffer cells, KCs) in thioacetamide (TAA) induced liver injury remains largely unknown. In this study, we evaluated the role of hyperglycemia in regulating NLRP3 inflammasome activation by inhibiting autophagy induction in KCs in the TAA-induced liver injury model. Type I diabetes/hyperglycemia was induced by streptozotocin (STZ) treatment. Compared to the control group, hyperglycemic mice exhibited a significant increase in intrahepatic inflammation and liver injury. Enhanced NLRP3 inflammasome activation was detected in KCs from hyperglycemic mice, as shown by increased gene induction and protein levels of NLRP3, caspase-1, ASC and IL-1β, compared to control mice. NLRP3 inhibition by its antagonist CY-09 effectively suppressed inflammasome activation in KCs and attenuated liver injury in hyperglycemic mice. Furthermore, inhibited autophagy activation was revealed by transmission electron microscope detection, decreased LC3B protein expression and p-62 protein degradation in KCs isolated from TAA-stressed hyperglycemic mice. Interestingly, inhibited AMPK but enhanced mTOR activation was found in KCs from TAA-stressed hyperglycemic mice. AMPK activation by its agonist AICAR or mTOR signaling knockdown by siRNA restored autophagy activation, and subsequently, inhibited NLRP3 inflammasome activation in KCs, leading to ultimately reduced TAA-induced liver injury in the hyperglycemic mice. Our findings demonstrated that hyperglycemia aggravated TAA-induced acute liver injury by promoting liver-resident macrophage NLRP3 inflammasome activation via inhibiting AMPK/mTOR-mediated autophagy. This study provided a novel target for the prevention of toxin-induced acute liver injury.

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DOI: 10.1002/eji.201970400

P1832

Aminooxy-acetic acid inhibits experimental autoimmune uveitis by modulating the balance between effector and regulatory lymphocyte subsets

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A small molecular compound, aminooxy-acetic acid (AOA), has been shown to modulate experimental autoimmune encephalomyelitis (EAE). The current study was designed to investigate whether AOA could have a similar effect on the development of experimental autoimmune uveitis (EAU) and to further explore underlying mechanisms of action of this drug.
EAU was induced in C57BL/6J mice by immunization with interphotoreceptor retinoid binding protein peptide 651-670 (IRBP651-670). AOA (500µg/d) or vehicle was administered by intraperitoneal injection from day 10 to 14 after EAU induction. The severity was assessed by clinical and histological scores. The integrity of the blood retinal barrier was detected with Evans Blue. Frequencies of splenic Th1, Th17 and Foxp3^{+}Treg cells were examined by flow cytometry. The production of cytokines and mRNA expression was detected by ELISA and RT-PCR. The expression of p-Stat1 and NF-κ B was detected by Western blotting.
AOA was found to markedly inhibit the severity of EAU, as determined by clinical and histopathological examinations. AOA partially maintained BRB integrity. Functional studies showed a decreased frequency of inflammation cells and cytokines. Functional studies showed that AOA inhibited the expressions of p-Stat1 and NF-κ B.
This study shows *that AOA inhibits the severity and development of EAU by modulating the balance between regulatory and pathogenic lymphocyte subsets.*

P1833

The effect and mechanism of palmatine on DSS-induced ulcerative colitis

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Activation of NLRP3 inflammasomes is crucial in the pathological process of Ulcerative colitis (UC), which could be negatively regulated by PINK1/Parkin-driven mitophagy. Palmatine is an herb derived isoquinoline alkaloid with potent anti-inflammatory and anti-bacteria activities. In present study, we evaluated the effect of palmatine on dextran sulfate sodium (DSS)-induced mice colitis and examined whether its effect is exerted by promoting mitophagy-mediated NLRP3 inflammasome inactivation. The result showed that palmatine (40, 100 mg/kg) significantly prevented bodyweight loss and colonic shortening in DSS mice and reduced the disease activity index and histopathologic score. The levels of MPO, IL-1β, TNF-α and the number of F4/80+ cells in colon of DSS mice were remarkably decreased by palmatine. Moreover, palmatine suppressed NLRP3 inflammasomes activation, but enhanced the expression of the mitophagy-related proteins involving LC3, PINK1 and Parkin in colonic tissue of DSS mice. These effects were consistent with the in vitro data revealing that palmatine inhibited the activation of NLRP3 inflammasomes, while promoted the expression and mitochondrial recruitment of
PINK1 and Parkin in THP-1 cell differentiated macrophages. Furthermore, the effect of palmatine on THP-1 cells was neutralized by a mitophagy inhibitor Cyclosporin A (CsA) and PINK1-siRNA. In

DOI: 10.1002/eji.201970400

parallel, CsA significantly attenuated the therapeutic effect of palmatine in DSS mice, illustrating that the anti-colitis effect of palmatine is
closely related to mitophagy. Taken together, the current results demonstrated that palmatine protected mice against DSS-induced colitis by facilitating PINK1/Parkin-driven mitophagy and thus inactivating NLRP3 inflammasomes in macrophage.

P1834

Role of inflammasomes in host innate immune response and defense against *Pasteurella multocida* infection

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Pasteurella multocida (*P. multocida*) is a gram-negative bacterium that is responsible for a variety of diseases in birds and mammals, including humans. We have previously reported that the *P. multocida* serotype A strain PmCQ2 causes severe lung pneumonia in bovines. Transcriptomic analysis showed that many genes related to the immune response were significantly upregulated in the lungs of mice infected with *P. multocida* compared with uninfected mice. However, the mechanism by which *P. multocida* induces host inflammatory cytokine secretion is poorly understood. In this study, the mechanism of caspase-1 activation and subsequent IL-1β secretion in macrophages infected with *P. multocida* was elucidated. The nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome was shown to be involved in inducing this cellular response. Compared with wild-type macrophages, *Nlrp3*$^{-/-}$ macrophages exhibited a clear decrease in caspase-1 activation and IL-1β secretion in response to *P. multocida* infection. Our results provide new insight into the host proinflammatory immune response against *P. multocida* and the critical involvement of the NLRP3 inflammasome in this activity.

P1835

Micheliolide inhibits NLRP3 inflammasome formation in macrophage by degrading IRAK4 and protects against ischemic stroke

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Background and Purpose: NLRP3 inflammasome in macrophage is activated after ischemic stroke and exacerbates stroke severity. It was previously found that micheliolide (MCL) inhibited NLRP3 signaling through suppressing NFκB pathway. Nevertheless, whether MCL could down-regulate formation of NLRP3 inflammasome and the therapeutic effect of MCL in ischemic stroke remains elusive. The current study evaluated the therapeutic effect of MCL in ischemic stroke and explored the impact of MCL on NLRP3 inflammasome in macrophage.
Methods: MCL (10mg/kg) was administered (i.v.) to mice 2h after transient middle cerebral artery occlusion (tMCAO, 1h). Stroke outcomes, neural inflammation, NLRP3 inflammasome formation of macrophage in stroke lesions were assessed. Underlying mechanism of the impact of MCL on NLRP3

DOI: 10.1002/eji.201970400

inflammasome formation was studied *in vitro* with primary cultured bone marrow derived macrophage of mice.
Results: MCL offered promising therapeutic effects on ischemic stroke. Mice displayed reduced infarct volumes, improved neurological functions and limited neural inflammation after MCL treatment. NLRP3 inflammasome formation in macrophage in stroke lesion was significantly inhibited by MCL treatment. Mechanistically, we found that activation of IRAK4 was vital in the process of NLRP3 inflammasome formation. MCL efficiently enhanced ubiquitination of IRAK4 thus promoting its degradation.
Conclusion: MCL displays potent therapeutic efficacy in ischemic stroke by suppressing post-stroke neural inflammation. IRAK4 plays vital role in the process of NLRP3 inflammasome formation in macrophage thus promoting post-stroke neural inflammation. Treatment of MCL enhances IRAK4 degradation thus inhibiting NLRP3 inflammasome formation.

P1836

Sinomenine attenuates Ang II induced podocyte autophagy, and also attenuates Ang II by podocytes induced via NETs

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Sinomenine, a pure alkaloid extracted from the Chinese medicinal plant, and sinomenine hydrochloride (SN) has been successfully used for the therapy of kidney diseases. Autophagy is a cytoprotective mechanism. However, excessive autophagy may lead to podocyte depletion. Angiotensin II (Ang II) signifcantly promotes podocyte autophagy. The present study evaluated the effect of SN in podocytes induced by Ang II. SN alone did not effect ROS generation or podocyte autophagy. SN attenuated Ang II-induced autophagy in podocytes. Furthermore, SN decreased the level of ROS generation in Ang II-induced podocytes via inhibition of NOX subunit p47-phox translocation to the membrane. To further defines whether neutrophil extracellular traps (NETs) have effect on podocyte injury, We observed NETs stimulate Ang II by podocytes.

P1837

Tumor associated macrophages (TAM) infiltration associate with IL-10 enriched microenvironments and epithelial-mesenchymal transition in invasive laryngeal carcinoma

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Laryngeal carcinoma (LC) is a highly invasive tumor associated with chronic inflammatory conditions. Tumour-associated macrophages (TAM) have lately emerged as a key inflammatory component which may contribute to head and neck carcinogenesis and epithelial-mesenchymal transition (EMT). Here, we aimed to explore the relationship between the TAM infiltration and EMT in invasive LC biopsies. For this, TAM markers (CD68, CD163), EMT markers (E-cadherin, β-catenin, vimentin, MMP-9) and

DOI: 10.1002/eji.201970400

IL-10 expression were evaluated by immunohistochemistry (IHC) in invasive LC (n=20), adjacent in-situ carcinoma (n=9) and normal epithelium (n=10). Nitric oxide (NO) and IL-10 levels, and MMP activity were assessed in LC plasmas (n=20) in comparison to healthy donors' plasmas (HD). We observed an abundant infiltration of TAM in invasive LC with a significant polarization of the macrophages towards and M2 profile (CD163/CD68, p≤0.05). Positive correlations were found between TAM density and vimentin expression (CD68+: r=0,53, p=0,01; CD163+: r=0,44, p=0,1) and MMP-9 expression (CD68+: r=0,56, p=0,01; CD163+: r=0,75, p=0,001). Importantly, tissues analysis showed presence of a strong expression of IL-10 in the tumor areas and the stroma, could be linked to CD68+ cells infiltration (r=0.73, p=0.0002). Markedly, stromal IL-10 expression strongly associated with E-cadherin loss (r=-0.61, p=0.004) and vimentin expression (r=0.64, p=0.002). Our results suggest that in invasive LC tumors, TAM infiltration associate with an IL-10 enriched immunosuppressive environment to promote EMT and tumor invasiveness.

P1838

IL-10/STAT3 signaling enhances expression of apoptosis inhibitor of macrophage (AIM) in macrophages

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Apoptosis inhibitor of macrophage (AIM) is a glycoprotein that modulates the signaling in inflammatory response including infection, cancer or other immune disease. Recent studies suggest that AIM enhances the M2 macrophage polarization similar to interleukin-10 (IL-10).
In Gene Expression Omnibus profile data, IL-10 enhanced AIM expression at gene level in human monocytes. Here, we investigated to validate this result in mouse bone-marrow derived macrophages (BMDM) treated with IL-10 and understand the pathway downstream of IL-10 that leads to AIM expression. We demonstrated that IL-10-induced increases in mRNA and protein expression of AIM in BMDM in a time-dependent manner. In addition, IL-10 enhanced phosphorylation of both of STAT1 and STAT3. Knockdown of STAT3 after the transfection of BMDM with STAT3-specific siRNAs or the STAT3-specific inhibitor 5,15-Diphenylporphyrin (DPP) reduced IL-10-induced AIM expression at protein level. However, knockdown of STAT1 had no effect. IL-10-induced STAT3 activity enhanced the AIM promoter activity by directly binding the promoter of AIM gene. Additionally, reduction of lipopolysaccharide (LPS)-induced IL-1β production by IL-10 was inhibited in BMDM from *AIM*$^{-/-}$ mice. Our data suggest the possibility that IL-10/STAT3/AIM pathway plays an essential role for anti-inflammatory response through reduction of IL-1 production.

P1839

Caspase-11 dependent-pyroptosis exacerbates sterile liver inflammation

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Sterile liver inflammation occurs in response to released damage-associated molecular patterns from cell death in the absence of pathogens. Caspase-11 non-canonical inflammasome, a cytosolic endotoxin (lipopolysaccharide: LPS) receptor, mediates pyroptosis of macrophages during sepsis. However, the role of caspase-11 in sterile liver inflammation (ischemia/reperfusion (I/R) injury) remains unknown. We sought to determine the mechanisms by which caspase-11 activation results in sterile

DOI: 10.1002/eji.201970400

inflammatory injury during liver I/R.
Here we found that liver tissue levels of activated caspase-11, downstream cleaved Gasdermin D (GSDMD) and pyroptosis were dramatically elevated in ischemic liver lobes after I/R compared with sham mice. Caspase-11$^{-/-}$ (casp-11$^{-/-}$) mice exhibited significant protection from hepatic I/R injury compared to Wild type (WT) mice (ALT: 1369±186 IU/L in casp-11$^{-/-}$, *vs* 2998±182 IU/L in WT). Tissue level of activated caspase-1, IL-1β and IL-18 were also significantly lower in casp-11$^{-/-}$ mice, compared to WT mice after hepatic I/R. Consistently, pro-inflammatory cytokines (IL-1β, IL-6 IL-12, TNF-α) are significantly reduced in casp-11$^{-/-}$ mice after liver I/R by Luminex® assay. Compares with caspase-11 FLOX mice, mice with caspase-11 depletion in myeloid cells or hepatocytes protected the liver from I/R injury. Interestingly, liver damage and inflammation significantly increased in GSDMD KO mice compared with WT after liver I/R. *In vitro*, caspase-11 is activated in macrophages in response to stimulation of exogenous DAMPs such as HMGB1 or Mitochondrial transcriptional factor A (TFAM) which is released from damaged hepatocytes during liver I/R.
In summary, Caspase-11 non-canonical inflammsome is activated and exacerbates organ injury and inflammation during liver I/R.

P2476

Endoglin+/αGal+ HepG2/dendritic fusion cell vaccine enhance antitumor immunity targeting tumor cells and neovascular endothelial cells in tumor

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Hepatocellular carcinoma (HCC) is a common malignant tumor with poor prognosis and high mortality. Here we developed a novel vaccine against HCC and neovascular endothelial tumor cells by (a) engineering HepG2 cells to express porcine α-1,3-galactosyltransferase (αGal) and the extracellular domain of endoglin (END), and then (b) fusing these cells with dendritic cells (DCs) from healthy volunteers. These END+/Gal+ -HepG2/DC fusion cells expressed whole tumor antigens, which stimulated cytotoxic T lymphocytes to proliferate and secrete IFN-γ. These primed T cells were selectively cytotoxic against αGal-producing and END-expressing cells, including cells involved in tumor angiogenesis. Primed and expanded T cells effectively inhibited tumor growth and prolonged survival of mice bearing human hepatomas. These results suggest that priming cytotoxic T cells with END+/Gal+-HepG2/DC fusion cells can effectively slow tumor growth and inhibit tumor angiogenesis, indicating strong clinical potential.

P2477

The effect of autophagy on the inflammatory response of macrophages infected with *Toxoplasma gondii*

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Inflammasomes played a crucial role in host defense against pathogens, however, intense inflammation could induce inflammatory diseases. Autophagy is a catabolic process which could regulate inflammation through many mechanisms, but less is known about the effect of autophagy on the inflammatory response in the context of *Toxoplasma gondii* infection. In this study, mice peritoneal macrophages were infected with *Toxoplasma gondii* after induction with Rapamycin or Bafilomycin A1, our results showed that the levels of LC3 and ASC could be induced by infection of *Toxoplasma gondii*, and ASC was decreased after Rapamycin treatment, meanwhile it was increased after

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DOI: 10.1002/eji.201970400

Bafilomycin A1 treatment, but IL-1β was decreased after the regulation of autophagy. Secondly, the mRNA of Arg1 and IL-10 were upregulated after the macrophages were infected with *Toxoplasma gondii*, while the mRNA of iNOS and IL-12 were upregulated with the decrease of Arg1 and IL-10 after Bafilomycin A1 treatment. Thirdly, the mRNA of SAG1 was increased after the regulation of autophagy. Our results suggested that the autophagy and inflammasome of macrophages could be induced by infection of *Toxoplasma gondii* which may indicated that autophagy play a negative regulatory on inflammation; the polarization of macrophages infected with *Toxoplasma gondii* were promoted to M1 instead of M2 while its autophagy was blocked; the proliferation of *Toxoplasma gondii* in macrophages was enhanced with the regulation of autophagy and its mechanism needs to be further investigated.
Keywords: *Toxoplasma gondii*, macrophages, autophagy, inflammasome

P2478

Zika virus infection induces acute kidney injury by activating NLRP3 inflammasome in mice

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Zika virus (ZIKV) is a newly emerging flavivirus that broadly exhibits in various bodily tissues and fluids, especially in the brain, and ZIKV infection often causes microcephaly. Previous studies have been reported that ZIKV infects renal cells and can be detected in the urine samples of infected individuals, however, whether ZIKV infection causes renal diseases and the underlying mechanisms remain poorly understand. Here, we identified that ZIKV infection resulted in acute kidney injury (AKI) and water re-absorption disorder in both newborn and adult mouse models, by increasing the levels of AKI-related biomarkers [e.g., serum creatinine (Src), kidney injury molecular -1 (Kim-1) and neutrophil gelatinase-associated lipocalin (NGAL)] and decreasing the expression of aquaporins, respectively. Furthermore, *in vivo* studies using ZIKV-infected newborn and adult mouse models demonstrated that ZIKV infection dramatically induced Nod-like receptor 3 (NLRP3) inflammasome activation in the kidney, therefore contributed to AKI. Notably, *in vitro* studies showed that overexpression of B-cell lymphoma-2 (Bcl-2) in human proximal tubular renal cells attenuated NLRP3 activation and renal apoptosis, thus reducing the renal cells injury induced by ZIKV infection. Overall, our study demonstrated that ZIKV infection induced AKI by enhancing NLRP3 inflammasome activation via suppressing Bcl-2, which may provide a potential therapeutic target for ZIKV-associated renal diseases and inflammatory damage.

P2479

Report of bla_{IMP-45} and armA co-carrying multidrug resistancePseudomonas isolates and characterization of their genetic environment

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The primary goal of this work is to discuss the mechanism of *Pseudomonas spp*. resistance to carbapenam and aminoglycoside antibiotics, which were recovered from our hospital. In a total of 487 non-replicate recovered isolates,three multidrug resistant *Pseudomonas aeruginosa* and three *Pseudomonas putida*,producing class B carbapenemase, were identified. They were all positive for Carba NP test.PCR screening followed by sequencing confirmed carriage of bla_{IMP-45} and *armA*,which confer resistance to β-lactams(except aztreonam) and aminoglycosides.. These two genes co-located in a Tn*1548*-associatedregion. PFGE disclosed that these isolates were not clonal closely related to

DOI: 10.1002/eji.201970400

each other, except two *P.aeruginosa*isolates were clonal related. S1-PFGE results showed thatthese isolates all carried plasmids of large size(300Kb~600Kb). This study showed that *Pseudomonas spp*. isolates co-carried bla_{IMP-45} and *armA* were disseminated in clinical settings. Spread of these genes may attribute to horizontal gene transfer of related entities. This is the first report of detection of bla_{IMP-45} and *armA in P. putida.*
Funding: Project supported by Youth fund project of hainan natural science fund 818QN326

P2480

Recombinant expression and antibody preparation of BPSS1617 protein in type Ⅲ secretion system of Burkhlderia pseudomallei

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The full-length coding sequence of BPSS1617 was amplified with PCR and cloned into the prokaryotic expression plasmid pET-22b.The recombinant plasmid was transformed into E.coli BL21 (DE3) strain for expression of the target protein, which, after purification, was used to immunize New Zealand white rabbits to obtain the polyclonal antibodies of BPSS1617. The specificity of the antibodies was evaluated by Western blotting,immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA) .The subcellular localization of BPSS1617 in Burkhlderia pseudomallei cells was investigated using the obtained antibodies. We
successfully obtain the recombinant BPSS1617 protein, and its specific polyclonal antibodies and identify that BPSS1617 is localized mainly in the cytoplasm of Burkhlderia pseudomallei cells.
Funding: Project supported by hainan natural science foundation 817398

P2481

A case report Splenic abscess caused by Burkholderia pseudomallei

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Melioidosis is an emerging infectious disease caused by Burkholderia pseudomallei. To our knowledge, there have been very few cases of splenic abscesses due to melioidosis in Hainan, China. The patient was a 55-year-old male farmer, who was admitted in our hospital with persistent left epigastric dull pain accompanied by chills and febrile. One month before, the patient presented with persistent abdominal pain. After received anti-infection therapy, the subjective symptoms eased slightly, but recently he suffered from intermittent abdominal pain again. The patient was treated by surgical excision and anti-infection therapy. Bacteria isolated from splenic pus were identified as B. pseudomallei by the Phoenix-100 system and indirect immunofluorescence. In endemic areas, B. pseudomallei should be considered as a causative organism of splenic abscess in patients withestablished risk factors. The isolation of B. pseudomallei from abscess sites is crucial to improve clinical outcomes by appropriateantimicrobial therapy coupled with surgical drainage.
Funding：Project supported by hainan natural science foundation 817398

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DOI: 10.1002/eji.201970400

P2482

Burkholderia pseudomallei-derived miR-3473 enhances NF-κB via targeting TRAF3 and isassociated with different inflammatory responses compared to Burkholderia thailandensis in murine macrophages

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We found that Bp and Bt had similar phenotypes in terms of intracellular replication, dissemination, TNF-α release and apoptosis in RAW264.7 macrophages or TC-1 pulmonary cell but in different level. After microRNA array analysis, we found some microRNAs were significantly expressed in macrophages treated by Bp. miR-3473 was one of them specificallyinduced, but not significantly changed in Bt-treated macrophages. In vivo, it was found that miR-3473 expression of total lungs cells from Bp-treated was higher than that from Bt-treated mice. And miR-3473 inhibitor was able to decrease the TNF-α release of mice and prolong the survival of mice with Bp infection. In sum, miR-3473 plays an important role in the differential pathogenicity of Bp and Bt via miR-3473-TRAF3-TNF-α network, and regulates TNF-α release, cell apoptosis and animal survival after Bp treatment. In this study, we have found a specific microRNA is related to bacterial virulence and provide insight into the mechanism for host-bacteria interaction, which suggests that potential oligonucleotides should be applied against bacterial infection.
Funding: This study was supported by hainan natural science foundation 817398

P2483

Development of tissue inflammation accompanied by NLRP3 inflammasome activation in rabbits infected with Treponema pallidum strain Nichols

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Objective: To investigate the expression of the NLRP3 inflammasome in the development of tissue inflammation in rabbits infected with *T. pallidum*.
Methods: Forty-five rabbits were randomly assigned to a blank group or an infection group, and the latter was divided into no BPG and BPG treatment subgroups. The development of lesions was observed, and biopsies of the injection site and various organs were obtained for NLRP3, caspase-1, and IL-1β mRNA analysis during infection. Blood was also collected for the determination of IL-1β concentration.
Results: Rabbits infected with T. pallidum exhibited NLRP3 inflammasome activation and IL-1β secretion in cutaneous lesions, showing a trend in elevation to decline; NLRP3 mRNA expression reached a peak at 18 d in the BPG treatment subgroup and 21 d in the no BPG treatment subgroup and returned to “normal” levels [vs. the blank group ($P > 0.05$)] at 42 d post-infection. The trend was reached a peak at 16 d in the BPG treatment subgroup and 18 d in the no BPG treatment subgroup. NLRP3, caspase-1, and IL-1 β mRNA expression levels were slightly different in different organs. NLRP3 inflammasome activation was also observed in the kidney, liver, lung, spleen and testis. IL-1 β expression was observed in the various organs; however, there was no detectable level of IL-1 β in the testes of the infected rabbits.
Conclusions: This study established a clear link between NLRP3 inflammasome activation and the development of tissue inflammation in rabbits infected with *T. pallidum*. BPG therapy imperceptibly adjusted syphilitic inflammation.

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DOI: 10.1002/eji.201970400

P2484

Insulin decreases the level of sTREM-1, TNFα, and HMGB1 in MODS rats with or without control of hyperglycemia after severe burn injury in vivo

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There is still no direct evidence whether insulin plays an anti-inflammatory effect after severe trauma by uncontrolling hyperglycemia in vivo. By adjusting the ratio of insulin and glucose combination, we used different dosage of insulin, observed the effect on the anti-inflammatory and major organ function protection of traumatic MODS rats with and without control of hyperglycemia. The results showed that the effect of anti-inflammation and protection of major organ function was positively correlated with the dosage of insulin rather than the control of hyperglycemia. The results suggested that by adjusting the ratio of insulin and glucose different dosage of insulin could be used to treat severe post-traumatic inflammatory pathological immune response, without limitation of blood glucose levels. In vivo effect of anti-inflammation of insulin do not depend on controlling traumatic hyperglycemia. Traumatic hyperglycemia itself might not be harmful to the body.

P2485

Knockdown of LOXL1 inhibits TGF-β1-induced proliferation and fibrogenesis of hepatic stellate cells by inhibition of Smad2/3 phosphorylation

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Liver fibrosis is pathological condition that seriously threatens human health. The lysyl oxidase (LOX) family has been reported to promote liver fibrosis. However, the effect of LOX-like 1 (LOXL1), a member of LOX family, on fibrogenesis of hepatic stellate cells (HSCs) remains unknown. The current study aimed to investigate the role of LOXL1 in liver fibrosis and the potential mechanism. We found that the mRNA and protein levels of LOXL1 were increased in transforming growth factor-beta 1 (TGF-β1)-stimulated human hepatic stellate cell line LX-2. Knockdown of LOXL1 inhibited the proliferation of TGF-β1-stimulated LX-2 cells. Knockdown of LOXL1 suppressed TGF-β1-induced expression of metalloproteinase type 1 (TIMP1), α-smooth muscle actin (α-SMA), and collagen type I (Col-I), as well as phosphorylation of Smad2 and Smad3 in LX-2 cells. In addition, the cell proliferation and fibrogenesis mediated by TGF-β1 stimulation and LOXL1 overexpression were abolished by knockdown of Smad2 and Smad3. Collectively, knockdown of LOXL1 suppressed cell proliferation and fibrogenesis in TGF-β1-stimulated HSCs via regulating the phosphorylation of Smad2/3.

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DOI: 10.1002/eji.201970400

P2486

Expression and significance of MT, NF-κB and HIF-1α in patients with inflammatory bowel disease

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Objective: To investigate the correlation and clinical significance of metallothionein (MT), nuclear factor-κB (NF-κB) and hypoxia-inducible factor-1α (HIF-1α) in colonic and peripheral blood of patients with inflammatory bowel disease.
Methods: From June 2017 to June 2018, the second affiliated hospital of Zhengzhou University was collected from the outpatients and hospitalized patients with active inflammatory bowel disease, including 30 patients with UC, 30 patients with CD, 30 cases of colon cancer surgical resection of adjacent colon tissue as a control, and 30 healthy volunteer serum samples were used as controls. The expression of MT and NF-κB in colonic mucosa was detected by immunohistochemical SABC, and the content of HIF-1α in peripheral blood were detected by ELISA.
Results: The expression of MT and NF-κB in colonic mucosa and the expression of HIF-1α in peripheral blood serum were statistically significant (P< 0.01). The expression of MT was higher than that of UC. In the CD group, the expression of NF-κB and HIF-1α was higher in the UC and CD groups than in the control group. In addition, MT was negatively correlated with NF-KB (r=-0.871, P< 0.01), and there was a significant negative correlation between the expression of MT and HIF-1α (r=-0.893,P ＜0.01). What's more, the expression of NF-κB and HIF-1α was positively correlated (r=0.814,P＜ 0.01).
Conclusions: There is a correlation between MT expression and the expression of NF-κB and HIF-1α. Down-regulation of MT may promote the expression of NF-κB and HIF-1α to participate in the pathogenesis of inflammatory bowel disease.

P2487

The expression of DAMP proteins HSP70 and cancer testis antigen SPAG9 in peripheral blood of patients with HCC and lung cancer

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There are different views of how the immune system participates in the reaction to cancer. Here, we evaluated expression of DAMP proteins HSP70 and cancer-testis antigen SPAG9 in patients with hepatocellular carcinoma (HCC) and lung cancer to explore tumor immunity. Our analysis showed that levels of HSP70 and SPAG9 antibody were significantly higher in serum of lung cancer and HCC patients than in serum from healthy subjects (P < 0.001), but there were no differences in levels of HSP70 antibody in patients and controls. Levels of serum SPAG9 antibody in newly diagnosed lung cancer patients were significantly higher than in treated lung cancer patients (P < 0.05), but there were no differences in levels of HSP70 or HSP70 antibody. Levels of serum HSP70 and SPAG9 antibody, but not HSP70 antibody, were also higher in hepatitis/cirrhosis patients than in healthy subjects (P = 0.005, P < 0.001). Levels of serum SPAG9 antibody were significantly higher in HCC patients than in hepatitis/cirrhosis patients, but there were no differences in HSP70 or HSP70 antibody levels. Finally, levels of serum HSP70 and SPAG9 antibody were significantly higher in HCC patients than in lung cancer patients (P < 0.05, P < 0.001). These results indicate that cancer-testis antigen SPAG9 induces a strong humoral immune response in cancer patients but HSP70 does not. These results show that SPAG9 has potential as a tumor-specific biomarker.

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DOI: 10.1002/eji.201970400

P2488

HSPA6 regulates the anti-HPV responses induced by hyperthermia in human keratinocytes

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Background: Human papillomavirus infection is a common clinical condition. A brand-new treatment method known as local hyperthermia has been proved to be effective against HPV infectious diseases.
Objective: This project aimed to study the underlying mechnism of hyperthermia and the effects of HSPA6.
Methods: HaCaT cell lines and CA tissues were studied in this research. A 44°C waterbath was used to mimic hyperthermia treatment. TMT-labelled mass spectrometry was used to study the proteomic alterations. RT-qPCR, western-blotting, IHC-staining and IF-staining were used to study the expression of HSPA6 and other molecules. Apoptosis, cell cycle and MTS assays were used to analyze functional changes. Bioinformatical analysis was performed with use of R 3.4.1.
Results: 1) 630 differentially expressed proteins were detected in HaCaT cells after hyperthermia, whose functions involved in metabolism regulation, apoptosis, stress responses, virus-host interaction and viral infection regulations, etc. 2) Hyperthermia induced HSPA6 expression in keratinocytes from all sources. HSPA6 deficiency damaged the resistance capacity of HaCaT cells towards hyperthermia treatment. 3) PCR-array showed that hyperthermia boosted anti-viral responses in HaCaT cells, which were bi-directionally regulated by HSPA6. 4) 44 °C hyperthermia caused 240 differentially acetylated proteins (DAPs) in HaCaT cells. HSPA6 deficiency caused a major influence to DAPs regarding RNA splicing/processing/metabolism, DNA replication and viral transcription regulation.
Conclusions: 1) 44 °C hyperthermia induces anti-HPV responses in keratinocytes in both proteomic and PTM levels. 2) HSPA6 is a potent protector, as well as a bi-directional anti-virus regulator for HaCaT cells in response to 44 °C hyperthermia.

P2489

Effect of Celastrus orbiculatus extract and Genistein on proliferation of 38B9 lymphoma cells in vitro

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Objective: To observe the regulation of Chinese herbal medicine *Celastrus orbiculatus* extract (COE) and *Genistein* (GEN) on the proliferation and secretion of inflammatory factors in 38B9 lymphoma cells *in vitro*.
Methods: 38B9 lymphoma cells were cultured *in vitro* and randomly divided into control group (CON), COE intervention group (COE), GEN intervention group (GEN) and western medicine *Cyclophosphamide* was used as positive control (CTX). The final intervention concentrations of the COE group, the GEN group, and the CTX group were 160 µg/mL, 80 µg/mL, and 25 µmol/L, respectively. At 24h, 36h, and 48h after intervention, cell apoptosis and cycle were measured by FCM. ELISA was used to detect the secretion of TNF-α, IL-6 and IL-10 inflammatory cytokines. qRT-PCR was used to detect the expression of inflammatory genes in cells.
Results: Compared with control group, the number of 38B9 lymphoma cells in each intervention group was decreased. Among them, CTX was the most significant, and the GEN inhibition rate was significantly higher than that of COE (P< 0.05). The G2-M phase decreased after COE and GEN intervention, and the S phase decreased after CTX intervention (P< 0.05). After COE and GEN

DOI: 10.1002/eji.201970400

intervention, the secretion of IL-6 in lymphoma cells from 38B9 lymphocytes was significantly decreased, IL-10 levels were significantly increased ($P < 0.05$), and the expression of TNF-α and IL-6 mRNA was significantly decreased ($P < 0.05$).
Conclusion: COE and GEN can inhibit the proliferation of 38B9 cells and decrease the the secretion of inflammatory cytokines.
Keyword: *Celastrus orbiculatus* Extract; *Glioblastoma*; Proliferation; inflammatory cytokines

P2490

Immunopathogenic effect of Campylobacter jejuni lipopolysaccharide on myelin of central nervous system in rats

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Objective: The aim of this study was to explore the role of *Campylobacter jejuni* (Cj) lipopolysaccharide (LPS) induced central neuropathy and the immunological pathogenesis.
Methods: Wistar rats were divided into control group, Cj-LPS group, LPS antibody group (Anti-LPS). Control group which mixture of normal saline and complete freund´s adjuvant (CFA). Cj-LPS (composed of LPS, CFA and saline).they were injected on rats. Neurological function was assessed and then rats were sacrificed at 4th week and 6th week. Pathohistological changes were observed by transmission electron microscopy (TEM). ELISA was used to detected myelin basic protein (MBP) and albumin (ALB) levels in rat serum and cerebrospinal fluid (CSF). And β-amyloid precursor protein (APP) and P-tau of brain and spinal cord were determined by confocal.
Results: Results indicated Cj-LPS could induce the damage of rats' brain and spinal, increased the neurologic function scores, concentrations of MBP and ALB, and the expressions of APP and P-Tau. Moreover, anti-LPS could reduce these expressions.
Conclusion: Cj-LPS damages the myelin of central nervous system in experimental rats, and this symptom may possibly through affect the expression of MBP, ALB, APP and P-Tau to induce the pathological damage of rats.

P2491

Negative regulation of MHC-I by Tim-3 contributes to infection tolerance

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T cell immunoglobulin and mucin protein 3 (Tim-3) is an immune checkpoint inhibitor that has therapeutic implications for many immune disorders. However, the mechanisms by which Tim-3 induces innate immune tolerance remain unclear. Here, we demonstrated that Tim-3 inhibits the expression of major histocompatibility complex class I (MHC-I) in macrophages at both the mRNA and protein levels by inhibiting the STAT1-NLRC5 signaling pathway. As a result, MHC-I-restricted antigen presentation by macrophages was inhibited by Tim-3 both in vitro and in an L. monocytogenes infection model in vivo. Systemic overexpression of Tim-3 or conditional knockout of Tim-3 in macrophages significantly exacerbated or attenuated CD8+ T cell tolerance and infection damage in L. monocytogenes-infected mice, respectively. We thus identified a new mechanism by which Tim-3 induces immune tolerance. Further studies on this pathway might shed new light on the mechanisms of infection tolerance and suggest new approaches for intervention.
Keywords: Tim-3; MHC-I; NLRC5; Antigen Presentation; Infection Tolerance

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DOI: 10.1002/eji.201970400

P2492

Clinical significance of serum immunoglobulin detection in patients with acute leukemia

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Objective: This study preliminarily explored the expression level of serum immunoglobulin in patients with acute leukemia and the correlation with clinical features and infection indicators.
Methods: A total of 123 newly diagnosed AL patients in our hospital from 2016 to 2018 were enrolled. All cases were divided into APL group (20 cases) , ANLL group (66 cases except APL) and ALL group (37 cases). The control group contained 50 healthy patients. The levels of IgG, IgA, IgM, κ(kappa) and λ(Lambda) in the serum of each group were determined by ELISA. The relationship between expression level of serum immunoglobulin and patient´s clinical characteristics such as gender, age, the count of peripheral white blood cells, hemoglobin, platelets, and the infection indexes C-reactive protein (CRP) and procalcitonin (PCT) were analyzed.
Results: The levels of IgG, IgA and LAMB in the ANLL group and the IgG level in the ALL group were significantly higher than the control group (P< 0.05). When the peripheral blood WBC>10×109/L or PLT>300×109/L, the average value of each immunoglobulin was higher than other groups; when PCT≥0.5ng/mL or CRP>10ng/L, immunoglobulin IgG and IgA were significantly increased (P< 0.05). In patients with infection occurred, the levels of immunoglobulins were higher than those in the uninfected group (P< 0.05).
Conclusion: The serum immunoglobulin level of patients with acute leukemia (except APL) is significantly higher than that of the healthy group, which can provide clinical diagnosis and infection assessment for patients with newly diagnosed AL.
Keywords: Acute leukemia; serum immunoglobulin; infection

P2493

Analysis and detection of drug resistance of non-fermenting bacteria during 2011-2016

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Objective: To investigate the distribution and drug resistance of non-fermenting bacteria in the various departments of the hospital, and to evaluate the method for detection of the carbapenemase by the non-fermenting strains , so as to provide scientific basis and methods for the control of nosocomial infection.
Methods: The results of non-fermenting bacteria were analysed by using WHONET 5.6 software in order to know the composition,distribution,drug resistance rate ,during 2011-2016.Then the Carbapenem Inactivation Method(CIM),Carba NP test(CNP),rapid Carba NP Test(RCNP) were performed for the resisitant strains to carbapenem in non- fermenting bacteria to detect whether they were carbapennemase producers.
Results: A total of 4526 strains of non-fermenting bacteria were isolated, among which Pseudomonas aeruginosa ,Acinetobacter baumannii respectively accounted for 48%(2194/4526), 44%(1989/4526);Distribution of non-fermenting bacteria in the ICU was the highest, followed by thoracic surgery, respectively 36%(1617/4526), 16%(688/4526); Pseudomonas aeruginosa and Acinetobacter baumannii of ICU to imipenem, meropenem resistance rate of >50%, In thoracic surgery,Pseudomonas aeruginosa and Acinetobacter baumannii resistance rates are lower than that of ICU; Pseudomonas aeruginosa to the carbapenems resistance rate was gradually decreased, while

DOI: 10.1002/eji.201970400

the Acinetobacter baumannii was not significant change,during 2011-2016. The sensitivity and specificity of CIM、CNP、RCNP were high, and the consistency of that was good.
Conclusions: The main strains of non- fermenting bacteria in the hospital are Pseudomonas aeruginosa and Acinetobacter baumannii , and that resistances are serious. Screening of carbapenem resistant strains by RCNP can improve the monitoring efficiency and control the spread of resistance genes.

P2494

Inonotus obliquus polysaccharides ameliorated inflammation of LPS-stimulated RAW264.7 macrophages involving inhibited NLRP3 inflammasome

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Inonotus obliquus polysaccharides (IOP) is the main chemical constituent of *Inonotus obliquus*, which shows antioxidant, anti-tumor and anti-inflammatory activities. Activation of NLRP3 inflammasome can cause overexpression and secretion of inflammation-associated cytokines and mediate inflammation. However, the anti-inflammatory roles of IOP in NLRP3 inflammasome in Lipopolysaccharide (LPS)-stimulated macrophages are still not well clarified. In this study, we used LPS induced an inflammatory cell model to explore the anti-inflammatory mechanism of IOP in RAW264.7 macrophages whether related to NLRP3 inflammasome. In our research, the RAW264.7 macrophages were divided into control group, LPS (1μg/mL) group, LPS+IOP (40μg/mL) group for the following experiments. We used RT-PCR to detect the expression of NLRP3 inflammasome in cells at mRNA level and found that the NLRP3, ASC and Caspase-1 were significantly reduced in IOP group. We performed WB to detect the expression of NLRP3 inflammasome in cells at protein level and found that the NLRP3, ASC and Caspase-1 were significantly decreased in IOP group. Meanwhile, ELISA was used to evaluate pro-inflammatory cytokines in the cell culture supernatant. Compared with the LPS group, IL-18 and IL-1β were significantly suppressed in IOP group. Above all, this study indicated that IOP could inhibit the RAW264.7 cell inflammation which induced by LPS and the possible mechanism may involve in inhibiting the NLRP3 inflammasome.

P2495

The expression change of inflammatory factors IL-1 and IL-6 in the uterine luminal fluid in ovariectomized estrogen-primed rats with lateral cerebral ventricle injection of GnIH/RFRP-3

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Objective: The study is to investigate the change of IL-1, IL-6 in the uterine luminal fluid in ovariectomized estrogen-primed rats with lateral cerebral ventricle injection of GnIH/RFRP-3.
Methods: GnIH/RFRP-3 was injected into the lateral ventricle of ovariectomized estrogen primed (OEP) rats. The uterine luminal fluid (ULF)was collected at 60 min, 120 min, 240 min and 360 min. The levels of IL-6 and TNF-αin serum were detected by ELISA.
Results: The expression of IL-6 and TNF-α in ULF was inhibited by microinjection of RFRP-3 into the lateral ventricle for 60 min, 360 min, which was statistically significant compared with the saline group ($P < 0.05$).
Conclusion: These results suggest that GnIH/RFRP-3 may inhibite the expression of IL-6 and TNF-α and regulate immune system through regulating the hypothalamic-pituitary reproductive axis.

DOI: 10.1002/eji.201970400

P2496

Identification of key genes and pathways involved in response to pain in goat and sheep by transcriptome sequencing

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Purpose: This study was to investigate the key genes and pathways involved in the response to pain in goat and sheep by transcriptome sequencing.
Methods: Chronic pain was induced with the injection of the complete Freund´s adjuvant (CFA) in sheep and goats. The animals were divided into four groups: CFA-treated sheep, control sheep, CFA-treated goat, and control goat groups (n = 3 in each group). The dorsal root ganglions of these animals were isolated and used for the construction of a cDNA library and transcriptome sequencing. Differentially expressed genes (DEGs) were identified in CFA-induced sheep and goats and gene ontology (GO) enrichment analysis was performed.
Results: In total, 1748 and 2441 DEGs were identified in CFA-treated goat and sheep, respectively. The DEGs identified in CFA-treated goats, such as C-C motif chemokine ligand 27 (CCL27), glutamate receptor 2 (GRIA2), and sodium voltage-gated channel alpha subunit 3 (SCN3A), were mainly enriched in GO functions associated with N-methyl-D-aspartate (NMDA) receptor, inflammatory response, and immune response. The DEGs identified in CFA-treated sheep, such as gamma-aminobutyric acid (GABA)-related DEGs (gamma-aminobutyric acid type A receptor gamma 3 subunit [GABRG3], GABRB2, and GABRB1), SCN9A,TRPV1, were mainly enriched in GO functions related to neuroactive ligand-receptor interaction, NMDA receptor, and defense response.
Conclusions: CCL27, GRIA2, and SCN3A may regulate the process of pain response during chronic pain in goats. GABA-related DEGs, SCN9A, and TRPV1 may modulate the process of response to pain in sheep. These DEGs may serve as drug targets for preventing chronic pain.

P2497

MicroRNA-22 suppresses cell proliferation, migration and invasion in oral squamous cell carcinoma by targeting NLRP3

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MicroRNAs (miRNAs) have been implicated as important regulators of carcinogenesis and tumor development. Recently, microRNA-22 (miR-22) has been reported to be a cancer-related miRNA in several types of tumors. In this study, we aimed to investigate the role of miR-22 in oral squamous cell carcinoma (OSCC). We found that miR-22 expression was significantly decreased in OSCC tissues compared with that in the adjacent noncancerous tissues. Furthermore, lentivirus-mediated miR-22 overexpression markedly reduced OSCC cell viability, migration and invasion, whereas miR-22 inhibitor promoted these parameters. Mechanistically, NLR family pyrin domain
containing three (NLRP3) was identified as a direct target of miR-22. miR-22 expression was inversely correlated with NLRP3 expression both in OSCC tissues and cell lines. Moreover, overexpression of miR-22 in OSCC cells could reverse the tumorpromoting
effect of the activated NLRP3 inflammasome and vice versus. Therefore, our results indicate that miR-22 may play a suppressive role in OSCC by targeting NLRP3, which offer new insights into the molecular mechanisms of the growth and metastasis of OSCC.

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DOI: 10.1002/eji.201970400

P2498

The expression of CD74 and macrophage migration inhibitory factor protein is upregulated in HBV-related hepatocellular carcinoma

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Background: Chronic HBV infection contributes to cancer development induced by pro-inflammatory cytokine production, such as CD74 and MIF.
Methods: We evaluated the level of CD74 by a capture enzyme-linked immunosorbent assay (ELISA) and the level of MIF protein was determined by Human Magnetic Luminex® Assays in 60 HBV-related hepatocellular carcinoma (HCC) patients.
Results: The mean concentration of CD74 was elevated significantly in tumor tissue (10.24 ng/mg) compared with non-tumor tissues (3.86 ng/mg). Meanwhile, the level of MIF was 63.49 ng/mg in tumor tissues, and 45.47 ng/mg in non-tumor samples. We found that elevated CD74 protein expression was associated with elevated serum AFP level and AST/ALT ratio, but elevated MIF protein was associated with Lymph node metastasis, serum AFP level and AST/ALT ratio. Only high level of MIF protein was associated with short-term survival months in HBV-related HCC patients. The elevated expression of MIF protein was associated with HBV >=10^5 (copies/ml) in liver cancer. The elevated level of MIF protein level was associated with the elevated level of CD74 protein in HBV-associated HCC progression.
Conclusion: The elevated level of CD74 and MIF Cytokines have been demonstrated to play a significant role in HBV-related HCC.

P2499

TAOK1 negatively regulates IL-17-mediated signaling and inflammation

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Interleukin 17(IL-17) is a cytokine which plays important roles in inflammation and autoimmune diseases. However, the regulation of its signaling transduction has not been well described. In this study, we report that thousand and one kinase 1 (TAOK1) functions as a negative regulator of IL-17-mediated signal transduction and inflammation. TAOK1 knockdown promotes the IL-17-induced cytokines and chemokines expression and the activation of MAPKs and p65.We further demonstrate that TAOK1 interacts with IL-17RA independent of its kinase activity, and TAOK1 dose dependently prevents the formation of IL-17R-Act1 complex. Consistent with this, TAOK1 deficiency exacerbates 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced experimental model of IBD likely by its promotion of IL-17-mediated signaling pathway. TAOK1 expression is decreased in the colons of ulcerative colitis (UC) patients. In conclusion, these findings suggest that TAOK1 is involved in the development of IL-17 related autoimmune disorders.

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DOI: 10.1002/eji.201970400

P2500

Relationship between *Xsp*I site polymorphisms of LDL-R gene and serum IL-2 and IL-10 in patients with hypercholesterolemia

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Background: Relationship has been identified in sporadic reports between polymorphisms and hypercholesterolemia. However, the relationship between inflammatory cytokines and polymorphism of low-density lipoprotein receptor (LDL-R) gene in hypercholesterolemia is unclear. This study aims to explore the relationship and significance between polymorphism of LDL-R gene and serum Interleukin-2(IL-2), IL-10 in patients with hypercholesterolemia.
Methods: PCR-RFLP and direct DNA sequencing assay were employed to determine polymorphism of LDL-R gene in 900 patients with hypercholesterolemia and 400 healthy cases. ELISA was applied to assay serum concentration of IL-2 and IL-10. Blood lipid indexes were tested in all cases.
Results: Compared with the healthy controls, level of IL-2 increased significantly, while IL-10 decreased significantly($P< 0.05$). Correlation analysis showed that IL-2 was positively correlated with total cholesterol(TC), LDL-c and genotype ($r= 0.542$, 0.410, 0.598, $P< 0.05$), and negatively correlated with HDL-c ($r=-0.352$, $P< 0.05$). Negative relationship also was found between TC, LDL-c, genotype and IL-10($r= -0.452$, -0.390, -0.613, $P< 0.05$), and positive correlation between HDL-c and IL-10 ($r=0.398$, $P< 0.05$). Multiple linear regression showed that genotypes and TC were independent factors affecting levels of IL-2 and IL-10 ($P< 0.05$).

P2501

Tim-4 inhibits NLRP3 inflammasome *via* LKB1/AMPKα pathway in macrophages

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The NLRP3 inflammasome is a molecular platform for IL-1beta and IL-18 production by triggering activation of inflammatory caspase-1. NLRP3 inflammasome activation in macrophages plays key roles in the pathogenesis of multiple liver diseases including non-alcoholic fatty liver disease (NAFLD). Tim-4, as the phosphatidylserine (PS) receptor, is expressed highly in macrophages, and macrophage Tim-4 inhibits inflammation under various conditions of immune activation. However, the precise role of Tim-4 in NLRP3 inflammasome regulation remains completely unknown. In this study, we confirmed that the expression of Tim-4 was increased in liver tissues, especially higher expression in liver macrophages in NAFLD mice models. *In vitro*, we found that Tim-4 could inhibit NLRP3 inflammasome activation and the inhibition was dependent on PS binding domain in the IgV domain. Mechanistically, Tim-4 induced the degradation of NLRP3 inflammasome components through activating AMPKα-mediated autophagy. Specifically, Tim-4 promoted AMPKα phosphorylation by interacting with LKB1 and AMPKα. In addition, PS binding motif was responsible for Tim-4 mediated AMPKα and LKB1 interaction. In conclusion, NAFLD microenvironments enhanced Tim-4 in macrophages suppresses NLRP3 inflammasome activation by activating LKB1/AMPKα mediated autophagy. Collectively, this study unveils the novel function of Tim-4 in suppressing NLRP3 inflammasome, which would shed new light on intervention of NAFLD or inflammatory liver diseases by targeting Tim-4.

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DOI: 10.1002/eji.201970400

Glycoimmunology

O057

Protective functions of Siglec-E and Siglec-9 in mouse airway inflammation and lung emphysema

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Sialic acid-binding Ig-like lectins E and 9 (Siglec-E in mice, Siglec-9 in humans) are expressed on neutrophils and monocytes and are implicated in regulation of inflammation and infection. However, the roles of Siglec-E and Siglec-9 in non-infectious inflammatory lung diseases, such as emphysema and COPD, are not known. We generated transgenic mice expressing human Siglec-9 and investigated its potential roles in lung inflammation and emphysema models. Siglec-9 transgenic (Tg) mice were generated using a BAC construct containing the human Siglec-9 promoter and genomic DNA. Western blot and flow cytometry confirmed that Siglec-9 expressing cells were predominantly Gr-1$^+$ cells and some CD11b$^+$ cells, as seen in humans. After back-crossing to the Siglec-EKO mice, Siglec-9Tg/Siglec-EKO mice (or Siglec-9Tg) were obtained and tested in LPS induced airway inflammation and neutrophil elastase induced emphysema models and compared with wild type (WT) and Siglec-EKO mice. Bronchoalveolar lavage and lung histology revealed that WT mice had a neutrophilic inflammatory response and emphysematous changes in the lung tissue. Siglec-EKO mice had significantly enhanced inflammation and emphysema with increased chord length. Citrullinated Histone H3, a neutrophil extracellular traps marker, was markedly increased in the lung tissue of Siglec-EKO mice by Western blot and immunofluorescence. In contrast, Siglec-9Tg mice showed inflammation and emphysema responses similar to those in WT mice. Thus, Siglec-E is protective in lung inflammation and emphysema; Siglec-9 substitutes for Siglec-E and restores anti-inflammatory functions *in vivo*. The interactions between Siglec-E and Siglec-9 in inflammatory lung diseases provide opportunities to understand and modulate these diseases.

O058

The Glycocode of the tumormicroenvironment in cancer, a novel immune check-point

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Alterations in glycosylation is a hallmark in pathogen recognition by the host which mediates cellular communication during inflammation but also the resolution-phase of inflammation. Homeostatic and inflammatory processes affect the glycan signature of glycoproteins expressed on the cell surface, on secretory proteins, due to up- and down-regulation of glycosylation genes. We study glycan signatures that are recognized by glycan binding receptors such as C-type lectins and siglecs expressed on diverse set of myeloid and lymphoid immune cells, that modify intracellular signalling and immunological outcome. In particular the micro-environmental glycosylation signatures that alter during inflammation and cancer are studied that drive immune responses towards immunity or tolerance and open new venues for immune interference.
We identified new mechanisms of immune tolerance through the modification of glycosylation of tumours (melanoma, pancreatic cancer and glioblastoma). The analysis of transcriptomic data from

DOI: 10.1002/eji.201970400

patients and cell lines allowed us to identify different clinically relevant glyco-subtypes of pancreatic cancer, characterized by specific glycosylation signatures and associated with Epithelial to Mesenchymal transition (EMT). This differential glycosylation during EMT alters the differentiation programmming of monocytes into tumor associated macrophages (TAMs) upon interaction with tumor cells. Our results show a clear impact of the glyco-code in pancreatic cancer progression and highlight its potential as diagnostic tools and targets for therapeutic strategies. Also in melanoma we identified the presence of sailic acids in the tumormicroenvironment to regulate increase of FoxP3 CD4+ T cells (Treg) and TAM illustrating immune-suppressive cues sialic acids impose on immune cells.

O059

Attenuated p38 O-GlcNAcylation promotes melanoma migration via inhibition of p38 phosphorylation

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O-GlcNAcylation is a dynamic O-linked glycosylation event which plays a crucial role in regulating cellular signaling, including cancer formation. However, the role of O-GlcNAcylation in the metastatic potential of melanoma is not fully elucidated. Here, we found that knockout OGT significantly inhibited B16 melanoma cell growth, migration and invasion. In addition, knockout OGT upregulated of p38 phosphorylation and downregulated of ERK1/2 phosphorylation. These imply that OGT promoted B16 melanoma migration via MAPK signaling pathway. Mechanistic studies revealed that there is an interaction between p38 and OGT. Moreover, p38 was directly modified by O-GlcNAcylation. These data indicated a correlation of p38 O-GlcNAcylation and its activation. However, how the O-GlcNAc modification on activation of p38 need further elucidated. Our findings reveal a regulatory mechanism in which O-GlcNAcylation of p38 may regulate activation/phosphorylation of p38 MAPK signaling pathway.

P0063

Gene expression profiles of peripheral CD4$^+$ T cells in patients with ovarian cancer and study on its glycolysis metabolism and regulation

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To investigate the differentially expressed profiles of mRNA in peripheral CD4$^+$T cells of ovarian cancer (OC) patients and healthy controls (HC), and further explore the characteristics and TLR8-mediated control of glucose metabolic that might involve the development of ovarian cancer. Gene expression profiles revealed significant differences in expression levels of 5175 genes, of which 3014 were up-regulated and 2161 were down-regulated. Further analysis indicated that the most significantly related pathway was metabolic pathway, and the signaling pathways regulating glucose metabolism including PI3K-AKT signaling pathway, mTOR signaling pathway and HIF-1 signaling pathway were also statistical differences. Thus, 8 glycolysis-related genes (mTOR, HIF1α, GLUT1, GPI, ENO1, PKM2, LDHα, PDK1) were identified, and the expression levels were all increased in peripheral CD4$^+$T cells of OC patients compared with benign ovarian tumor patients and HC.

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DOI: 10.1002/eji.201970400

Moreover, we showed that the ovarian cancer cell SKOV3 could increase the expression levels of 8 glycolysis-related genes in $CD4^+$T cells, promote the levels of glucose uptake and glycolysis, and the proportion of $CD4^+CD25^+Foxp3^+$ T cells was increased, the proliferation levels of $CD4^+$T cells were decreased, and enhanced the suppression to the proliferative of Naïve $CD4^+$T cells. However, TLR8 (Toll-like receptor 8) ligand ssRNA40 could mediate glycolysis metabolic control of $CD4^+$T cells cocultured with SKOV3. Our study indicated that the expression profiles of peripheral $CD4^+$T cells were significantly different in OC patients, and the most related pathway was metabolic pathway. Glycolysis metabolism might be key players in $CD4^+$T cells, which provide a new direction for immunotherapy of ovarian cancer.

P0064

Enhanced N-glycan branching on CD8 T cells exacerbates autoimmune diabetes in non-obese diabetic mice

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β1,6N-acetylglucosaminyltransferase V (Mgat5), is responsible for tetra-antennary N-glycans in the biosynthesis of glycoprotein and the resultant N-glycan branching serves as a backbone for additional glycosylation such as poly-N-aceyllactosamine (poly-LacNAc) structure. The N-glycan branching is involved in the pathological conditions such as malignancies, but the modulatory roles of N-glycan branching on T cells in autoimmune diabetes is not completely understood. Here, we report that the levels of N-glycan branching on T cells is positively correlated with the diabetogenesis in NOD mice. Since the levels of N-glycan branching is regulated during T-cell development and highly increases in pathogenic lymphocytes in pancreas, we generated established T cell-specific Mgat5 transgenic and mutant NOD mice. Interestingly, our results demonstrated that the diabetic incidence in Mgat5 transgenic mice was significantly higher and that of Mgat5 mutant mice markedly attenuated compared to control NOD mice. In adoptive transfer experiments, NOD/SCID mice that received control CD4 T cells plus transgenic CD8 T cells developed diabetes more rapidly than the mice received control or transgenic CD4 T cells plus control CD8 T cells, suggesting that the diabetogenicity in Mgat5 transgenic mice is a CD8 T cell-autonomous manner. Moreover, we generated Mgat5/NY8.3 doubly transgenic NOD mice expressing highly pathogenic MHC class-I-restricted 8.3 T cell receptor, and observed that these mice have higher diabetic incidence than wild-type NY8.3 transgenic NOD mice. Taken together, enhanced N-glycan branching on CD8 T cells exacerbates the development of diabetes in NOD mice.

P0651

Serum IgG glycan hallmarks of an autoimmune disease

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To facilitate higher rates of remissions for autoimmune diseases, therapy for which is expected to be most efficacious at the earliest and mildest stages, supportive biomarker information is necessary; however, none of the existing markers exhibits both high sensitivity and specificity. Here, we demonstrate the high performance of N-glycans on serum-IgG for a common autoimmune disease, systemic lupus erythematosus (SLE). On the basis of high sensitive TiO_2-PGC chip-based glycomic analyses of 227 SLE patients and 164 healthy controls, two N-glycans are identified as biomarkers with good capacity for predicting the diagnosis of SLE, which is a chronic severe disease with several

DOI: 10.1002/eji.201970400

antibodies and diverse manifestations ranging from glomerulonephritis to neurological disorders. Notably, these two N-glycan biomarkers are specific for SLE as they reveal no significant changes in other systematic autoimmune diseases that are easily-confused with SLE, such as rheumatoid arthritis (RA), primary Sjögren´s syndrome (pSS) and systemic sclerosis (SSc), for which differential specific biomarkers are identified. Furthermore, these N-glycan biomarkers correlate closely with disease activity and reflect therapeutic response. Alterations of the N-glycan biomarkers in SLE point to an increase of hybrid glycan and reduction of complex glycan, which might be resulted from the down-regulation of the enzyme N-acetylglucosaminyltransferase II (GlcNAcT II). The dysregulated N-glycan biosynthesis suggests new potential pathogenesis for this autoimmune disease. These results demonstrate the potential clinical significance of serum N-glycan biomarkers for the differentiation of easily-confused autoimmune diseases, and raise the prospect of efficient population screening and suggest new potential therapeutic targets in autoimmune conditions.

P0652

Endogenous sialidases desialyate T cells, and promote T cell activation and function

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The germinal center (GC) in lymphoid tissue is critical to antibody formation, including class-switching and somatic hypermutation to increase antibody affinity. These events require that antigen-presenting-cells (APCs) and B and T cells interacts through their surface proteins in the immune synapse. These proteins are glycosylated, a post-transcriptional modification that covalently attaches oligosaccharides onto the polypeptide and frequently contain sialic acid (SA) as the terminal carbohydrate unit. These highly negatively charged SA moieties diminish accessibility of surface molecules, hinder protein-carbohydrate or protein-protein interactions; therefore, their enzymatic removal—desialylation, by microbial neuraminidases or endogenous sialidases (NEUs) facilitates these interactions, and modulates relevant cellular functions. Historically GCs have been identified by their binding of peanut agglutinin, a lectin that recognizes the subterminal galactose on desialylated proteins.
Since hyposialylation is considered a hallmark of activated T cells, we hypothesized that GC formation requires the activation of NEUs that remove terminal SAs of T and perhaps B cells and APCs to promote their interactions. We show that ICAM-1 on APCs and LFA-1 on T cells, two important proteins in the immune synapse, are sialylated; and desialylation *in vitro* enhances T cells response to peptide-pulsed DCs. Further, during vaccination, administration of pan-sialidase inhibitor 2-DN reduces splenocytes hyposialylation and suppresses antibody production. We conclude that NEUs play a critical role in GC formation and function through desialylating key molecules in the immune synapse. The ability to modulate sialidase activity may find clinical application by increasing cellular interactions in the GC during immunizations or decreasing them during hyperinflammatory states.

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DOI: 10.1002/eji.201970400

P1249

Study on the biological effects of vascular endothelial cells by dengue virus type Ⅱ infection

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Despite the unclear underlying mechanism, dengue virus (DENV) infection is known to increase human endothelial cell permeability. In this study, we cultured DENV-infected pHUVECs in vitro and performed a comparative analysis on the biological characteristics of the in vitro pHUVECs and those cultured in a traditional manner. In vitro culture of pHUVECs maintaining biological elasticity and hardness was conducted using polyacrylamide gel (PAAG). The expression of characteristic markers in VECs and the endothelial area were analyzed with the immunofluorescence (IF) technique and flow cytometry. The proliferation of pHUVECs was determined on the basis of cell count and the BrdU assay. Real-time PCR and Western Blotting were performed to identify the differences in CX40 expression between the two approaches. The sugar content of cell surface was measured by Glycosaminoglycan Assay. Compared to those cultured with the traditional method, the PAAG-based pHUVECs have a smaller area (per cell) and a lower proliferation rate. In contrast, the expression of CX40 and the content of glycosaminoglycan are comparatively high in the PAAG-based pHUVECs. This indicates that the biological characteristics of pHUVECs vary as the basal conditions (elasticity, harness) change when different culture methods are adopted.
This work is also financially supported by Science Foundation of Hainan Province (NO.20158310).

P1250

Bioengineered immune therapeutics targeting O-GlcNAcylated NF-kappaB c-Rel to treat autoimmune diabetes

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Type 1 diabetes is an autoimmune disease associated with hyperglycemia. Increased glucose flux enhances the hexosamine biosynthetic pathway and intracellular posttranslational modification of proteins by the sugar N-acetyl glucosamine (GlcNAc) in a process called O-GlcNAcylation. We discovered that hyperglycemia increases the O-GlcNAcylation of the transcription factor, nuclear factor kappaB (NF-kB) c-Rel at serine 350. O-GlcNAcylation of c-Rel activates c-Rel-dependent transcription of proautoimmune cytokines in T cells and inhibits the expression of T regulatory cell specific transcription factor FOXP3. These reciprocal effects caused by c-Rel O-GlcNAcylation may act as positive feed back accelerator of autoimmunity under hyperglycemia. Hence, blocking the function of O-GlcNAcylated c-Rel will have dual benefits in controlling autoimmune diabetes by diminishing the T cell-mediated autoimmunity and enhancing the T regulatory cell function to suppress autoimmunity. We developed a novel peptoid, called peptoid3, by molecular modeling and de novo synthesis, that specifically blocks the function of O-GlcNAcylated c-Rel. We found that peptoid3 treatment significantly decreased T cell receptor-induced, O-GlcNAcylation-dependent expression of proautoimmune cytokines and enhanced FOXP3 expression in T cells. Peptoid3 treatment selectively affected autoimmunity-associated genes and did not exhibit toxicity on survival or proliferation of T cells. Broad inhibition of hexosamine biosynthetic pathway or NF-kB will cause many side effects due to their ubiquitous importance in multiple biological functions. Therefore, inhibitors of O-GlcNAcylated

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DOI: 10.1002/eji.201970400

NF-kB c-Rel function may prove long-sought-after specific molecular therapeutic to diminish autoimmunity in type 1 diabetes.

P1840

Strong correlation between KPNA2 gene expression and diabetes-related liver cancer identified by co-expression analysis

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, and its pathogenesis remains unclear. Many studies have confirmed that diabetes is an independent risk factor for liver cancer, but the underlying mechanism between HCC and diabetes has not fully elucidated. Therefore, we used bioinformatics methods to analyze the differentially expressed genes of liver cancer and type 2 diabetes to reveal the potential pathophysiological relationship. In our study, the differentially expressed genes of HCC in datasets GSE45436, GSE112790 and GSE121248 were used to construct a co-expression network by WGCNA in dataset GSE9843. The most relevant module that was related to CTNNB1 mutation was screened and Hub genes that show most connections in the network as indicated by their high KME (eigengene connectivity) value were screened. Then we found KPNA2 by the intersection of Hub genes and the differentially expressed genes of type 2 diabetes in dataset GSE7014. According to Oncomine database, we found that KPNA2 was overexpressed in hepatocellular carcinoma compared with normal tissues on transcriptional level. Patients of hepatocellular carcinoma with low KPNA2 expression have longer survival time than patients with high expression of KPNA2 in survival analysis.

P2502

Phosphoglyceric acid mutase-1 contributes to oncogenic mTOR-mediated tumor growth and confers non-small cell lung cancer patients with poor prognosis

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Abnormal glucose metabolism (Warburg effect, also known as aerobic glycolysis) is considered to be one of the important features of tumors, which confers the advantage of tumor cell survival and proliferation. Due to frequent mutations of oncogenes and tumor suppressors, the over-activated mammalian target of rapamycin (mTOR) pathway can induce the Warburg effect in tumor cells. Our study reports the role and mechanism of a glycolytic enzyme, phosphoglycerate mutase-1 (PGAM1), in mTOR signaling pathway mediated Warburg effect. mTOR promotes the expression of PGAM1 by hypoxia-inducible factor 1α (HIF1α) mediated transcriptional activation. Blocking PGAM1 inhibits mTOR-dependent glycolysis, reduces tumor cell proliferation and prolongs mouse survival. In human non-small cell lung cancer (NSCLC) tissues, PGAM1 expression is positively correlated with mTOR pathway activity, and PGAM1 is a poor prognostic factor for patient survival. Our findings indicate that PGAM1 is a downstream effector of the mTOR signaling pathway and that the mTOR-PGAM1 signaling cascade may be one of the causes of the Warburg effect of tumor cells. PGAM1 can be used as a novel prognostic biomarker for NSCLC and a therapeutic target for tumors.

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DOI: 10.1002/eji.201970400

P2503

Elevated core-fucosylated IgG is a new marker for hepatitis B virus-related hepatocellular carcinoma

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Immunoglubulin G (IgG) and its abnormal glycosylations are associated with carcinogenesis. The present study investigates the relationship between cancer-derived IgG and clinicopathological characteristics in hepatocellular carcinoma (HCC) and assesses the value of serum N-glycosylated IgG in diagnosing and monitoring hepatitis B virus (HBV)-related HCC. Tissue microarray analysis of 90 HCC tissues showed that HCC patients with IgG immunopositivity had higher levels of core-fucosylated a fetoprotein (AFP-L3), larger tumors, and a higher incidence of portal vein tumor thrombus. HCC patients presented a significantly increased fraction of Lens culinaris agglutinin binding IgG (core-fucosylated IgG, IgG-L3) among total serum IgG. The clinical diagnostic performance of serum IgG-L3% was evaluated in 3 case-control studies.IgG-L3% had better general diagnostic performance than AFP in the training set and validation cohort 1 . In validation cohort 2, where we aimed to assess the efficiency of IgG-L3% in patients with AFP-negative HCC, the diagnostic accuracy of IgG-L3% was 72.54-73.60%. Finally, a longitudinal evaluation based on 31 HCC patients demonstrated that IgG-L3% decreased in 24 patients after curative surgery. HCC patients with higher IgG-L3% had poor survival during a 3-year follow up. We conclude that HCC-derived IgG is correlated with progressive behavior of HCC. Therefore, elevated core-fucosylated IgG is a new diagnostic and prognostic marker in HBV-related HCC.

Non-coding RNAs in Immune Regulation

O161

MicroRNAs of the miR-17~92 family inhibit macrophage activation and inflammation

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Production of pro- and anti-inflammatory cytokines by macrophages is tightly controlled by multiple layers of mechanisms including those involving miRNA-mediated regulation of post-transcriptional events. By genetically deleting all three individual miRNA clusters in the miR-17~92 family, we found that this family of miRNAs inhibit macrophage activation by promoting expression of a key anti-inflammatory cytokine IL-10 and thus suppress production of pro-inflammatory mediators such as TNF. Consistent with the heightened inflammatory responses *in vitro*, myeloid-specific ablation of miR-17~92 family miRNAs *in vivo* led to exacerbated obesity under the condition of a high-fat diet and poor survival in the DSS-induced colitis model. Moreover, miRNA-deficient adipose tissue macrophages and intestinal macrophages exhibited enhanced levels of TNF and reduced levels of IL-10. Mechanistically, miR-17~92 family miRNAs sustained IL-10 production in macrophages by promoting transcription of the *Fos* gene encoding a key transcription factor driving *Il10* expression. The positive effects of miR-17~92 family miRNAs on Fos expression are secondary to negative regulation of *Fos* by transcription factor YY1 that is a direct target of miR-17~92 family miRNAs. Taken together, these results identified miR-17~92 family miRNAs as crucial regulators of the balance between pro- and anti-inflammatory cytokines and illustrated key roles for three clusters of the miR-17~92 family in cooperatively regulating macrophage activation and inflammatory responses.

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DOI: 10.1002/eji.201970400

O162

Long noncoding RNA AVAN promotes antiviral innate immunity by interacting with TRIM25 and enhancing the transcription of FOXO3a

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Accumulating evidence has shown that long noncoding RNAs (lncRNAs) are involved in several biological processes, including immune responses. However, the role of lncRNAs in innate immune responses remains largely unexplored. Here, we identify an uncharacterized human lncRNA, AVAN (antivirus and activate neutrophil), that is significantly up-regulated upon virus infection. Mechanistically, cytoplasmic lncRNA-AVAN directly binds tripartite motif containing 25 (TRIM25) and enhances the association of TRIM25 and RIG-I and the ubiquitylation of RIG-I, thereby promoting TRIM25- and RIG-I-mediated antiviral innate immune signaling. We also found that nuclear lncRNA-AVAN positively regulates the transcription of forkhead box O3A (FOXO3a) *in cis* by associating with its promoter and inducing chromatin remodeling to maintain neutrophil activation and chemotaxis. Collectively, these findings highlight the potential clinical implications of lncRNA-AVAN as a key positive regulator of antiviral innate immune response and a promising target for developing broad antiviral therapeutics.

O163

miRNA-21 overexpression promotes T follicular helper cells mediated autoimmune response in systemic lupus erythematosus

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Previous studies have reported the increased numbers of circulating T follicular helper (Tfh) cells in patients with systemic lupus erythematosus (SLE), which play an important role in the pathogenesis of SLE. However, the mechanism of abnormal differentiation of Tfh cells in SLE is not yet clear. Here we observed an increase in the expression levels of miR-21, CXCR5, PD1, BCL6 and IL21 in $CD4^+$ T cells of SLE patients. miR-21 expression levels positively correlated with SLEDAI scores in SLE patients. In vitro overexpression of miR-21 significantly promoted Tfh differentiation, in contrast, downregulation of miR-21 inhibited Tfh differentiation under Tfh cells polarized-condition. In vivo inhibition of miR-21 in MRL/lpr mice by miR-21 Antagomir significantly decreased the percentage of Tfh cells and activated B cells. Compared to the negative control, alleviated skin and renal lesions, decreased serum levels of antinuclear antibodies and anti-nuclear ribonucleoprotein antibodies were observed in MPL/lpr mice treated with miR-21 Antagomir. Our data demonstrated that miR-21 overexpression contributes to the development of SLE by promoting Tfh cells mediated autoimmune response. MiR-21 antagomir may be a promising therapeutic strategy for alleviating autoimmune response in SLE.

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DOI: 10.1002/eji.201970400

O164

The long-non-coding RNA Snhg1 is essencial for memory CD8 T cell establishment

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Memory CD8 T cells can survive in the body for a long time, mediating rapid and enhanced immune response, which are the key immune cells against viral infection and tumor. Studies of recent years have shown that long non-coding RNAs (LncRNAs) show much higher cell lineage specificity compared with mRNAs in T cell development, differentiation and function. However, it remains unclear what and whether LncRNAs play important role in the differentiation of memory CD8 T cells. Our RNA-seq analysis uncovers lncRNA Snhg1 required for memory CD8 T cell differenciation. Snhg1 pull-down mespectrum discovers the membrane trafficking protein Vps13D essencial for memory CD8 establishment. For the mechanism, we found Snhg1/Vps13D ablation block CD127 trafficking from ER-Golgi to cell membrane, and then impair Tmem through Stat3-Tcf1. Transgenic Tcf1 rescues Snhg1/Vps13D ablation phenotype to memory CD8 T cell. Given that memory CD8 T cells play a key role in antagonizing virus infection and tumor, this study will provide certain theoretical basis and technical support for targeting LncRNAs to enhance memory CD8 T cells' immunotherapy.

O165

HBV infection promotes proliferation, migration and invasion of HCC via up-regulating miR-181a/362/382/19a targeting PTEN

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Persistent replication of HBV can lead to different degrees of hepatitis, cirrhosis and even hepatocellular carcinoma (HCC). The mechanism how HBV affects the development and progression of HCC is very complicated and needs to be explored. Studies have reported that microRNAs (miRNAs) are involved in the regulation of numerous important biological processes, such as cell proliferation, apoptosis, differentiation and epigenetic changes. Given that abnormal expression of miRNAs is closely related to human tumors, we explored the potential role of miRNAs in HCC by performing miRNA expression profiling between HepG2 and HepG2.215. We noted miR-181a/382/362/19a were markedly overexpressed and highly associated with HBV-infected HCC. Using an integrated approach, we uncovered that phosphatase and tensin homolog (PTEN), which is a powerful tumor suppressor and whose deficiency is a key event in the pathogenesis and progression of human cancers, was a novel target of miR-181a/382/362/19a. Next, we found the expression of PTEN was obviously reduced in HBV-infected HCC tissues/adjacent noncancerous liver tissues (ANLTs) in comparsion with the HBV-uninfected HCC tissues/ANLTs. In the following experiments concerning cell biological behaviors, we observed miR-181a/362/382/19a could augment the proliferation, migration and invasion potentialities of hepatocellular carcinoma cells by targeting PTEN. Furthermore, we discovered miR-181a/362/382/19a could promote the activation of PI3K/AKT signaling pathway in hepatoma cells via regulating PTEN. Altogether, **our findings indicate HBV infection promotes the proliferation, migration and invasion of hepatoma cells by up-regulating miR-181a/362/382/19a targeting PTEN and activating PI3K/AKT signaling pathway**, providing new strategies for HBV infection-related HCC with miR-181a/362/382/19a and PTEN as potential targets.

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DOI: 10.1002/eji.201970400

O166

miR-149-3p reverses CD8 T cell exhaustion by decreasing inhibitory receptors and promoting cytokine secretion in breast cancer cells

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Breast cancer is the most common cause of cancer death in women. Blockade of immune checkpoint proteins has been considered one of the most effective breast cancer immunotherapies. Aberrant miRNA expression leading to post-transcriptional induction of pathological inhibitory receptor expression and activity may lead to cancer cell escape from immune surveillance. It is not clear how miRNAs regulate immune checkpoint proteins in breast cancer. In this study, we explored the function of miRNA in regulating CD8 T cell exhaustion. The downregulated miRNAs were screened in PD-1 overexpressed CD8+ T cells using miRNA array in breast cancer mouse homograft. It reveals that miR-149-3p was among 5 such miRNAs and the only one of the five with a sequence predicted to bind the 3´UTRs of mRNAs encoding T cell inhibitor receptors PD-1, TIM-3, BTLA, and Foxp1. Treatment of CD8+ T cells with a miR-149-3p mimic reduced apoptosis, attenuated changes in mRNA markers of T cell exhaustion, and downregulated mRNAs encoding PD-1, TIM-3, BTLA, and Foxp1. On the other hand, T cell proliferation and secretion of effector cytokines indicative of increased T cell activation (IL-2, TNF-α, IFN-γ) were upregulated after miR-149-3p mimic treatment. Moreover, treatment with a miR-149-3p mimic promoted the capacity of CD8+ T cells to kill targeted 4T1 mouse breast tumor cells. When the cells were treated with an inhibitor of miR149-3a, opposite effects were observed. Collectively, these data show that miR-149-3p can reverse CD8+ T cell exhaustion and reveals it to be a potential antitumor immunotherapeutic agent in breast cancer.

O167

Decreased long intergenic noncoding RNA P7 predicts unfavorable prognosis and promotes tumor proliferation via the modulation of the STAT1-MAPK pathway in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common neoplasm and is a leading cause of cancer-related death. Despite advances in the diagnosis and management of HCC, its prognosis remain unfavorable. Accumulating evidence has shown that long intergenic noncoding RNAs (lincRNAs) play central roles in the development of HCC. In this study, we identified a long intergenic noncoding RNA referred to as lincRNA P7 in HCC and explored its clinical significance and biological functions in HCC. The expression level of lincRNA P7 was significantly aberrantly deceased in HCC cancer tissues and cells lines. Gain- and loss-of-function experiments revealed that overexpression of lincRNA P7 significantly inhibited the proliferation of HCC-derived cancer cells, whereas lincRNA P7 knockdown promoted cell growth. Mechanistically, lincRNA P7 blocked Erk1/2 signaling and repressed activation of the STAT1 pathway. In nude mouse models, we show that overexpression of lincRNA P7 effectively repressed HCC xenograft tumor growth in vivo . Moreover, a clinical investigation demonstrated
that down-regulated lincRNA P7 expression correlated with liver cirrhosis, Hepatitis B virus (HBV) infection, clinical stage of the tumor and recurrence. A Kaplan-Meier survival analysis showed that the expression of lincRNA P7 was significantly related to overall survival (P = 0.003) and recurrence-free

survival (P = 0.031). Collectively, our findings suggested that the down-regulation of lincRNA P7 predicts poor clinical outcomes for HCC patients and might be a powerful candidate prognostic biomarker and target in HCC.

O168

MicroRNA-31 promotes T-cell pathogenicity in chronic graft-versus-host disease

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Chronic graft-versus-host disease (cGVHD) remains a major cause of non-relapse mortality following allogeneic hematopoietic stem-cell transplantation (allo-HCT). Certain microRNAs (miRs) play critical roles in T-cell responses. Using oligonucleotide array, we found miR-31 was dramatically up-regulated in T cells under alloantigen-driven vs. homeostatic proliferation following bone marrow transplantation (BMT) in mice. Dysregulation of miR-31 is implicated in cancer, however how miR-31 impacts allogeneic T-cell response is unknown. In three murine models of cGVHD, the recipients transferred with donor T cells deficient for miR-31 exhibited significantly alleviated disease, reflected by attenuated fibrosis in skin and reduced airway resistance, elastance and pathological damage in lung as compared with those of WT T cells. MiR-31-deficient T cells showed defects in proliferation and survival in allogeneic recipients; they differentiated more towards Tregs but fewer to Th17 cells. Recipients of miR-31-deficient graft displayed improved immune reconstitution but reduced pathogenic follicular T helper cells. Furthermore, miR-31 enhances HIF1α signaling (glycolysis promotor), GLUT1 upregulation and glucose uptake, but inhibits lipid droplet accumulation in allo-reactive T cells after BMT, indicating that miR-31 promotes metabolic switch from fatty acid β-oxidation to aerobic glycolysis in allo-reactive T cells. Importantly, miR-31-deficient T cells were capable of controlling leukemia relapse as efficiently as WT T cells, indicating miR-31 is dispensable for T cells to mediating graft-versus-leukemia (GVL) activity after allogeneic-BMT. Administration of anti-miR-31 at day 21 post-BMT attenuated cGVHD manifestation in the recipients. Taken together, miR-31 represents a promising therapeutic target for the control of cGVHD while sparing GVL effects after allo-HCT.

P0065

Long noncoding RNA ITPRIP-1 positively regulates the innate immune response through promotion of oligomerization and activation of MDA5

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Emerging evidence indicates that long noncoding RNAs (lncRNAs) regulate various biological processes, especially innate and adaptive immunity. However, the relationship between lncRNAs and the interferon (IFN) pathway remains largely unknown. Here, we report that lncRNA ITPRIP-1 (lncITPRIP-1) is involved in viral infection and plays a crucial role in the virus-triggered IFN signaling pathway through the targeting of melanoma differentiation-associated gene 5 (MDA5). LncITPRIP-1 can be induced by viral infection, which is not entirely dependent on the IFN signal. Besides, there is no coding potential found in the lncITPRIP-1 transcript. LncITPRIP-1 binds to the C terminus of MDA5,

DOI: 10.1002/eji.201970400

and it possesses the ability to boost the oligomerization of both the full length and the 2 caspase activation and recruitment domains of MDA5 in a K63-linked polyubiquitination-independent manner. Amazingly, we also found that MDA5 can suppress hepatitis C virus (HCV) replication independently of IFN signaling through its C-terminal-deficient domain bound to viral RNA, in which lncITPRIP-1 plays a role as an assistant. In addition, the expression of lncITPRIP-1 is highly consistent with MDA5 expression, indicating that lncITPRIP-1 may function as a cofactor of MDA5. All the data suggest that lncITPRIP-1 enhances the innate immune response to viral infection through the promotion of oligomerization and activation of MDA5. Our study discovers the first lncRNA ITPRIP-1 involved in MDA5 activation.

P0066

Tumor suppressive miR-6775-3p inhibits ESCC progression through forming a positive feedback loop with p53 via tumor antigen MAGE-A family proteins

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Accumulating evidences indicate that microRNAs (miRNAs) play vital roles in multiple diseases, including cancer. In the present study, we showed that miR-6775-3p plays a tumor suppressive role in esophageal squamous cell carcinoma (ESCC). High expression miR-6775-3p is associated with good clinical outcomes of ESCC patients. Over-expression of miR-6775-3p inhibited tumor growth and liver metastasis of ESCC xenograft tumors. Enforced expression of miR-6775-3p inhibited ESCC cell proliferation, migration and invasion. KEGG pathway analysis revealed that miR-6775-3p was associated with the genes on “pathway in cancer”. Mechanically, miR-6775-3p inhibited the expression of tumor antigens MAGE-A family through direct binding the 3'UTR region of MAGE-A mRNAs, and attenuated MAGE-A-inhibited transcriptional activity of tumor suppressor p53. In addition, miR-6775-3p also directly inhibits its host gene SLC7A5 which has been reported to play oncogenic roles in cancer progression. Interestingly, miR-6775-3p and its host gene SLC7A5 were directly transcriptionally induced by p53. **Thus, for the first time, our study proposed a novel positive feedback regulation between miR-6775-3p and p53 via tumor antigen MAGE-A family, which plays crucial role in ESCC progression.**

P0067

Circular RNA ciRS-7 accelerates ESCC progression through acting as a miR-876-5p sponge to enhance tumor antigen MAGE-A family expression

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As the most well-known circular RNA, ciRS-7 (also termed CDR1as) has been reported to act as a miR-7 sponge, resulting in reduced miR-7 activity and increased miR-7-targeted transcripts. Here, we showed that ciRS-7 is up-regulated in esophageal squamous cell carcinoma (ESCC), and is associated with the poor clinicopathological parameters of ESCC patients. Moreover, over-expression of ciRS-7 increased the proliferation, migration and invasion of ESCC cells. Mechanistic studies revealed that ciRS-7 contains nineteen miR-876-5p binding sites and acts as a miR-876-5p sponge. Over-expression of ciRS-7 resulted in the reduced tumor-repressive function of miR-876-5p on its downstream target tumor antigen MAGE-A family. In animal experiments, enforced

DOI: 10.1002/eji.201970400

ciRS-7 increased ESCC tumor growth and metastasis through targeting miR-876-5p/MAGE-A family axis. **Collectively, our study provided novel evidence that ciRS-7 accelerates ESCC progression by acting as a miR-876-5p sponge to enhance tumor antigen MAGE-A family expression.**

P0068

MiR-1165-3p suppresses Th2 differentiation via targeting IL-13 and PPM1A in a mouse model of acute asthma

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Background: Previously, we demonstrated that microRNA-1165-3p (miR-1165-3p) was significantly reduced in Th2 cells and that miR-1165-3p was a surrogate marker for atopic asthma. Little is known about the mechanisms of miR-1165-3p in the regulation of Th2-dominated allergic inflammation.
Materials and Methods: $CD4^+$ naïve T cells were differentiated into Th1 or Th2 cells *in vitro*. MiR-1165-3p was upregulated or downregulated using lentiviral systems during Th1/Th2 differentiation. *In vivo*, the lentiviral particles with the miR-1165-3p enhancer were administered by tail vein injection on the first day of an HDM-induced acute asthma model. Allergic inflammation and Th1/Th2 differentiation were routinely monitored. To investigate the potential targets of miR-1165-3p, biotin-miRNA pull-down products were sequenced, and the candidates were further verified with a dual-luciferase reporter assay. The roles of a target protein phosphatase, Mg^{2+}/Mn^{2+}-dependent 1A (PPM1A), in Th2 cell differentiation and allergic asthma were further explored.
Results: The miR-1165-3p enhancer suppressed Th2 cell differentiation *in vitro*. In contrast, miR-1165-3p silencing promoted Th2 cell development *in vitro*. In the HDM-induced model of acute asthma, miR-1165-3p upregulation was accompanied by reduced airway hyperresponsiveness, serum IgE, airway inflammation and Th2 cell polarization. IL-13 and PPM1A were the direct targets of miR-1165-3p. The expression of IL-13 or PPM1A was inversely correlated with that of miR-1165-3p. PPM1A regulated the STAT and AKT signaling pathways during Th2 differentiation. Moreover, plasma PPM1A was significantly increased in asthma patients.
Conclusions: Our data suggest that miR-1165-3p negatively regulates Th2 cell differentiation by targeting IL-13 and PPM1A in allergic asthma.

P0069

The Pseudogene Olfr29-ps1 Promotes the Suppressive Function and Differentiation of Monocytic MDSCs

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Long non-coding RNA (lncRNA) plays a critical role in many biological processes, such as cell differentiation and development. However, few studies about lncRNAs regulating the differentiation and development of myeloid-derived suppressor cells (MDSCs) exist. In this study, we identified a lncRNA pseudogene, Olfr29-ps1, which was expressed in MDSCs and upregulated by proinflammatory cytokine IL6. The Olfr29-ps1 in vertebrates is conserved, and the similarity between the Olfr29-ps1 and human OR1F2P sequence is 43%. This lncRNA promoted the immunosuppressive function and differentiation of monocytic (Mo-)MDSCs in vitro and in vivo. It directly sponged miR-214-3p to downregulate miR-214-3p, which may target MyD88 to modulate the differentiation and

DOI: 10.1002/eji.201970400

development of MDSCs. The functions of Olfr29-ps1 was dependent on IL6-mediated N6-methyladenosine (m6A) modification, which not only enhanced Olfr29-ps1, but also promoted interaction of Olfr29-ps1 with miR-214-3p. Thus, our results demonstrated that the pseudogene Olfr29-ps1 may regulate the differentiation and function of MDSCs through a m6A-modified Olfr29-ps1/miR-214-3p/MyD88 regulatory network, revealing a mechanism for the regulation of myeloid cells and also providing potential targets for antitumor immunotherapy.

P0070

The role of miR-20a-5p in immune inflammatory response by regulating PP2Ac in systemic lupus erythematosus

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Background: Protein phosphatase 2A (PP2A) is Ser/Thr phosphatase, and the catalytic C subunit of PP2A (PP2Ac) is increased in T cells of patients with systemic lupus erythematosus (SLE). PP2A/C expression in SLE is controlled both epigenetically and genetically. Here we present additional mechanistic studies to investigate the effect of miR-20a-5p/PP2Ac signaling pathway on the expression of downstream protein phosphorylation and on the regulation of immune inflammatory response in SLE.
Methods: The effects between miR-20a-5p and the target gene *PPP2CA* of PP2Ac was verified by the dual luciferase reporter gene assays. The mechanisms involved in miR-20a-5p regulation of PP2Ac were analyzed PP2Ac, T-AKT, p-AKT (Ser473), p-AKT (Thr308) and ICOS by western blot and real-time polymerase chain reaction. A clinical case-control study was performed to investigate the relationship between miR-20a-5p/PP2Ac pathway and clinical features of SLE patients.
Results: Dual luciferase reporter gene assay confirmed that miR-20a-5p could bind to the 3´UTR of *PPP2CA* gene and directly regulate the expression of *PPP2CA* gene. Cell function experiments confirmed that miR-20a-5p could target and regulate the expression of PP2Ac, which consequently affected the downstream p-AKT (Ser473), p-AKT (Thr308) phosphorylation levels and immune inflammatory response. Clinical studies showed that the expression of miR-20a-5p decreased and the expression of *PPP2CA* mRNA increased SLE patients. The expression levels of miR-20a-5p and *PPP2CA* mRNA in SLE patients were all associated with the presence of pleurisy, pericarditis, anti-SSA/Ro antibodies, as well as plasma IL-6 and IL-17 levels.
Conclusion: Our results indicate a functional link between miR-20a-5p-mediated PP2A/C regulation and immune inflammatory response ability.

P0071

The diagnosis value of inflammatory-related MicroRNA as biomarkers in Ankylosing Spondylitis

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The Study aimed to clarify whether plasma miR-146a, miR-15a, miR-20a, miR-125a-3p, miR-125a-5p, miR-125b-5p, miR-148a, miR-149a, miR-499 and miR-155a, expressed differently between in Ankylosing Spondylitis(AS) patients and healthy control, and to provide new risk and prognosis biomarkers for AS.
In this case-control study, 44 AS patients and 56 healthy controls(HCs) from July 2015 and July 2016 were consecutively enrolled. Peripheral whole blood from all samples were used to extract total RNA

DOI: 10.1002/eji.201970400

and serum was used to test related clinical parameters including ALT, AST, Cholesterol, HDL-C, LDL-C, TG, GGT, ALP, CRP, ESR, IgG, IgA and IgM. The expression levels of miRNAs were detected by quantitative real-time PCR(qPCR). And the analysis of miRNAs differences between the groups and the correlations with clinical parameters were analyzed by SPSS 21.0.
The results showed: miR-146a, miR-125a-3p, miR-125a-5p, miR-125b-5p and miR-155a were found significantly decreased in AS patients compared to HCs. miR-499a were highly expressed in AS patients(P< 0.05). According to Receiver operating characteristic(ROC) curve, miR-146a(AUC = 0.644, 95%CI: 0.540-0.739), miR-125a-5p(AUC = 0.628, 95%CI: 0.574-0.772) and miR-125b-5p(AUC = 0.687, 95%CI: 0.61-0.81) have good specificity for diagnosis of AS(P< 0.05). In the patients with AS, miR-499a and CRP have a significant positive correlation(r=0.307, P=0.048). There are negative correlations between the level of miR-146a and IgA (r=-0.341, P=0.023), and also between miR-155a and CRP(r=-0.308, P=0.047).
In conclusions: miR-155a, miR-146a and miR-499a correlates with the disease activity of AS. The expression levels of miR-146a, miR-125b-5p, miR-499a and miR-155a might be used as new diagnosis biomarkers for AS.

P0072

The functional and mechanistic studies of miRNA-133b and its target NCAPH in NSCLC

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Objective: Lung cancer is the leading cause of caner related deaths worldwide, and characterizing the underlying mechanisms or identifying potential novel biomarkers for NSCLC is very important. By applying a new method called Cross-Value Association Analysis (CVAA) in TCGA solid tumor database, our previous work had identified many tumor related novel genes, such as condensin family member NCAPH.Recently, we identified many differential expressed miRNAs comparing the transcriptome data generated from para-cancerous or cancerous NSCLC tissues. We carried on a series of studies to investigate the role of miR-133b in the development and progression of non-small cell lung cancer.
Methods: Molecular biological and biochemical methods, such as construction of vectors, proliferation and migration of tumor cells in vitro, construction of double luciferase reporter vectors, etc.
Results: **miRNA-133b was identified to be downregulated in the cancerous cell lines, tissues and exosomes isolated form peripheral blood in cancer patients.**Furthermore, we found that **over-expression of miRNA-133b or NCAPH knockdown in NSCLC A549 cells inhibited cancerous cell proliferation and migration.**We also found that NCAPH high expression in NSCLC tumor tissue. Interestingly, we provided evidences showing **miR-133b overexpression or NCAPH depletion decreased cancerous cell stemness marker genes expression and Wnt/b-catenin signaling pathway, indicating that miRNA-133b targeting to NCAPH regulates NSCLC progression possibly through regulating lung cancer stem cells in a Wnt/b-catenin dependent mechanism.**
Conclusion: Mir-133b is expected to become a new target for clinical application of NSCLC.

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DOI: 10.1002/eji.201970400

P0073

Mechanisms of breast cancer exosomes regulating CD73 expression in infiltrating γδT2 cells by long non-coding RNA SNHG16

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Purpose: Existed evidence indicate breast cancer could be regulated by immune microenvironment. Here we aim to identify the role of infiltrating γδT cells in breast cancer.
Methods: FCM was used to identify the phenotype of infiltrating γδT2 cells. Furthermore, in vitro experiments were performed to identify breast cancer derived exosomes regulate the CD73 expression of γδT2 cells. Finally, Lnc-RNA was screened by gene chip and bioinformatics analysis, and the molecular mechanism of SNHG16 ceRNA function was further clarified by western-blot and fluorescein reporter gene assays.
Results: FCM showed the number of Vδ2 cells were significantly higher in breast cancer. In vitro cytotoxic experiments showed Vδ2 cells derived from breast cancer tissue revealed strong immunosuppressive rather than cytotoxic function. Co-culture Vδ2T cells with triple negative breast cancer cell lines found the expression of CD73 in Vδ2T cells increased significantly with non-contact fashion. Similar results were obtained by co-culturing exosomes from triple negative breast cancer lines with Vδ2T cells. The lncRNA chip found that the expression of SNHG16 upregulated in the exosomes of triple negative breast cancer cell lines. Furthermore, we demonstrated SNHG16 function as ceRNA via competently binds to SMAD5 binding site miR-16-5p, thus activating SMAD5 pathway and promoting the expression of CD73. In addition, TCGA database indicated high expression of SNHG16 was significantly correlated with poor prognosis in breast cancer.
Conclusion: CD73+ γδT2 cells are important immunosuppressive cells in breast cancer. TNBC cells secrete exosome and depend on SNHG16 to regulate the expression of CD73 in γδT2 cells.

P0074

Circular RNA sequencing indicates circ-IQGAP2 and circ-ZC3H6 as the non-invasive biomarkers in primary Sjögren's syndrome

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Primary Sjögren's syndrome (pSS) is a complex systemic autoimmune disease that primarily presents with dysfunction of the exocrine glands and is marked by xerostomia and xerophthalmia. Circular RNAs (circRNAs) are usually stable noncoding RNAs that can act as microRNA sponges and modulate transcription. Emerging data have implied that circRNAs have potential as biomarkers of autoimmune diseases; however, no known associations have been identified between circRNAs and pSS. To characterize the expression profiles of circRNAs in pSS, RNA sequencing was carried out, and two candidate biomarkers, circ-IQGAP2 (hsa_circ_0129656) and circ-ZC3H6 (hsa_circ_0001062), were discovered. Circ-IQGAP2 and circ-ZC3H6 had significantly upregulated expression in the minor salivary glands small salivary glands (MSGs) of pSS patients, and this elevated expression was confirmed by qPCR in plasma exosomes extracted from 35 pSS and 20 non-pSS subjects. In addition, there were no significant differences in the host genes *IQGAP2* and *ZC3H6* between pSS and non-pSS subjects. Furthermore, the predicted target miRNAs and mRNAs of circ-IQGAP2 and circ-ZC3H6 were involved in biological processes associated with the immune response, including the proliferation, differentiation and activation of immunocytes. Remarkably, the expression of these circRNAs showed the significant correlations with both clinical features including IgG level and focus score. ROC analysis showed that the indexes comprised of both two circular RNAs and clinical

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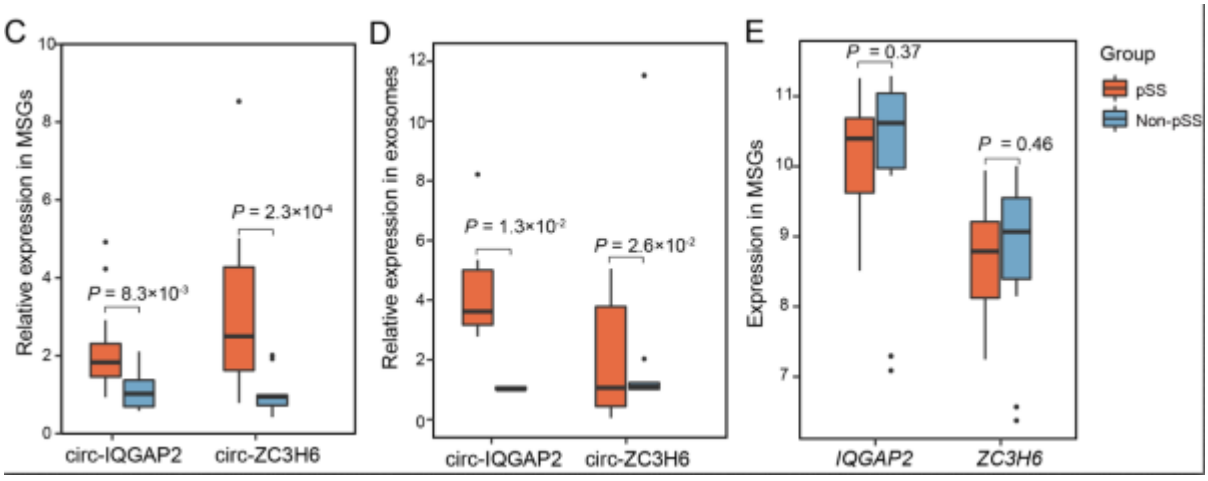
DOI: 10.1002/eji.201970400

features had more effective discrimination to distinguish pSS from non-pSS than those without both circRNAs, with high mean areas under the curve of 0.961 in the MSGs and 0.929 in the plasma exosomes. Taken together, this study indicated the potential roles of circ-IQGAP2 and circ-ZC3H6 as noninvasive biomarkers for the diagnosis of pSS and provided clues for further functional studies.



[Figure 1]

P0075

LncRNA PICSAR promotes cell proliferation, migration and invasion of fibroblast-like synoviocytes by sponging miRNA-4701-5p in rheumatoid arthritis

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Recently, long non-coding RNAs (lncRNAs) have drawn an increasing attention because of the pivotal roles they played in various types of autoimmune diseases, including rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLSs), a prominent component of hyperplastic synovial pannus tissue, are the primary effector cells in RA synovial hyperplasia and invasion which can lead to joint destruction. Although the important biological functions of lncRNAs have been gradually explored, their underlying mechanism in RA-FLS remains unclear. Here, we conducted the lncRNA microarray to screen differentially expressed lncRNAs and mRNAs, then analyzed the results by bioinformatics and statistical methods. We identified a long intergenic non-protein-coding RNA162 (LINC00162), also known as lncRNA PICSAR (p38 inhibited cutaneous squamous cell carcinoma associated lincRNA), has significantly higher expression in RA-FLSs by quantitative real-time PCR (qRT-PCR). Simultaneously, PICSAR knockdown can negatively regulate the proliferation, migration and invasion of FLSs. Furthermore, we conducted molecular biological analysis to predict miRNAs which have a potential binding site for PICSAR. Among these miRNAs, the expression of miR-4701-5p was significantly increased after knockdown of PICSAR and overexpression of miR-4701-5p lowered the level of PICSAR in RA-FLSs. Ultimately, luciferase reporter assay was adopted to validate the interaction of lncRNA PISCAR and miRNA-4701-5p. We also demonstrate that overexpression of miR-4701-5p can ulteriorly suppress cell proliferation, migration and invasion after PICSAR knockdown. Based upon these findings, PISCAR may exert an essential role in promoting synovial invasion and joint destruction by sponging miRNA-4701-5p in RA and that targeting lncRNA PISCAR may have therapeutic potential on patients with RA.

DOI: 10.1002/eji.201970400

P0076

Expression alteration of selected long non-coding RNAs in macrophages in response to Brucella abortus infection

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Macrophages eliminate pathogenic microorganisms through a regulated expression of several inflammatory mediators. However, *Brucella abortus*, a bovine and human infecting pathogen, can modulate the physiology of these cells to escape to their microbicidal mechanisms. Its strategy of immune evasion depends on the secretion of protein effectors that inhibit the production of pro-inflammatory factors of the host. The expression of inflammatory factors can be regulated by non-coding RNAs (ncRNA), being the long ncRNAs (lncRNA) the largest subgroup of these regulatory elements that actively participate in the gene expression of several cellular processes, including response to infection.
Because the role of these RNAs in infection with *B. abortus* is completely unknown, we approached this issue in our work quantifying the relative expression levels of eight lncRNA expressed in macrophages infected with the pathogenic strain *B. abortus* 2308, with the objective of knowing if this pathogen modulates its expression and, thus, the pro-inflammatory response. The results showed that at the 1st and 4th hour post-infection, *B. abortus* 2308 suppresses the expression of several lncRNAs such as lincRNA-cox2, LncRNA-MALAT1 and LncRNA-GAS5, which are associated with the transcription of pro-inflammatory genes or the induction of the differentiation of macrophages towards a M1 phenotype. In addition, after the 1st hour post-infection *B. abortus* 2308 showed a high expression of Lethe and LincRNA-EPS, both lncRNA known for their repressor role of the inflammatory response. These data help to understand the hypothesis that *Brucella* uses a "stealth" strategy to persist within its host.
Financed by FONDECYT 1180122.

P0077

BLiCR, a long noncoding RNA required for optimal germinal center B cell-expression and function of BCL6

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BCL6 is the master transcriptional factor for B cell differentiation into germinal center (GC) B cells, helping sustain proliferation and limit terminal differentiation into plasma cells. GCs are essential for diversification and affinity maturation of antibody responses and the generation of longlived memory B cells and plasma cells. Dysregulation of BCL6 expression and germinal center reactions have been found to lead to various B cells lymphomas and contribute to B cell-derived autoimmunity. It is therefore not surprising that BCL6 expression is tightly regulated via a combination of transcriptional factors and epigenetic modifications. Here we have discovered an additional layer of complexity: a long noncoding RNA (lncRNA) that we have named *Blicr*, which acts to enhance and maximise Bcl6 expression. We show that *Blicr* is located within a super-enhancer region only found in B cells and is highly expressed in human germinal center B cells (GCBs) and the GC-derived human B cell lymphoma cell line, Ramos. In addition, single molecular RNA fluorescence in situ hybridization (RNA FISH) shows that *Blicr* is mainly localized in nucleus in GC B cells. Knockdown of *Blicr* reduces BCL6 protein and impairs cell cycle and proliferation. Furthermore, several Bcl6 target genes also appeared deregulated by *Blicr* knockdown. These results suggest that *Blicr* is crucial for maximal expression of

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DOI: 10.1002/eji.201970400

B cell-expressed BCL6. Our work suggests that manipulation of this long-non-coding RNA to increase or decrease its expression may be respectively used to boost vaccination or to treat GC-derived lymphomas.

P0653

NF-κB-driven miR-34a impairs Treg/Th17 balance *via* targeting Foxp3

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The regulatory T cells (Tregs), a subset of T cells with its specific transcription Foxp3, are a unique cell type for the maintenance of immune homeostasis by controlling effector T cells (Teffs) responses. It is common that a defect in Tregs with Treg/Teff disorder causes autoimmune diseases. However, the precise mechanisms are not thoroughly revealed. Here, we report that miR-34a could attenuate *Foxp3* gene expression *via* targeting the its 3' untranslated region (3' UTR). The hsa-miR-34a increased in peripheral blood mononuclear cells (PBMCs) from rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) patients, and displayed a positive correlation with some serum markers of inflammation. In addition, mmu-miR-34a levels were downregulated in TGF-β-induced Tregs but upregulated in Th17 cells induced *in vitro* compared to activated $CD4^+$ T cells. It has been demonstrated that elevated miR-34a disrupting Treg/Th17 balance *in vivo* contributed to the progress of pathogenesis of collagen induced arthritis (CIA) mice. Furthermore, IL-6 and TNF-α were responsible for the upregulation of miR-34a and downregulation of Foxp3, which was reverted by the addition of NF-κB/p65 inhibitor BAY11-7082, thus indicating that NF-κB/p65 inhibited Foxp3 expression in an miR-34a-dependent manner. Moreover, IL-6 or TNF-α-activated p65 could bind on the *miR-34a* promotor and enhance its activity, resulting in upregulation of its transcription. Taken together, we show that NF-κB activated by inflammatory cytokines, such as IL-6 and/or TNF-α, reduces Foxp3 levels *via* regulating miR-34a expression, which provides a new mechanistic and therapeutic insight into the ongoing of autoimmune diseases.

P0654

MiR-15a/16 deficiency induces M1 macrophages and regulatory $CD19^+Tim\text{-}1^+$ cells in tumors

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M1 macrophages participate in pro-inflammatory and anti-tumor processes by producing cytokines, while regulatory B cells negatively regulate immune responses by producing cytokines. MiR-15a/16 is considered as a tumor suppressor gene and its biological activity has attracted wide study. Our study found that, in young miR-15a/16 knockout mice (8 weeks) transplanted with hepatic cancer (H22) cells or treated with dextran sulfate sodium (DSS), the frequency of M1 cells in tumors or colonic tissues was higher than control group, and the proportion of M1/M2 macrophages was also increased. It indicates that miR-15a/16 may promote polarization of macrophages from M2 to M1. Meanwhile, the frequency of IL-10-producing $CD19^+Tim\text{-}1^+$ cells was increased in both young miR-15a/$16^{-/-}$ mice transplanted with H22 cells and old miR-15a/$16^{-/-}$ mice (15-18 months) with the onset of B-cell leukemia. The cells down-regulated the function of effector $CD4^+CD25^{low}$ T cells depending on IL-10 production, and adoptive transfer of $CD19^+Tim\text{-}1^+$ cells promoted tumor growth in mice. Bioinformatics analysis shows that miR-16 targets the 3´-untranslated region (3´-UTR) of STAT3 mRNA. We further confirm that miR-15a/16 deletion can affect M1 polarization by activating the STAT3 and NF-κB signaling pathways in macrophages, and induce IL-10-producing Breg cells by up-regulating STAT3

DOI: 10.1002/eji.201970400

expression. Our research enriches the understanding of the biological role of miR-15a/16 and provides more experimental data for the development of clinical therapeutic drugs based on miR-15a/16.

P0655

MicroRNA-5112 targets IKKγ to regulate the activation of murine bone marrow-derived dendritic cells induced by flagellin

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Dentritic cells (DCs), the most potent antigen-presenting cells, can be activated by the recognition of the foreign pathogens via Toll-like receptor (TLRs) resulting in the release of downstream proinflammatory cytokines and up-reguation of costimulatory molecules. microRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by either translational inhibition or mRNA degradation. The regulation role of miRNAs in DCs activation induced by flagellin (ligand of TLR5) remain unknown. Here, we identify differentially expressed miRNAs in BMDCs between flagellin treatment and medium alone using miRNA microarray. We found that flagellin stimulation could downregulate miR-5112 expression in BMDCs as well as spleen DCs (*in vitro* and *in vivo*). Forced the miR-5112 expression could decrease the inflammatory cytokines production and accompanied with a reduction of IKKγ in BMDCs induced by flagellin. miR-5112 also could inhibit the inflammatory response induced by flagellin *in vivo*. IKKγ was proven to be directly targeted by miR-5112 in TLR5 signal pathway. Our results suggest that reduction of miR-5112 is a potential positive feedback loop to enhance inflammatory cytokine production in flagellin-activated BMDCs.

P0656

Hepatic miR-143 prevents hepatocellular carcinoma carcinogenesis via suppressing STAT3 activation

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Nonresolving chronic inflammation induces the carcinogenesis of hepatocellular carcinoma (HCC), and IL-6 production and effect in the liver are of prior importance in this process. The regulatory mechanisms of IL-6 effect have currently attracted much attention. We previously found that microRNA-143 (miR-143) was abundantly expressed in the liver but significantly decreased in HCC tissues, and its roles in HCC carcinogenesis remain unknown. Here, we found that miR-143 deficiency promoted HCC carcinogenesis, which was dependent on IL-6. The production of IL-6 in the liver was not influenced, but IL-6 effector signaling and downstream STAT3 phosphorylation were significantly enhanced by miR-143 deficiency. Furthermore, we found that miR-143 could suppress STAT3 activation by enhancing the binding between SOCS3 and STAT3. Thus, we propose that hepatic miR-143 prevents HCC carcinogenesis via suppressing IL-6 effector signaling.

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DOI: 10.1002/eji.201970400

P0657

The role of miR-338-5p in deep vein thrombosis via regulating IL-6

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Rationale: Deep venous thrombosis (DVT) is one of the most common venous thromboembolic (VTE) disorder which have a high mortality. Elevated Interleukin-6 (IL-6) expression has been identified to be involved in the DVT formation. However, the molecular mechanisms leading to the elevated IL-6 in DVT remain unclear.
Methods: miRNA expression in DVT was profiled by miRNA microarray analysis. The upstream miRNA regulators of IL-6 were predicted by *in silico* target prediction tools. Expression of IL-6 was examined by quantitative real-time PCR (qRT-PCR) and enzyme-linked immunoassay (ELISA) and *miR-338-5p* level was evaluated by qRT-PCR in the peripheral blood mononuclear cells (PBMCs). Dual-luciferase reporter assay was used to identify the interaction between *miR-338-5p* and *IL-6*. Murine model of DVT was developed and the co-localization of *miR-338-5p* and *IL-6* in thrombosis was demonstrated by florescent in situ hybridization (FISH). The biological effect of *miR-338-5p* on IL-6 was demonstrated both *in vitro and in vivo.*
Results: Microarray and qRT-PCR results showed that miR-338-5p was significantly negatively correlated with IL-6. *In vitro* experiments showed that overexpressed *miR-338-5p* reduced IL-6 expression, while inhibited *miR-338-5p* increased IL-6 expression. Moreover, *in vivo* studies found that DVT mice with agomiR-338-5p delivery resulted in decreased IL-6 expression and alleviated thrombus formation, whereas antagomiR-338-5p acted inversely. Dual luciferase reporter assay identified that there is direct binding between *miR-338-5p* and *IL-6*.
Conclusions: These findings suggested that decreased *miR-338-5p* promotes DVT formation by enhancing IL-6 expression. *miR-338-5p* may be explored as a promising diagnostic marker and therapeutic target for DVT.

P0658

RNA-binding protein human antigen R regulates microRNA biogenesis via enhanced Drosha expression

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MicroRNA biogenesis changes significantly when an organism responds to various adverse stimuli. Drosha functions as the key regulator of miRNA biogenesis and plays an essential role in the cellular response to an external inflammatory stimulus. The expression of Drosha is dynamically regulated. The RNA-binding protein HuR is a well-known positive regulatory factor, and the level of this protein increases when cells encounter stress. In this study, miRNA sequencing was employed to observe the genome-wide miRNA profiles in small interfering RNA-treated HuR-silenced intestinal epithelial cells line IEC-6. We found that 370 of 372 miRNAs were downregulated in the HuR-silenced IEC-6. However, HuR silencing did not affect the P-element induced wimpy testis-interacting RNA biogenesis, which is Drosha independent. By western blotting and qPCR, we found that exogenous HuR overexpression increases Drosha mRNA and protein expression but does not affect its mRNA stability. Drosha abundance is controlled post-transcriptionally by HuR via direct association with the 3′-untranslated regions of Drosha mRNA. Interestingly, knocking down endogenous HuR enhanced the

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DOI: 10.1002/eji.201970400

association of Drosha mRNA with Argonaut (Ago)-containing complexes and increased the colocalization of Drosha mRNA with processing bodies; the downregulation of Drosha induced by HuR silencing was eliminated by the knockdown of Ago1, Ago2, or RCK. Moreover, HuR indirectly enhanced Drosha transcription by increasing the level of c-Myc. Thermoplegia leads to the development of pyroptosis by promoting inflammatory factor release. In a thermoplegia model, intestinal epithelial cells HuR levels increased, thereby resulting in enhanced Drosha expression, which regulated the miRNA biogenesis and involved in cell pyroptosis.

P0659

Nicotine exerts dual effects in inflammatory bowel diseases by shifting Th1/Th2 balance toward Th1 through miR-124-IL-6R pathway

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Although it is generally believed that smoking protects against Th2-type ulcerative colitis (UC) and worsens Th1-type Crohn´s disease (CD), the mechanism remains elusive. Nicotine is the main component of bidirectional regulation of tobacco on inflammatory bowel disease (IBD). Our previous studies have revealed that nicotine protects against UC through specifically elevating miR-124 in the colon, while others reported that miR-124 is also highly expressed in CD, but it worsens CD. The bidirectional regulation of miR-124 on IBD is parallel to that of nicotine. We speculate that nicotine might play a dual role in IBD by activating miR-124 and regulating Th1/Th2 balance. Results showed that miR-124 overexpression protected UC mice with a Th1 polarization in lymphocytes and colon tissue, while miR-124 knockdown showed opposite effect. Similar results were obtained in human lymphocytes. Furthermore, miR-124 knockdown could abolish Th1 polarization effect of nicotine on UC mice and human lymphocytes. IL-6R interference could significantly attenuate the role of miR-124 in Th1 polarization, indicating that it is a downstream target of miR-124 on Th1/Th2 balance. Collectively, these data indicate that miR-124-IL-6R pathway mediates the dual role of nicotine in IBD by shifting Th1/Th2 balance toward Th1.

P0660

LncRNA Dnmt3aos regulates Dnmt3a expression leading to aberrant DNA methylation in macrophage polarization

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Long non-coding RNAs (lncRNAs) play key roles in various biological processes. However, the roles of lncRNAs in macrophage polarization remain largely unexplored. In this study, thousands of lncRNAs were identified that are differentially expressed in distinct polarized bone marrow-derived macrophages (BMDMs). Among them, Dnmt3aos (DNA methyltransferase 3A, opposite strand), as a known lncRNA, locates on the antisense strand of Dnmt3a. Functional experiments further confirmed that Dnmt3aos were highly expressed in M(IL-4) macrophages and participated in the regulation of Dnmt3a expression, and played a key role in macrophage polarization. The DNA methylation profiles

DOI: 10.1002/eji.201970400

between the Dnmt3aos knockdown group and the control group in M(IL-4) macrophages were determined by MeDIP-seq technique for the first time, and the Dnmt3aos-Dnmt3a axis-mediated DNA methylation modification-regulated macrophage polarization related gene IFN-γ was identified. Our study will help to enrich our knowledge of the mechanism of macrophage polarization and will provide new insights for immunotherapy in macrophage-associated diseases.

P0661

MiRNA-29a promotes B cell overactivation by upregulating CRKL in systemic lupus erythematosus

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Objective: Systemic lupus erythematosus (SLE) involves the production of autoantibodies by overactivated B cells. We aimed to investigate the expression of miR-29a in SLE and to determine the role of miR-29a in SLE B cell hyperactivity and its contribution to SLE pathogenesis.
Methods: We used hybridization chips to detect peripheral blood B cell miRNA expression profiles from 66 SLE patients and 10 healthy controls (HCs). Peripheral blood mononuclear cell (PBMC) miRNA profiles from 3 SLE patients and 3 HCs were detected using next-generation sequencing. miRNAs that were up- or down-regulated in both analyses were identified, and qRT-PCR was used to validate candidate miRNAs. A miR-29a inhibitor was introduced into healthy control PBMCs, and IgG levels were detected by enzyme-linked immunosorbent assay (ELISA). Bioinformatics analysis was used to predict the target of miRNA-29a among dysregulated mRNAs. We also demonstrated the direct and transcriptional regulation of *CRKL* by miR-29a using dual luciferase reporter assays and confirmed the expression of CRKL mRNA and protein using next-generation sequencing and western blotting.
Results: The expression of miR-29a was significantly downregulated in B cells from SLE patients. Transfection of miR-29a inhibitors into healthy PBMCs increased IgG production by B cells. In B cell lines, low expression of miR-29a promoted increased levels of CRKL, suggesting that miR-29a regulates B cell antibody production and is possibly dependent on the regulation of CRKL expression.
Conclusion: MiR-29a may affect the secretion of IgG antibody in B cells by regulating CRKL, suggesting that miR-29a plays a role in B cell hyperactivity.

P0662

Effect of lncRNA-125 on mouse and human macrophage polarization and during Mtb infection

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Tuberculosis (TB) is one of the leading fatal infectious diseases world-wide and also accounting for significant morbidity especially in developing countries. The bacteria responsible for this disease,

DOI: 10.1002/eji.201970400

Mycobacterium tuberculosis (Mtb), can subvert the host defence by skewing macrophage activation towards a less microbicidal alternative activated state to avoid classical effector killing functions. However, the molecular details underlying this evasion mechanism are barely understood. Our group explores host factors and pathways that are subverted by Mtb to increase its persistence and survival within macrophages. Amongst those host factors, long non-coding RNAs (lncRNAs) are emerging as important regulators of immune cells activation and response to pathogens. However, in comparison to the number of studies elucidating the role of lncRNAs in cancer, studies in TB have received minimal attention. In collaboration with our partners from the RIKEN Omics Science Center, Yokohama, Japan, we performed genome-wide transcriptome analysis [Cap Analysis Gene Expression (CAGE)] of IFN-ϒ stimulated (classically activated) macrophages and IL-4/13 stimulated (alternatively activated) macrophages. We have identified 151 differentially expressed long non-coding RNAs (lncRNAs) that might play functional roles in macrophage polarization. In particular, lncRNA-125 was significantly downregulated during Mtb infection. This project aims at functionally validating lncRNA-125 in polarized and/or Mtb-infected murine and human macrophages using chemically engineered antisense oligonucleotides (GapmeRs), address the question as to whether and how Mtb targets lncRNAs to elicit their evasion mechanisms, hence identifying lncRNAs, particularly lncRNA-125, as new host factors to be further investigated for TB diagnostics and therapies.

P0663

IL-4/IL-13-inducible lincRNA-MIR99AHG regulates macrophage polarization and acts as a promoter of Mtb growth

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) kills millions of people worldwide every year, and there is an urgent need for targeting host-pathogen interaction as a strategy as pathogens acquire resistance to current antimicrobials. Long intergenic nonocoding RNAs (lincRNAs) are important rgulators of gene expression. Although long noncoding RNAs (lncRNAs) are expressed in immune cells their functional role in gene regulation and bacterial infections remains under-studied. Here, we identify an immunoregulatory, lincRNA-MIR99AHG, which is upregulated in macrophages upon IL-4/IL-13 stimulation and downregulated upon Mtb infection. To evaluate functions of this lincRNA, we employed loss of function experiments. Knockdown of MIR99AHG impeded intracellular Mtb growth in mouse and human macrophages and also reduced proinflammatory cytokines. The deletion of lincRNA-MIR99AHG impaired the expression of the neighbouring gene coxsackie virus and adenovirus receptor (CXADR). Transcriptome analysis of macrophages from mice together with loss of function experiments, showed a role of MIR99AHG as a potential regulator of macrophage polarization and promoter of Mtb growth. Furthermore, MIR99AHG shows these effects by interacting with hnRNPA2/B1. Together, these findings identify MIR99AHG as a negative regulator of excessive inflammation to promote Mtb growth.

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DOI: 10.1002/eji.201970400

P1251

CircDYM ameliorates depressive-like behavior by targeting miR-9 to regulate microglial activation via HSP90 ubiquitination

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Circular RNAs (circRNAs), highly expressed in the central nervous system, are involved in various regulatory processes and implicated in some pathophysiology. However, the potential role of circRNAs in psychiatric diseases, particularly major depressive disorder (MDD), remains largely unknown. Here, we demonstrated that circular RNA DYM (circDYM) levels were significantly decreased both in the peripheral blood of patients with MDD and in the two depressive-like mouse models: the chronic unpredictable stress (CUS) and lipopolysaccharide (LPS) models. Restoration of circDYM expression significantly attenuated depressive-like behavior and inhibited microglial activation induced by CUS or LPS treatment. Further examination indicated that circDYM functions as an endogenous microRNA-9 (miR-9) sponge to inhibit miR-9 activity, which results in a downstream increase of target-HECT domain E3 ubiquitin protein ligase 1 (HECTD1) expression, an increase of HSP90 ubiquitination, and a consequent decrease of microglial activation. Taken together, the results of our study demonstrate the involvement of circDYM and its coupling mechanism in depression, providing translational evidence that circDYM may be a novel therapeutic target for depression.

P1253

Exosomes derived from miR-143-overexpressing mesenchymal stem cells suppress lung cancer progression by targeting KRAS

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Mesenchymal stem cells (MSCs) derived exosomes (MSC-exo) are attractive candidates for clinical therapeutic applications. However, the effect and mechanisms of MSC-exo in lung cancer remain unclear. In the present study, we found that MSC-exo could significantly suppress lung cancer cell growth in A549 and H1299. However, MSC-exo showed the ability to promote tumor cell migration and invasion. Further investigation showed that MSC-exo could promote EMT of A549 through Smad and non-Smad pathways. Knocking down TGF-β1 expression in MSCs could deactivate these pathways, abrogate the EMT promoting effect and enhance the tumor suppression effect of MSC-exo. It has been reported that dysregulation of miR-143 was associated with lung cancer progression, and the expression of miR-143 was negatively correlated with TGF-β1 in tumors. Our previous study found that knocking down TGF-β1 expression could up-regulate miR-143 expression in MSCs. We wondered whether overexpression of miR-143 in MSCs could also revert the EMT promoting effect and suppress lung cancer cells effectively and safely. It was exciting to find that exosomes from miR-143-overexpressing MSCs (miR-143-MSC-exo) could more markedly inhibit tumor cell growth than MSC-exo as control. Further study indicated that miR-143 could enhance the pro-apoptosis ability of MSC-exo by targeting KRAS in A549 cells. In A549 subcutaneous xenograft model, either miR-143-MSC-exo or MSC-exo treatment via tail vein injection could reduce the tumor size of lung cancer, but with the treatment of miR-143-MSC-exo, a greater growth suppression effect was observed. These findings suggest a method by which MSC-exo may be safely employed in lung cancer therapy.

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DOI: 10.1002/eji.201970400

P1254

SNHG5 inhibite EMT progresssion of lung adenocarcinoma through regulating localization of MTA2 and Twist1

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Long noncoding RNAs (lncRNAs) are emerging as important regulators in cellular processes. In this study, we aimed to investigate the effects of the long non-coding RNA, SNHG5 in epithelial-mesenchymal transition (EMT) of lung adenocarcinoma (LAD) and explore the underlying mechanisms. RT-qPCR results showed that the expression of SNHG5 and CDH1 were both significantly lower in lung adenocarcinoma (P< 0.001; P< 0.001), while N-cadherin and Vimentin were significantly higher (P< 0.001; P< 0.001). Overexpression of SNHG5 has no effect on proliferation of A549, but suppress its migration and invasion. Knockdown of SNHG5 could increase its migration and invasion. Western blot and immunofluorescence showed SNHG5 expression was positively correlated with E-cadherin protein expression, but negatively correlated with N-cadherin and Vimentin in lung cancer cell line A549. Overexpression of SNHG5 suppressed the increase of cell migration,and expression of Snail, SLUG and ZEB1 in the process of EMT of A549 induced by TGF-β1. Immunofluorescence demonstrated that overexpression of SNHG5 inhibited the entry of MTA2 and Twist into the nucleus of A549 cells, resulting in the decrease of MTA2 and Twist expression in the nucleus, while the increase of MTA2 and Twist expression in the cytoplasm. Overall, this study showed SNHG5 could suppress the EMT process of lung cancer by inhibiting the subcellular localization of MTA2 and Twist1 in nucleus, highlighting the potential of SNHG5 as a novel target therapy for lung adenocarcinoma.

P1255

The role of H19 Rs217727 polymorphism in hepatocellular carcinoma cell apoptosis

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Background: The objective of the current study was to explore the role of H19 rs217727 polymorphism in the control of hepatocellular carcinoma (HCC).
Method: The Student´s t test, Cox regression, and Kaplan-Meier analyses were used to analyse whether the H19 rs217727 polymorphism played an important role in the development of HCC. Real time polymerase chain reaction (PCR) and western blot analysis were carried out to measure the levels of H19, microRNA (miR)-675, FAS-associated death domain (FADD), caspase-8, and caspase-3 among H19 CC, CT, and TT groups, as well as in cells transfected with H19/si-H19, or miR-675 mimic/inhibitor. The MTT assay, colony formation assay, and flow cytometry assay were performed to detect the effect of H19/miR-675 on cell viability, cell colony formation and cell apoptosis.
Result: T allele of H19 rs217727 polymorphism apparently increased the survival rate of patients with HCC. Meanwhile, H19 enhanced miR-675 expression but reduced the mRNA and protein levels of FADD, caspase-3, and caspase-8. The T allele of H19 rs217727 polymorphism apparently increased the apoptotic rate of HCC cells. Moreover, H19 upregulated the expression of miR-675 whereas

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DOI: 10.1002/eji.201970400

reducing the expression of FADD, caspase-3, and caspase-8. Finally, H19 and miR-675 promoted cell proliferation and cell colony formation but repressed cell apoptosis.
Conclusion: the polymorphism of H19 rs217727 was associated with HCC via the H19/miR-675/FADD/caspase-8/caspase-3/apoptosis signaling pathway.
[This work was supported by grant from Henan natural science foundation(No.182300410343); grant from NSFC(No.81071724)]

P1256

Hypoxia-induced miR-191-C/EBPβ signaling regulates cell proliferation and apoptosis of fibroblast-like synoviocytes from patients with rheumatoid arthritis

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Background: Hypoxia plays an important role in the proliferation of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS), leading to pathology of RA. This study was conducted to evaluate hypoxia-induced-microRNAs (hypoxamiR) in RA-FLS and its role in the function of RA-FLS.
Methods: RA-FLS were cultured under normoxia (21% O_2) or hypoxia (3% O_2) condition, followed by a microRNA (miRNA) array analysis. The upregulation of miR-191 by hypoxia was confirmed in RA-FLS and FLS from osteoarthritis (OA) patients by quantitative real-time polymerase chain reaction (RT-PCR). Transfection of miR-191 mimic and inhibitor was used to investigate the function of miR-191 in RA-FLS. The functional targets of miR-191 were predicted by bioinfomatics, and then validated by reporter gene assay.
Results: A subset of miRNAs was identified to be induced by hypoxia including miR-191. The upregulation of miR-191 was found to be specific in hypoxic RA-FLS, compared to hypoxic OA-FLS. We observed that miR-191 in RA-FLS increased cellular proliferation via promoting G_1/S transition of the cell cycle and suppressed cell apoptosis induced by cell starvation. Bioinformatical analysis and experimental assays identified CCAAT/enhancer binding protein β (C/EBPβ) as a target gene of miR-191 in RA-FLS. Enforced expression of C/EBPβ rescued the cellular phenotypes induced by miR-191. In addition, an inverse correlation between the C/EBPβ level and hypoxia stimulation was found in RA-FLS, and overexpression of C/EBPβ could partly rescue the hypoxia-induced cell proliferation.
Conclusion: We demonstrated the miR-191-C/EBPβ signaling pathway mediating the hypoxia-induced cell proliferation in RA.

P1257

Role of LncRNA AK085865 in regulating macrophage polarization during allergic asthma and its mechanism

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Background: LncRNA has been reported to play an important role in cellular immune responses, but few studies have examined the role of lncRNA in regulating macrophage polarization in Allergic Asthma(AA).

DOI: 10.1002/eji.201970400

Objective: To explore the role of lncRNA AK085865 in AA and its mechanism.
Methods: Firstly, Df1-induced asthma model was established in WT and KO Mice. Histopathological changes were observed by HE and PAS staining, inflammatory cytokines were detected by ELISA, macrophages and innate lymphoid cells were detected by flow cytometry.
Results: We found that lncRNA AK085865 can promote the polarization of macrophages to M2. Furthermore, we found that the severity of AA in AK085865 KO mice was significantly reduced, and the proportion of M2 macrophagesand ILC2s was also significantly down-regulated.
Conclusions: This study highlights the AK085865 knockdown can reduce the polarization of M2 macrophages, thereby reducing the proportion of ILC2s in AA.
Keywords: AA, lncRNA, macrophages, innate lymphoid cells.

P1258

Screening and identification of specific circRNA in tissues and gastric juice of patients with gastric cancer

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CircRNA expression profiles for gastric cancer (GC) were screened using tissue samples from 5 GC patients with different TNM stages and its corresponding adjacent non-tumor tissues as controls. Results showed A total of 445 circRNAs, including 69 (15.51%) upregulated and 376 (84.49%) downregulated circRNAs, were found significantly aberrantly expressed in gastric cancer tissues. The top ten upregulated in gastric cancer tissues were hsa_circRNA_040698, hsa_circRNA_053294, hsa_circRNA_056670, hsa_circRNA_005152, hsa_circRNA_033572, hsa_circRNA_100451, hsa_circRNA_101692, hsa_circRNA_405965, hsa_circRNA_102485 and hsa_circRNA_016503. while the top ten downregulated ones were hsa_circRNA_049637, hsa_circRNA_103514, hsa_circRNA_402565, hsa_circRNA_100703, hsa_circRNA_003653, hsa_circRNA_103512, hsa_circRNA_007148, hsa_circRNA_008882, hsa_circRNA_101965, and hsa_circRNA_067967. The hot-point chromosomes were chr1, chr3, chr4, chr6, and chr11. Hsa_circRNA_100703 was significantly downregulated in 77.1% (27/35) gastric cancer tissues. Its levels in gastric cancer tissues were related to tumor stage (P = 0.035), distal metastasis (P = 0.046), tissue carcinoembryonic antigen (P = 0.001), and carbohydrate antigen 19-9 expression (P = 0.025). More importantly, hsa_circRNA_100703 can stably exist in human gastric juice; and its nature meets the requirements of clinical detection. Our study uncovered the circRNA expression profile in human gastric cancer. Moreover, some circRNAs can stably exist in human body fluid, and has the potential to be used as novel biomarkers for the screening of high-risk gastric cancer patients.
Keywords: Biomarker, circular RNA, clinical significance, expression profile, gastric cancer
Funding Information: This study was supported by grants from the Social Development Research Project of Hainan Province (No. ZDYF2017118)

P1259

Critical role of alternative M2 skewing in mir-155 deletion-mediated protection of colitis

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Inflammatory bowel disease (IBD) is associated with dysregulation of both innate and adaptive immune response in the intestine. MicroRNA (miR)-155 is frequently expressed and functions in many immune cell types. Besides its function in adaptive immunity, miR-155 is a key regulator of the innate immune response in macrophages, dendritic cells, and even in epithelia cells. Although the roles of miR-155 within T and B lymphocytes in colitis have been reported, its function in innate immune cells has not been thoroughly examined. In this study, the dextran sulfate sodium (DSS)-induced colitis model was established in wild-type (WT) and miR-155−/− mice. Our results showed that miR-155 deficiency in macrophages recapitulated the alleviated colitis feature of miR-155−/− mice and appeared to skew toward the alterative M2 phenotype. Notably, the predominance of M2 in colon can result in dampened intestinal immune cell proliferation and inhibit CD4 T cell polarization toward Th1 and Th17. Moreover, C/EBPβ and SOCS1 were demonstrated as two key functional targets in this process. We also provided evidence for use of miR-155 inhibitor to treat colitis. Collectively, the findings highlight the central role of alternative M2 skewing for miR-155 function in colitis and reveal that macrophages might be a main target for therapeutics.

P1260

miRNAs expression profile in early rheumatoid arthritis and validation of hsa-miR-361-5p as biomarker for early diagnosis

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Rheumatoid arthritis (RA) is an autoimmune disease with a prevalence of 0.5 to 1.1%. The early detection increases the remission possibilities in 40% to 60% of patients. Anticitrullinated protein antibodies (CCP) have a sensitivity of 72% and a specificity of 92% however, are not exclusive for RA. Therefore, there is a need of new early RA biomarkers. miRNAs contribute to rheumatoid arthritis (RA) and these molecules are very likely to be used in early RA as biomarkers. For detection of microRNAs overexpressed on RA, whole blood samples were collected and preserved with 1ml of RNA later. The subjects were classified on CTRL (healthy controls), CCP+ (positives to CCP antibodies), eRA (early rheumatoid arthritis) and cRA (established rheumatoid arthritis), according to the characteristics of the natural illness history. RNA from whole blood was extracted and 100ng of RNA was hybridized in Agilent Human miRNA Microarray with probes for 1205 miRNAs (G4870A). Data were analyzed with GeneSpring software. 253 human miRNAs showed differential expression among all groups ($p < 0.05$). Microarray validation was performed by RT-qPCR with stem-loop primer, specific for mature miRNAs. The hsa-miR-361-5p was overexpressed in eRA compared to the other groups, consistent with the microarray data. In ROC analysis, hsa-miR-361-5p had a specificity of 92.3% and a sensitivity of

DOI: 10.1002/eji.201970400

85.7% (AUC>0.9, $p < 0.05$). We identified a specific transcriptional fingerprint of miRNAs early RA and identify important regulatory pathways. Also hsa-miR-361-5p is overexpressed in early RA showing potential as a biomarker.

P1841

MicroRNA-7 suppresses CD4$^+$ T cell activation and function and contributes to immune-mediated liver injury

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The biological role of microRNA-7 has not been reported in the activation and function of CD4$^+$ T cells. Here, we show that, compared with wild type (WT) mice, miR-7-deficient (miR-7^{def}) mice exhibit a shorter survival time course, accompanied by elevated total cell number, activation and proliferation, and cytokine production of CD4$^+$ T cells. Moreover, miR-7 expression is significantly upregulated in activated CD4$^+$ T cells. And the activation and function of CD4$^+$ T cells from miR-7^{def} mice increase significantly in response to stimulation of TCR signaling in vitro. Notably, In vivo studies show that miR-7^{def} mice develop more serious pathology of liver tissue in response to ConA-induced acute liver injury (ALI), accompanied by enhanced activation, proliferation, and cytokine production of CD4$^+$ T cells. Importantly, adoptively transferred CD4$^+$ T cells from miR-7^{def} mice significantly promote liver pathology in the ALI model. In conclusion, our data reveal a previously unknown role of miR-7 as a novel critical negative regulator in CD4$^+$ T cell activation and function, which contributes to the understanding of CD4$^+$ T cell biological function and spur the development of therapeutic strategies against CD4$^+$ T cell-related immune diseases.

P1842

LincRNA-p21 knockdown reversed tumor-associated macrophages function by promoting MDM2 interaction with p53 and ameliorated breast cancer development

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Tumor-associated macrophages (TAMs) are important regulators of the complex interplay between the immune system and breast cancer. TAMs fuel the cancer progression and metastasis by reprogramming their specific functional phenotype in cancer settings. Therefore, it is important to address the mechanisms of shaping specific functional phenotype of TAMs in tumor milieu. In the present work, we found that lincRNA-p21 significantly up-regulated in 4T1 educated macrophages by LncRNA microarray analysis. LincRNA-p21 knockdown facilitated macrophages polarization into pro-inflammatory M1 in tumor microenvironment, and promoted anti-tumor response, such as inducing breast cancer cells apoptosis, inhibiting the tumor cells migration and invasion. Furthermore, lincRNA-p21 knockdown in macrophages benefited Mouse Double Minute 2 (MDM2) interaction with p53, facilitated the activation of NF-κB and STAT3 and then promoted the functional shift of macrophages in tumor microenvironment. *In vivo*, lincRNA-p21 knockdown in tumor infiltrated macrophage could ameliorate breast cancer development. Our data indicated that lincRNA-p21 was a key regulator of TAMs function in tumor milieu. Our data also suggested that lincRNA-p21 may be a novel therapeutic target of tumors characterized by TAMs infiltration.

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DOI: 10.1002/eji.201970400

P1843

Effect of the miRNA-21/FOXO1/ADAR1 negative feedback loop on macrophage activation syndrome

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Macrophage activation syndrome (MAS) is a life-threatening condition that results in a cytokine storm. The mice model of MAS suggests that continuous high expression of IFN-γ and decreased expression of IL-10 are the main mechanisms of pathogenesis. Here, we established a MAS model with repeated CpG stimulation in C57BL/6 mice. Macrophages from MAS mice and control C57BL/6 mice were separated using magnetic beads. Changes in mRNA and miRNA levels were detected by RNA sequencing and verified by qPCR analysis. We found that the levels of several mRNAs and miRNAs related to macrophage polarization, including IFN-γ and IL-10, significantly changed, which suggests that macrophage polarization involved in the pathological process of MAS and subsequent occurrence of hemophagocytic syndrome. We discovered that the miR-21/FOXO1/ADAR1 negative feedback loop plays a key role in macrophage activation. After CpG stimulation, the miR-21 level significantly increased, the transcription factor FOXO1 was downregulated, and the downstream RNA-editing enzyme ADAR1 reduced. Conversely, overexpression of ADAR1 increased the probability of mutation of A to G in pre-miRNA-21 and decreased the production of mature miR-21. In vivo experiments demonstrated that an antagomir to miRNA-21 upregulated the expression of IL-10 and downregulated IL-6. Overexpression of ADAR1 by adenovirus can effectively reduce IFN-γ and upregulate IL-10. The antagomir to miR-21 and ADAR1 overexpression could reduce inflammatory infiltration of the liver, spleen, and lymph nodes; promote blood cell recovery; and downregulate ferritin and upregulate fibrinogen production. In conclusion, intervening in the miRNA-21/FOXO1/ADAR1 negative feedback loop may have a therapeutic effect on MAS.

P1844

Function of lncRNA and RNA-protein interaction in viral infection and innate immune response regulation

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RNA protein interaction plays an important role in the whole process of life evolution. As the discovery of tremendous lncRNA transcripts in mammal and other eukaryotic transcriptome, lots of interests have been attracted to the functional identification of lncRNAs or RNA binding proteins in recent years. They are increasingly revealed to be signaling regulators, enzymatic modulators, RNA sensors and RNA modifiers in many aspects.
Our research interests focus on the role of noncoding RNAs and RNA interacting proteins in innate immune regulation, especially in antiviral immune responses. Through comparing the transcriptome of different macrophages, we identified several types of lncRNAs with different expression patterns during viral infection, regarding their IFN-dependence and IFN-independence with decreased or increased expression. Through protein interacting RNA profiling, we identified IRF3, TBK1 and cGAS as lncRNA targeting molecules in immune responses. Specifically, we found that viral induced

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DOI: 10.1002/eji.201970400

lncRNA-ACOD1 facilitates viral replication in both mouse and human cells through directly binding host metabolic enzyme GOT2 and promoting its catalysis. LncRNA-GMR (GSH-Modification related RNA) is proved to be an antiviral lncRNA with activity to strengthen TBK1 signaling through modulating its GSH modification, but its expression is depressed by viral infection. However, in another case, lncRNA-PIR (Positive IRF3 Regulator) is induced in viral infection as a positive feedback regulation which could promote IRF3 activity through influencing its protein interacting to eliminate viral infection. Our work reveals the molecular mechanisms of lncRNA-mediated regulation of viral replication and antiviral signaling, which are potential targets for developing broad-acting antiviral therapeutic strategies.

P1845

Long non-coding RNA linc-AAM contributes to macrophage activation and adaptive immune response by facilitating immune response gene expression

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Long intergenic noncoding RNAs (lincRNAs) are specifically expressed in immune cells. However, their functions in immunity, especially in adaptive immunity, are largely unexplored. Here, we identify an immunoregulatory lincRNA *linc-AAM* highly expressed in activated macrophages. Transcriptome analysis of *Linc-AAM*-knockdown macrophages combined with bone marrow derived macrophages from *Linc-AAM*-knockout mice and gain-of-function experiments revealed a role of *Linc-AAM* in activating macrophages and promoting the expression of immune response genes (IRGs). *Linc-AAM*-knockout mice had reduced IRGs expression levels in spleen and thymus as well as impaired antigen-specific immune responses in ovalbumin-immunized model. *Linc-AAM* is transcribed in a p65-dependent manner. *Linc-AAM* interacted with heterogeneous nuclear ribonucleoprotein L (hnRNPL) *via* two *CACACA* motifs resulting in the dissociation of hnRNPL from histone H3 at the IRGs locus, and bound to the promoter of IRGs to facilitate transcription. These findings identify *Linc-AAM* as a transcription enhancer of IRGs and adaptive immunity assistor, highlighting the crucial role of lncRNAs in regulating immune response.

P1846

miR-146a-5p contributes to decreased apoptosis of spleen cells via upregulating BAG-1 during Echinococcus multilocularis infection

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Echinococcus multilocularis is an important zoonotic parasite, responsible for alveolar echinococcosis in human beings. As an immune organ, the spleen plays a key role in host immune responses during *E. multilocularis* infection. Here we showed that the apoptotic levels of the spleen cells significantly decreased in mice 2 month and 3 month post infection of *E. multilocularis* ($p < 0.05$). To decipher the mechanism behind this, the miRNA profiles of these spleen cells were defined and found several miRNAs including miR-146a-5p were dysregulated. Bioinformatic analysis revealed that there were several potential miR-146a-5p targets related to apoptosis including BAG-1, an antiapoptotic protein. Both miR-146a-5p and BAG-1 was validated to be down-regulated and up-regulated by qPCR in mice 2 month and 3 month post infection of *E. multilocularis*, respectively. Moreover, BAG-1 was further validated to be up-regulated at the protein level by Western blot in mice 2 month and 3 month post

DOI: 10.1002/eji.201970400

infection of *E. multilocularis*. The results of dual fluorescent reporting assay verified that miR-146a-5p was able to bind to the 3' UTR of BAG-1, suggesting that BAG-1 is its real target. In mouse spleen cells, miR-146a-5p over-expression led to a significant increase of apoptosis ($p < 0.05$), while miR-146a-5p low-expression induced a remarkable decrease of apoptosis ($p < 0.001$). Taken together these results indicate that downregulation of miR-146a-5p is attributed to a decreased apoptosis of the spleen cells via upregulating BAG-1, thus affecting immune responses against *E. multilocularis*.

P1847

Role of mircoRNAs in immune-mediated necrotizing myopathy through regulation of myogenic differentiation-associated genes

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Objectives: Immune-mediated necrotizing myopathy (IMNM) is characterized by different stages of myofiber necrosis and regeneration on muscle biopsy. MircoRNAs (miRNAs) regulate various biological processes including myogenesis.The aim of this study was to examine IMNM-related miRNAs and their target genes, as well as potential mechanisms involved in the onset of IMNM.
Methods: Multiple miRNAs were screened and qRT-PCR was conducted in muscle biopsy samples of IMNM patients and healthy controls to validate differentially expressed miRNAs in IMNM. Bio-informatic analysis was used to predict target genes of miRNAs and double-luciferase reporter assay to confirm. Several tests including qRT-PCR, immunofluorescence, western blot analysis were performed in vitro and in vivo to explore possible pathogenesis of IMNM.
Results: The levels of miR-23a-3p, miR-146a-5p and miR-150-5p were significantly down-regulated in IMNM patients compared with healthy controls, and the predicted target gene of the three differentially expressed miRNAs was RNA binding protein, HuR, which can stabilize mRNA of MyoD and Myogenin. HuR mRNA expression was significantly decreased in muscle tissue of IMNM patients compared with normal controls. Immunofluorescence histochemical staining in muscle tissue section of IMNM showed that HuR expressed in the nucleus of mature myocytes and in the cytoplasm of regenerating muscle cells. Furthermore, at the onset of differentiation of human myoblasts, HuR cytoplasmic abundance increased dramatically, and returned to a predominantly nuclear presence upon completion of myogenesis.
Conclusion: MiR-23a-3p, miR-146a-5p and miR-150-5p can influence human myoblast differentiation by regulating the expression of HuR and possibly participate in the pathogenesis of IMNM.

P1848

The role of MiR-374c-5p in URSA by targeting IL-6

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Objective: To elucidate the potential role of microRNA miR-374c-5p (miR-374c-5p) in unexplained recurrent spontaneous abortion (URSA) by regulating interleukin-6 (IL-6) and explore the underlying

DOI: 10.1002/eji.201970400

mechanism.
Methods: IL-6 expression in the blood and decidua of URSA and norma pregnancy (NP) subjects were detected by qRT-PCR and ELISA. MiRNA profiling analysis of the PBMC from URSA patients and NP subjects was performed by using microarray. miR-374c-5p expression was confirmed by qRT-PCR. Hela cells were transfected with miR-374c-5p mimics and inhibitor. URSA mice model were treated with miR-374c-5p Agomir. The direct binding between miR-374c-5p and IL-6 was detected by Dual- luciferase reporter assay and FISH.
Result(s): Compared with NP women, IL-6 expression increased markedly in PBMC and decidua while miR-374c-5p decreased significantly in URSA patients. After the transfection of miR-374c-5p mimics, IL-6 expression down-regulated obviously in HeLa cells, while transfection of miR-374c-5p inhibitor acted oppositely. Study *in vivo* demonstrated that IL-6 expression decreased in the decidual of miR-374c-5p overexpressed mice and the embryo absorption rate reduced significantly at the same time.
Conclusion(s): Decreased miR-374c-5p is involved in URSA by enhancing IL-6 expression .miR-374c-5p/IL-6 might be a promising target for URSA therapy.
Key Words: unexplained recurrent spontaneous abortion, miR-374c-5p, IL-6, maternal-fetal immune tolerance, non-coding RNA

P1849

MiR-1246 promotes metastasis and invasion of A549 cells by targeting GSK-3β–mediated Wnt/β-catenin pathway

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Purpose: MicroRNAs (miRNAs) are a group of small non-coding RNAs involved in different cancers, including lung cancer. Here, we aim to investigate the expression profiles of circulating miRNAs and their roles contributed to the progress of lung cancer.
Materials and methods: The levels of circulating miRNA in lung cancer patients were investigated by miRNAs assay. Then we predicted the target genes of aberrantly expressing miRNAs by searching genetic databases. Based on the A549 cells transfected with miR-1246 mimics or miR-1246 inhibitor, we further measured the roles of miR-1246 involving in the epithelial-mesenchymal transition (EMT), migration and invasion capacities of lung cancer cells in vitro. Finally, we detected the effects of miR-1246 on glycogen synthase kinase-3β (GSK-3β)/β-catenin pathway by immunofluorescence and Western blot, respectively.
Results: We identified that 14 miRNAs were aberrantly expressed in the serum of lung cancer patients. Among them, miR-1246 was the most up-regulated. The cell assays indicated that miR-1246 significantly increased the migration and invasion capabilities of A549 lung cancer cells. Meanwhile, immunofluorescence analysis revealed that miR-1246 promoted EMT process of A549 cells accompanying with decreasing E-cadherin expression, while increasing vimentin and transforming growth factor β (TGF-β) expression. Furthermore, an online tool predicated that miR-1246 might bind to 3′-untranslated region of GSK-3β, which was confirmed by overexpression and knockdown of miR-1246 assays.
Conclusion: Taken together, the study illustrates that miR-1246 regulates Wnt/β-catenin pathway through targeting GSK-3β/β-catenin, which partly contributing to tumor metastasis. MiR-1246 may play an essential role in the diagnosis and therapeutic of lung cancer.

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DOI: 10.1002/eji.201970400

P1850

Hsa-miR-200b-3p negatively regulates angiogenesis in hepatocellular carcinoma by targeting ERG expression in endothelial cells

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Introduction: HCC is accompanied by strong angiogenesis. MiR-200b-3p, an anti-angiogenic regulator in hypoxia, is down-regulated in HCC, however, the role of miR-200b-3p in cancer angiogenesis remains unclear. ETS-related gene (ERG) is an essential transcription factor for endothelial cells, in which miR-200b-3p targeting site exists. In the present study, we hypothesized that down-regulated miR-200b-3p in HCC cells may affect ERG expression in endothelial cells, resulting in increased angiogenesis in the cancer microenvironment.
Method: Expressions of ERG and miR-200b-3p in human HCC tissues were studied by IHC and qRT-PCR. HUVECs were transfected with miRNA-200b-3p expression vector and ERG level was determined by western blot. We also measured miR-200b-3p expression in HUVECs, HCC cell lines and exosomes secreted from HCC cell lines. Moreover, HUVECs were cultured in tumour conditioned media and ERG level and angiogenic property were analysed. Dual luciferase reporter assay was done to show ERG is a direct target of miR-200b-3p.
Result: Clinical HCC tissues showed higher ERG expressions and in contrast lower miR-200b-3p expression than those from adjacent non-cancer tissues.Overexpression of miR-200b-3p in HUVECs decreased ERG protein expression in the cells. MiR-200b-3p was secreted in exosomes from HCC cell lines (Hep3B, HepG2 and HLE). Conditioned medium from miR-200b-3p overexpressing HLE cells reduced ERG expression and tube forming capacity of HUVECs. Dual luciferase reporter assay demonstrated that ERG was a direct target of miR-200b-3p.
Discussion: Downregulated miR-200b-3p in HCC could promote angiogenesis by increasing ERG expression in endothelial cells. MiR-200b-3p can be a potential target for controlling angiogenesis in HCC.

P1851

miRNA exosomal biomarkers for identification of active tuberculosis disease

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Tuberculosis which is caused by bacilli *Mycobacterium tuberculos*is is the major cause of morbidity since antiquity and still responsible for huge number deaths in developing countries after having complete anti tuberculosis therapy cure due to the emergence of drug resistant (MDR and XDR) tuberculosis cases. Thus to identify early possible signatures of disease with respect to drug resistant will help in rapid identification and eradication of disease severity.
There are combination of diagnostic assay are available for detection of tuberculosis like chest X-ray, sputum microscopy, culture and PCR-based diagnostics but none have higher specificity and can not discriminate between active v/s latent TB. A rapid point-of-care test that discriminates active TB or incipient disease from truly latent M.tb infection remains the “holy grail” of TB diagnostics research. In addition, a test that is able to predict treatment failure, often a sign of drug resistance, early would improve patient outcome and reduce the spread of drug resistant TB.
Exosomes are nanovesicles cargoes of 30-150nm size carrying nucleic acids ,proteins and lipids,among them certain small non coding RNA such as miRNA are present which are immunomodulatory in nature. By undertaking testing of **miRNA expression profiles as a biomarker**

DOI: 10.1002/eji.201970400

of TB disease we will provide important information regarding the best **approach to develop a new point-of-care test to aid both TB diagnosis and to predict response to treatment and can use as miRNA based therapy for TB disease.**

LB122

Long noncoding RNA lincRNA-EPS controls host antiviral innate immunity through binding with PKR

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Long noncoding RNAs (lncRNA) are generally expressed in immune cells and participate in multiple biological processes. LincRNA-EPS was certified to restrain inflammatory responses through targeting NF-kB pathway in macrophages and endotoxic-shock mice model. However, it is unclear whether lincRNA-EPS plays a role in host antiviral immunity. In our study, we have found that lincRNA-EPS knockdown and knockout macrophages manifested upregulated basal and induced level of interferon stimulated genes (ISGs) to prevent virus replication and viral particles production. The same conclusion could be obtained in gain-of-function and rescue experiments, which was reversed in the case that lincRNA-EPS was overexpressed. Meanwhile, *lincRNA-EPS*$^{-/-}$ mice protected itself from organ injury and lethality in the condition of acute Vesicular Stomatitis Virus (VSV) infection. Moreover, lincRNA-EPS was found to bind with the interferon-induced Ser/Thr protein kinase (PKR) to regulate type I interferon signaling pathway. Once interfering PKR expression, the antiviral effect mediated by lincRNA-EPS deficiency was lost significantly. Together, lincRNA-EPS was demonstrated to inhibit antiviral immune activity to maintain host immune balance.

P2504

The landscape and implications of chimeric RNAs in cervical cancer

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Background: Gene fusions and fusion products have been proven to be ideal biomarkers and drug targets for cancer.Even though a comprehensive study of cervical cancer has been conducted as part of the Cancer Genome Atlas (TCGA) project, few recurrent gene fusions have been found, and none above 3% of frequency.
Methods: We believe that chimeric fusion RNAs generated by intergenic splicing represent a new repertoire of biomarkers and/or therapeutic targets. However, they would be missed when only genome sequences and fusions at DNA level are considered. We performed extensive data mining for chimeric RNAs using both our and TCGA cervical cancer RNA-Seq datasets. Multiple criteria were applied. We analyzed the landscape of chimeric RNAs at various levels, and from different angles.
Findings: The chimericRNA landscape changed as different filterswere applied. 15 highly frequent (N10%) chimeric RNAs were identified. LHX6-NDUFA8 was detected exclusively in cervical cancer tissues and Pap smears, but not in normal controls. Mechanistically, it is not due to interstitial deletion, but a product of cis-splicing between adjacent genes. Silencing of another recurrent chimera, SLC2A11-MIF, resulted in cell cycle arrest and reduced cellular proliferation. This effect is unique to the chimera, and not shared by the two parental genes.
Interpretation: Highly frequent chimeric RNAs are present in cervical cancers. They can be formed by intergenic splicing. Some have clear implications as potential biomarkers, or for shedding new light on the biology of the disease.

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DOI: 10.1002/eji.201970400

P2505

Seed targeting with tiny anti-miR-1297 inhibits EMT in melanoma cells

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MicroRNAs (miRNAs) are small, noncoding RNAs that have tissue- and cell-specific expression. They have the ability to regulate the malignant proliferation and transformation of tumour cells. The research focussed on the expression and role of miR-1297 in melanoma. We firstly found that miR-1297 is up-regulated in melanoma tissues and cell lines. Functionally, phosphatase and tension homology deleted on chromsome ten gene (PTEN) was used as a potential target for miR-1297 and detected using Western blotting and immunohistochemistry (IHC). We then used chemical synthesis of anti-miR1297 to explore the influence on melanoma cells and examined the effects on A375 cell proliferation using MTT and western blotting methods. The results showed that anti-miR-1297 transfected A375 cells could inhibit the growth. Furthermore, transfection with anti-miR-1297 reduced PTEN protein expression and partially restrained A375 cells proliferation, migration and reversed Epithelial-Mesenchymal Transition (EMT) progression. In conclusion, we tentatively put forward that miR-1297 might be the key oncomiR in melanoma, and seed-targeted anti-miR-1297 might serve as a new tactic for miR-1297-based therapies.

P2506

Knockdown of LncRNA PVT1 inhibits glioma progression by regulating miR-424 expression

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Plasmacytoma variability translocation 1 (PVT1), an oncogene, has been reported to be highly expressed in many tumors, including human glioma, gastric cancer, and non-small cell lung cancer. Functionally, it could also regulate the development of tumor cells. However, its specific roles and pathogenesis in human gliomas are still not clear. This study investigated the function and mechanism of PVT1 knockdown in the proliferation and malignant transformation of human gliomas. We firstly examined the expression levels of PVT1 and miR-424 in human glioma tissues and cell lines. We also used gene manipulation techniques to explore the effects of PVT1 knockdown on cell viability, migration, invasion, and miR-424. We found that PVT1 knockdown effectively inhibited cell viability, migration and invasion of human glioma cells and increased miR-424 expression. Based on the negative correlation between PVT1 and miR-424, we then confirmed the direct interaction between PVT1 and miR-424 using RNA immunoprecipitation (RIP) and luciferase reporter assays. Further, we established a xenograft nude mouse model to determine the role and mechanism of PVT1 on tumor growth in vivo. In addition, PVT1 knockdown was shown to promote miR-424 in vivo. In summary, the present study demonstrated that PVT1 knockdown could negatively regulate miR-424 to inhibit human glioma cell activity, migration and invasiveness. PVT1 knockdown could negatively regulate miR-424 to inhibit cellular activity, migration and invasiveness in human gliomas, which explained the oncogenic mechanism of PVT1 in human gliomas. It also suggested that PVT1 might be a novel therapeutic target for human gliomas.

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DOI: 10.1002/eji.201970400

P2507

Lnc-Lsm3b feedback terminates innate immune response against RNA virus

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Innate RNA sensor RIG-I recognizing "nonself" viral RNA while discriminating against "self" RNA from the host is critical in the initiation of antiviral type I interferons (IFNs) production. As one of interferon-stimulated genes (ISGs), RIG-I expression is markedly induced upon interferons stimulation. Interestingly, continued up-regulation of RIG-I protein does not correlate with shutdown of type I IFNs production at late stage of antiviral response. What molecules can timely terminate RIG-I-triggered type I IFNs production and maintain immune homeostasis needs to be further identified. By RNA-seq and functional screening of RIG-I-binding lncRNAs in RNA virus-infected macrophages, we identified that long noncoding RNA lnc-Lsm3b can directly bind to RIG-I but cannot activate RIG-I ATPase activity. Importantly, lnc-Lsm3b, significantly upregulated by type I IFNs in the cytoplasm at the late antiviral response, can compete with viral RNAs to bind to RIG-I and feedback inhibit RIG-I-triggered antiviral signaling cascades. Mechanistically, multiple GA-rich sequences of lnc-Lsm3b that formed multivalent double-stranded RNA stem-loop structure were required for its high affinity binding to RIG-I. The binding of lnc-Lsm3b to RIG-I restricted RIG-I protein conformation shift, thus making RIG-I lose ability of TRIM25 binding, CARD ubiquitination, and then preventing RIG-I oligomerization for initiation of downstream antiviral signaling. These data define a new feedback mechanism for suppressing the prolonged activation of RIG-I-initiated signaling and overproduction of type I IFNs. Our results further demonstrate that host lnc-Lsm3b interaction contributes to the maintenance of immune homeostasis, suggesting a potential perspective for the control of inflammatory autoimmune diseases.

P2508

Lnccxcl2 limits lung inflammation by suppressing Cxcl2 expression

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Virus-induced cell death can trigger severe inflammatory damage by recruiting neutrophils into injured tissue. Tissue-located myeloid cells, such as macrophages and dendritic cells are the main sensors of dead cells in the regional tissue to initiate neutrophil recruitment by secreting multiple chemokines, such as CXCL1 and CXCL2. Epithelial cells as the first defense line in tissues, especially in lung and intestine, also produce chemokines during lethally viral infection, thus tight regulation of the production of these chemokines by epithelial cells is more critical to prevent inflammation and subsequent tissue damage. LncRNA-mediated regulation of gene expression has been reported to be involved in many immune responses, however, whether lncRNA participate in the regulation of chemokine production and function has not been well studied. Here we identified a long-noncoding RNA, which we termed it as *Lnccxcl2*, limited the expression of *Cxcl2* in mouse lung epithelial cells (MLE-12 cells). *Lnccxcl2*-deficient MLE-12 cells exhibited increased expression of *Cxcl2* and enhanced recruitment of neutrophils during viral infection. Rescuing the expression of *Lnccxcl2* in deficient MLE-12 cells

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DOI: 10.1002/eji.201970400

reversed the increased expression of *Cxcl2* and enhanced neutrophils recruitment. Mechanistically, *Lnccxcl2* bound to the promoter region of *Cxcl2* and maintained the repressed chromatin state of the same region, consequently inhibited the transcription of *Cxcl2*. Our results demonstrate a new epigenetic way to control inflammation during viral infection, providing a new method to control tissue damage in infectious and other inflammatory diseases.

P2509

Exosomal miRNAs in gastric cancer: liquid biomarkers and clinical application

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Gastric cancer (GC) is a highly prevalent disease that is responsible for most cancer-related deaths. The poor prognosis of GC patients can be attributed to delayed diagnosis. Therefore, there is an urgent need to search for effective screening methods to improve diagnosis of early-stage disease and for innovative treatment approaches. miRNAs are endogenous noncoding small RNAs with a length of 20-22 nt that regulate post-transcriptional degradation of target gene mRNA. miRNAs are stable in the circulation if they are encapsulated into exosomes and taken up by receptor cells, which causes specific changes in the biological behavior of the receptor cells. Tumor cells can achieve crosstalk with the microenvironment through the uptake and release of exosomal miRNAs. Thus, cancer-derived exosomal miRNAs in tumors may provide detailed information about the tumor biology of each individual patient. Exosomal miRNAs can be secreted by almost all cells and exist various biological fluids, such as blood, urine and saliva, giving them an advantage as noninvasive and convenient disease biomarkers. Moreover, exosomal miRNAs can be used as therapeutic targets and drug delivery agents in clinical practice. In this review, we summarize new aspects of exosomal miRNAs in the diagnosis, prognosis and treatment of GC, especially in chemoresistance, and show how miRNAs are enclosed in the exosomes.

P2510

MicroRNA mediated degradation of lncRNA Sros1 promotes innate response to intracellular bacteria by enhancing STAT1 expression

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Interferon-γ (IFN-γ) signaling pathway is essential for the macrophage innate immune response to intracellular bacteria. To identify new regulators of IFN-γ signaling in macrophages, we uncovered microRNAs that influence *Listeria* monocytogenes (LM) infection of macrophages. We discovered that the microRNA miR-X is upregulated upon infection, promoting IFN-γ-mediated LM clearance in macrophages by indirectly enhancing STAT1 expression. We reveal that miR-X targets a previously unknown, long non-coding RNA (Suppressive non-coding Rna Of Stat1, Sros1) for degradation in

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DOI: 10.1002/eji.201970400

macrophages. Further, we show that Sros1 RNA binds to the RNA binding protein C-protein, and that loss of C-protein reducing STAT1 expression. Sros1-deficient mice exhibited resistance to LM challenge, with strengthened IFN-γ signaling. Our work suggests that miR-X-mediated degradation of Sros1 RNA facilitates C-protein promoting STAT1 expression, thus promoting IFN-γ-mediated innate immunity. These findings shed light on the complex regulatory networks of cytokine-initiated innate responses in host-pathogen interactions.

P2511

Scaffold Lnczc3h7a promotes TRIM25-mediated K63 ubiquitination of RIG-I to strengthen the antiviral innate immune response

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Discrimination of self and non-self RNAs is always the crucial issue in immunity. Viral or bacterial RNAs are able to activate innate immune responses. However, the role of self-RNAs played in innate immunity remain unclear. The E3 ubiquitin ligase TRIM25 is novel RNA-binding protein, which mediates Lysine 63-linked ubiquitination of RIG-I to facilitate type I interferon (IFN) production and antiviral immunity. Here, we identify a novel lncRNA, Lnczc3h7a, which is binds to TRIM25 upon vesicular stomatitis virus (VSV) infection and acts as a potent regulator to facilitate RIG-I-mediated antiviral immune responses. Deficiency of Lnczc3h7a dramatically represses RIG-I antiviral signaling pathway and the production of type I IFNs to RNA viruses *in vitro* and *in vivo*. Lnczc3h7a binds to TRIM25 and RIG-I, serving as a scaffold to stabilize the RIG-I/TRIM25 complex and facilitating TRIM25 catalyzing K63-linked ubiquitination of RIG-I and antiviral responses. Furthermore, unlike non-self viral RNAs, Lnczc3h7a fails to trigger the activation of RIG-I, specially binds to RIG-I homodimers which are already activated by viral RNAs. Our findings reveal a host lncRNA as a key regulator not a stimulator to facilitate RNA sensor-mediated innate immune responses, and provide a novel mechanism of self-RNA function in innate immunity.

P2512

Nuclear *Lncsavrm1* suppresses type I interferon production by inhibiting TDP43 cleavage

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Type I interferons (IFNs) play a key role in antiviral innate immune responses. However, the production of IFNs needs to be precisely regulated as excessive or insufficient production of IFNs can be harmful to host. Long noncoding RNAs (lncRNAs) participate in the regulation of diverse biological processes, and the functions of lncRNAs depend on their subcellular localization and abundance. Here we identified a lncRNA *Lncsavrm1* which was abundant in the nucleus and significantly down-regulated after vesicular stomatitis virus (VSV) infection in macrophages. Deficiency of *Lncsavrm1*

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DOI: 10.1002/eji.201970400

sufficiently enhanced antiviral responses through promoting type I IFNs production both *in vitro* and *in vivo*. Mechanistically, *Lncsavrm1* directly bound to TDP43 and inhibited the cleavage of TDP43 upon viral infection, then specifically promoted the degradation of IRF3 and consequently suppressed the production of type I IFNs. Our study identifies an abundant nuclear lncRNA as a new negative regulator of antiviral innate responses, providing a new potential target for treating viral infection and related diseases.

P2513

LncRNA NR0 associated with H.pylori infection promotes the progression of gastric cancer by binding to NF45/NF90

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Recently, long noncoding RNAs (lncRNAs) have been reported to play a pivotal role in the occurrence and progression of cancer. The object of this study was to screen lncRNAs that are dysregulated in gastric cancer and to investigate their potential functions. Global expression of lncRNAs in gastric cancer and adjacent normal tissues of patients was profiled using a microarray assay. We identified an lncRNA NR0, this lncRNA was transcribed independently of the coding region of NR6A1 and was determined to be markedly upregulated in human gastric carcinoma relative to their corresponding normal gastric tissues by quantitative RT-PCR (qRT-PCR) analysis of tissues from 52 gastric carcinoma patients. We also found that infected with H.pylori elevated the expression of IL-8, IL-1β and TNF-α, simultaneously, we found lncRNA NR0 is upregulated. Further results demonstrated that lncRNA NR0 facilitated the proliferation and migration of gastric cancer cells in vitro and promoted tumor growth in a mouse model of human gastric cancer. Our results also indicated that lncRNA NR0 might function in gastric cancer by binding with NF45/NF90. Further studies indicated that the 5′-end of lncRNA NR0 promoted the binding of NF45/NF90, and thus promote the stability of the compound (NF45/NF90) and its client protein. These results expanded our understanding of lncRNA NR0 as a new avenue for therapeutic intervention against gastric cancer progression.

P2514

MicroRNAs in keratinocytes contributes to skin regeneration by suppressing the inflammatory response

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Objective: In the process of cutaneous wound healing, inflammation promotes scar formation. Early in gestation, wounds in fetal mammalian skin do not trigger inflammation in response to damage and heal without scarring. We aim to detect the expression of microRNAs in mid- and late- gestational fetal keratinocytes and explored the role of differentially expressed microRNAs in epidermal keratinocytes in inhibiting skin scar formation by regulating inflammation.
Methods: The different expression of miRNAs was detected by next-generation sequencing from RNA which was extracted from mid- and late- gestational fetal keratinocytes. Bioinformatics analyses were used to identify candidate novel human miRNAs. Real-time qRT-PCR and western blot were used to detect the effects of miRNAs on regulating inflammatory response and extracellular matrix. *In vivo* wound healing experiments were used to examine the effects of miRNAs on regulating inflammatory factors and scar formation in wounds. The potential target genes of miRNAs were predicted and demonstrated by bioinformatics analyses, luciferase reporter assays and western blot.

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DOI: 10.1002/eji.201970400

Results: A novel miRNA candidate, seq-915_x4024, was identified by bioinformatics analyses. Besides, seq-915_x4024 and miR-149 regulated the expression of inflammatory factors and extracellular matrix *in vitro*. Using *in vivo* wound healing experiments, we demonstrated that overexpression of seq-915_x4024 and miR-149 in keratinocytes suppresses the inflammatory response and scar formation. Furthermore, bioinformatics analyses, luciferase reporter assays and western blot demonstrated that IL1α, IL1β, IL6, IL8 and TNF-α are targets of miR-149 or seq-915_x4024.
Conclusion: Overexpression of seq-915_x4024 and miR-149 in keratinocytes suppresses scar formation by targeting the inflammatory factors.

P2515

Long non-coding RNA HOXA transcript antisense RNA Myeloid-specifc-HOXA1 axis downregulates the immunosuppressive activity of Myeloid-Derived suppressor cells in lung cancer

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HOXA transcript antisense RNA myeloid-specifc 1 (HOTAIRM1) is a long non-coding RNA that has been shown to be a key regulator of myeloid cell development by targeting HOXA1. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that possess immunosuppressive function. However, the impact of HOTAIRM1 on the development of MDSCs remains unknown. In this study, we demonstrated that HOTAIRM1 was expressed in MDSCs and that overexpression of HOTAIRM1 could downregulate the expression of suppressive molecules in MDSCs. In addition, HOTAIRM1 levels were observed to be decreased in the peripheral blood cells of lung cancer patients compared with those of healthy controls. By analyzing HOTAIRM1 expression levels in different types of lung cancer, we found that HOTAIRM1 was mainly expressed in lung adenocarcinoma. Finally, it was confrmed that HOTAIRM1 could enhance the expression of HOXA1 in MDSCs and that high levels of HOXA1, the target gene of HOTAIRM1, could delay tumor progression and enhance the antitumor immune response by downregulating the immunosuppression of MDSCs. Taken together, this study illustrates that HOTAIRM1/HOXA1 downregulates the immunosuppressive function of MDSCs and may be a potential therapeutic target in lung cancer.

P2516

Long noncoding RNA GCRL1 mediates gastric malignancy through miRNA-885-3p and cyclin-dependent kinase 4

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Gastric cancer (GC) is one of the most common malignancy and the third leading cancer-related death in China. Long non-coding RNAs (lncRNAs) have been implicated in numerous tumors including gastric cancer, however, the mechanism of many functional lncRNAs is still unclear. In this study, we identified the abundantly expressed lncRNA, RP11-290F20.3, in gastric cancer cells and patient tumor tissues. We named this lncRNA as Gastric Cancer Related LncRNA1 (GCRL1), which could regulate gastric cell proliferation and metastasis, both *in vitro* and *in vivo*. Mechanistically, miRNA-885-3p (miR-

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DOI: 10.1002/eji.201970400

885-3p) could inhibit the cell proliferation and metastasis in gastric cancer by negatively regulating the expression of cyclin-dependent kinase 4 (CDK4) at the posttranscriptional level. Further, GCRL1 promoted the cell proliferation and metastasis by sponging miR-885-3p and hence, positively regulating CDK4 in gastric cancer cells. Taken together, our results demonstrate a novel regulatory axis of malignant cell proliferation and invasion in gastric cancer, comprising GCRL1, miR-885-3p, and CDK4, which may serve as a potential therapeutic target in gastric cancer.

P2517

Long non-coding RNA AK085865 ablation confers susceptibility to viral myocarditis by regulating macrophage polarization

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Rationale: Accumulating evidence indicates that macrophages represent the major inflammatory infiltrates and regulators of macrophage polarization may also exert pivotal functions in the development of coxsackievirus B3 (CVB3)-induced viral myocarditis (VM). However, despite the importance of this process to VM, the mechanisms underlying macrophage polarization remain to be explored.
Objective: Here, we sought to identify novel and functionally important long non-coding RNAs (lncRNAs) during macrophage polarization and investigate their function and contribution to VM.
Methods and Results: Global lncRNA profiling identified several dysregulated transcripts. Among them, the lncRNA AK085865 was found to be mostly differentially expressed during macrophage polarization. Knockdown of AK085865 diminished phenotype expression of M2 macrophages while promoting polarization to the M1 phenotype. Moreover, AK085865$^{-/-}$ mice had increased susceptibility to CVB3-induced VM. We observed striking bias toward M1 macrophages, whereas the M2 population was decreased in AK085865$^{-/-}$ VM mice. We found that AK085865 specifically interacts with interleukin enhancer binding factor (ILF) 2. In addition, we confirmed that AK085865 was involved in the ILF2/ILF3 complex-mediated miRNA processing pathway through functioning as a negative regulator.
Conclusions: Collectively, our findings uncover a critical role for AK085865 in the regulation of macrophage polarization *in vitro* and *in vivo*, identifying a new player in the development of VM and providing a potential, clinically significant therapeutic target.

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DOI: 10.1002/eji.201970400

P2518

The role and mechanism of Lnc-028 in the polarization of macrophages associated with gastric cancer immune microenvironment

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Tumor-associated macrophages were isolated from gastric cancer tissues and paracancerous tissues, and the expression of NR_028 was detected by RT-qPCR. The experiment results showed that NR_028 is increased in gastric cancer tissues.The expression of cytokines in M0, M2 and SM2 macrophage culture supernatants was detected by ELASA. The supernatant was used to stimulate gastric cancer cells AGS, BGC-823 and SGC-7901. The experiment results showed that M2 and SM2 supernatant promote the proliferation, migration and invasion of gastric cancer cells.THP-1 cell line stably knocking down NR_028 was established, and the tail vein was injected into the tumor-bearing nude mice of gastric cancer cells to detect the metastasis of gastric cancer cells. M2 macrophages with low expression of NR_028 inhibited the metastasis of gastric cancer cells in nude mice, indicating that it may be involved in the malignant phenotype and invasion and metastasis of gastric cancer.Most of NR_028 is localized to the nucleus of M2 macrophages. RNA-pulldown combined with Western blot showed that NR_028 may bind to Arg-1 and CHI1L3 proteins.

P2519

microRNA-181b suppresses the metastasis of lung cancer cells by targeting sex determining region Y-related high mobility group-box 6 (Sox6)

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The aim of the study was to measure the expression of microRNA (miR)-181b in patients with lung cancer, investigate its biological function and elucidate the underlying mechanisms associated with the development of lung cancer. miR-181b expression in tissues was measured via RT-qPCR. After A549 cells were transfected with miR-181b mimic or si-Sox6, the proliferation, migration and cell cycle distribution of A549 were evaluated using cell counting kit-8 assay, transwell assay and flow cytometry. The levels of cell cycle-related proteins and Sox6 were analyzed by western blotting. Gene targets of miR-181b were predicted via bioinformatics analysis and verified using a dual-luciferase reporter gene assay. Expression of miR-181b was significantly downregulated in lung cancer tissues ($P< 0.05$), and was inversely correlated with the degree of cell differentiation and clinical stages of lung cancer (both $P< 0.05$). Additionally, the expression of miR-181b was significantly lower in adenocarcinoma compared with squamous cell carcinoma in the lungs ($P< 0.05$). Overexpression of miR-181b significantly decreased the protein level of Sox6 and significantly suppressed the cell proliferation and metastasis (both $P< 0.05$); this effect was also observed in A549 cells transfected with si-Sox6. The luciferase activity of a Sox6 3´-untranslated region-based reporter construct was significantly lower when transfected with miR-181b ($P< 0.05$), which suggests that Sox6 is a direct target of miR-181b. The results of the present study suggest that miR-181b may function as a tumor inhibitor in the development of lung cancer via targeting Sox6 to decrease the proliferation and metastasis of lung cancer cells.

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DOI: 10.1002/eji.201970400

P2520

IL-1β-mediated up-regulation of WT1D via miR-144-3p and their synergistic effect with NF-κB/COX-2/HIF-1α pathway on cell proliferation in LUAD

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IL-1β is an important mediator of "inflammation-cancer" transformation through IL-1β/NF-κB/COX-2/HIF-1α signaling pathway, whereas certain portion of patients with lung adenocarcinoma (LUAD) still suffer from rapid tumor progression in clinical practice, indicating the occurrence of potential bypass. Real-time polymerase chain reaction was applied to examine the expressions of mir-144-3p, WT1, NF-κB, COX2 and HIF-1α at the mRNA level in 127 LUAD samples and corresponding adjacent tissues. miR-144-3p mimic and antagormiR were used to trigger activation and suppression of miR-144-3p in A549 cells, respectively. MTT assay and Western blotting analysis were carried out to evaluate the cell proliferation. Stable clones with over-expression or knockdown of WT1 were generated with plasmid or shRNA by lentiviral vector technology in H1568 and H1650 NSCLC cell lines, respectively. Dual luciferase reporter assay was performed to validate the effect of miR-144-3p on WT1D. Xenograft model was established for in vivo experiment, and TCGA data were extracted for validation. miR-144-3p could suppress the WT1D expression at the post-transcriptional level, hence regulating cell proliferation in LUAD. WT1 and COX-2 were independent prognostic factors of LUAD patients. In addition, inhibition of IL-1β/miR-144-3p/WT1D and IL-1β/NF-κB/COX-2/HIF-1α pathways using miR-144-3p mimic and Celecoxib, respectively, displayed synergistic suppressive effect on cell proliferation in LUAD. A de novo IL-1β/miR-144-3p/WT1D axis was involved in proliferative regulation of LUAD. Moreover, simultaneous blockade of both IL-1β/miR-144-3p/WT1D and IL-1β/NF-κB/COX-2/ HIF-1α pathways might have synergistic suppressive effect on cell proliferation in LUAD.

P2521

LncRNA *CamK-A* regulates Ca^{2+}-signaling-mediated tumor microenvironment remodeling

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Cancer cells entail metabolic adaptation and microenvironmental remodeling to survive and progress. Both calcium (Ca^{2+}) flux and Ca^{2+}-dependent signaling play a crucial role in this process, although the underlying mechanism has yet to be elucidated. Through RNA screening, we identified one long noncoding RNA (lncRNA) named *CamK-A* (lncRNA for calcium-dependent kinase activation) in tumorigenesis. *CamK-A* is highly expressed in multiple human cancers and involved in cancer microenvironment remodeling via activation of Ca^{2+}-triggered signaling. Mechanistically, *CamK-A* ctivates Ca^{2+}/calmodulin-dependent kinase PNCK, which in turn phosphorylates IkBa and triggers calcium-dependent nuclear factor kB (NF-kB) activation. This regulation results in the tumor microenvironment remodeling, including macrophage recruitment, angiogenesis, and tumor progression. Notably, our human-patient-derived xenograft (PDX) model studies demonstrate that targeting *CamK-A* robustly impaired cancer development. Clinically, *CamK-A* expression coordinates with the activation of CaMK-NF-kB axis, and its high expression indicates poor patient survival rate, suggesting its role as a potential biomarker and therapeutic target.

DOI: 10.1002/eji.201970400

Highlights:

- **LncRNA *CamK-A* promotes the Warburg effect**
- ***CamK-A* is involved in Ca^{2+} signaling by regulating CaM kinase**
- ***CamK-A*-CaMK axis induces tumor microenvironment remodeling through NF-kB signaling**
- ***CamK-A* may be a potential therapeutic target for cancer treatment**

P2522

MicroRNA-29a regulates neural stem cell neuronal differentiation by targeting PTEN

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Neural stem cells (NSCs) are self-renewing, pluripotent, and undifferentiated cells which have benefits as candidates for central nervous system (CNS) injury. However, the transplanted NSCs usually maintain their undifferentiated characteristics, or differentiated potentially along the glial lineage after transplantation. MicroRNAs (miRNAs) are small, non-coding RNAs that play key roles in cell differentiation. However, it is unknown whether miR-29a could play a role in the process of NSC´s differentiation. Primary NSCs were derived from rat embryonic cortex. Lentiviral vector-mediated miR-29a (LV-miR-29a) and negative control (LV-null) were infected into NSCs. Quantitative real-time PCR was used to detect expression of miR-29a and PTEN. Immunocytochemistry was used to stain neurons, astrocytes, and oligodendrocytes. Dual luciferase assay to study the interaction between miR-29a and PTEN. The current study showed that the expression of miR-29a was upregulated during NSC differentiation, while the expression of PTEN was downregulated during NSC differentiation. After infection with LV-miR- 29a, MAP-2-positive neurons significantly increased, and GFAP-positive astrocytes significantly decreased. Furthermore, we demonstrated that PTEN is a molecular target of miR-29a. miR-29a promote the neuronal differentiation and decrease the astrocytes differentiation of NSCs via targeting PTEN. This may give insight into a novel mechanism of NSC differentiation and provide a promising therapeutic target.
Keywords: Astrocytes, Differentiation, MicroRNA-29a, Neural stem cells, Neurons, PTEN

P2523

miR-183-5p inhibits occurrence and progression of acute myeloid leukemia via targeting Erbin

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Erbin has been shown to have significant effects on the development of solid tumors. However, little is known about its function and regulatory mechanism in hematological malignancies. The biological function of Erbin on cell proliferation was measured in vitro and in vivo. The predicted target of Erbin was validated by dual-luciferase reporter assay and rescue experiment. We found that overexpression of Erbin could inhibit the cell proliferation and promote the cell differentiation of acute myeloid leukemia (AML) cells, whereas depletion of Erbin could enhance the cell proliferation and block the cell differentiation in AML cells in vitro and in vivo. Besides, miR-183-5p was identified as the upstream regulator that negatively regulated the Erbin expression. The results were confirmed by

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DOI: 10.1002/eji.201970400

dual-luciferase reporter and RNA pull-down assay. Furthermore, we found that miR-183-5p negatively regulated Erbin, resulting in enhanced cell proliferation of AML cells via activation of RAS/RAF/MEK/ERK and PI3K/AKT/FoxO3a pathways. The activation of RAS/RAF/MEK/ERK and PI3K/AKT/FoxO3a pathways was mediated by Erbin interacting with Grb2. These results were also validated by rescue experiments in vitro and in vivo. All above-mentioned findings indicated that the miR-183-5p/Erbin signaling pathway might represent a novel prognostic biomarker or therapeutic target for treatment of AML.

P2524

long non-coding RNA IFNG-AS1 decrease CD4$^+$ T cells activation by regulating the MHC II molecule in myasthenia gravis

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Activation of CD4$^+$T cells play an important role in Myasthenia Gravis (MG) pathogenesis by recognizing the peptide antigens presented via the major histocompatibility complex (MHC) class II molecules, after having been activated those cells would secrete different cytokines to stimulate B cells to produce antibodies, previous study confirmed that the MHCII molecular gene polymorphism is closely related to the onset of MG. Our previous studies have shown that long non-coding IFNG-AS1 could downregulate the activation of CD4$^+$T cells and decrease the expression of MHC II molecules (HLA-DRB etc.) in MG patients. However, which gene type of MHC II is the specific target of IFNG-AS1 remains unknown, even mechanisms of how IFNG-AS1 could regulate MHC II molecules and inhibit CD4$^+$T cell activation are still unclear. In this paper, we found IFNG-AS1 RNA was found to bind chromosome remodeling complex hnRNPK and hnRNPU, a component of the histone H3 lysine 4 methyltransferase complexes, and to alter histone 3 methylation at the HLA-DR. Thus, this lncRNA regulates epigenetic marking of HLA-DR-encoding chromatin,expression of HLA-DR, and effect the present peptide antigens.

P2525

Identification of a novel lncRNA, DJC2-LncRNA, related to the terminal differentiation of myeloid cells

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Long non-coding RNA(lncRNA) is involved in cell differentiation and somatic regulation at multiple levels and is closely related to disease. In our study, we have identified a novel LncRNA by rapid-amplification of cDNA ends (RACE) experiment, named DJC2-LncRNA, due to its sequence containing intron retentions of the DnaJ homolog subfamily C member 2 gene. Its expression was significantly increased with the differentiation of myeloid immune cells such as human monocyte-derived DCs, and myeloid leukemia cell lines such as NB4 and THP-1 by all-trans-retinoicacid (ATRA). Through laser scanning confocal microscopy we can see the difference in the distribution of this lncRNA over time in and outside the nucleus, which indicates that its function may be time and location specific. Down-regulation of its expression by siRNA interference can up-regulate the expression of DC mature markers such as CD58, CD86 and CD273.So, it may play an important regulatory role in the terminal differentiation of myeloid cells.

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DOI: 10.1002/eji.201970400

P2526

miRNA-219 alleviate the inflammation caused by spinal cord injury through inhibiting the expression of NEUROD4

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To investigate the potential role of miRNA-219 in spinal cord injury and its possible mechanism. We observed the changes of hindlimb grip recovery among mice model of spinal cord injury. Microarray was performed to get differentiatal expression of miRNAs in the mice. qRT-PCR was used to detect the expressions of miRNA-219, IL3, MCP-1 and IL16, after spinal cord injury. Subsequently, miRNA-219 was overexpressed to examine its influence on inflammation and grip strength after spinal cord injury. Bioinformatics technology was employed to predict target gene of miRNA-219. The binding of miRNA-219 and its target gene was verified by dual luciferase reporter gene assay.we found that the strength of hindlimb grip began to recover 7 days after spinal cord injury. The results of microarray and qRT-PCR confirmed that miRNA-219 level decreased with increase of injury time, while the levels of IL3 (interleukin-3) inflammatory genes MCP-1 (monocyte chemoattractant protein-1), TNF-α (tumor necrosis factor alpha) and IL16 (interleukin-16) were significantly increased. However, overexpression of miRNA-219 in the mice significantly reduced the level of inflammation. Finally, we found that miRNA-219 can bind to the NEUROD4 3´UTR, which was further confirmed by dual luciferase reporter assay. Spinal cord injury could lead to decreased expression of miRNA-219. The existence of miRNA-219 could reduce the inflammation by inhibiting NEUROD4 and promote the recovery of spinal cord.

P2527

Circulating miR-92b and miR-375 for monitoring chemoresistance and prognosis of patients with small cell lung cancer

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Objective: miRNAs have been reported to be stably detectable in plasma and function as potent biomarkers in various cancers. This study aimed to evaluate the expression of candidate circulating miRNAs in patients with small cell lung cancer (SCLC) in order to identify potential non-invasive biomarker for SCLC.
Methods: Quantitative reverse-transcriptase PCR was performed to evaluate the expression level of selective plasma miRNAs in SCLC patients at the stage of diagnosis, disease remission and drug resistance, respectively. The correlations between miRNAs expression and clinicopathological factors of SCLC was analyzed. Diagnostic value of selected miRNAs was evaluated by the area under the receiver operating characteristic curve (AUC).
Results: Expressions of five miRNAs (miR-92b, miR-146a, miR-375, miR-1224 and miR-1246) were significantly up-regulated in the sera of SCLC patients after emergent chemoresistance. Receiver operating characteristic (ROC) curves analysis showed that AUC of miR-92b and miR-375 were 0.785 and 0.791, respectively. The results demonstrated that these two miRNAs had better diagnostic accuracy for monitoring drug resistance. In addition, miR-92b and miR-375 levels were decreased after effective chemotherapy. Furthermore, Kaplan-Meier survival analysis confirmed that high expression of miR-92b and miR-375 were closely related to shorter progression-free survival (PFS) of SCLC patients.

DOI: 10.1002/eji.201970400

Conclusions: These findings indicated that circulating miR-92b and miR-375 might be effective non-invasive biomarkers for monitoring chemotherapy resistance and prognosis of SCLC.

P2528

miR-17-5p promotes migration and invasion in breast cancer cells by repressing netrin 4

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Netrin 4 (NTN4) is downregulated in breast cancer (BC) and can inhibit the migration of BC cells. miRNAs dysregulation plays prominent roles in BC tumorigenesis. However, the function of miR-17-5p, its relationship with NTN4 and its underlying functional mechanism in BC is unclear and was investigated in the current study. Compared with normal breast samples, **miR-17-5p was upregulated in BC specimens** in The Cancer Genome Atlas (TCGA). A clinical analysis based on TCGA showed that **miR-17-5p expression correlated with BC tumor stage, lymph node status, estrogen receptor and progesterone receptor status.** A wound-healing assay and Transwell assay implied that **miR-17-5p upregulation promotes BC cell migration and invasion**. Reverse transcription-quantitative PCR and ELISA showed that **NTN4 mRNA and protein were both downregulated after miR-17-5p was overexpressed in Hs578T cells, whereas miR-17-5p inhibition had the opposite effect in MCF-7 cells**. We also performed a dual-fluorescent reporter assay, the results of which demonstrated that **miR-17-5p represses NTN4 expression by directly targeting the 3' untranslated region of NTN4 mRNA**. In summary, miR-17-5p considerably promotes BC cells migration by suppressing NTN4 expression, and may therefore offer a potential therapeutic target for BC.

P2529

A novel protein encoded by a circular RNA circPPP1R12A promotes tumor pathogenesisand metastasis of colon cancer via Hippo-YAP signaling

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It has been well established that circular RNAs (circRNAs) play an important regulatory role during tumor progression. Recent studies have indicated that even though circRNAs generally regulate gene expression through miRNA sponges, they may encode small peptides in tumor pathogenesis. However, it remains largely unexplored whether circRNAs are involved in the tumorigenesis of colon cancer (CC).
We firstly screened the expression profiles of human circRNAs in CC tissues and found that the expression of hsa_circ_0000423 (termed as circPPP1R12A) was significantly increased in CC tissues. We also found that circPPP1R12A was mostly localized in the cytoplasm of CC cells.Kaplan-Meier analysis showed that patients with higher levels of circPPP1R12A had a significantly shorter overall survival. By gain- and loss-of-function approaches, the results suggested that circPPP1R12A played a critical role in proliferation, migration and invasion of CC cells. Furthermore, we showed that circPPP1R12A carried an open reading frame (ORF), which encoded a functional protein (termed as circPPP1R12A-73aa). Next, we found that PPP1R12A-C, not circPPP1R12A, promoted the proliferation, migration and invasion abilities of CC *in vitro* and *in vivo*. Finally, we identified that circPPP1R12A-73aa promoted the growth and metastasis of CC via activating Hippo-YAP signaling pathway.

DOI: 10.1002/eji.201970400

In the present study, we illustrated the coding-potential of circRNA circPPP1R12A in the progression of CC. Moreover, we identified that circPPP1R12A-73aa promoted the tumor pathogenesis and metastasis of CC via activating Hippo-YAP signaling pathway. Our findings might provide valuable insights into the development of novel potential therapeutic targets for CC.

P2530

Paraspeckle participates in IL-6-induced HCC progression by nuclear retention of PRDX5 and GSTP1

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Paraspeckle is a kind of highly structured and organized nonmembranous nuclear body. lncRNA-nuclear paraspeckle assembly transcript 1 (NEAT1) is essential for assembling of paraspeckles, acting as the scaffold for enrichment of RNA-binding proteins including NONO, SFPQ and PSPC1 which are also vital for the structural maintenance of paraspeckles. We found that the formation of paraspeckle increases upon viral infection or proinflammatory stimuli, and that the expression of both NEAT1 and NONO increases in hepatocellular carcinoma (HCC) samples. However, the role of paraspeckle in HCC progression, especially when related to inflammation, is largely unknown. Here we reported that IL-6 signaling increased paraspeckle formation in HCC cell lines. IL-6-induced STAT3 phosphorylation as well as IL-6-promoted *in vitro* cell invasion, cell cycle progression and survival of HCC cells were suppressed by destruction of paraspeckle formation with silencing NEAT1_2 or NONO. Mechanistically, by binding and trapping peroxiredoxin-5 (PRDX5) mRNA in nucleus and subsequently decreasing protein level of PRDX5 which can inhibit STAT3 phosphorylation by interacting with STAT3, paraspeckle promoted IL-6-induced STAT3 phosphorylation. Furthermore, IL-6 stimulation also preserved glutathione S-transferase P (GSTP1) in paraspeckles, which otherwise inhibits DNA damage and apoptosis through its detoxification and anti-oxidation function. The trapping of both PRDX5 mRNA and GSTP1 protein in paraspeckles contributes to the increased DNA damage under IL-6 stimulation in HCC cells. Therefore, our data indicates that paraspeckle promotes IL-6/STAT3 signaling-induced HCC progression by nuclear retention of both mRNA and proteins. The formation of paraspeckle maybe a potential target for HCC therapeutics.

P2531

The role of LINC00355 in gastric cancer

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Long noncoding RNAs (lncRNAs) are known to regulate the development and progression of various cancers. However, few lncRNAs have been well characterized in gastric cancer. Here, we identified the lncRNA LINC00355 upregulated substantially via TCGA database analysis and overexpression of LINC00355 correlated with poor prognosis. ISH confirmed that LINC00355 expression was higher in GC tissues rather than paired adjacent normal tissues, and it mainly localized in the nuclei rather in the cytoplasm. The expression of LINC00355 was higher in the serum of GC patients compared with healthy controls. In vitro data showed that LINC00355 knockdown significantly inhibited proliferation of

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DOI: 10.1002/eji.201970400

GC cells and induced cell cycle arrest at the S and G2/M phase. RNA immunoprecipitation assay confirmed that LINC00355 binds to EZH2, a core component of polycomb repressive complex 2. Further analysis indicated that LINC00355 may regulate cell cycle progression by epigenetically promoting the expression of p27. Based on the above results, we hypothesize that LINC00355 is an oncogenic lncRNA that regulates GC progression by epigenetically regulating the expression of p27, suggesting that dysregulated lncRNAs may serve as key regulatory factors in GC progression.

P2532

miR-125b-5p and miR-99a-5p downregulate human γδT-cell activation and cytotoxicity

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As an important component of innate immunity, human circulating γδ T cells function in rapid responses to infections and tumorigenesis. MicroRNAs (miRNAs) play a critical regulatory role in multiple biological processes and diseases. Therefore, how the functions of circulating human γδ T cells are regulated by miRNAs merits investigation. In this study, we profiled the miRNA expression patterns in human peripheral γδ T cells from 21 healthy donors and identified 14 miRNAs that were differentially expressed between peripheral αβ T cells and γδ T cells. Of the 14 identified genes, 7 miRNAs were downregulated, including miR-150-5p, miR-450a-5p, miR-193b-3p, miR-365a-3p, miR-31-5p, miR-125b-5p and miR-99a-5p, whereas the other 7 miRNAs were upregulated, including miR-34a-5p, miR-16-5p, miR-15b-5p, miR-24-3p, miR-22-3p, miR-22-5p and miR-9-5p, in γδ T cells compared with αβ T cells. In subsequent functional studies, we found that both miR-125b-5p and miR-99a-5p downregulated γδ T cell activation and cytotoxicity to tumor cells. Overexpression of miR-125b-5p or miR-99a-5p in γδ T cells inhibited γδ T cell activation and promoted γδ T cell apoptosis. Additionally, miR-125b- 5p knockdown facilitated the cytotoxicity of γδ T cells toward tumor cells in vitro by increasing degranulation and secretion of IFN-γ and TNF-α. Our findings improve the understanding of the regulatory functions of miRNAs in γδ T cell activation and cytotoxicity, which has implications for interventional approaches to γδ T cell-mediated cancer therapy.

P2533

MicroRNA-146a overexpression impairs the positive selection during T cell development

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MicroRNAs play crucial roles in modulating immune system. miR-146a, a potent feedback suppressor of NF-κB signaling, was shown to limit the innate immune response and myelopoiesis in a knockout mouse model. Here, we observed high lymphopoiesis demonstrated as mild splenomegaly and severe lymphadenopathy in a miR-146a transgenic mouse model. Overexpression of miR-146a resulted in enhanced proliferation and reduced apoptosis of T cells. More activated CD4+ T cells or effector memory T cells were observed in transgenic mice even under physiological conditions. Importantly, as one of the key steps to generate central tolerance, the positive selection of thymocytes is impaired in transgenic mice, resulting in more CD4+CD8+ double-positive thymocytes but fewer CD4+CD8− and CD4−CD8+ single-positive thymocytes. The maturation of selected CD4−CD8+ thymocytes was also impaired, leading to more severe loss of CD4−CD8+ than CD4+CD8− thymocytes in thymus of transgenic mice. Gene expression profiling analysis identified nine positive selection-associated genes, which were downregulated in transgenic mice, including genes encoding major histo-

DOI: 10.1002/eji.201970400

compatibility complex class I/II molecules, IL-7 receptor α chain, and Gimap4, whose downregulation may contribute to the impairment of positive selection. Gimap4 was verified as a novel target of miR-146a. These findings further extend our understanding of the function of miR-146a in T cell biology and identify a novel regulatory mechanism underlying the positive selection during T cell development.

P2534

miR-21 contributes to human amniotic membrane-derived mesenchymal stem cell growth and human amniotic membrane-derived mesenchymal stem cell-induced immunoregulation

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Background: Some microRNAs (miRNAs) have been reported to participate in the regulation of immune responses. Our aim was to investigate the effects of miR-21 on the biological characteristics, immunoregulatory properties, and potential mechanisms of hAM-MSCs.
Methods: hAM-MSCs were isolated from the placental amnion membrane of a newborn. Cell proliferation, cell cycle, apoptosis were measured by CCK-8 and flow cytometric assays . The expression of mesenchymal-specific antigens vimentin and stage-specific embryonic antigen-4 (SSEA-4) were identified by immunofluorescence staining. Tumor necrosis factor alpha (TNF-a), monocyte chemotactic protein-1 (MCP-1), and interleukin-10 (IL-10) expressions in the cocultured supernatant of hAM-MSCs and peripheral blood mononuclear cells (PBMC) were detected via enzyme-linked immuno- sorbent assays (ELISA).
Results: The immunofluorescent staining results showed that vimentin and SSEA-4 were positive in hAM-MSCs. CCK-8 assays revealed that miR-21 over- expression significantly promoted hAM-MSC proliferation. Cell cycle analyses revealed that the number of hAM-MSCs-miR-21 cells during the synthesis phase (S phase) was significantly increased. miR-21 over- expression also significantly inhibited apoptosis in hAM-MSCs. The ELISA analyses revealed that miR-21 overexpression enhanced the inhibitory effect of hAM-MSCs on the secretion of TNF-a and MCP-1 as well as the promotive effect on the secretion of IL-10 in PBMC cocultured with miR-21-hAM-MSCs. In addition, miR- 21 downregulation reduced the inhibitory effect of hAM-MSCs on the secretion of TNF-a, MCP-1, and the promotive effect on the secretion of IL-10 in PBMC cocultured with anti-miR-21-hAM-MSCs.
Conclusions: Our data showed that miR-21 promoted hAM-MSCs proliferation, inhibited apoptosis, and was involved in controlling the immunoregulatory capacity of hAM-MSCs.

Immunogenetics and Immunoepigenetics

O305

Transcriptional regulation of recombination activating gene 1, the key molecule for V(D)J recombination reactions of immunoglobulin and T cell receptor

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Recombination activating gene 1 (*RAG1*) as a key component of the Rags multiprotein complex that mediates the DNA cleavage phase during V(D)J recombination. The V(D)J recombination assembles a diverse repertoire of immunoglobulin and T-cell receptor genes in developing B and T-lymphocytes.

DOI: 10.1002/eji.201970400

The recombination mediated by *RAG1* is also involved in the dominant mutational process and the predominant driver of oncogenic genomic rearrangement, and is further responsible for leukemic clonal evolution. In this study, the significant *RAG1* increase is observed in the subsets of B- cell leukemia patients, and the high expression of *RAG1* is observed to be correlated with high proliferation markers. We also analyzed the association of *RAG1* expression with the common oncogenic genetic defects in leukemia and found that patients with higher expression of *RAG1* had significantly higher rate of *IKZF1* deletion and *CRLF2* mutations compared to that with low expression. *IKZF1*-encoded protein, IKAROS, is the key transcription factor for lymphocyte development, and we also found that IKAROS directly binds to the *RAG1* promoter and regulates *RAG1* expression. CK2 inhibitor by increasing IKAROS activity significantly suppresses *RAG1* expression by increasing IKAROS binding to the *RAG1* promoter in patient samples. Taken together, these results demonstrate that *RAG1* high expression may have oncogenic effect in leukemia. Our data for the first time proved that IKAROS directly suppresses *RAG1* expression, and also suggest that IKAROS may work with *RAG1* together to regulate the immunoglobulin class switch and T-cell receptor rearrangement.

O306

A subset of somatic mutations in switched memory B cells are generated from interchromosomal templates

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Previous work from our lab has detected the contribution of a gene conversion-like process, termed templated mutagenesis, to somatic hypermutation in the germinal center reaction. These studies found that pairs of somatic mutations in close proximity (8bp) frequently (60-80% of unique clusters) had a cognate template in the IgHV germline repertoire, regardless if the mutated sequence was an IgHV gene itself, or whether it was an exogenous transgene with little overt homology to the germline IgHV repertoire. Here, using a computational pipeline termed TRACE (Template Recognition via Analysis of Monte Carlo Experiments), we demonstrate that large homologous templates (20-38bp) also exist in the genome and account for clusters of mutations. TRACE analysis of somatically-mutated IgHV repertoires from switched memory B cells of four healthy human donors revealed that 5-10% of the total somatic mutation burden is derived from such tracts. Further, we find the use of IgHV pseudogenes as templates both from intrachromosomal locations as well as interchromosomal locations. HI-C analysis of interchromosomal contacts between the IgH locus and sites identified by TRACE revealed a significant increase in contact between these sites in germinal center B cells as compared to naïve B cells (KS Test, $p< 0.001$, $n=4$). ATAC-seq analysis of B cell populations revealed that predicted interchromosomal donors were preferentially located in sites within 1kb of open chromatin ($p< 0.001$, $n=4$). Together, this data suggests that the pattern of somatic mutagenesis during the germinal center reaction is, in part, influenced by homologous motifs located in distant genomic sites.

DOI: 10.1002/eji.201970400

O307

UBC9 deficiency selectively impairs the functionality of common lymphoid progenitors (CLPs) during bone marrow hematopoiesis

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Hematopoietic development occurs in the bone marrow, and this process begins with hematopoietic stem cells (HSCs). Ubc9 is a unique E2-conjugating enzyme required for SUMOylation, an evolutionarily conserved post-translational modification system. Here, we show that a conditional Ubc9 deletion in the hematopoietic system caused decreased thymus weight and reduced lymphocyte to myeloid cell ratio. Importantly, Ubc9 deletion in the hematopoietic system only selectively impaired the repopulation capacity of common lymphoid progenitors (CLPs) in the bone marrow and perturbed their potential to differentiate into lymphocytes, thereby decreasing the number of T/B cells in the periphery. Ubc9 was found to be required for CLP viability, and therefore, Ubc9 deficiency rendered CLPs to undergo apoptosis and attenuated their proliferation. Thus, Ubc9 plays a critical role in the regulation of CLP function during hematopoietic development in the bone marrow.

O308

DNA methylation inhibitor is a promising strategy on K27M-mutant pediatric High-grade gliomas

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Pediatric High-grade glioma (pHGG) in children is a devastating disease and the leading cause of pediatric death by brain tumor, which has a 5-year survivor rate is less than 1%. Current care mainly involves surgery and radiation without available chemotherapeutic agents in pHGG. Thus, there is an urgent need to develop new and more efficacious therapies. Genetic and epigenetic alterations have been widely reported in pHGG, which connecting mutations in chromatin regulation, especially the most common mutations of histone H3 (*H3F3A*) at amino acid 27 (lysine to methionine (K27M), which highly correlated with tumor aggressiveness and poor survive of patients. This alteration represents an attractive target for diagnostic and treatment purposes. Our results have indicated that the cancer cells with *K27M* mutations are extremely sensitivity to DNA hypomethylating agent (HMA) because of its defects in remethylation process and maintaining gene silencing during treatment and result in prolonged anti-proliferation effects. During treatment, expression of up-regulated genes, including tumor suppressors, were positively correlated with nucleosome accessibility and negatively correlated with gene promoter DNA methylation. Alternatively, expression of down-regulated genes, such as oncogenes, were negatively correlated with nucleosome accessibility and positively correlated with gene body DNA methylation. Furthermore, it can further up-regulate endogenous retroviruses (ERVs) and immune response genes to reactivate immune pathways at K27M-mutant cells by comparing wild type cells. Taken together, these results suggest that K27M-mutant pHGG could be a therapeutic target of HMA and also provide rationale for combination treatments of HMA with immune checkpoint therapies in pHGG patients.

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DOI: 10.1002/eji.201970400

O309

Germline-encoded TCR-MHC contacts promote TCR V gene bias in neonatal T cell repertoire

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A large number of highly variable T cell receptors (TCRs) are expressed in each individual, enabling T cells to recognize antigen as peptides bounded to major histocompatibility complex (MHC) proteins. It remains controversial whether germline-encoded TCR repertoire is shaped by MHC polymorphism and, if so, what is the preference between different MHC alleles and TCR specificity. Here, we applied quantitative trait locus (QTL) mapping to test the associations between MHC polymorphism and TCR beta chain V (TRBV) genes usage in a cohort of 201 Chinese newborns. Our results showed that 14.6% (7/48) of TRBV genes are in association with nucleotide and amino acid variants of the MHC alleles, many of which showed spatial proximities in structural analysis of TCR-pMHC complexes. These results show for the first time that MHC variants bias TRBV gene usage in newborns of Chinese ancestry and indicate that germline-encoded contacts influence TCR-MHC interactions in the neonatal T cell repertoires.

O310

Immunological and clinical significance of major histocompatibility complex class I-related chains A (MICA) alleles in gastric cancer patients

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Gastric Cancer is one of the leading causes of death in Chile and worldwide. Natural Killer cells play a key role in the antitumor response mediated by NKG2D receptor, one of whose main ligands is MICA (MHC class I polypeptide-related sequence A). Although MICA is expressed by gastric adenocarcinoma (GA) also it is released and modulates negatively to receptor. The MICA gene is highly polymorphic, originating alleles that encode protein variants with differential characteristics; released by exosomes or susceptible to proteolytic shedding, therefore with different treatment strategies. To study the immunological and clinical significance of MICA variants, we performed massive sequencing of MICA in fifty patients with GA. The combination of 3 single nucleotide variations (SNV) 129Val, 175Ser and 251Arg associated with soluble MICA release, were present in 80%, 48% and 52% of patients, respectively. To identify the MICA alleles, we analyze thirty-seven patients by the sequence-based typing (PCR-SBT) method. We found that 46%, 26%, 14%, 8% of patients were MICA*002, MICA*008, MICA*009, and MICA*004 allele respectively. The patients with the MICA-129 Met/Met genotype mainly had a tumor size larger 5 cm while that patients with MICA-129 Val/Val had a lower tumor size. However, patients with metastasis shown a MICA-129 Val/Val or Val/Met genotype. Our findings suggest that the presence of SNVs in the *MICA* gene, in patients with GA, and some alleles might be related with immune evasion strategies and the establishment of tumors. Furthermore, the identification of MICA alleles would allow personalize the selection of the immunotherapy.

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DOI: 10.1002/eji.201970400

O311

Negative elongation factor complex enables macrophage inflammatory responses by controlling anti-inflammatory gene expression

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Studies on macrophage gene expression have historically focused on events leading to RNA polymerase II recruitment and transcription initiation whereas the contribution of the post-initiation steps to macrophage activation remains poorly understood. Here, we report in resting macrophages widespread promoter-proximal RNA polymerase II pausing, marked by broad co-localization with the negative elongation factor (NELF) complex and facilitated by PU.1 binding near transcription start sites. Upon inflammatory stimulation, over 50% of activated transcriptome is regulated by polymerase pause-release and a transient genome-wide NELF dissociation from chromatin, unexpectedly, independent of CDK9, a presumed NELF kinase. Genetic disruption of NELF in macrophages dramatically enhanced *de novo* transcription of the AP-1 factor-encoding *Fos* and *Jun* and, consequently, AP-1 targets including a critical anti-inflammatory cytokine *Il10*. Consistently, augmented transcription of *Il10* in NELF cKO attenuated the production of pro-inflammatory mediators and ultimately macrophage-mediated inflammation. Together, our findings establish a previously unappreciated role of post-initiation regulator, NELF, in constraining transcription of inflammation inhibitors such as IL-10 thereby facilitating inflammatory macrophage activation.

O312

HLA B27 allele positivity in the referred patients to a tertiary private medical centre of Bangladesh

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Background: Human Leukocyte Antigen B27 (HLA-B27) (subtypes B*2701-*2759) is a class I surface antigen encoded by the B locus in the major histocompatibility complex (MHC) is strongly associated with ankylosing spondylitis (AS) and other associated inflammatory diseases, referred to collectively as 'spondyloarthrosis'. The Prevalence of HLA-B27 varies regionally. This is the first report to determine the positivity of HLA-B27 in referred patients in a tertiary medical centre of Bangladesh.
Methods: The study was cross-sectional and collected from 9285 referred patients to Popular Diagnostic Centre Ltd., Dhaka from October, 2015 to December, 2018. All the patients referred for the complaints with low back pain, joint pain, eye pain etc. has been included. The test was performed in LightCycler 480 (Roche Applied Sciences, Switzerland) platform using LightMix HLA-B27 kits from TIB MolbiolGmbh (Germany).

DOI: 10.1002/eji.201970400

Results: A total of 9285 referred patients were enrolled with age ranging from 21 to 68 years. Of the total patients, 5185 were male (56%) and 4100 were female (44%). Overall, 2647 (29%) has been detected positive and 6638 (71%) were negative for HLA-B27. However,73% (1943) of male referred patients and 27% (704) of female patients were found positive. The study showed that male patients were significantly more affected than female patients (OR=2.89; p< 0.001).
Conclusion: This is the first study to evaluate the positivity of HLA B-27 among Bangladeshi population. The study concluded that male patients were predominant than female subject. Epidemiological studies with multicentric large cohort may be undertaken to see the prevalence of HLA-B27 in Bangladesh.

LB006

STR based chimerism monitoring post hematopoietic stem cell transplantion

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Introduction: The monitoring of donor chimerism for evaluating graft outcome following Hematopoietic Stem Cell Transplant (HSCT) is essential. Quantitative evaluation was based on Short Tandem Repeats (STRs) variability. However, the population specific genetic propensity of STRs (in context of graft outcome informativeness) is largely unknown in North Indians.
Material & Methods: A total of 206 patients (56.2% malignant, 43.7% non-malignant diseases) underwent HSCT at AIIMS, New Delhi, were followed regularly (2016-2019). Molecular chimerism was monitored using multiplex STR-PCR based AmpFlSTR Identifiler Kit which included 15 STRs located on chromosomes 2, 3, 4, 5, 7, 8, 11, 12, 13, 16, 18, 19, 21, X and Y. The fragment analysis was performed on 3130xl genetic analyzer using Gene Mapper software. Percentage informativity for each STR locus was dependant on the potential to differentiate between patient and their respective donors.
Results: Informativity was highly variable among the analyzed STRs with a mean of 9 informative markers among recipient-donor pairs. The informativity ranged from 56-72%. Percent informative index of D8S1179 was observed to be the highest while; D5S818 and D16S539 were least informative. A new set of STRs (including 2 new markers, ACTBP2 and ApoIII) are under evaluation for possible replacement of AmpFISTR kit.
Conclusion: Here we report the informativeness of these STRs based on observed heterozygosity. We need to elucidate whether the in-use STRs are best option for Indian population or an alternate set of markers specific should be identified. The trends observed in both malignant and non-malignant cohorts will be presented.

LB044

Immunogenetic correlates of HIV in North Indian population

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Genomic architecture of specialized cohorts *viz*. viremic controllers, elite controllers, exposed uninfected individuals, rapid and slow progressor individuals indicate population specific genetic correlates of HIV/AIDS vulnerability. We performed candidate gene based studies to explore the genetic predisposition to HIV infection in the North Indian population. Particularly, we analyzed the genes that influence i) HIV cell entry, ii) viral replication as well as iii) pro and anti inflammatory cytokines. Our major findings are (a) Well known protective variant CCR5 delta 32 is rare (< 1%), and CCR5 promoter haplotypes were found associated with susceptibility and development of AIDS, (b)

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DOI: 10.1002/eji.201970400

No association of CCR2 64I, CCL3L1 copy numbers and *APOBEC3B* deletion (29.5 kb) with HIV susceptibility and/or resistance, (c) Among the observed seven TIM-1 exon-4 haplotypes, significantly higher CD4 counts were observed in D3-A +ve HIV patients, (d) TRIM5 α -exon 2 variant 43Tyr-allele and haplotypes carrying this allele were associated with resistance to HIV infection, e) low expressing allele *01 of NFKBIL1 gene and APOBEC3(H) haplotypes carrying rs139292 (N15del) and rs139297 (G105R) were associated with HIV susceptibility and (f) Significantly higher allelic frequencies of IL-1α -889 T and IL-4 -1098 T were observed in HIV patients, while IL-1α -889 CC, IL-4 -1098 GG and IL-6 nt565 AA genotypes were observed significantly lower as compared healthy uninfected controls. The study represents distribution of immunoregenetic variants and their influence on HIV/AIDS outcome in North Indian population.

LB080

Association of -871 C>T promoter *TNFSF13B* (BAFF) gene polymorphism in periodontal disease

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Periodontal disease (PD) is characterized by the destruction of normal supporting dental tissues by an inflammatory process. Large infiltrations of B cells, specifically plasma cells are the hallmark of periodontitis lesions. B cell activating factor (BAFF) promotes the survival, proliferation and maturation of B cells. BAFF -871 C>T polymorphism (rs9514828) have been related with autoimmunity and host response to pathogens as minor T allele is associated with high BAFF expression. However, the role of BAFF in periodontal disease has not been completely elucidated. We analyzed rs9514828 polymorphism in 260 healthy controls (HC), 42 acute apical abscess (AAA), 76 primary apical periodontitis (pAP), 31 secondary apical periodontitis (sAP) patients and determined their associations with the risk of diseases. Genotyping was performed by PCR-RFLP technique and soluble BAFF (sBAFF) levels were measured with ELISA test. Although no differences in the genotype/ allele frequencies between HC and patients with AAA and pAP were found, minor allele T significantly decrease the risk of sAP ($p< 0.05$, OR=0.5, 95%CI:0.025-0.99) vs HC. sBAFF serum levels was elevated in PD patients compared to HC ($p< 0.05$) and diminished in sAP ($p< 0.05$). sBAFF correlate (r_s=0.5132) with periodontal bone resorption in AAA patients. These results suggest that rs9514828 could be a susceptibility marker for sAP in western Mexican population. Diminished sBAFF could result in aggravation of PD in sAP patients. However, excessive sBAFF levels in AAA patients could be detrimental to the host. Subsequent studies in Mexican patients are needed to elucidate it.

Supported by grant PRODEP UDG-PTC-1480

DOI: 10.1002/eji.201970400

LB081

Association of CD26/dipeptidyl peptidase IV level with disease activity and bone erosion in rheumatoid arthritis

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Dipeptidyl peptidase IV (DPP-IV, CD26) plays many roles in the pathogenesis of several autoimmune and inflammatory diseases. The current study evaluated the association of DPP-IV enzymatic activity and its gene expression with disease activity and bone erosion in rheumatoid arthritis. Blood samples were collected from 20 rheumatoid arthritis patients and 40 healthy volunteers. Patients were divided into four subgroups using DAS28 index. CD26 gene expression levels were analyzed in peripheral blood mononuclear cells by quantitative reverse transcription-polymerase chain reaction. Additionally, the enzymatic activity of this molecule in serum was determined using Gly Pro-p-nitroanilide as substrate. Digital radiography was applied to obtain images for bone erosion assessment. No significant difference in serum DPP-IV activity level was seen between patients and controls ($p = 0.140$). However, patients exhibited an increase in CD26 mRNA expression (1.68 times) when compared to controls ($p = 0.001$). Moreover, a strong positive correlation between CD26 gene expression and DAS28 index as well as bone erosion in the hands was observed ($r = 0.71$, $p = 0.002$ and $r = 0.61$, $p = 0.049$, respectively). This study demonstrated that CD26 mRNA expression in rheumatoid arthritis patients is associated with disease activity and bone erosion, suggesting a potential role for this molecule in the immunopathology of rheumatoid arthritis and bone erosion.

P0078

Regulatory role of Tet2 in innate immune response to infection

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Tet2 acts as a critical tumor suppressor in myeloid malignancies mostly in an enzymatic activity-dependent manner, and is also important for resolving inflammatory response via enzymatic activity-independent way. Myelopoiesis is a common host immune response during acute and chronic infections; however, its epigenetic mechanism needs to be identified. Whether Tet2 is involved in pathogen infection-induced myelopoiesis need to be further investigated. In our study, we find that Tet2 can increase the numbers of mature myeloid cells such as monocytes, neutrophils or mast cells in blood or tissues in mouse models of acute abdominal sepsis and chronic parasite infection. Mechanistic study revealed that Tet2 represses expression of SOCS3, a negative regulator of JAK-STAT signalling pathway, not through oxidation of DNA methylation but via decreasing the level of 5-methylcytosine (5-mC) in mRNA of SOCS3. For the underlying mechanism of 5-mC in regulating mRNA stability, we preliminarily found that methylated cytosines, including ones in the 3′ untranslated region of Socs3, influenced double-stranded RNA formation for Adar1 binding, probably through cytosine methylation-specific readers, such as RNA helices. Our study reveals a previously unknown regulatory role of Tet2 mediating mRNA demethylation and promoting mRNA editing at an epitranscriptomic view, and also promoting myelopoiesis during infection at an immunological view. Moreover, dysregulation of mRNA cytosine methylation, mRNA editing and JAK-STAT signaling for myelopoiesis in Tet2 mutant individual may add insight to the development of malignant diseases.

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DOI: 10.1002/eji.201970400

P0079

Genetic polymorphism of rs9277535 in HLA-DP associated with rheumatoid arthritis and anti-CCP production in a Chinese population

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Objective: HLA-II molecules are critical in triggering human immune response, especially in activating CD4+ T cells. HLA-DP, belonging to HLA-II molecules, draws increasing attention for its role in presentation of viral antigen and autoantigen to T cells. Researches reported single nucleotide polymorphism (SNP) of HLA-DP associated with HBV infection and autoimmune diseases such as SLE. However, little is known about the relationship between HLA-DP and rheumatoid arthritis (RA). Rs9277535 is located in 3' UTR region of HLA-DPB1, a subunit of HLA-DP, and was reported to affect HLA-DP mRNA expression. In the present study, we explore the relationship between gene polymorphism of rs9277535 in HLA-DPB1 and RA susceptibility and progression.
Methods: Samples from 254 patients with RA and 391 age and sex matched healthy controls were collected and genotyped by a polymerase chain reaction-high resolution melting (PCR-HRM) assay. Serological tests (anti-CCP, rheumatoid factor, C-reactive protein, anti-keratin antibody) were detected by laboratory assays.
Results: Strong association was observed between SNP rs9277535 in HLA-DP and RA susceptibility (allele frequency distribution: OR=1.409, 95%CI=1.121-1.773, P=0.004). Further validation was provided by disease model analysis (recessive model: OR=1.889, 95%CI=1.194-2.990, P=0.008; dominant model: OR=1.464, 95%CI=1.050-2.041, P=0.025; additive model: OR=2.208, 95%CI=1.335-3.652, P =0.003). Allele A was correlated to increased risk of RA. Serological test results demonstrated patients carrying allele A of rs9277535 had elevated serum anti-CCP antibody level.
Conclusions: The present study provided evidence that HLA-DP gene polymorphism associated with RA susceptibility. Allele A of rs9277535 in HLA-DP correlated to increased risk of RA and elevated serum anti-CCP level.

P0080

Tet2-mediated DNA 5-hydroxymethylcytosine regulates processing of pathogen antigen for anti-infection immunity

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5-methylcytosine (5-mC), the most widely studied type of DNA methylation, is catalysed and maintained by the DNA methyltransferase (DNMT) family members. TET cytosine dioxygenase family members mediate oxidation of 5-mC into 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), which is critical for active DNA demethylation. Oxidation products of DNA methylation generated by TET proteins have their own characteristic genomic distributions and transcription regulation functions. In particular, 5-hmC is relatively stable and abundant, and it appears to play a distinct regulatory role during cell lineage specification. In our study, we find that Tet2-mediated 5-hmC can regulate transcription activity of GILT which processes disulfide bond-containing antigen of pathogen during infection for both innate and adaptive immunity. Loss of Tet2 delays escape of infected *L. monocytogenes* from the macrophage phagosome for replication, and impairs cross-presentation of disulfide bond-containing viral antigens for $CD8^+$ T cells activation. The mechanistic study preliminarily reveals that Tet2-mediated 5-hmCs can act with specific regulator to

DOI: 10.1002/eji.201970400

regulate transcription of GILT. This study reveals a novel regulatory role of 5-hmC as an independent base to regulate antigen processing for antigen presenting cells.

P0081

A novel HPV peptides-nano vaccine, designed by immunoinformatics and nanomaterials

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Human papillomavirus (HPV)-caused cervical cancer is the second common female cancer globally. The development of effective and economical HPV vaccine is pursued urgently, due to affordability issues and type-restricted protection of the marketed vaccines. Persistent infection with HPV16 has much association with the development of cervical cancer. L1, the major capsid protein of HPV, has been well investigated as a potential vaccine candidate.
In this study, varied immunoinformatics and computational tools were employed to design a novel peptide based nano vaccine of HPV. Three immunodominant epitope domains (266-288aa, 49-65aa, 311-335aa) from the L1 of HPV 16 with potential to promote Th, CTL, B-cell, and INF-gamma responses were selected. Three immunodominant epitope domains (266-288aa, 49-65aa, 311-335aa), with potential to promote Th, CTL, B-cell, and INF-gamma responses, were selected from the L1 of HPV 16. Different segments were joined together by proper linkers to construct the protein of which the physicochemical, structural, and immunological characteristics were evaluated. Moreover, modeling, refinement, and validation were performed to assure a high quality 3D structure of the protein. Docking and molecular dynamics studies demonstrated an appropriate and stable state of the protein. Finally, the protein was conjugated to gold nanoparticles (AuNPs) through direct reaction between mercapto group of uniquecysteines and AuNPs, rendering pro-AuNPs, i.e. the novel peptide based nano vaccine of HPV.
This study presents a new vaccine design strategy based on bioinformatics, nanomaterials and immunology.
This work was supported by University Science and Technology Innovation Team of Henan Province （19IRTSTHN006）

P0082

Immunoglobulin kappa chain, a novel molecule of myocardial cells

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Immunoglobulins (Igs) play an important role in host defense and were initially thought to be expressed solely by B cells. However, growing evidence indicated that non B cells can also produce Igs (non B-Ig). In this study, we for the first time identified functional Igκ in cardiomyocyte of both human and mice. We first identified Igκ transcript in cardiomyocyte of μMT mice (B cell deficient mice) and HL-1(mice myocardial cell line) as well as human iPS-CM(induced pluripotent stem cell-derived cardiomyocytes), then we analyzed the VκJκ recombination pattern, and found that unlike the B cell-derived Ig that showed diversity, the cardiomyocyte-derived Ig showed restricted VκJκ recombination pattern, which suggested that the function of the cardiomyocyte-derived Ig is different from that of B-Ig. The localization analysis revealed that Igκ mainly located on the intercalated disc (ICD) of heart tissue of both μMT mice and human autopsy specimen as well as human iPS-CM. Interestingly, the expression and secretion of Igκ was induced and upregulated during hypoxia. More importantly, cardiomyocyte-specific knockout of Igκ disrupts normal structure of ICD and leads to severe

DOI: 10.1002/eji.201970400

constriction and conduction dysfunction. However, the mechanism remains unclear. Our data indicate for the first time that Igκ is expressed by cardiomyocytes in mice and human. The physiological and pathological function of cardiomyocyte derived Igκ needs further investigation.

P0084

A putative intronic enhancer regulates LILRB1 gene in human NK cells

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The inhibitory receptor leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) has a widespread expression in human hematopoietic system and interacts with major histocompatibility complex class I to maintain the balance of immune responses. We previously described that *LILRB1* polymorphisms are associated with LILRB1 expression patterns on individual Natural Killer (NK) cells and human cytomegalovirus (HCMV) infection in transplant patients. Thus, understanding how polymorphisms influence LILRB1 expression could help improve management of HCMV in transplant patients. Although lymphocytes and monocytes are known to use distinct promoters to transcribe the LILRB1 gene, molecular mechanisms resulting in the various LILRB1 expression patterns in individuals and different immune cells are not well understood. We did not observe notable differences in activity and methylation frequency of the lymphocyte-specific promoter in donors with different *LILRB1* haplotypes. Interestingly, there is a putative enhancer spanning about 3kb in the first intron marked by specific histone modifications in lymphocytes but not in monocytes. CRISPR knockout of the putative enhancer in a human NK cell line significantly decreased LILRB1 expression. We found the transcription factor Yin and Yang 1, which is known to regulate enhancer/promoter interactions, binds with both LILRB1 promoters and the putative enhancer by chromatin immunoprecipitation. Furthermore, results using chromosome conformation capture provide strong evidence that the putative enhancer is in physical contact with both known *LILRB1* promoters in NK cells. These results shed light on the regulation mechanism of *LILRB1* in lymphocytes and provide a foundation to investigate the allele-specific regulation of *LILRB1* in NK cells.

P0665

GATA3-mediated epigenetic regulation of SEMA7A contributes to recruitment of macrophages in breast carcinoma and tumorigenicity in a murine breast cancer model

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GATA3 is a transcription factor that maintains mammary luminal epithelial cell fate. Loss of its expression in breast cancer correlates with a poor prognosis in clinic patients. Previous studies showed its functional link with suppression of EMT and other intrinsic mechanisms in breast tumor cells. However, if GATA3 also involves in the remodeling of cancer immuno-microenvironment is unknown. Here, we analyzed the TCGA dataset in breast cancer and found that GATA3 expression levels are negatively associated with CD68 expression levels in breast cancer. In a murine breast cancer model, we found that overexpression of GATA3 in murine breast cancer cell line 4T1 inhibits recruitment of macrophages in xenografts. Mechanistically, GATA3 recruits histone methytransferase EHMT2 to regulate H3K9 dimethylation status on SEMA7A genomic locus, and consequently epigenetically repress SEMA7A expression in tumor cells. Mice implanted with SEMA7A silenced 4T1 tumor cells showed decreased recruitment of macrophage in xenografts compared to the control group. Furthermore, reintroduction of SEMA7A in GATA3-overexpressing 4T1 cells increased recruitment of macrophage. Finally, we further confirmed the prognostic value of

DOI: 10.1002/eji.201970400

GATA3/EHMT2/SEMA7A axis in a clinical cohort. The discovery of a GATA3/EHMT2/SEMA7A axis regulates the breast cancer immuno-microenvironment and opens up a novel therapeutic intervention in breast cancer.

P0666

Occult hepatitis B virus infection and related mechanism in pregnant women with anti-HBc positive

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Background: Occult hepatitis B infection (OBI) is characterized by serum hepatitis B surface antigen (HBsAg) negative and hepatitis B virus (HBV) DNA positive (HBsAg-/HBV DNA+).Occult hepatitis B infection in pregnant women has been scarcely investigated, especially China is one of the areas with high prevalence of chronic hepatitis B.
The aim of this study was to explore the prevalence and mechanism of occult hepatitis B virus infection in pregnant women with the anti-HBc positive. Materials A total of 416 pregnant women with the anti-HBc positive from Second affiliated hospital of anhui medical university were enrolled. The clinical data and serum samples were collected from the pregnant women. HBsAg and anti-HBc were detected with enzyme-linked immunosorbent assay(ELISA) and chemiluminescence microparticle immunoassay. DNA was detected using nested PCR, the HBV DNA from HBV DNA-positive subjects was extracted and amplified by nested PCR, and the PCR products were sequenced.
Results: Hepatitis B virus DNA was detected in 63 of 416 HBsAg-negative subjects with a median HBV DNA level of 17.95 (0, 366.25) log IU/ml, and the prevalence of OBI in the anti-HBc positive pregnant women was 15.14%. Sequencing showed that the HBV genotype is a mix of B and C . There are many amino acid substitutions (L22S, P70S, R79C, R75H, M103V, S113L, S136P, C139R, D144G, N146D, I57F,etc) after comparing the sequences.
Conclusions: These mutations may influence HBsAg detection by damaging the binding of antibodies to antigens. But multiple types of mutants co-exist with wild-type HBV and interact to maintain low viral loads.

P0667

A GATA3-SEMA3B axis suppresses metastasis of breast cancer by inhibiting recruitment of regulatory T cells

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The transcription factor GATA3 plays an essential role in differentiation of mammary luminal epithelial cells and maintenance of mammary gland homeostasis. Previous studies demonstrated that GATA3 suppresses metastasis of breast cancer cells by inhibition of EMT process. However, if the immuno-microenvironment also involves in GATA3-mediated suppression of breast cancer metastasis is unknown. Here, by analyzing the TCGA dataset in breast cancer, we found that the expression level of GATA3 is negatively associated with the expression level of FoxP3. To investigate the causative link between the expression patterns of GATA3 and FoxP3 in breast cancer tissues, Using a murine breast cancer cell line 4T1 to establish a lung metastasis model, we showed that overexpression of GATA3 inhibits breast cancer lung metastasis and represses recruitment of Foxp3-positive Treg cells in metastatic lesions. Mechanistically, SEMA3B, a secreted semaphorin family member, is a direct

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DOI: 10.1002/eji.201970400

downstream target transcriptionally activates by GATA3. We showed that overexpression of SEMA3B in 4T1 tumor cell significantly inhibits lung metastasis of breast cancer and represses recruitment of Foxp3-positive Treg cells in metastatic cancer. Moreover, knockdown of SEMA3B in GATA3-overexpressing tumor cells partially attenuates GATA3-mediated inhibition of breast cancer lung metastasis, and consequently decreases recruitment of Treg cells in cancer tissues. Furthermore, our *in vitro* assay also demonstrated chemo-repellent characteristics of SEMA3B for primary isolated FoxP3-positive Treg cells. Our data provide new insights into the role of GATA3 signaling for inhibition of breast cancer metastasis by upregulating SEMA3B and consequently remodeling of the cancer microenvironment by repressing recruitment of Treg cells.

P0668

Impact of the leukocyte immunoglobulin-like receptor A3 on osteoblast differentiation

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Objective: Functional leukocyte immunoglobulin-like receptor A3 (LILRA3) is an high-risk gene in susceptibility to rheumatoid arthritis (RA) . Serum levels of LILRA3 in RA patients were positively correlated with the markers of bone destruction in patients with RA, such as sharp score. The aim of this study is to investigate the effect of LILRA3 on osteoblast differentiation in vitro, and to better understand the role of LILRA3 in the pathogenesis of RA.
Method: The MC3T3-E1 cell line can differentiate into mature osteoblast under the culture condition of α-MEM medium containing ascorbic acid and β-glycerophosphate. The above cultured MC3T3-E1 cells were stimulated with 0, 1 or 5μg/mL of LILRA3 recombinant protein. To characterize the formation of calcium nodules, the staining for alizarin were performed after 21 days. The mRNA expression of key molecules in osteoblastogenesis, including Osterix, Alkaline Phosphatase (ALP), Bone Sialoprotein (BSP) and Osteocalcin (OCN), were measured by q-PCR on day14 and day21, respectively.
Results: On day 14 after stimulation with recombinant LILRA3, the expression of osteoblast-related genes, including ALP, BSP and OCN, were downregulated in a dose-dependent manner ($P < 0.05$). On day 21 post stimulation, the OCN mRNA expression was decreased ($P< 0.05$) and the formation of calcium nodules was almost inhibited in MC3T3-E1 cell line.
Conclusion: Our results indicate that LILRA3 may act as a suppressor on osteoblast differentiation by downregulating the expression of osteoblast-related genes and hindering the formation of calcium nodules.
Keywords: LILRA3, Rheumatoid Arthritis, Osteoblast Differentiation

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DOI: 10.1002/eji.201970400

P0669

Multiple plasma metals, gene polymorphisms and serum C-reactive protein: a metal-metal and gene-metal interaction study

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Background: Few studies have evaluated the associations of multiple metals with C-reactive protein (CRP). Furthermore, whether plasma metals would interact with each other, and interact with the CRP gene polymorphisms in relation to CRP is unknown.
Objectives: To explore the associations of multiple plasma metals with serum CRP, and to test the interaction between metals, and the gene-metal interactions on the levels of serum CRP.
Methods: We included 3210 participants in Dongfeng-Tongji cohort, China, and measured 23 plasma metals concentrations and serum CRP. We selected 2 CRP-related SNPs (rs3093059 and rs769449) in the present study. For metals which were associated with the concentrations of CRP, we further test the interactions between metals on CRP, and the gene-metal interactions on CRP.
Results: After multivariate adjustment, plasma copper and copper/zinc (Cu/Zn) ratio were found to be positively associated with serum CRP, whereas selenium was negatively associated with serum CRP (both $P< 0.001$). Moreover, we found selenium and zinc would attenuate the positive association between high plasma copper and CRP (P for interaction< 0.001). Furthermore, we found the 2 CRP-related SNPs would modify the associations between copper and CRP (all P for interaction< 0.001), whereas no gene-metal interactions were found for selenium.
Conclusion: Our results suggest that serum CRP was positively associated with plasma concentration of copper and Cu/Zn ratio, and inversely associated with selenium. Plasma zinc, selenium and CRP-related variants would modify the associations between plasma copper and serum CRP.

P0671

T-cell-activation-induced expression of proprotein convertase FURIN enzyme is dependent on the predicted super-enhancer region

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T cell activation leads to changes in cytokine production, cell proliferation, and differentiation, which are regulated by protein synthesis and maturation. Proprotein convertases are key enzymes that proteolytically process and convert the precursor proteins into mature bioactive form. The prototype proprotein convertase FURIN is induced upon TCR activation, and its expression is upregulated by the Th1 hallmark cytokine IL-12. T-cell expressed FURIN is critical for the maintenance of peripheral immune tolerance and protective host responses against intracellular pathogens.
Vahedi et al. (Nature 2015) investigated super-enhancers in mouse T helper cell subtypes to identify genes that determine Th cell fate. In Th1 cells, FURIN was among the top genes encompassing the

DOI: 10.1002/eji.201970400

highest super-enhancer architecture (p300 binding) upstream the promoter. This implies that FURIN expression in Th1 cells could be uniquely genetically regulated and important for the determination of Th1 cell fate.
To understand the role of the putative FURIN super-enhancer region, we generated T cell lines lacking the p300 binding sites upstream the *Furin* promoter. These cell lines show a decreased level of FURIN mRNA expression in response to T cell activation. Moreover, cells lacking the predicted FURIN super-enhancer region express distinct cytokine patterns compared to the wild-type control cells.
Further studies are needed to reveal the mechanisms by which the super-enhancer-dependent regulation of FURIN expression influences cytokine expression profiles. FURIN is an important regulatory factor in T-cell-dependent host responses. Therefore, understanding the FURIN regulation T cells may provide novel insights into the immune-mediated pathologies, including autoimmune diseases and immunodeficiences.

P1261

Primary analysis of the characteristics of BCR H chain CDR3 repertoires in the peripheral blood of twins

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The adaptive immune system's capability to protect the body requires a highly diverse lymphocyte antigen receptor repertoire. Adaptive immunity is provided by B and T cells bearing B-cell receptors (BCRs) and Ig-like T-cell receptors (TCRs), respectively. The influence of genetic factors on the development of BCRs is still unclear. Here, we analyzed the BCRs of three pairs of monozygotic twins and three pairs of dizygotic twins by next-generation sequencing technology to assess the effects of genetic factors on that. We found that the diversity of monozygotic twins BCRs and length distribution of the complementarity determining region 3（CDR3）are similar to dizygotic twins. Furthermore, the amino acid sequences of monozygotic twins are similar to that of dizygotic twins. All amino acids are extract at high frequency from arginine, aspartic acid, tyrosine, glycine and alanine. However, V-J pairing of monozygotic twins in the pseudogene and out of frame sequences are more similar than dizygotic twins. These data increase our understanding of BCRs and show that BCRs are primarily affected by genetic factors before experiencing antigen.

P1262

Primary analysis of the characteristics of TCR β chain CDR3 repertoires in the peripheral blood of twins

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The immune repertoires are formed under the influence of two forces: individual genetic traits and the environment. There are some studies have reported that the repertoires of twins are more similar, however, the repertoires being more similar at which development stage and corresponding mechanisms are still unclear. We analyzed the TCR β-chain CDR3 repertoires of three pairs of monozygotic twins (MZ) and three pairs of dizygotic twins(DZ) at different development stages and compared the differences of repertoires five years before and after by high-throughput sequencing. We found that there is no significant difference in diversity among MZ, DZ, and non-twins (NZ); the V gene usage is more similar before thymus selection, but the J gene usage and V-J pairing are more similar after thymus selection. In terms of amino acid, there are more overlap sequences between the

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DOI: 10.1002/eji.201970400

twins; however, there is no significant difference in amino acid usage and length distribution among MZ, DZ, and NZ. These data show that the TCR repertoires are affected by genetic factors or environment factors at different stages: the V gene usage being more susceptible to genetic factors ; the J gene usage and V-J pairing being affected by thymus selection; while twins have more same amino acid sequences that recognize the same antigen. These findings provide a deeper insight into the mechanism of TCR repertoires generation.

P1263

Sublytic C5b-9 induces IL-23 and IL-36a production by glomerular mesangial cells via PCAF-mediated KLF4 acetylation in rat Thy-1 nephritis

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Sublytic C5b-9 formation on glomerular mesangial cells in rat Thy-1 nephritis (Thy-1N), a model of human mesangioproliferative glomerulonephritis, is accompanied by the production of proinflammatory cytokines, but the relationship between sublytic C5b-9 and cytokine synthesis and the underlying mechanism remains unclear. To explore the problems mentioned above, in this study, we first examined the levels of proinflammatory ILs (e.g., IL-23 and IL-36a) as well as transcription factor (KLF4) and coactivator (PCAF) in the renal tissues of Thy-1N rats and in the glomerular mesangial cell line (HBZY-1) stimulated by sublytic C5b-9. Then, we further determined the role of KLF4 and PCAF in sublytic C5b-9-induced IL-23 and IL-36a production as well as the related mechanism. Our results showed that the levels of KLF4, PCAF, IL-23, and IL-36a were obviously elevated. Mechanistic investigation revealed that sublytic C5b-9 stimulation could increase IL-23 and IL-36a synthesis through KLF4 and PCAF upregulation, and KLF4 and PCAF could form a complex, binding to the IL-23 or IL-36a promoter in a KLF4-dependent manner, causing gene transcription. Importantly, KLF4 acetylation by PCAF contributed to sublytic C5b-9-induced IL-23 and IL-36a transcription. Besides, the KLF4 binding regions on IL-23 or IL-36a promoters and the KLF4 lysine site acetylated by PCAF were identified. Furthermore, silencing renal KLF4 or PCAF gene could significantly inhibit IL-23 or IL-36a secretion and tissue damage of Thy-1N rats. Collectively, these findings implicate that the KLF4/PCAF interaction and KLF4 acetylation by PCAF play a pivotal role in the sublytic C5b-9-mediated IL-23 and IL-36a production of Thy-1N rats.

P1264

Identification of CCP1 interacted proteins in mouse testis by immunoprecipitation combined with LC/MS-MS

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CCP1 (Cytosolic carboxypeptidase 1) is identified as a member of M14 subfamily of cytosolic carboxypeptidase. CCP1 autosomal recessive mutation results in Purkinje cell degeneration, male sterility and female sub-fertility. The mechanism of male sterility has not been revealed yet. Therefore, it is important to identify CCP1 interacting proteins in mouse testis for clarifying the function of CCP1 in male reproductive system. Here, we used coimmunoprecipitation and LC/MS-MS to identify CCP1 interacting proteins from Balb/C mouse testis in a proteome-wide scale. Subsequently, the bioinformatics analyses using GO, PANTHER and KEGG databases were used to explore the CCP1 interacting proteins function and involved pathways. Finally, we analyzed interactions between CCP1 and its interacting proteins by STRING database. As a result, we identified 325 proteins interacting

DOI: 10.1002/eji.201970400

with CCP1 in mouse testis. Most of them had hydrolase and binding activities with nucleic acids and proteins, which were mainly involved in inflammation process mediated by chemokine and cytokine signaling pathway, nicotinic acetylcholine receptor signaling pathway and Parkinson disease related pathways. The Actb(Actin, cytoplasmic 1), Tubb4b (Tubulin beta-4B chain), Prkaca (cAMP-dependent protein kinase catalytic subunit alpha) and Gnai2 (Guanine nucleotide-binding protein G(i) subunit alpha-2) were the main related proteins. Notably, Tubb4b was in the core of the interacting network. Our study identified CCP1 interacted proteins in proteome-wide scale, and it surely will provide deep insight of CCP1 function and probable related pathways in mouse testis.

P1265

Variation in complement factor H affects complement activation in immunoglobulin a vasculitis with nephritis

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Immunoglobulin A vasculitis with Nephritis (IgAVN) and IgA nephropathy (IgAN) are widely considered as related diseases. Considerable evidences support the notion of involvement of complement activation in both IgAVN and IgAN. Our previous studies identified a genetic variant in complement factor H (*CFH*), rs6677604, as an IgAN-susceptible variant by genome-wide association study (GWAS), and further confirmed its linkage to *CFHR3-1Δ* and proved its influence on complement activation and thereby on IgAN susceptibility. To explore the role of rs6677604 in complement activation of IgAVN, we enrolled 632 patients with IgAVN, 1178 patients with IgAN and 902 healthy controls in the present study. The genotype of rs6677604 was measured by TaqMan allele discrimination assays or was extracted from our previous GWAS data. The frequency of the rs6677604-A allele was significantly higher in IgAVN than in IgAN. However, no significant differences were observed between IgAVN and the controls. Higher complement factor H (FH) levels were observed in IgAVN than IgAN, and positive correlation between circulating FH and C3 levels was present in IgAVN. In both IgAVN and IgAN, rs6677604-A was associated with less intensity of glomerular C3 deposits. In agreement with the higher frequency of rs6677604-A in IgAVN, the glomerular C3 deposits of patients with IgAVN were less intense than those in IgAN. Our findings suggest that genetic variation in *CFH* (rs6677604) is involved in the phenotype of complement activation in both IgAVN and IgAN. Moreover, rs6677604 might contribute to the difference of complement activation intensity between IgAVN and IgAN.

P1266

Experience of a register of recurrent hereditary fevers in a Moroccan pediatric rheumatology unit

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Autoinflammatory syndromes are hereditary diseases of which most frequent are the familial mediteranean fever “FMF”, the mevalonate kinase deficiency "MKD", and the periodic syndrome associated with the TNF receptor "TRAPS". They have in common a clinical profile of recurrent fevers

DOI: 10.1002/eji.201970400

interspersed with free intervals and biological inflammation during crisis periods.
The study aim is to caracterise the epidemiological, clinical, biological, genetic and therapeutic profiles of FMF, MKD and Muckle Wells syndrom in a Moroccan patients.
We retrospectively analyzed data from patients referring to a pediatric rheumatologic moroccan unit, for recurrent fever attacks, between 2006 and 2017. In total, we diagnosed 20 patients (11 females, 9 males) of whom 14 children with FMF, 5 with MKD and 1 child with MW syndrom. The average age of 10 ½ years. All patients were moroccan from various geographical origins. Consanguinity notion was noted in 48% of the cases.
The main clinical presentation of these patients are recurrent fever (81,90%), arthralgia (90,95%), cutaneous manifestations (73,81%), abdominal pain (78,57%), headache (11,90%), myalgia (4,76%), and also deafness, muteness and l conjunctivitis (5%).
Genetic study was carried in 16 of our patients according to the diagnosis. The mutations found in FMF are M694V (50%), M694I (21,43%), A744 (14,29%), E148Q (7,14%) and P369 (7,14%). As for the MKD, V337I was found in 1 patient (20%).
The prevalence of AID is still underestimated in Morocco, due to the rarity of cases, the lack of availability of biological and genetic tests to the general public.

P1267

Significance of the human *TERT* gene polymorphisms and telomere length in patients with non-Hodgkin's lymphoma, comparison with healthy controls and human cancer cell lines

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Human telomerase reverse transcriptase (*TERT*) gene encodes the catalytic subunit of telomerase that is essential for maintenance of telomere length. We aimed to find out whether variability within the *TERT* gene could be associated with telomere length in patients with with non-Hodgkin's lymphoma (NHL) compared to healthy controls and human cancer cell lines cultured *in vitro.*
The *TERT* (rs2736100, rs2853669, rs2853690, rs33954691, rs35033501) single nucleotide polymorphisms and MNS16A repeats were evaluated in 75 NHL patients and 100 healthy controls. Telomere length of target genomic DNA samples from 12 human cancer cell lines, peripheral leukocytes in 68 NHL patients and 156 healthy donors was assessed.
The rs2853690-*CC* homozygosity was slightly more common among patients than controls (OR=1.916, p=0.082). The rs33954691-*T* allele was more often detected in patients in stage I than in more advanced NHL stages at diagnosis (p=0.014), and controls (OR=2.613, p=0.001). Patients carrying the MNS16A VNTR-243 variant (previously found to be associated with a less favourable response to therapy and to be more frequent among non-responders) characterized with shorter telomeres in peripheral leukocytes (p=0.002). The rs285669-*AA*-positive patients presented with shorter telomeres than *AG*-heterozygous (p=0.036) and *GG*-homozygous (p=0.009) patients. *In vitro* cultured cancer cell lines characterized with shorter telomere length as compared to NHL patients (p=0.002) and controls (p< 0.001).
These results imply that the *TERT* polymorphisms may play a prognostic role in patients with NHL.

This work was supported by the TARGETTELO project No. STRATEGMED3/306853 from the National Centre for Research and Development, Warsaw, Poland.

DOI: 10.1002/eji.201970400

P1852

BAFF and *APRIL* variants associated with susceptibility to IgA nephropathy and lupus nephritis

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Objectives: IgA nephropathy (IgAN) and lupus nephritis (LN) are the two most common autoimmune kidney diseases. Amounting evidence supported that genetics plays an important role in their etiology. Recent genome-wide association studies (GWAS) suggested there have been shared genetic basis between these two different diseases. *TNFSF13B* (also known as B cell activating factor, *BAFF*) variants have been associated with systemic lupus erythematosus (SLE), and *TNFSF13* (also known as a proliferation-inducing ligand, *APRIL*) variants with IgAN. This study was to associate BAFF and APRIL gene variants with IgAN and LN by cross-phenotype analyses, aiming to identify the causal variant.
Methods: In a Chinese population including 2,837 IgAN patients, 6,498 healthy controls, and 498 lupus nephritis patients. We performed GWAS followed by locus-specific association testing in IgAN and LN.
Results: In IgAN, we observed that 2 SNPs (rs3803800 and rs9901675) in the *TNFSF13* region and 2 SNPs (rs9587560 and rs7982100) in the *TNFSF13B* region were significantly associated with IgAN, after multiple correction. In LN, only rs2075230 in *TNFSF13* survived in association instead of *TNFSF13B* variants. The associated variants in IgAN showed no association in LN, and *vice versa*. In sub-phenotype analysis, significant associations were observed between rs3803800 and serum IgA level, and between rs7982100 and galactose deficient IgA1 level.
Conclusion: In Chinese, we observed shared *APRIL* gene associations in IgAN and LN, but the associated variants were disease specific. We did not replicate genetic associations between *BAFF* and LN.

P1853

Variants in *CFHR1* gene contribute to complement activation in IgA nephropathy

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IgA nephropathy (IgAN) is the most common primary glomerulonephritis which is associated with the activation of complement system. Recent IgAN-GWAS study identified 1q32 as the IgAN susceptible locus, which contains *CFH* and five *CFHR*s genes. Our previous study proved *CFHR3-1Δ* affected complement activation and thereby associated with IgAN. Besides *CFHR1* deletion, three completely linked coding variants in *CFHR1* were reported to form two FHR-1 allotypes, FHR-1*A and FHR-1*B. Previous studies found that the two FHR-1 allotypes associated with the susceptibility of aHUS, a disease caused by uncontrolled activation of the complement system. Here we explored the association of *CFHR1* coding variants with the genetic susceptibility and complement activation in IgAN. A total of 1114 biopsy-proven primary IgAN patients and geographically matched unrelated healthy individuals were recruited. The genotypes of *CFHR1* variants and copy numbers of *CFHR1* gene were analyzed by Sanger sequencing and MLPA, respectively. Demographic, clinical and pathological information of enrolled patients were collected from medical records. Six different types of FHR-1 were identified, including del/del, del/B, del/A, BB, AB and AA. Compared with healthy controls, patients with IgAN carried more FHR-1*A allotype (48.8% vs 43.2%, $p< 0.001$). Moreover, IgAN patients who carried FHR-1*AA presented with higher intensity of C3 deposition ($p=0.005$). However,

DOI: 10.1002/eji.201970400

patients with different *FHR-1* types showed no significant difference in eGFR, proteinuria and hypertension. In conclusion, our study found that FHR-1*AA contributed to more intensive renal local complement activation, and served as a risky factor to IgAN susceptibility.

P1854

Glu^{496}to Ala and Gln^{460}to Arg Polymorphism in the P2X7 receptor impairs ATP-induced IL-1β release from human THP-1 cell in high uric acid background

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Objective: ATP acts on the sputum receptor P2X ligand-gated ion channel 7 (P2X7R) as a second signal to induce gouty arthritis, establishing a wild-type, Glu^{496}to Ala and Gln^{460}to Arg THP-1 cell line stably expressing P2X7R in high uric acid background. Analyze the functional study of P2X7R.
Method: After transfection, divided into three groups: MSU (labeled M), MSU+ATP (labeled MA), and unstimulated control group (labeled C) were added to the wild type or mutant types. Detection of IL-1β protein expression level by ELISA and Caspase-1 and NLRP3 mRNA expression levels by qRT-PCR.
Result: 1. In group M, The protein expression level of IL-1βof wild-type was higher than that of Glu^{496}to Ala and Gln^{460}to Arg, the difference was significant (93.56±1.147pg/ml vs 12.31±0.5800pg/ml, P< 0.0001;93.56±1.147pg/ml vs 10.70±0.7049pg/m, P< 0.0001, respectively). The expression of Caspase-1 and NLRP3 mRNA of wild-type was higher than Glu ^{496}to Ala and Gln^{460}to Arg , but the difference was not significant (P>0.05).
2.In group MA. The protein expression level of IL-1β, Caspase-1 and NLRP3 mRNA of wild-type was higher than that of ^{1}Glu ^{496}to Ala and ^{2}Gln^{460}to Arg. The difference was significant (^{1}87.12±2.632pg/ml vs 28.24±6.427pg/ml, P=0.0001; 1.003±0.0763 vs 0.1389±0.0077, P=0.0078; 0.9971±0.090 vs 0.4430±0.0077, P=0.0173,respectively;^{2}87.1±2.632pg/ml vs 10.25±0.4701pg/ml, P< 0.0001; 1.003±0.0763 vs 0.0575±0.0081, P=0.0065; 0.9971±0.090 vs 0.6243±0.0087, P=0.0486.respectively).
3.In group C, The protein expression level of IL-1β, Caspase-1 and NLRP3 mRNA of wild-type, were not different from Glu ^{496}to Ala and Gln^{460}to Arg (P>0.05).
Conclusion: Glu ^{496}to Ala and Gln^{460}to Arg down-regulate the functional status of P2X7R in high uric acid background, which reduces the secretion of IL-1β, NLRP3 and Caspase-1.

P1855

ALKBH5 inhibits human extravillous trophoblast motility in vitro

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Background/Aims: Emerging reports suggest that ALKBH5 regulate multiple disease pathogenesis. However, the mechanisms and functions of ALKBH5 in preeclampsia remain unknown. This study aims to explore the effects of ALKBH5 in migration and invasion of trophoblast cells.
Methods: The cloning vector (pEX-1-ALKBH5) and pEX-1-control vector were transfected into HTR cells, determining the optimal transfection concentration by RT-qPCR. after over-expression of ALKBH5, transwell assay were performed to examine the migration and invasion of HTR cell, western blot were used to detected levels of EMT markers,MMP9 and FOXM1.
Result: We found transfection of pEX-1-ALKBH5 could obviously inhibit HTR cells migration and invasion. The epithelial marker was increased, while the mesenchymal markers and MMP9 were

DOI: 10.1002/eji.201970400

decreased. Besides, over-expression of ALKBH5 could decrease FOXM1 levels.
Conclusion: Our results have demonstrated a novel mechanism by which ALKBH5 inhibits HTR cells, which may play an important role in the occurrence and development of preeclampsia, and FOXM1 maybe its target.

P1856

An exome-wide association study of Han Chinese identifies multiple *HLA* missense variants and a novel *LRRC4C* variant predisposing to iga nephropathy

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Objectives: IgA nephropathy (IgAN) is one of the most common primary glomerulonephritis of considerable genetic predisposition. Genome-wide association studies have identified about 13 loci for IgAN. However, the majority of them reside in non-coding sequences. The contributions of coding variants have not yet been systematically evaluated.
Methods: We performed a large-scale exome-wide study in 640 IgAN cases and 4295 healthy controls in a Han Chinese population using a custom exome array.
Results: After stringent quality control, genotype data for 33592 autosomal biallelic variants in 628 cases and 4089 controls qualified for genetic association tests. We observed 22 *HLA* SNPs and 1 non-*HLA* SNP (minor allele frequency >5%) with suggestive association ($p< 5\times10^{-5}$). An intronic variant in *GABBR1* showed the most significance (3.61×10^{-10}). And 6 variants belongs to missense variants (1 in *POM121L2*, 3 in *UBD*, and 2 in *HLA-DQA1* gene). An intergenic variant in 3´ of *LRRC4C* showed novel genetic associations with IgAN with p of 2.51×10^{-6}.
Conclusion: Our study reveals additional loci and novel genes for genetic predisposition to IgAN, which may shed novel biological mechanism.

P1857

Complementing role of survivin and VEGF in the hypoxia mediated angiogenesis as putative therapeutics for breast cancer

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Breast cancer is frequent malignancy in females and accounts for 1.38 million new cancer cases. Besides role of various genetic mutations and risk factors in pathogenesis of breast cancer, discovery of key genes to establish progression and metastasis in breast cancer is need of hour. The pathophysiology of breast cancer is multicascade process and angiogenesis plays a key role in its tumorigenesis and metastasis. Cutting-edge studies have revealed that VEGF is important mediator of angiogenesis that initiates tumor cell motility, invasion and metastasis via the hyper-activation of the PI3K/AKT pathway. In addition, VEGF is closely associated with survivin to play crucial role in tumor angiogenesis. Survivin, the 16.5 KDa anti-apoptotic intracellular protein highly expressed in most of the solid malignancies drastically enhances cytokinesis, cycle progression and inhibits apoptosis, which is strongly correlated with poor outcome in breast cancer. Further, the high expression of VEGF leads to the deep neovascularization within the breast tumor and has been demonstrated to be associated with a poor prognosis. Besides, the induction of hypoxia within the tumors via the stabilization of HIF1-alpha promotes elevated expression of VEGF and survivin. The role of survivin and VEGF has been reported in plethora of studies yet key factors associated with angiogenesis that regulate their expression in the metastasis of breast cancer remains to be elucidated. Therefore

DOI: 10.1002/eji.201970400

interplay of the elevated expression of survivin and its splice variants with VEGF and its interaction in hypoxia mediated angiogenesis is explored here as a putative therapeutic target for breast cancer.

P1858

Increased expression of CD40 and CD40 ligand in acute coronary syndrome patients: association with genetic variants in western Mexican population

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Acute coronary syndrome (ACS) belongs to the clinical entities characterized by obstruction of a coronary artery. In its etiology, inflammation contributes significantly trough the activation of immune cells by co-stimulatory molecules as CD40 and CD40L. The purpose of this work was to explore the changes of mRNA and circulating soluble levels of CD40-CD40L in ACS patients and association with genetic variants in a western Mexican population.
320 ACS patients and 300 individuals with similar age than cases without ischemic cardiopathy were recruited. Genotype determination was made using TaqMan SNP genotyping assays. *CD40* and *CD40L* mRNA expression in whole blood samples were quantified using TaqMan Gene Expression Assays. Soluble levels were measured in plasma by enzyme-linked immunosorbent assay.
We did not find evidence of association between on *CD40* (rs1883832, rs4810485 and rs11086998) and *CD40L* (rs3092952 and rs3092920) genetic variants and susceptibility to ACS nor changes in their mRNA expression (p>0.05), although rs1883832 and rs4810485 affected significantly sCD40 circulating levels (p=0.005; p=0.015, respectively). Circulating soluble levels were higher in ACS patients; specifically sCD40L was higher in male patients with unstable angina compared to female patients (p=0.016).
Our results showed genetic variants affect soluble levels of CD40 although the *CD40* and *CD40L* polymorphisms are not associated with susceptibility to ACS in western Mexican population. *CD40L* mRNA expression, sCD40 and sCD40L plasma levels were higher in ACS patients even when they are under pharmacological treatment, which suggests an active role of the CD40-CD40L system in the immunopathogenesis of acute coronary syndrome.

P1859

Genetic variants of inflammation-modulating cytokines in HIV infected individuals

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Background and aim: Cytokines regulate inflammation. Mechanisms underlying regulation of inflammation are not clearly understood. This study investigated genetic variants of inflammation-modulating cytokines and their relationship with inflammation in HIV.
Materials and methods: 284 adults comprising of individuals 187 receiving ART, 32 ART naïve were recruited from an HIV care and treatment centre while 68 uninfected samples were collected from blood donors. Genotyping was done using ARMS PCR and PCR-RFLP techniques. Levels of cytokines and inflammation biomarkers were measured by ELISA.
Results and conclusion: Genotypes of IL-1Beta and IL6 gene were associated with hsCRP plasma levels. Polymorphisms of IL-6 and IL-1β may determine the production of the cytokine in question in

DOI: 10.1002/eji.201970400

the presence of HIV infection but not in the HIV uninfected individuals. Carriers of IL-6 GG and IL-1β CC had significantly higher median plasma levels of the respective cytokines. IL-10 genotypes were not associated with hsCRP levels. Observed differences in plasma levels between HIV infected and uninfected could be due to the different inflammation and immune activation processes from stimulated cells exposed to the virus.

P2535

Function of fat mass and obesity associated gene FTO in regulation of flag-ghrh mRNA expression

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In this study, the effects of m6A modification on the transcription and translation of genes were verified by the cells with overexpressing Flag-Ghrh and knocking out FTO in vitro. First, we constructed the overexpression vector of Flag-Ghrh pENTR -Flag-Ghrh and adenovirus pAd-CMV-SP-Flag-Ghrh-P2A-GFP-SV40, and the overexpression vectors were verified Flag-Ghrh gene expression in the 293A, HeLa and C2C12 cells. Constructed lentivirus and adenovirus with FTO knockdown by shRNA and CRISPR/Cas9. The mRNA and protein levels of Flag-Ghrh were detected by q-PCR and Western Blot assays after knocking out FTO gene in HeLa cells. The results showed that knocking out the methylase FTO increased the m6A methylation level of Flag-Ghrh mRNA, thereby increasing the expression levels of Flag-Ghrh mRNA and Flag-Ghrh protein. It provides an important basis for studying the m6A modification of mRNA.

P2536

Research on vitamin D pathway related gene's variations and susceptibility to allergic rhinitis

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Recently the regulatory effect of vitamin D metabolism on immune diseases has been gradually discovered. The metabolic process of vitamin D involves many key regulators, such as Vitamin D binding protein(GC gene), Vitamin D receptor(VDR gene) . The genetic variation of these genes may affect the expression and activity of their proteins, thus forming individual differences and affecting the occurrence and development of diseases. However the effect of genetic variation of vitamin D metabolic pathway related genes on allergic rhinitis(AR) in Hainan has not been reported. Therefore, 94 patients with AR and 113 healthy controls in Hainan were selected for applying RCT research to study the relationship between genetic variation of key genes in vitamin D signaling pathway and AR in Hainan. 9 polymorphic loci (rs7041, rs4588, rs222016 of vitamin D binding protein coding gene GC, rs12794714 of CYP2R1 gene of cytochrome P450 family, rs731236, rs2228570, rs1544410, rs7975232, rs11568820 of vitamin D receptor gene) were selected in this study, among which three loci includingwere significantly different in Hainan healthy population and in the Northernchina population. There are significant differences in distribution frequencies of rs731236, rs1544410 and rs1568820 between Hainan healthy cases and the Chinese population in NCBI database, which indicates that the climate of tropical islands in Hainan has affected the mutations. The genotype GG at rs222016 locus of GC gene had resistance to AR in Hainan ($P < 0.05$). The association between the polymorphism of the other eight SNPs and AR in Hainan was not found in this study.

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DOI: 10.1002/eji.201970400

P2537

Circular RNA expression profiles of peripheral blood mononuclear cells in hepatocellular carcinoma patients by sequence analysis

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Circular RNAs (circRNAs) are a large class of noncoding RNAs that have potential regulatory roles in disease pathogenesis and progression. Recently, circRNAs have been found to be expressed in hepatocellular carcinoma (HCC) tissues and involved in the development and metastasis of HCC. However, the significance of circRNAs in PBMCs of HCC patients remains unclear. In this study, RNA sequencing analysis was performed to identify circRNAs from four HCC patients and three healthy controls to determine the expression pattern of circRNAs in the PBMCs and the circRNAs´ molecular regulatory networks in HCC pathogenesis. A total of 58 circRNAs were found to be significantly changed in the PBMCs of HCC patients compared with those of the healthy cases. Six random representative circRNAs were further validated by real-time RT-PCR in 72 samples of PBMCs from HCC patients and 30 control subjects. Chi-square test indicated that one of the up-regulated circRNA candidates-circ_0000798-was correlated with clinical variables. Highly expressed circ_0000798 was associated with poor overall survival of HCC patients. Receiver operating characteristic curve analysis further revealed that circ_0000798 was discriminating HCC patients from healthy controls. Finally, the predicted competing endogenous RNA network of circ_0000798 showed that it might act as a "sponge" of target microRNAs, that would subsequently regulate the expression of target genes in PBMCs. In summary, this is the first study to comprehensively identify dysregulated circRNAs in PBMCs of HCC patients, and its findings suggest that dysregulated circ_0000798 in PBMCs has potential as a convenient biomarker for diagnosing or prognosticating HCC.

P2538

Genome-wide profiling of nucleosome position and chromatin accessibility in single cells using scMNase-seq

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Nucleosome organization is important for chromatin compaction and accessibility. Profiling nucleosome positioning genome-wide in single cells provides critical information to understand the cell-to-cell heterogeneity of chromatin states within a cell population. Single-cell micrococcal nuclease sequencing (scMNase-seq) is a method of detecting genome-wide nucleosome positioning and chromatin accessibility simultaneously from a small number of cells or single cells. To generate scMNase-seq libraries, single cells are isolated by FACS sorting, lysed and digested by MNase. DNA is purified, end-repaired and ligated to Y-shaped adaptors. Following PCR amplification with indexing primers, the subnucleosome- and mononucleosome-sized DNA fragments are recovered and sequenced on Illumina platforms using paired-end 50 pattern. On average, 0.5 to 1 million unique reads are obtained for each single cell. Library preparation of scMNase-seq takes only 2 days, which requires only very basic molecular biology techniques and does not require sophisticated lab equipment.

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DOI: 10.1002/eji.201970400

P2539

Identification of SUV39H2 as a potential oncogene in lung adenocarcinoma

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SUV39H2 (suppressor of variegation 3-9 homolog 2) as a methyltransferase of histone 3 lysine 9, regulates transcriptional repression, maintains heterochromatin, and plays a key role in DNA repair. Studies have found that SUV39H2 is upregulated in cancers, and SUV39H2 is involved in tumor development and drug resistance. However, the significance of SUV39H2 in lung adenocarcinoma has not been explored. In our study, we screened out potential genes with abnormal expression in lung adenocarcinoma in the GEO and TCGA databases by bioinformatics analysis, and then analyzed and clustered these genes. We found that SUV39H2 was up-regulated in lung adenocarcinoma, and the overexpression of SUV39H2 was mainly associated with altered copy number. Patients with SUV39H2 overexpression had shorter overall survival time. We also confirmed its mRNA and protein expression levels in lung adenocarcinoma tissues. Subsequently, we evaluated the altered gene expression profile and possible pathways of SUV39H2 by second-generation sequencing RNA-seq analysis. We found that TPM4, STOM and OPTN may be affected by SUV39H2, in which OPTN is regulated by SUV39H2. It was then verified by a series of molecular biology experiments such as RT-qPCR and WB. At the same time, we used in vitro invasion assay and colony formation assay, as well as constructed mouse models of in situ tumors and metastases, which demonstrating the potential carcinogenic effects of SUV39H2. In vitro and in vivo experiments with SUV39H2 knockdown indicate the potential role of SUV39H2 in carcinogenesis and metastasis. SUV39H2 may be a potential oncogene in lung adenocarcinoma, mediating tumorigenesis and metastasis.

P2540

Study on the level of coagulation indexes and its clinical significance in 190 patients with lung cancer

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Objective: To analyze the relationship between the changes of coagulation parameters and the clinicopathological features of lung cancer patients.
Methods: A total of 190 newly diagnosed patients with lung cancer from January 2017 to December 2017 were selected from the First Affiliated Hospital of Xinjiang Medical University.The plasma thromboplastin time(TT)and prothrombin time(PT), activated partial thromboplastin time(APTT),fibrinogen(FIB),D-dimer (D-D),platelet count(PLT) levels,and patient-related clinical pathology data were collected.
Results: Compared with female patients,the APTT and PT were prolonged and the FIB was increased in male patients(P< 0.05); compared the D-D levels in patients aged ≥60 years and < 60 years old,suggesting that the older the age, the higher the D-D level. Compared with negative patients, the level of FIB and D-D were significantly increased in patients with positive lymph node invasion(P< 0.05). The patients with distant metastasis not only had higher levels of FIB and D-D than those without distant metastasis, and their PLT levels were significantly higher (P< 0.05); the mean values of FIB and D-D in adenocarcinoma patients, compared with squamous cell carcinoma patients, were increased (P< 0.05). The mean values of FIB and D-D in patients with stage III-IV tumors ,compared with the stage I-II, suggesting that the later the tumor stage, the higher the D-D and FIB levels(P< 0.05).

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DOI: 10.1002/eji.201970400

Conclusion: Coagulation dysfunction has a certain correlation with tumor cell growth and migration. Determination of coagulation index in lung cancer patients can provide relevant evidence for clinical evaluation of the disease.

P2541

Hypomethylation regulation of the gene expression levels of CD40: Implications for neural tube defects

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Background: Neural tube defects (NTDs) are a series of complicated congenital malformations in central nervous system, and its mechanism is not yet clear. CD40 gene is a member of tumor necrosis factor receptor (TNFR) superfamily. Its encoded protein is essential for mediating a series of immune, inflammatory and apoptotic responses, which have been demonstrated to be involved in NTDs, but the study of CD40 in NTDs has not been reported.
Methods: In this study, real-time PCR was performed to measure the differential mRNA level of CD40 and its ligand, CD40L, in the spinal cord tissues of NTDs and healthy controls. And we reanalyzed the previous DNA methylation chip data by using different methods and found several differential methylation sites of CD40. Then, pyrosequencing was used to verify the methylation levels of these sites between NTDs and controls group.
Results: The expression level of CD40 and CD40L mRNA were found significantly increased in the spinal cord tissues of NTDs and SBs, *p* value were 0.0162 and 0.0480, respectively, while the difference was not found in CHCs, compared to controls. Further verification showed hypomethylation status in TSS200-1500 region of CD40 in NTDs compared to controls. **Conclusion:** Our findings suggest that the spinal cord tissues in NTDs showed a increased mRNA level of CD40 and CD40L, and the inflammation pathway where they belong might be involved in the etiology of NTDs. The high expression of CD40 in NTDs may due to the DNA hypomethylation.
Keywords: CD40, Neural tube defects, Gene expression, DNA methylation

P2542

Association of atypical chemokine receptor 1 polymorphism with the erythrocyte chemokine scavenging

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Objective: Erythrocyte atypical chemokine receptor 1 (ACKR1) can scavenge chemokines in whole blood. ACKR1 gene consists of two major alleles: *FY*A* and *FY*B*. The aim of this study was to determine the association of ACKR1 polymorphism with the erythrocyte chemokine scavenging.
Methods: The ACKR1 were genotyped by 5´-nuclease assay in healthy Chinese Han individuals, while erythrocyte chemokine scavenging function and ACKR1 expression from the same samples were measured using erythrocyte chemokine binding assays and quantitative flow cytometry respectively.
Results: The erythrocyte chemokine scavenging of CXCL8 was significantly lower in the individuals with the *FY*A/ FY*A* genotype compared to those with *FY*A/ FY*B* genotype (*P*=0.016). Similar result was also observed in erythrocyte chemokine scavenging of CCL2 (*P*=0.038). The expression of ACKR1 on erythrocyte surface in the individuals with the *FY*A/ FY*A* genotype was significantly higher compared to those with *FY*A/ FY*B* genotype (*P*=0.021).

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DOI: 10.1002/eji.201970400

Conclusions: ACKR1 polymorphism is associated with the differential erythrocyte chemokine scavenging. It is probable that a change in ACKR1 structure caused by ACKR1 polymorphism is responsible for the differential erythrocyte chemokine scavenging.

P2543

TDIF1 promotes non-small cell lung cancer development through P53/HDAC1/2 axis

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TdT-interacting factor 1 (TdIF1) is a ubiquitously expressed DNA- and protein-binding protein that directly binds to terminal deoxynucleotidyl transferase (TdT) polymerase. Little is known about the functional role of TdIF1 in cancer cellular signaling, nor has it previously been identified as aberrant in any type of cancer. We report here for the first time that TdIF1 is abundantly expressed in clinical lung cancer patients and that high expression of TdIF1 is associated with poor patient prognosis. We further established that TdIF1 is highly expressed in human non-small cell lung cancer (NSCLC) cell lines compared to a normal lung cell line. shRNA-mediated gene silencing of TdIF1 resulted in the suppression of proliferation and anchorage-independent colony formation of the A549 adenocarcinoma cell line. Moreover, when these TdIF1-silenced cells were used to establish a mouse xenograft model of human NSCLC, tumor size was greatly reduced. These data suggest that TdIF1 is a potent regulator of lung tumor development. Several cell cycle-related and tumor growth signaling pathways, including the p53 and HDAC1/2 pathways, were identified as participating in the TdIF1 signaling network by in silico analysis. Microarray, transcriptome and protein-level analyses validated p53 and HDAC1/2 modulation upon TdIF1 downregulation in an NSCLC cellular model. Moreover, several other cell cycle regulators were affected at the transcript level by TdIF1 silencing, including an increase in CDKN1A/p21 transcripts. Taken together, these results indicate that TdIF1 is a bona fide tumor-promoting factor in NSCLC and a potential target for therapy.

P2544

Mysm1 epigenetically regulates the immunomodulatory function of adipose-derived stem cells in part by targeting miR-150

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Adipose-derived stem cells (ASCs) are highly attractive for cell-based therapies in tissue repair and regeneration because they have multilineage differentiation capacity and are immunosuppressive. However, the detailed epigenetic mechanisms of their immunoregulatory capacity are not fully defined. In this study, we found that Mysm1 was induced in ASCs treated with inflammatory cytokines. ASCs with Mysm1 knockdown exhibited attenuated immunosuppressive capacity, evidenced by less inhibition of T cell proliferation, more pro-inflammatory factor secretion, and less nitric oxide (NO) production in vitro. Mysm1-deficient ASCs exacerbated inflammatory bowel diseases but inhibited tumor growth in vivo. Mysm1-deficient ASCs also showed depressed miR-150 expression. When transduced with Mysm1 overexpression lentivirus, ASCs exhibited enhanced miR-150 expression. Furthermore, Mysm1-deficient cells transduced with lentivirus containing miR-150 mimics produced less pro-inflammatory factors and more NO. Our study reveals a new role of Mysm1 in regulating the

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DOI: 10.1002/eji.201970400

immunomodulatory activities of ASCs by targeting miR-150. These novel insights into the mechanisms through which ASCs regulate immune reactions may lead to better clinical utility of these cells.

P2545

Characterization of major histocompatibility complex (MHC) class I and class II genes in northern pig-tailed macaques (Macaca leonina)

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The northern pig-tailed macaque (*Macaca leonina*) has been identified as an independent species from the pig-tailed macaque group. We have previously reported that this species macaque is a promising animal model in HIV/AIDS pathogenesis and vaccine studies due to susceptibility to HIV-1. However, the major histocompatibility complex (MHC) genetics in this animal remain poorly understood. Here, using a sequencing-based typing method, we identified and characterized the MHC genes including three class I loci (A, B, and I) and ten class II genes (*DPA*, *DPB*, D*QA*, *DQB*, *DRA*, *DRB, DMA*, *DMB*, *DOA and DOB*) in northern pig-tailed macaque for the first time. The MHC in this macaque was designated *Malo* following the nomenclature. A total of 40 MHC-I and 80 MHC-II alleles were identified and near 70% of them were never reported from other macaque species. The MHC-A and -B loci were both found to be duplicated and a single *DRA* and multiple *DRB* genes were observed as rhesus macaques or southern pig-tailed macaques. In addition, the patterns of polymorphism, positive selection and phylogenetic relationship between northern pig-tailed macaques and other macaque species were detected. Furthermore, three MHC alternative splicing variants were found. In all, we performed a compressive analysis on northern pig-tailed macaque MHC genes for the very first time. The characterization of full-length MHC class I and class II alleles in this study significantly improves understanding of the immunogenetics of northern pig-tailed macaques and provides the groundwork for future animal model studies.

P2546

Methylation of IFI44L gene for differential diagnosis of SLEwith tumor, hepatitis and myositis

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First,the purpose To detect the degree of methylation of IFI44L in PBMC-DNA of patients with tumor,hepatitis and myositis, and to distinguish it from SLE
Second,the materials Specimen source:491 whole blood samples were collected from clinically diagnosed patients,including 127 dermatomyositis,37 systemic vasculitis,44 leukemia,46 multiple myeloma,51 breast cancer,55 lymphoma,46allergic purpura,44 psoriasis and 41 hepatitis.The specimens were all from the Department of Clinical Laboratory,Department of Rheumatology,Hematology and Dermatology of the Second Xiangya Hospital of Central South University.The age distribution ranged from 15 to 72 years old.148 youth healthy control population.
Third, the method 1,DNA extraction 2,methylation specific high resolution dissolution curve method (MS-HRM) operation
Fourth, the experimental results Disease and healthy people:myositis (positive:17,negative:110),systemic vasculitis(0,37),leukemia(0,44),multiple myeloma(2,44),breast cancer(0,51), lymphoma(0,55),allergic purpura(1,45), psoriasis(0,44), hepatitis(1,40),and healthy people (8,140).
V. Analysis of experimental results and conclusions IFI44L methylation degree 25% evaluation of

DOI: 10.1002/eji.201970400

PBMC-DNA detection efficacy of myositis, systemic vasculitis, leukemia, multiple myeloma, breast cancer, lymphoma, allergic purpura, psoriasis, hepatitis and healthy people. Detection sensitivity and specificity: myositis 13.3%, 86.7%; systemic vasculitis 0%, 100%; leukemia 0%, 100%; multiple myeloma 4.4%, 95.6; breast cancer is 0%, 100%; lymph Tumor 0%, 100%; allergic purpura 2.2%, 97.8%; psoriasis 0%, 100%; hepatitis 2.5%, 97.5%; healthy people 4.6%, 95.4%. There was no significant difference between the above disease groups and the healthy control group. The degree of methylation of IFI44L at 25% can be used for differential diagnosis of SLE and tumor, hepatitis and other connective tissue diseases.

P2547

Study on the correlation between PDCD1 gene polymorphism and systemic lupus erythematosus in northern Han population

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To investigate the relevance of programmed cell death-1(PDCD1) gene single nucleotide pioymorphism and system lupus erythmatosus in mordern Han population.293 cases with SLE in northern Han and 292 healthy cases were selected, the detection of genotype and alleles were by polymerase chain reaction-ligase detection ,and turther sequencing was performed. PDCD1gene rs2227981 polymorphic loci were mainly CC genotype in the case group and control group ,there was no ststistical significance between the two groups; PDCD1gene rs2227982 polymorphic loci were mainly CT genotype in the case group and control group, there was no ststistical significance between the two groups; PDCD1gene rs11568821 polymorphic loci were mainly GG genotype in the case group and control group, there was no ststistical significance between the two groups. At the same time, haplotype analysis showed that haplotypes distribution in case group and control group did not significantly. Racial differences do not exist in PDCD1 gene rs2227981, rs2227982 and rs11568821, polymorphic loci of PDCD1gene sites rs2227981, rs2227982 and rs11568821 may be not the genetic factors of SLE.

B Cell Development and Activation

O185

Opposing roles for the related ETS-family transcription factors Spi-B and Spi-C in regulating B cell differentiation and function

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Cell fate decisions of antigen-specific B cell clones to differentiate into either antibody-secreting plasma cells or memory B cells, are regulated by transcription factors. The E26-transformation-specific transcription factor Spi-B is expressed throughout B cell development and is downregulated upon plasma cell differentiation. Spi-C is highly related to Spi-B and has similar DNA-binding specificity, but has a more restricted expression pattern. Spi-B is required for B cell proliferation in response to BCR or toll like receptor engagement, while the function of Spi-C is poorly understood. In this study, we investigated the roles of Spi-B and Spi-C in regulation of B cell differentiationand function. *Spib*$^{-/-}$ B cells differentiated into plasma cells in an accelerated manner under T cell-dependent conditions and

DOI: 10.1002/eji.201970400

had an altered pattern of gene expression including *Bach2*, a key regulator of secondary antibody responses and plasma cell differentiation. Deletion of one allele of *Spic* in *Spib*$^{-/-}$ B cells partially restored normal plasma cell differentiation and gene expression. Genome-wide chromatin immunoprecipitation sequencing analysis showed that Spi-B and Spi-C directly interacted with sites in the *Bach2* gene. Reporter gene analyisis showed that *Bach2* is transcriptionally activated by Spi-B and repressed by Spi-C. Interestingly, Bach2 also repressed *Spic*, suggesting mutual cross-antagonism between these two transcription factors. Deletion of one allele of *Spic* rescued defective secondary antibody responses in *Spib*$^{-/-}$ mice in response to immunization with T cell dependent antigen. In summary, these studies show that Spi-B and Spi-C are important regulators of B cell differentiation into antibody-secreting cells.

O186

Cmtm7 knockout inhibits B-1a cell development at transitional (TrB-1a) stage

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CMTM7 is a member of CMTM (CKLF-like MARVEL transmembrane domain-containing) family proteins, which link classical chemokines and the transmembrane-4 superfamily (TM4SF). CMTM7 was first identified as a tumor suppressor that inhibits the EGFR-Akt pathway. It also links membrane IgM (mIgM) and BLNK, and initiates BLNK-mediated signal transduction in B lymphoma cell lines. In this sudy, we identified that CMTM7 is essential for B-1a cell development. Following *Cmtm7* knockout, numbers of B-1a cells reduced markedly in all investigated tissues. Using bone marrow and fetal liver adoptive transfer model as well as conditional knockout mice, we showed that reduction of B-1a cells resulted from B cell-intrinsic defects. Due to B-1a cell loss, *Cmtm7*-deficient mice produced less IgM and IL-10, and were more susceptible to bacterial sepsis. Self-renewal and homeostasis of mature B-1a cells in *Cmtm7*$^{-/-}$ mice were not impaired, suggesting the effect of *Cmtm7* on B-1a cell development. Further investigations demonstrated that the function of *Cmtm7* in B-1a cell development occurred at the specific transitional B-1a stage (TrB-1a). In addition, the residual B-1a cells attenuated the phosphorylation of BCR signaling compounds, Syk, Btk and PLCγ2, after BCR cross-linking. They also exhibited altered Igμ repertoire exemplified by loss of V_H11. Thus, *Cmtm7* controls B-1a cell development at transitional stage.

O187

Integration of T-helper signals and BCR signaling mediates enhanced plasma cell differentiation of human memory B-cells through regulation of CD45 phosphatase activity

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Memory B-cells (MBC) effectively differentiate into antibody-secreting cells (ASC) generating protective antibodies, however, the exact mechanism is still unresolved. We reported that T-helper

DOI: 10.1002/eji.201970400

signals upregulate B-cell receptor-signaling through increasing CD45 phosphatase activity potentiating the activity of Src-family kinases. We now show that high CD45 phosphatase activity and B-cell receptor-signaling in MBCs is essential for their effective differentiation towards ASCs through enhancing the expression of transcription factors IRF4 and BLIMP1. Increasing T-cell help lead to incremental increase both in CD45 phosphatase activity and ASC-differentiation, and CD45 worked as a sensor of received T-cell help defining high-affinity germinal center plasma cell precursors. Mechanistically, T-helper signals upregulate CD45 phosphatase activity through increasing the surface binding of a CD45 ligand, Galectin-1. This study connects T-helper signals with BCR-signaling through CD45 phosphatase activity regulation and provides a mechanism for efficient ASC-differentiation of MBCs.

O188

Dock8 regulates BCR signaling and activation of memory B cells via WASP and CD19

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DOCK8 deficiency is an autosomal recessive-combined immunodeficiency characterized by recurrent infections, elevated serum IgE, severe allergic symptoms and autoimmunity. The role of Dock8 in B cell development and function has been revealed, but the role of DOCK8 on BCR signaling and function of memory B cells still remains elusive. In this study, we generated a **Dock8 knockout mouse model** and collected peripheral blood mononuclear cells from **Dock8 patients** to study the effect of **Dock8 deficiency on the BCR signaling and activation of memory B cells** with confocal microscopy and total internal reflection fluorescence microscopy.We found that the activation of key positive upstream BCR signaling molecules-pCD19 and pBtk is reduced. And the total protein and activated levels of WASP are decreased in Dock8 deficient mouse B cells.Our previous research has shown that WASP positively regulates cd19 transcription, furthermore, we found that Dock8 regulates cd19 transcription.The magnitude of BCR clustering, B cell spreading and BCR signalosome recruitment are severely disrupted in memory B cells of Dock8 patients compared to that of HC memory B cells and close to the levels of naïve B cells. Overall, our study provides a novel mechanism that **Dock8 regulates BCR signaling by regulating cd19 transcription** and the underlying mechanism of noncompetence of memory B cells in Dock8 patients.

O189

WASP couples Mst1 to regulate B cell development and B cell receptor signalin

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Mst1 is a serine/threonine kinase involved in cell survival, proliferation, apoptosis, and tumorigenesis. In mice, Mst1 regulates actin dynamics required for T cell adhesion and migration, which correlates with thymic egress and entry into lymphatic tissue. The role of Mst1 in B cells and how it may control actin-dependent processes has not been well characterized. WASP deficiency only moderately affects development and BCR signaling, suggesting WASP likely couples with other molecules. We investigated whether Mst1 couples with WASP to regulate B cell development and activation. Experimenting on Mst1/WASP double knockout (DKO) mice, we found a severedefect in bone marrow B cell development, BCR signaling, and early B cell activation. Furthermore, we found are reciprocal regulation between Mst1 and WASP in WASP and Mst1 KO mice, whereby, most importantly, Mst1 inhibits the expression and activation of WASP via increasing expression of WASP-interacting protein

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DOI: 10.1002/eji.201970400

(WIP). Interestingly, we also found an increased activation of Mst1 in Wiskott-Aldrich syndrome (WAS) patients, which may shed light on the mechanism involved in the development of lymphoma. Overall, our study provides a partner of WASP to regulate B cell development and BCR signaling as well as the reciprocal regulating molecular mechanism of one another.

O190

Critical roles of endonucleases Regnase-1 and Regnase-3 in early lymphopoiesis

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Transcriptional regulatory networks play a central role in differentiation of hematopoietic cells. However, it remains unclear how post-transcriptional machineries control hematopoiesis. Regnase-1 is an RNase critical for controlling inflammatory responses in innate and adaptive immunity. It forms a family consisting of three other genes with the conserved RNase and CCCH-type zinc finger domains. Here we show that Regnase-1 and its family member, Regnase-3 (encoded in the Zc3h12c gene), cooperatively regulate early stage lymphopoiesis in the bone marrow. Unlike Regnase-1, Regnase-3 did not regulate inflammatory responses in macrophages. Instead, we found that **doubly-deficient (DKO) bone marrow cells had a severe loss of B cell progenitors**, which was not observed in mice lacking either of these genes. On the other hand, double deficiency did not impair myelopoiesis. Competitive reconstitution experiments showed that **DKO cells had a profound defect of early-stage lymphopoiesis as early as at LMPP stage, whereas there was a mild increase of myeloid as well as megakaryocyte/erythroid lineages**, suggesting that Regnase-1 and Regnase-3 promote lymphopoiesis in a cell-intrinsic manner. Of note, RNA-seq analyses of LMPP revealed that DKO cells had altered transcriptome that was skewed toward myeloid- and megakaryocyte/erythroid-biased gene expressions. Intriguingly, we found that LPS in-vivo treatment, a mouse model for acute myelopoiesis, downregulated Regnase-1 protein expression in lymphoid progenitors. Taken together, we demonstrate that post-transcriptional regulation by Regnase-1 and Regnase-3 is critical for early stages of lymphopoiesis by controlling transcriptome biases of hematopoietic progenitors.

O191

Lymph node B cells promote tumor pre-metastatic niche formation by pathogenic IgG

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The pre-metastatic niche in secondary organs is critical for subsequent tumor metastasis. However, the role of B cell-mediated humoral immunity in promoting tumor pre-metastatic niche formation remains further investigation. In a spontaneous lymph nodes (LN) metastasis model of mouse breast cancer, we find that the primary tumor induces significant accumulation of B cells in tumor-draining LNs prior to tumor cell metastasis. And both B cell recruitment and proliferation may contribute to their accumulation in LNs. These B cells are mainly located in the LN germinal centers and produce abundant pathogenic IgG. Functionally, the B cells can promote breast cancer metastasis to LNs by pathogenic IgG, which promotes the activation of NF-κB pathway and CXCR4/SDF1α axis-mediated metastasis. Mechanistically, these pathogenic IgG induces tumor-intrinsic HIF-1α and COX-2 activation contributing to the upregulation of CXCR4 and SDF1α respectively. HIF-1α promotes

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DOI: 10.1002/eji.201970400

chemokine receptor CXCR4 expression on tumor cells and COX-2-mediated PGE2 secretion induces LN matrix cells to secrete the chemokine SDF1α, thus promoting the pre-metastatic niche formation in LNs. Therefore, our findings yield additional insights into newly proposed roles for B cells and antibodies in the formation of pre-metastatic niche and organotropic metastasis, suggesting new potential targets for the diagnosis and treatment of cancer.

O192

Determination of *Jak3* and *Ikzf3* as recurrent driver genes cooperating with deletions of genes encoding Spi-B and PU.1 in B cell acute lymphoblastic leukemia

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Precursor B-cell acute lymphoblastic leukemia (B-ALL) is associated with recurrent mutations in genes encoding transcription factors that drive B cell proliferation or development. The transcription factors Spi-B (encoded by *Spib*) and PU.1 (encoded by *Spi1*) regulate the development and function of B cells. We previously showed that deletion of both genes (ΔSpib/Spi1) leads to 100% incidence of B-ALL in mice. ΔSpib/Spi1 mice develop leukemia around 18 weeks of age, suggesting that additional driver mutations are required to cooperate with the initial Spi-B and PU.1 knockouts to drive leukemogenesis. We hypothesized that recurrent secondary driver mutations are necessary for the development of B-ALL in ΔSpib/Spi1 mice. Using whole exome sequencing and RNA sequencing on thymic leukemias isolated from mice, we determined that 3/8, 5/8, and 5/8 leukemias had high-frequency variants in *Jak1, Jak3,*and *Ikzf3*, respectively. *Jak* variants were predicted to be gain-of-function while *Ikzf3* variants were predicted to be loss-of-function. Mutational signature analysis demonstrated that ΔSpib/Spi1 leukemias had a high frequency of C->A transversions, suggesting 8-oxoguanine DNA damage. Retroviral transduction of pro-B cells with vectors encoding mutated *Jaks* resulted in a proliferative advantage and promoted leukemia. Treatment of leukemia cells with Ruxolitinib, a JAK inhibitor, prevented IL-7-dependent proliferation. In conclusion, we have shown that *Jak3* and *Ikzf3* are recurrently mutated in our mouse model and that gain-of-function *Jak* variants promote leukemia development.

LB007

Moroccan classification of the common variable immunodeficiency

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The heterogeneity of the common variable immunodeficiency called for a clinical classification as well as the biological relevance of B lymphocytes. A European multicentre trial was launched to develop a classification scheme based on B-cell phenotyping by flow cytometry and the clinical manifestations indicated in these patients. Establish a clinical and immunophenotic classification of Moroccan cases with a CVID.20 patients with CVID in this study who met ESID/PAGID inclusion criteria.Phenotyping of B-cell sub-populations is performed by flow cytometry. A reduction in switched memory B lymphocytes has been confirmed in most patients, associated with an increased risk of splenomegaly and granulomatous disease. An expansion of CD21low B lymphocytes has marked patients with splenomegaly.

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DOI: 10.1002/eji.201970400

Based on these findings and the pathogenic consideration of B cell differentiation, we suggest an improved classification for CVID (EUROclass / MAGHclass), separating patients with almost absent B cells, and those who are significantly reduced to switched memory B cells.
Keywords: CVID, Lymphocyte B, EUROclass, MAGHclass

LB045

Pathogenic CARD11 mutations impact B-cell development and differentiation through a non-canonical pathway

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Pathogenic CARD11 mutations cause aberrant NF-κB activation, which is presumably responsible for multiple immunological disorders. However, whether there is an NF-κB independent regulatory mechanism contributing to CARD11 mutations related pathogenesis remains undefined. By using three distinct genetic mouse models, *Card11* KO mouse model mimicking primary immunodeficiency, CARD11 E134G point mutation mouse model representing BENTA disease, and the mouse model bearing oncogenic K215M mutation, here we show CARD11 has a non-canonical function as a negative regulator of the AKT-FOXO1 signal axis, independent of NF-κB activation. Although BENTA disease related E134G mutant elevates NF-κB activation, we unexpectedly find that E134G mutant mice phenotypically copy *Card11* KO mice, in which NF-κB activation is disrupted. Mechanistically, E134G mutant causes exacerbated AKT activation and reduced FOXO1 protein in B cells similar to that in *Card11* KO cells. Moreover, the oncogenic CARD11 mutant K215M reinforces the importance of the non-canonical function of CARD11. In contrast to E134G mutant, K215M obtains a stronger inhibitory effect on AKT activation and more stabilized FOXO1. Likewise, E134G and K215M mutant exhibit the converse impacts on B cell development and differentiation. Our results demonstrated that CARD11 orchestrates dual signaling pathways in B cells. The dysregulated non-canonical, regulatory function on AKT-FOXO1 signal axis caused by pathogenic CARD11 mutants also plays an indispensable role.

LB082

A standardized and reproducible method for expansion and activation of human primary B cells

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The frequency of B cells in peripheral blood is low, therefore expansion is necessary to obtain sufficient numbers for experimental set-ups. B cells express co-stimulatory surface receptors, such as CD40, that mediate interactions with T cells which results in further B cell expansion.
To overcome the difficulties and limitations of a reliable B cell expansion, the B Cell Expansion Kit was developed. Using the Kit expansion rates of up to 20 times can be achieved within 14 days of cultivation. At the same time, the combination of premium grade cytokines with minimal lot-to-lot variation and optimized xeno-free StemMACS HSC Expansion Medium ensure high reproducibility.
The key of the Kit is the interaction of B cells and CD40-Ligand. CD40-Ligand, a member of the TNF superfamily, is transiently expressed on activated $CD4^+$ T cells. This interaction is involved in B cell differentiation and proliferation, isotype class-switching, and protection from apoptosis. To increase the biological activity of the CD40-Ligand and, thus, maximize expansion, the Kit contains also a cross-linking antibody.
Here we provide experimental evidence for the reproducible expansion of B cells. For this purpose,

DOI: 10.1002/eji.201970400

CD19$^+$ B cells were isolated, expanded with the B Cell Expansion Kit over a period of 14 days and the subset distribution, the activation state as well as the cytokine secretion were analyzed. Besides this, the comparison of the standard protocol and an addition of MACS premium grade human IL-21 showed an earlier onset of expansion, induction of mature B cells formation and an altered cytokine secretion profile.

P0085

The roles of B cell TLR signaling in promoting germinal center reaction

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Although TLR signaling in B cells has been implicated in promoting the germinal center (GC) reaction in viral infections and autoimmune diseases, the underlying mechanism is poorly understood. We previously reported that a bacterial phage Qb-derived virus-like particle could induce GC reaction via stimulating TLR/MyD88 signaling selectively in B cells. Using this new model antigen, we recently discovered that there are at least two mechanisms by which B cell TLR signaling can promote GC reaction. First, activating B cell TLR signaling can strongly enhance the initial proliferation of antigen-specific B cells, and it also can induce Bcl-6 expression in a fraction of the activated B cells, thus increasing the number of precursor cells for GC reaction. Interestingly, these effects take place mainly at the beginning stage of B cell activation. Second, TLR signaling in B cells can promote generation of Tfh cells by enhancing CD4$^+$ T cell proliferation and differentiation. Intriguingly, under this circumstance, it was antigen-specific B cells, rather than dendritic cells, were the dominant antigen-presenting cells that primed naïve CD4$^+$ T cell activation. In this meeting, we will present our recent results regarding the roles of B cell TLR signaling in anti-viral and autoimmune GC reaction. The mechanism revealed in these works will provide insight for future utilization of this signaling pathway in designing novel vaccine and for treating autoimmune diseases.

P0086

Regulatory roles of AIM2 on differentiation and activation of B cells

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Experimental studies found that B-cells expressed high levels of AIM2 in human tonsils. Therefore, we further explore the effect of AIM2 on differentiation and activation of B cells. CD19+CD138+ B-cells expressed significantly higher levels of AIM2 compared to CD19+ B-cells in human tonsils. Immunohistochemical analysis revealed that CD19+ B-cells around germinal center expressed high levels of AIM2. Flow cytometry analysis suggested that the expression of AIM2 in peripheral blood B cells of SLE patients was lower than that of normal controls. After stimulating naïve B cells with LPS, a more detailed analysis of different B cells (memory B-cells, plasma B-cells, naïve B-cells) demonstrated that AIM2 was preferentially expressed in naïve B-cells, whereas other B cells expressed lower levels of AIM2 and memory B-cells expressed least. In CD19^{cre}AIM2$^{f/f}$ model, the expression of CD3 and CD19 in spleen was down regulated and the activation of T cells was enhanced. In Rag2 mouse model induced by KLH, the expression of GC B cells was down regulated with the induced expression of CD3 and the reduced expression of CD4. Together, these observations indicate that AIM2 is highly expressed in naïve B cells and promotes differentiation of B cells.

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DOI: 10.1002/eji.201970400

P0087

Antibody secreting cells require EZH2 for maximal metabolism, antibody production, and repression of the B cell transcriptional program

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The differentiation from naïve B cells to antibody secreting cells(ASCs) is the key part of the humoral immune response. Epigenetic remodeling is required during ASCs terminal differentiation; however, little is known about direct functions of epigenetic enzymes in ASCs. Enhancer of zest 2 (EZH2), a methyltransferase, mediates the silence of some genes through the formation of H3K27me3. A tamoxifen-inducible deletion was employed *in vivo* to analyze EZH2´s role in ACSs terminal differentiation and function independent of T cell help and a germinal center. Following stimulation with non-protein antigen LPS or protein antigens influenza PR8, *Ezh2*-deficient ASCs were reduced in number and up regulated inflammatory pathways, B cell lineage transcription factors, and Blimp-1 repressed genes; but failed to induce metabolic pathways associated with oxidative phosphorylation and glycolysis. Genes that normally gained H3K27me3 were dysregulated and demonstrated increased chromatin accessibility at those loci in the absence of EZH2. EZH2 was required for maximal antibody secretion as ASCs demonstrated reduced mitochondrial respiration and glucose metabolism in the absence of EZH2. Together, these data demonstrate the critical role of EZH2 in facilitating epigenetic changes that regulate terminal differentiation and antibody production of ASCs.

P0088

Human gingiva-derived mesenchymal stem cells treat lupus nephritis through targeting on B cells via CD39-CD73 signal pathway

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B lymphocytes play a critical role not only in host defense but also in autoimmune diseases. Although there are some already established biological therapeutic approaches or agents targeted on B cells to treat autoimmune diseases, not all of them have satisfactorily therapeutic effects on autoimmune and inflammatory diseases. Mesenchymal stem cells (MSCs), especially bone marrow-derived MSCs (BMSCs), have emerged as a new way to solve the issue because of their immunomodulatory and tissue regeneration properties. Nonetheless, BMSCs hold the drawbacks such as difficult assessment, dysfunction of autologous BMSCs and tumorigenesis. Compared with BMSCs, human gingiva-derived MSCs (GMSCs) are more superior in preventing and/or treating autoimmune diseases In this study, we demonstrated that administration of GMSCs significantly treated lupus by decreasing proteinuria levels, frequency of plasma cells, levels of autoantibodies and lupus nephritis severities. We further observed GMSCs in fact directly targeted on B cells that includes direct suppression of activation and proliferation, plasmocyte differentiation and autoantibody production in inflammatory milieu. The

DOI: 10.1002/eji.201970400

blockage of CD39/CD73 pathway dramatically abrogated the suppressive capacities of GMSCs *in vitro* and *in vivo*. Collectively, manipulation of GMSCs provides a promising strategy on treatment of patients with systemic lupus erythematosus (SLE) and other B cell-associated autoimmune diseases.

P0089

Fcμ receptor promotes the survival and activation of marginal zone B cells and protects mice against bacterial sepsis

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The marginal zone B cells (MZB) are located at the interface between the circulation and lymphoid tissue and as a gatekeeper play important roles in both innate and adaptive immune responses. We have previously found that MZB are significantly reduced in mice deficient in the IgM Fc receptor (FcμR) but how FcμR regulates the development and function of MZB remains unknown. In the present study, we found that both marginal zone precursor (MZP) and MZB were decreased in *FcμR*$^{-/-}$ mice. The reduction of MZP and MZB was not due to impaired proliferation of these cells but rather due to their increased death. Further analysis revealed that *FcμR*$^{-/-}$ MZB had reduced tonic BCR signal, as evidenced by their decreased levels of phosphorylated SYK and AKT relative to WT MZB. MZB in *FcμR*$^{-/-}$ mice responded poorly to LPS *in vivo* as compared to MZB in WT mice. Consistent with the reduced proportion of MZB and their impaired response to LPS, antibody production against the type 1 T-independent Ag, NP-LPS, was significantly reduced in *FcμR*$^{-/-}$ mice. Moreover, *FcμR*$^{-/-}$ mice were highly susceptible to *Citrobacter rodentium*-induced sepsis. These results reveal a critical role for FcμR in the survival and activation of MZB and in protection against acute bacterial infection.

P0090

The RAG1's non-core region suppresses genomic instability in *BCR-ABL1*$^{+}$ B-cell lymphoblastic leukeamia

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Antigen receptor genes are assembled by controlled, large-scale deletions and inversions. Errors in this process produce aberrant genomic rearrangements, which can fuel the development of T- and B-cell malignancies. RAG1'non-core region in preserving genomic stability and to what extent leukaemia can be prevent remain poorly defined. We took advantage of the bone marrow transplantation system and established two mouse model of *BCR-ABL1*$^{+}$ B-lymphoblastic leukeamia in wild type (WT) mice and core RAG1(cR1) mice which amino acid 1-383 are deleted. The results showed that leukaemic cells from cR1 mice demonstrated stronger potential for lymphomagenesis, such as significantly declined in survival rate and cell apoptosis than WT. In genome-wide analysis, the wrong rearrangements of Ig locus in leukaemia cells from cR1 (6, average) is more numerous than WT (1, average). The leukeamia cells displayed one or few predominant rearrangements, indicating a clonal or oligoclonal origin. In addition, off-target rearrangements from cR1 leukaemia (5194, average) is significant more than WT (2155, average). Deletion is the predominant DNA structure mutation and mutations are diversified in cR1 leukaemic cells. The number of translocation from cR1(1251, average) is also more than WT (350, average). Off-target rearrangements scattered across tumor genomes. These results showed that the deletion of RAG1's non-core region increased the genomic instability in tumor cells.

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DOI: 10.1002/eji.201970400

P0091

OTUB1 is an essential regulator of B-cell development

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Deubiquitination is emerging as a central player during the development of the immune system and the establishment of a protective immunity. By performing comparative expression profiling analysis, we observed that OTUB1 is one of the most commonly expressed deubiquitinases (DUB) in both patient-derived normal B-cells and B-cell lymphomas.
Using mouse genetics, we identified OTUB1 as a key regulator of splenic B-cell differentiation. We found that the B-cell specific ablation of OTUB1 results in a ~4-fold accumulation of Marginal Zone B-cells (MZB) and a concomitant decrease in Follicular B-cells (FoB) in the spleen. This misbalance in the MZB/FOB ratio correlates with (i) a two-fold increase in the proportion of circulating mature B-cells; (ii) a drastic disorganization of the spleen with a mis-localization of MZB within and outside the follicles. Importantly, altering OTUB1 levels correlates with a dysbiosis and an enrichment of *Alistipes*bacteria in the gut microbiota. These data suggest that OTUB1 plays a key role in B-cell differentiation and the humoral response.
Using CRISPR-generated knock-in mouse models, we observed that the inactivation of OTUB1 catalytic site results in a similar misbalance in the MZB/FOB ratio as observed previously. To define the substrate(s) that OTUB1 targets during B-cell differentiation, we mapped the interactome of both wild-type OTUB1 and its inactivated mutant by mass spectrometry. We observed that OTUB1 interacts with several proteins involved in cell-cell adhesion and communication. Altogether, our data point toward a key role for OTUB1 in splenic B-cell differentiation by modulating key intracellular signaling cascades.

P0093

Dissecting the dynamic regulation of Histone H2B post-translational modifications at DNA double-stranded breaks during antibody class switch recombination

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Antibodies play key roles in humans by protecting against pathogen infection. Activated germinal center B cells mutate and rearrange their Immunoglobulin (Ig) genes to produce high affinity antibodies of different classes through two specialized genetic events, somatic hypermutation and class switch recombination (CSR). Activation-induced cytidine deaminase (AID) induces DNA double-stranded breaks (DSBs) within Ig genes to induce CSR through a deletional recombination event that allows B cells to undergo isotype switching without altering the antigen-binding specificity of an antibody, thus generating antibodies with different effector functions. The unproductive repair of these programmed DSBs causes chromosomal translocation and leads to the development of B cell lymphoma. Histone post-translational modifications (PTMs) at DSBs are crucial for sensing DNA damage as they recruit various DNA repair factors to DSBs. We have previously shown the monoubiquitination (ub) and deubiquitination of histone H2B at lysine 120 (H2BK120) are involved in DSB repair and CSR. It has been recently suggested that in response to DSBs, H2BK120 undergoes a different type of histone PTM, namely, acetylation (ac). However, little is known regarding the functional consequence of H2BK120ac in DNA repair and CSR. Here, *using chromatin immunoprecipitation we have characterized the dynamic regulation of these histone PTMs at site-specific DSBs in mouse CH12F3-2 and human Ramos cells. The role of SAGA chromatin-modifying*

DOI: 10.1002/eji.201970400

complex in deubiquitinating and acetylating H2BK120 has also been investigated. Our results show that PTMs of H2BK120 represent a potential novel DSB repair pathway in DNA damage response and CSR.

P0094

Antibodies encoded by FCRL4-bearing memory B cells preferentially recognize commensal microbial antigens

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FCRL4, a low-affinity IgA receptor with strong immunoregulatory potential, characterizes a unique subpopulation of mucosa-associated tissue-based memory B cells (Bmem). In contrast to healthy volunteers, dysregulated FCRL4$^+$ Bmem were reported in circulation of individuals with chronic infectious diseases including malaria, HCV and HIV. In an effort to gain further insight into these unique Bmem we investigated the antibody repertoire encoded by FCRL4-bearing Bmem of healthy individuals. In this study, we performed a comparative analysis of antibody repertoires of FCRL4$^+$ and FCRL4$^-$ Bmem isolated from human tonsil samples by high-throughput sequencing approaches as well as by single cell antibody cloning. We observed that FCRL4$^+$ Bmem had lower levels of somatic mutations in their antigen receptor sequences compared to FCRL4$^-$ Bmem while displaying comparable frequencies of variable gene family usage. Importantly, antibodies derived from FCRL4$^+$ Bmem showed increased reactivity to commensal microbiota, a characteristic that was not accompanied by increased auto-reactivity or poly-reactivity. Our study links expression of the immunoregulatory FCRL4 molecule with increased recognition of commensal microbial antigens.

P0095

Antibody repertoire analysis using next-generation sequencing reveals enhanced formation of poly-reactive antibodies in the absence of pre-B cell receptor

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Although the molecular mechanisms that yield the extremely large antibody repertoires are precisely understood, comprehensive description of the global antibody repertoire generated in individual bodies has been hindered by the lack of powerful measures. To obtain holistic understanding of the antibody-repertoire space, we used next-generation sequencing (NGS) to analyze the deep profiles of mouse and human antibody repertoires. During B cell development in bone marrow the pre-B cell receptor (pre-BCR), consisting of the μ heavy chain (μHC) and the surrogate light chain (SLC), plays important roles during B cell development. The formation of the pre-BCR is considered a prerequisite for B cell development; however, a significant number of peripheral mature (leaky) B cells exist in SLC-deficient mice. The antibody repertoires of leaky B cells thus reflect the absence of pre-BCR function. We dissected the antibody repertoire features of the leaky B cells using NGS and primary component analyses. Analysis of the CDR-H3 sequences of μHC repertoires revealed that certain bone marrow B cells with particular CDR-H3 profiles undergo clonal expansion in leaky B cells. Part of these CDR-H3s contain arginine(s) in the middle of the CDR-H3 loop in SLC-deficient mice, whereas few arginine(s) exist in this middle loop in WT CDR-H3s in the absence of clonal expansion. This CDR-H3 feature in

DOI: 10.1002/eji.201970400

leaky B cells presumably reflects the role of the pre-BCR in poly-reactive antibody regulation, since arginine(s) are often found in the antigen-binding site of poly-reactive antibodies.

P0672

Blnk/Hdac9 maintains the nuclear localization of Foxo1 through supressing BCR-ABL1 kinase activity in *BCR-ABL1*$^+$ B-cell acute lymphoblastic leukemia

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The BCR-ABL1-positive B-cell acute lymphoblastic leukemia (BCR-ABL1$^+$ B-ALL) encoding constitutively active BCR-ABL1 tyrosine kinase, which activate its downstream signaling pathways and drive malignant transformation. However, the molecular mechanism of regulation of BCR-ABL1 tyrosine kinase activity has not been elucidated in BCR-ABL1$^+$B-ALL. Here, we demonstrate that the tyrosine kinase activity of BCR-ABL1 induce γH2AX, a biomarker of genome instability, through supressing the expression of Blnk, Foxo1 and Hdac9 in pro-B ALL cells established by transfecting pro-B cells with BCR-ABL1-expressing retrovirus, which is proved through increased expression of Blnk, Foxo1 and Hdac9 in pro-B ALL cells after tyrosine kinase inhibitor(TKI) treated. We establish the Blnk/Foxo1/Hdac9 knock-out pro-B ALL cell lines by using CRISPR-Cas9 technology, our results show that kinase activity of BCR-ABL1 is enhanced along with dramatically increased proliferation and reduced sensitivity to TKI induced apoptosis, and higher expression of γH2AX demonstrate BCR-ABL1 contribute to genome instability in Blnk/Foxo1/Hdac9 knock-out pro-B ALL cells. Furthermore,we demonstrate that Foxo1 phosphorylation that are regulated by Blnk via supressing PI3K-AKT pathway, on the other hand, the nuclear localization of Foxo1 is maintained by Hdac9 through reducing Foxo1 acetylation to make it hard to been phosphorylated in pro-B ALL cells. We conclude that Blnk/Hdac9 and BCR-ABL1 regulate the nuclear localization of Foxo1 with antistatic function on each other to regulate cell proliferation and genome instability in BCR-ABL1$^+$ pro-B-ALL.

P0673

High mobility group box 1 enhances Toll-like receptor 9 expression in B cells in myeloperoxidase-ANCA-associated vasculitis

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Objective: High mobility group box 1 (HMGB1) played pathogenic role in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Recent findings demonstrated that Toll-like receptor 9 (TLR9) was involved in B cell tolerance breaking of autoimmune disease, including AAV. The current study aimed to investigate the effect of HMGB1 on TLR9 in B cells of AAV.
Methods: 32 patients with myeloperoxidase (MPO)-AAV in active stage were recruited. Intracellular TLR9 expression in various B cell subpopulation of the whole blood was detected by flow cytometry and the correlation with clinical data was analyzed. To explore the effect of HMGB1 on B cell, peripheral blood mononuclear cells (PBMCs) from AAV patients were isolated. After stimulated with HMGB1, TLR9 expression in various B cell subpopulation and proliferation ratio of B cells were analyzed by flow cytometry.
Results: Intracellular TLR9 expression in B cells, memory B cells and plasmablasts correlated with erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). In particular, TLR9 expression in plasma cells correlated with ESR (r=0.501, p=0.007), CRP (r=0.501, p=0.004), serum creatinine (r=0.427, p=0.015), eGFR (r=-0.382, p=0.031) and Birmingham Vasculitis Activity Score (r=0.397,

DOI: 10.1002/eji.201970400

p=0.033). Compared with the control group, TLR9 expression in plasma cells and the proliferation ratio of live B cells by HMGB1 stimulation were significantly upregulated (234.26±137.24 *vs.* 119.27±78.33, p< 0.05;15.73%±6.18% *vs.* 8.81%±4.69%, p< 0.05).
Conclusion: TLR9 expression in plasma cells was associated with disease activity of MPO-AAV. HMGB1 could enhance TLR9 expression and B cell proliferation. These indicated a role of HMGB1 on TLR9 in B cells in MPO-AAV.

P0674

Protein 4.1R regulates the fate of primary B cells by regulating canonical NF-κB signaling

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During immune response, B cells can undergo differentiation to IgM secreting plasma cells differentiation (PCD) or enter the memory pathway characterized by class switch recombination (CSR). Both of these processes occur in activated B cells, which are reported relate to membrane-association proteins and adaptors. Protein 4.1R acts as an adaptor, linking membrane proteins to cytoskeleton, involving cell activation, differentiation and cytokine secretion. However, the effect of 4.1R on regulating B cell fate remains unclear. Here, we show an important association between B cell fate and 4.1R. In vitro, primary B cells were stimulated by LPS combined with IL-4, results showed that 4.1R deficiency ($4.1R^{-/-}$) compared with wildtype ($4.1R^{+/+}$) B cell augment expression of activation-induced cytidine deaminase (AID) and germline, resulting in switch IgM to IgG1, increased $IgG1^{+}$ B cells. Interestingly however, IgG1 and IgM secretion and $CD138^{+}$ B cells were reduced. Which suggesting 4.1R regulates B cell activation signals to determine B cell fate. Mechanical analysis, during the whole process, 4.1R regulates canonical NF-κB rather than non-canonical NF-κB to promote the expression of Pax5, Bcl6 and IRF4, and reduce Blimp-1 expression, which leads to B cell activation, proliferation and up-regulate of CSR while down-regulate PCD. Further experiments indicate that over-activation of canonical NF-kB may induce apoptotic signaling to cause PCD apoptosis reduce PCD number. Our results suggest that 4.1R acts as a B cell fate regulator via inhibiting the canonical NF-κB pathway.

P0675

C-terminal deletion-induced AID condensation leads to immunodeficiency

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Activation-induced cytidine deaminase (AID)-initiated DNA lesions are required for antibody class switch recombination and somatic hypermutation to diversify antibody repertoire. The inactivation of AID leads to immunodeficiency termed as Hyper-IgM syndrome. We found that C-terminal-deleted AID leads to immunodeficiency despite strong cytidine deaminase activity and demonstrated that C-terminal loss causes AID to form condensates, insulating its mutagenic activity from genomic targets. AID C-terminal tail inhibits protein condensation induced by its N terminus and an Arginine-rich patch. Moreover, C-terminal-deleted mutants sequestrate wild-type AID to condensates in sub-nuclear compartments, resulting in dominant-negative phenotype. These findings showed that phase-separated mutagenic activity of AID C-terminal deletion mutants causes dominant negative immunodeficiency diseases.

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DOI: 10.1002/eji.201970400

P0676

CD10-positive granulocytes in bone marrow decreased significantly in myelodysplastic syndrome patients than aplastic anemia and iron deficient anemia patients

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Myelodysplastic syndromes (MDS) are highly heterogeneous myeloid neoplasms, and a large number of patients are difficult to diagnose and classify by blood and bone marrow examination. As a surface marker of granulocyte, studies have shown CD10 can be used to define the degree of granulocyte maturation in MDS patients. However, whether it can be used for differential diagnosis of MDS and other hematological diseases, such as aplastic anemia (AA), iron deficient anemia (IDA) has not been reported till now. In this study, we found CD10-positive granulocytes in bone marrow decreased significantly in MDS patients than AA and IDA patients, indicating the index has clinical diagnostic and differential diagnostic value.

P0677

Analysis of the association between candidate single nucleotide polymorphisms and multiple myeloma susceptibility

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Background: Multiple myeloma (MM) is an extremely complex plasma cell malignancy and the pathogenesis of this malignant disease is still unclear. Genome-wide association analyses (GWASs) have identified numerous susceptibility gene of cancer, whereas it is necessary to verify among different ethnic-groups.
Purpose: Here, we aim at analyse the association of candidate single nucleotide polymorphisms and multiple myeloma risk in Han Chinese.
Method: The SNP loci of candidate gene include the *HLA-I* (rs6457327), *HLA-II* (rs2647012 and rs7755224), *CXCR5* (rs4938573), *ETS1* (rs4937362), *LPP* (rs6444305), *NCOA1* (rs79480871) and *LRP1B* (rs61070260). Genomic DNA was extracted from whole blood samples of 827 MM patients and 709 healthy controls, polymerase chain reaction (PCR) and matrix-assisted laser deionization-time-of-flight mass spectrometry (MALDI-TOF MS) were used for the amplification and genotyping of gene, and the results were analyzed by MassArray Typer software. Allele frequency distribution of MM patients and the control group was evaluated by the chi-square test and Fisher´s exact test, P value less than 0.05 was statistically significant.
Results: Seven SNPs (rs6457327, rs2647012, rs4938573, rs4937362, rs6444305, rs79480871 and rs61070260) of the 8 were successfully genotyped and one (rs7755224) was failed. Allele frequencies of rs79480871 and rs61070260 were associated with susceptibility in MM patients (P=9.2×10-5; P=3.937×10-37).
Conclusion: NCOA1 (rs79480871) and LRP1B (rs61070260) genes polymorphisms are associated with susceptibility to MM.

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DOI: 10.1002/eji.201970400

P0678

The biological significance of PD-1/PD-L1 signal on regulatory B cells in invasive breast cancer

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Accumulating evidence suggests that Regulatory B cells (Bregs) play important roles in inhibiting the immune response in tumors. The PD-1 and PD-L1 were important molecular to maintains the balance of the immune response and immune tolerance. This study aims to evaluate the induction of PD-1/PD-L1 signaling on Bregs in invasive breast carcinoma (IBCa). In an effort to explore the role of PD-1/PD-L1 signaling on Bregs in human breast cancer etiology, we examined the expression of CD19 and PD-1 in breast tumor tissues, the presence of B cells, PD-1^{+}B cells and Bregs in peripheral blood of patients with breast tumor, the levels of sPD-L1 and IL-10 in serum of patients and assessed their relationship. We found the expression of CD19 and PD-1 on TILs was significantly higher in IBCa than that in breast fibroadenoma tissues (FIBma). Meanwhile, the proportion of PD-1^{+}B cells and Bregs in peripheral blood of BC was significantly higher than that of FIBma. And the levels of sPD-L1 and IL-10 in serum of BC were higher than that of FIBma, and the positive correlation of sPD-L1 and IL-10, IL-10 and Bregs, sPD-L1 and Bregs were observed. We further demonstrated that sPD-L1 could induce Bregs differentiation and IL-10 mRNA in a dose-dependent manner *in vitro*. Finally, we showed that the induction of Regulatory T cells by Bregs was further shown to promote proliferation and inhibit the apoptosis of tumor cells. Together, these results are suggestive of PD-1/PD-L1 signaling induces the expansion and differentiation of Bregs.

P0679

Autoreactive CD80^{high} MZ B cells are resistant to gamma radiation-induced apoptosis

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Marginal zone (MZ) B cells are CD21^{high}CD23^{low} B cells, which respond rapidly against blood-borne pathogens. We previously showed that there are a CD80^{high} subpopulation of MZ B cells that contain anti-type II collagen (CII) B cells and are more abundant in DBA/1 mice than in C57BL/6 mice. Here, we observed that the CD80^{high} MZ B cells were maintained relatively stable after 2 Gy irradiation whereas significant proportions of CD80^{low} MZ and follicular B cells died after the irradiation. The number of the CD80^{low} MZ B cells was recovered in about 2 months. The radio-resistance of the CD80^{high} MZ B cells appears to depend on BCR-mediated signaling since the radio-resistance was decreased upon treatment with ibrutinib, a BTK inhibitor, in the in vitro culture of MACS-purified splenic B cells and also on T cells since anti-CD40 stimulation increased the radio-resistance. Upon addition of unstimulated or stimulated CD4^{+} T cells, stimulated CD4^{+} T cells enhanced the survival of both CD80^{high} and CD80^{low} MZ B cells. We performed mRNA gene expression analysis to find the differences between CD80^{high} and CD80^{low} MZ B cells. Developmentally, the CD80^{high} MZ B cells were almost absent in 2 weeks-old mice similarly to CD80^{low} MZ B cells. We observed that irradiation accelerated the CIA progression and the increase in serum concentration of anti-type II collagen (CII) Abs upon immunization with CII in the DBA/1 mice. In summary, the CD80^{high} MZ B cells are thought to be a MZ B cell subpopulation distinct from the CD80^{low} MZ B cells.

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DOI: 10.1002/eji.201970400

P0680

Peritoneal B1a and marginal zone B cells upregulated LAG-3 upon toll-like receptor stimulation and ADAM10/17 regulated level of LAG-3 on B cells

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Lymphocyte activation gene 3 (LAG-3/CD223) is one of immune checkpoint molecules. LAG-3 is expressed on many kinds of immune cells, including activated CD8+, CD4+, NK cells, and dendritic cells, and known for having inhibitory roles in those immune cells. A recent study suggested that LAG-3 was also expressed on activated B cells. But the significance of the LAG-3 expression on B cells is still unclear in terms of its role, regulation, and expression level in each B cell subsets. In this study, we studied LAG-3 expression in various subsets of B cells upon Toll-like receptor (TLR) stimulation and its regulation. Among B cell subsets, peritoneal B1a cells highly upregulated LAG-3 upon the TLR3, 4, 7, and 9 simulation. Marginal zone B cells (MZB) also increased LAG-3 expression when they were stimulated by TLR 4 ligand, lipopolysaccharide (LPS). Given that peritoneal B1a cells and MZB cells are innate-like B cells, the expression of LAG-3 may be related to their characteristics of innate-like B cells. When we measured level of LAG-3 on peritoneal B1a cells at different times after LPS stimulation, the LAG-3 expression increased at first, but 24 hours later, its level decreased. And we found that this change of LAG-3 expression was modulated by a disintegrin and metalloproteinase 10/17 (ADAM10/17) similarly to the regulation of LAG-3 on T cells. In conclusion, we supposed that the balance between synthesis and shedding by ADAM10/17 was important for the expression of LAG-3 on B cells upon TLR stimulation.

P0681

Ubiquitination of BCR-mediated signaling protein by Pellino 1 in B cell development

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B lymphocytes (Bcells) express a variety of B cell receptors (BCRs), Toll-like receptors (TLRs) and cytokine receptors that leading to enhancements in B cell survival, proliferation, cytokine and antibody production, and development. These receptors synergize with signals and augment both innate and adaptive immune functions of B cells. Therefore, dysregulated B cell signaling can function as a primary driver of many human diseases such as autoimmune disorders, leukemia and lymphoma. Ubiquitination is an enzymatic post-translational modification that plays a critical role in the overall regulation of signaling proteins in B cell signaling transduction cascades. Pellino 1 (Peli1) is a signal-responsive E3 ubiquitin ligase acting as a critical mediator for a variety of immune receptor signaling pathways through catalyzing ubiquitin chains of several key molecules linked to lysine 48 or 63. Peli1 expression is highly suppressed in normal or non-pathologic conditions. Notably, Peli1 expression and activation is regulated by receptor-mediated signaling and under certain pathogenic condition such as asthma and diffuse large B cell lymphoma. However, the biological roles of BCR signaling-induced Peli1 in B cell development, proliferation and function remain unclear. We developed transgenic and knock-out mice models to investigate altered expression of Peli1 and could induce abnormalities in regulation of B cell development, tolerance, or function. Using these mouse models, this study includes the relationship between the deregulated induction of Peli1 and the abnormal B cell development and activation.

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DOI: 10.1002/eji.201970400

P1268

Evaluation of tofacitinib on B cell subsets in patients with rheumatoid arthritis

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Objective: Tofacitinib is an oral JAK inhibitor for the treatment of rheumatoid arthritis (RA). Altered B cell subsets have been reported in RA patients pathology . This study was undertaken to evaluate the effects of tofacitinib on B memory subsets in RA patients.
Methods: Levels of B subsets(CD19+cells, CD19+IgD+CD27+, CD19+IgD+CD27−, CD19+IgD-CD27−, CD27+IgD-CD27+) in the PB of 8 RA patients were analyzed by using flow cytometry in baseline and after Tofacitinib 3 month treatment.
Results: Tofacitinib treatment resulted in an decrease in CD19+ B cells, CD19+IgD-CD27−and CD27+IgD-CD27+B cells (Fig1).
Conclusion: Our findings indicated that monitoring of CD19+ B cells, CD19+IgD-CD27−and CD27+IgD-CD27+B cells appeared to be adequate to assess RA disease activity in tofacitinib-treated patients with RA.

P1269

CUL7 E3 ubiquitin ligase mediates the degradation of activation-induced cytidine deaminase (AID) and regulates the immunoglobulin class switch recombination in B-lymphocytes

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Activation-induced cytidine deaminase (AID) initiates class switch recombination and somatic hypermutation in immunoglobulin (Ig) genes. The activity and protein levels of AID are tightly controlled by various mechanisms. In this study, we found that CUL7 E3 ubiquitin ligases specifically mediated AID ubiquitination. CUL7 overexpression or knockdown influenced the decay of AID, affecting AID protein levels and subsequently IgA class switch in CH12F3 cells, a mouse B lymphocyte cell line. Further analysis indicated that CUL7 mediated AID ubiquitination by forming a complex with FBXW11. In a *CUL7*$^{fl/fl}$*CD19*$^{cre+}$ mouse model, we demonstrated that CUL7 knockout significantly enhanced AID protein levels in B-cells in the germinal center and increased both the IgG1 and IgA class switch. Collectively, our results reveal a subtle regulation mechanism for tightly controlling AID protein levels. The manipulation of this pathway may be useful for regulating AID abundance and efficiency of Ig class switching, and is therefore a potential target for developing immunologic adjuvants for vaccines of various pathogens such as human immunodeficiency virus type 1 (HIV-1) and influenza viruses.

P1270

Scaffold attachment factor A acts as a positive regulator for RAG-mediated V(D)J recombination

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DOI: 10.1002/eji.201970400

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Generation of immunoglobulin (Ig) and T cell receptor (TCR) diversity through RAG-mediated V(D)J recombination is the foundation of adaptive immunity. Understanding the molecular regulation of RAG-mediated V(D)J recombination remains a long term research topic in immunology. Using proximity dependent biotin identification (BioID) and GST-pull down assays, we found that scaffold attachment factor A (SAFA) interacts with RAG1. Immunofluorescence microscopy results showed that SAFA associated with RAGs and SAFA truncates altered RAGs location on the nuclear skeleton. In plasmid-based recombination assay in HEK293T cells, overexpression of SAFA enhances RAG-mediated V_{H1-8} recombination; knockdown of SAFA in HEK293T cells reduced RAG-mediated V_{H1-8} recombination; re-expression of SAFA restored RAG-mediated V_{H1-8} recombination. These results showed for the first time that SAFA acts as a positive regulator for RAG-mediated V(D)J recombination.

P1271

Methyltransferase Nsd2 ensures germinal center selection by promoting adhesive interactions between B cells and follicular dendritic cells

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Antibody affinity maturation, which is an antigen based selection process for B cells, occurs in germinal centers (GCs). GCB cells must efficiently recognize, acquire, and present antigens from follicular dendritic cells (FDCs) to receive positive selection signals from T helper cells. Previous studies showed that GCB cells undergo adhesive interactions with FDCs, but the regulatory mechanisms underlying the cell adhesions and their functional relevance remain unclear. Here, we identified H3K36me2 methyltransferase Nsd2 as a critical regulator of GCB cell-FDC adhesion. Nsd2 deletion modestly reduced GC responses but strongly impaired B cell affinity maturation. Mechanistically, Nsd2 directly regulated expression of multiple actin polymerization-related genes in GCB cells. Nsd2 loss reduced B cell adhesion to FDC-expressed adhesion molecules, thus affecting both B cell receptor (BCR) signaling and antigen acquisition. Overall, Nsd2 coordinates GCB positive selection by enhancing both BCR signaling and T cell help.

P1272

PGE2 protect the survival and antibody secretion of plasma cells under ER stress through EP4 receptor

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Prostanglandin E2 is produced from arachidonic acid by the cyclooxygenase enzymes and prostanoid synthases in response to various infection and its four G-protein coupled receptor EP1-EP4 are widely expressed on plenty of cell types. Here, by studying on EP4f/fMb1cred mice, we reported that **deficiency of EP4 receptor led to fewer viable plasma cells(PCs) and lower frequency of IgM-secreting cells after stimulation with LPS in vitro or after immunization with TI antigen.** Notably, the PGE2's impact on hurting on the survival of PCs was supposed to be through EP4 receptor specifically and **PGE2 signal by EP4 receptor only influenced the survival of PCs, not affecting the initial activation of B cells and their terminal differentiation to PCs.** In mechanism, during the exposure to endoplasmic reticulum(ER) stress, **EP4-deficient PCs showed suppressed AKT phosphorylation and Xbp-1/Grp94/Bip expression as well as an increasing level of**

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DOI: 10.1002/eji.201970400

phosphorylated IRE1 and its downstream JNK. Later this elevated IRE1-JNK signal could initiate cell apoptosis by activating Caspase family. And the bad survival of EP4 deficient PCs could be rescued by addition of JNK inhibitor or AKT phosphorylation producer. Consistently, the function of PGE2-EP4 signal on IgM secretion was confirmed in a pneumonia infection mouse model. In summary, our data originally revealed that the protective role PGE2-EP4 signal played in affecting the survival of IgM-PCs, and the precise modulation on PGE2 signal through EP4 receptor may give us a potential clue to get better first line defense in bacterial infection.

P1273

High expression of NEK2 mediated by p53 contributes to progression and relapse of multiple myeloma

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Multiple myeloma (MM) is a B cell malignancy. Previously, we have shown NEK2 associated with aggressive cancer behavior and poor prognosis in MM. However, we don't know how NEK2 was activated in MM. To elucidate the mechanisms of abnormal high expression of NEK2, NEK2 expression were analyzed using FISH,PCR-sequencing and MSPCR. The results show that about chromosome amplification of NEK2 and deletion of p53 contribute to NEK2 high expression in MM but not mutation or methylation. When knock-down the p53 expression with CRISPR technique, NEK2 was upregulated in p53-/- MM cell lines and HEK293 cells both on mRNA and protein level ($P< 0.01$). Meanwhile, when p53 deletion and NEK2 overexpression occur simultaneously,the phenomena of asymmetric mitosis and multipolar division are more obvious ($P< 0.001$) and the subcutaneous tumorigenesis is significantly greater than that of the single overexpression of NEK2 and deletion of p53 group ($P< 0.001$), suggested that the double hit of p53 deletion and NEK2 overexpression increases the chromosomal instability and enhances the tumorigenic ability. ChIP and fluorescence reporter system analysis show that p53 could bind to the promoter region of NEK2 and regulate its transcriptional expression. Further CGH analysis of the deletion of p53 expression in HEK293 cells can cause 1q21.4-44 amplification of the chromosome region of the NEK2 directly, which further confirmed by FISH. In summary, p53 deletion and NEK2 overexpression induced drug resistance, proliferation and chromosomal instability in MM. p53 could regulate NEK2 expression by binding to the promoter region of NEK2 or causing NEK2 amplification.

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DOI: 10.1002/eji.201970400

P1274

NEDD4-1 overexpression sensitizes multiple myeloma cells to bortezomib via ubiquitinating Akt

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Multiple myeloma (MM) is a hematological malignancy characterized by monoclonal expansion of plasma cells in the bone marrow and/or extramedullary sites. Proteasome inhibitor bortezomib (Bor) is one of the most effective drugs for treatment of MM which may eventually develop acquired-resistance to Bor during long-term treatment. Several researches show that E3 ubiquitin ligases (E3s) primarily determine the substrate specificity of ubiquitin proteasome system and play an essential role in Bor resistance of MM. NEDD4-1 is a founding member of the NEDD4 family of E3 ligases and is involved in the proliferation, migration, invasion, and drug sensitivity of cancer cells. Our current study aims to explore the role and underlying mechanism of NEDD4-1 in acquired resistance of Bor in MM. Clinically, low NEDD4-1 expression has been linked to poor prognosis in patients with multiple myeloma. Functionally, NEDD4-1 knockdown (KD) resulted in bortezomib resistance in myeloma cells in vitro and in vivo. Overexpression (OE) of NEDD4-1, but not an enzyme-dead NEDD4-1-C867S mutant, had the opposite effect. Mechanistically, pAkt-Ser473 levels and Akt signaling were elevated and decreased by NEDD4-1 KD and OE, respectively. NEDD4-1 ubiquitinated Akt and targeted pAkt-Ser473 for proteasomal degradation. More importantly, the NEDD4-1 KD-induced upregulation of Akt expression sensitized myeloma cells to growth inhibition after treatment with an Akt inhibitor. Collectively, our results suggest that high NEDD4-1 levels may be a potential new therapeutic target in multiple myeloma.

P1275

The chromatin remodeling complex Ino80 regulates the antibody immune response

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Chromatin remodeling regulates RNA transcription via changing DNA accessibility and protein recruitment. Cellular processes like proliferation and differentiation largely depend on genome-wide transcriptional changes induced by those complexes.
Ino80 is a major chromatin remodeling complexes that shapes the chromatin by modifying the histone landscape. The Ino80 complex is well-conserved from yeast to humans. It is involved in the regulation of transcription, DNA replication and repair; however, tissue-specific functions in mammals are hardly understood.
We study the importance of the Ino80 complex to mount antibody immune responses using conditional knockout mice lacking the Ino80 ATPase activity. Remarkably, a loss of the Ino80 function in mature B cells renders those mice incompetent to form antibodies upon immunization. On the one hand, we find that Ino80 enhances cytokine responsiveness at the onset of an immune response, thereby regulating germinal center entry and the expression levels of the activation-induced deaminase (AID) enzyme, which induces class-switch recombination and somatic hypermutation. On the other hand, Ino80 mediates a metabolic switch required for terminal differentiation from activated germinal center B cells to antibody-secreting plasma cells.
Collectively, we show that Ino80 is involved in the regulation of the most important hallmarks of B cell biology: germinal center formation, class-switch recombination and plasma cell differentiation.

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DOI: 10.1002/eji.201970400

P1276

O-GlcNAcylation of c-Myc dynamically regulates pre-B cell proliferation

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O-Linked β-N-acetylglucosamine (O-GlcNAc) modification regulates the activity of hundreds of nucleocytoplasmic proteins involved in a wide variety of cellular processes, such as gene expression, signaling, and cell growth; however, the mechanism underlying regulation of B cell development and function by O-GlcNAcylation remains largely unknown. Here, we demonstrate that changes in cellular O-GlcNAc levels significantly affected the growth of large pre-B cells transiently expressing pre-B cell receptors comprising heavy chains with surrogate light chains on their surface membrane. Large pre-B cells rapidly proliferate to allow expansion of functional clones that express successfully rearranged heavy chains at the pro-B stage during early B cell development. In our study, overall O-GlcNAc levels linked to the glucose-uptake rate in these proliferative pre-B cells were highly induced as compared with those in pro-B cells. Mechanistically, inhibition of O-GlcNAcylation in pre-B cells markedly downregulated c-Myc expression, resulting in cell cycle arrest via blockade of E- and A-type cyclin expression. Additionally, restriction of glucose availability to pre-B cells reduced cellular O-GlcNAc levels, as well as c-Myc expression, and subsequently decreased cell proliferation. Importantly, the population of large pre-B cells in the bone marrow was severely impaired by administration of an O-GlcNAc inhibitor to mice. These results strongly suggested that O-GlcNAcylation-dependent expression of c-Myc represents a new regulatory component of pre-B cell proliferation, as well as a potential therapeutic target for the treatment of pre-B cell-derived leukemia.

P1277

Wiskott-Aldrich syndrome protein (WASP) plays a critical role in regulating the signaling and Ag-presentation function of the B cell receptor in germinal center B cells

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Wiskott-Aldrich syndrome protein (WASP)-deficiency causes a severe immune disorder with impaired humoral immune responses. Previous studies suggested a role for WASP in affinity-maturation but not B cell expansion in germinal center responses. This study examined the cellular mechanism by which WASP regulates the affinity maturation of germinal center B cells (GCBs). GCBs are positively selected in the light zone (LZ) by interactions with antigen presented by follicular dendritic cells through the B cell receptor (BCR) and with cognate T cells through antigen capture, processing and presentation. Using Sheep Red Blood Cell (SRBC) immunized wild-type (wt) and $WASP^{-/-}$ mice as the models, we found that $WASP^{-/-}$ mice had a reduced percentage of GCBs with the LZ surface mark. LZ B cells from immunized $WASP^{-/-}$ mice exhibited impaired spreading, BCR clustering and early signaling, when interacting with membrane-bound antigen. Although $WASP^{-/-}$ LZ B cells internalized soluble antigen through the BCR in comparable kinetics and levels as wt LZ B cells, they generated a significantly lower level of antigen-loaded MHC class II complexes on the cell surface and interacted with cognate $CD4^{+}$ T cells much less efficiently than wt LZ B cells. Our results suggest that WASP is critical in regulating early BCR signaling, antigen presentation, and the interaction of GCBs with T cells, all the critical steps for B cell affinity maturation.

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DOI: 10.1002/eji.201970400

P1278

Protein kinase CK2 is a critical regulator of B-cell development and differentiation

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Protein kinase 2 (CK2) is a serine/threonine kinase composed of two subunits: two catalytic subunits, CK2α and CK2α´, and the regulatory subunit CK2β. It is overexpressed and overactive in B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphomas, leading to inappropriate activation of NF-κB, JAK/STAT and PI3K/AKT/mTOR signaling. However, how CK2 functions in normal B-cell development and differentiation is not known. We generated mice with specific deletion of CK2α in B-cells (CD19-Cre) and these mice exhibited cell-intrinsic expansion of marginal zone B-cells (MZB) at the expense of type 1 transitional B-cells, with no changes in follicular B-cells. CK2α-deficient transitional B-cells showed enhanced activation of NOTCH2, a transcriptional regulator required for MZB cell development. Following T cell-dependent immunization, CK2α-deficient cells promoted germinal center formation while the generation of plasma cells was antagonized. These data demonstrate that CK2α is an important regulator of B-cell development and differentiation.

P1279

Generation of AID conditional knockout mice

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Activation-induced cytidine deaminase (AID) initiates class-switch recombination and somatic hypermutation of immunoglobulin genes. In addition to this function, AID is also implicated in epigenetic regulation in pluripotent stem cells and in oncogenesis of lymphoid and non-lymphoid origins. To examine role of AID in specific cell types, we developed conditional knockout of AID in mice and serve it freely available to the community. We took so-called “three-loxP strategy”. *Aicda* gene consistis of 5 exons and catalytic domain is encoded by exons 2 and 3. The targeting vector contains loxP-RFP-frt-Neo-frt-loxP before exon 2 and single loxP after exon 3. Heterozygously targeted mice were crossed with Flp mice to obtain loxP-RFP-frt-loxP-exon 2-exon 3-loxP configuration [Aicda-RFP]. We confirmed expression of RFP in B cells of geminal centers of spleen and intestine-associated lymphoid tissue. After crossing Aicda-RFP mice with Cre mice driven by tissue-nonspecific alkaline phosphate promoter, we could obtain partial and complete deletion, namely: loxP-exon 2-exon 3-loxP [Aicda-FL] and deletion of exons 2 and 3 [Aicda-KO]. Homozygous mice were obtained for each genotype and were checked for AID activity by serum IgG ELISA and in vitro class-switch assay. AID activity was normal for Aicda-FL but partially and completely absent for Aicda-RFP and Aicda-KO, respectively. Aicda-FL mice would be useful for the studies of AID function in subpopulation of B cells and in non-lymphoid cells.

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DOI: 10.1002/eji.201970400

P1860

The lack of the CD38 and CD19 genes impact in the development of splenic immature B cells from mice

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CD38 and CD19 proteins play an important role in the regulation of immune system. Firstly, CD38 it is an ectoenzyme and receptor possessing key physiological roles. It has been suggested that the CD38 absence exacerbates the development of an autoimmune phenotype in a lupus-prone mouse or the Cd38$^{-/-}$ mice. Secondly, CD19 is an essential molecule in the BCR signaling. Several reports show that the lack of CD19 develop an immunodeficient phenotype in *Cd19*$^{-/-}$ mice. Likewise, some reports have suggested an association between the CD38-CD19 signaling pathways, however is still unclear the molecular mechanism for this interaction. In order to solve if there is any collaboration or requirement of both receptors, we have generated a new murine model deficient in the expression of both CD38 and CD19 genes. Our first outcome shows that the double knockout mice decreased reproductive ability and survival. Then, the results shown that immature transitional 1 and 2 B cells were decreased on these mice, whereas the marginal zone B cells were completely absented in the double knockout mice. Altogether, our results suggested that the simultaneous absence of CD38 and CD19 genes affect the development of immature splenic B cells. In addition, we have to analyzed the origin of the development defect concerning to the reproductive ability and the survival rates. This model could allow us to observe the interaction in the signaling pathways between CD38 and CD19 and it could be a useful model for the study of immunodeficiency and autoimmunity.

P1861

Generation of a B-1 lymphocyte-specific conditional knockout mouse model *Bhlhe41*$^{\text{tdTomato-Cre}}$

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B-1 lymphocytes play indispensable roles in defensing a wide range of pathogens prior to adaptive immune response arising. Although several transgenic mouse models targeting different stages of B lymphocytes have been established e.g. *Cd19-Cre* and *Mb1-Cre*, a B-1 lymphocyte specific conditional knockout mouse model for illuminating the role of a gene of interest in B-1 lymphocytes is still missing. In this study, we aimed to generate a B-1 lymphocyte-specific knockout mouse model by expression of Cre recombinase under the control of the promoter of *Bhlhe41,* which is preferentially expressed in B-1 lymphocyte and microglia in immune system. A *tdTomato-2A-Cre-polyA* cassette was inserted to the locus of *Bhlhe41* by CRISPR-Cas9 technology. *Bhlhe41*$^{\text{tdTomato-Cre/+}}$ *Rosa26*$^{\text{EYFP/+}}$ mice were generated for determining Cre-mediated recombination efficiency. The accuracy of insertion of *tdTomato-2A-Cre-polyA* cassette to Bhlhe41 locus was confirmed by significantly reduced peritoneal B-1a lymphocytes in *Bhlhe41*$^{\text{tdTomato-Cre/tdTomato-Cre}}$ mice. Flow cytometric analysis of tdTomato in immune cells from *Bhlhe41*$^{\text{tdTomato-Cre/+}}$ confirmed the specific expression of BHLHE41 in B-1 lymphocytes rather than other B subsets. Further analysis of EYFP in immune cells from *Bhlhe41*$^{\text{tdTomato-Cre/+}}$ *Rosa26*$^{\text{EYFP/+}}$ mice revealed larger than 90% recombination in splenic and peritoneal B-1 lymphocytes while less than 10% recombination in other B subsets. Hence, we

DOI: 10.1002/eji.201970400

generated a B-1 lymphocyte-specific conditional knockout mouse model *Bhlhe41*$^{tdTomato\text{-}Cre}$, which can be used for studying the function of a specific gene in B-1 lymphocyte-mediated immune responses.

P1862

Interleukin (IL)-21 promotes the differentiation of IgA$^+$ B cells in porcine Peyer's patch via activation of JAK1-STAT3 signaling pathways

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Secretory Immunoglobulin A (sIgA) is a critical antibody to prevent the invasion of pathogens via mucosa. Previous publications reported that some cytokines promote the class switch recombination (CSR) from IgM$^+$ to IgA$^+$ B cells in mice and humans. To evaluate the functions of porcine cytokines on intestinal B cells, a panel of porcine cytokines was employed to stimulate IgM$^+$ B lymphocytes derived from swine Peyer's patches. The results showed that IL-21 accelerated IgA production of B cells. Further investigations demonstrated that IL-21 promoted proliferation and inhibited apoptosis of IgM$^+$ B cells. In addition, the expression of circle transcription α (*CTα*), a hallmark of active IgA CSR, was also upregulated by IL-21. Further evidence indicated that IL-21 facilitated the differentiation of antibody-secreting cells, showing increased *IRF7* and *PRDAM1* expression and downregulated *AID, BCL6,* and *PAX5* expressions in B cells. Moreover, we found that IL-21 promoted the differentiation of IgA$^+$ B cells via JAK1-STAT3 signaling pathways, showed a significant decrease of IgA production when JAK1-STAT3 pathways were inhibited. Taken together, these results suggested that IL-21 promoted the differentiation of IgA$^+$ antibody-secreting cells through activation of JAK1-STAT3 signaling pathways in IgM$^+$ B lymphocytes. The findings obtained from this study could contribute to the extension of the mucosal immunology on swine IgA CSR and improvement of mucosal vaccines for the pig industry.

P1863

Pathogenic IgG$^+$ plasma cell polarization fosters immune privilege and dismantles hepatoma immunotherapy

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Plasma cell subset composition, generation, and functional and clinical relevance in human local tissues are not known. Here, we identify pathogenic plasma cell maturation in human hepatocellular carcinoma (HCC). B cells undergo unique somatic hypermutation and selective class switch recombination to IgG in HCC. Inflammatory macrophages, upon encountering activated CD4$^+$ T cells, acquire ability to generate pathogenic IgG$^+$ plasma cells via CXCR3/CXCL10/ERK interaction, and in turn shapes immune landscape to dismantle effector T cell immunity and foster hepatoma progression. Interestingly, we demonstrate that an epigenetic reprogramming strategy, which has shown great potential for cancer therapy in the SCID T-cell transfer model, chiefly amplifies pathogenic IgG$^+$ plasma cell polarization in immune-competent hosts. Therapeutic strategy combining epigenetic reprogramming with B cell depletion, FcγR blockade, or Syk-Btk inhibition successfully elicits antitumorigenic T cell responses.

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DOI: 10.1002/eji.201970400

P1864

Immunoglobulin heavy variable gene mutation and micro-RNA expression in Burkitt's lymphoma at a referral hospital in western Kenya

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Objective: Burkitt's lymphoma (BL) is a virus associated childhood B-cell cancer common in eastern Africa. Continued survival of B-cells in germinal centres depend on expression of high affinity immunoglobulins (Ig) to complementary antigens by somatic hypermutation of Ig genes. Cellular microRNAs, non-coding RNAs have been reported to play role in cell cycle regulation. . Both viral antigen dependent mutation and micro-RNA expression maybe involved in BL pathogenesis. We describe immunoglobulin heavy variable (IgHV) rearrangement and micro-RNA expressions in BL tumours.
Methods: Genomic DNA were extracted and purified from BL tissue blocks at Moi Teaching and Referral Hospital, before amplification using IgHV consensus primers and sequencing. The sequences were then aligned with germline alleles in IMGT/V-QUEST® database. Total RNA extracted from tissue blocks and cell lines were used to determine relative expression of hsa-*miR*-34a and *hsa-miR*-127.
Results: In all tumours, allele alignment scores and number of mutations range were 89.2-93.2%, 15-24 respectively. The range of IgHV amino acid changes were higher in EBER-1$^+$ (15-25) than EBER-1$^-$ (9-15). In MYC$^+$ tumours, the relative expression were: *hsa-miR*-127(2.09); *hsa-miR*-34a (2.8) and MYC$^-$ *hsa-miR*-127 (1.2), *hsa-miR*-34a (1.0).
Conclusions: B-cell in BL contained somatic mutated IgHV gene and upregulated cellular microRNAs with possible pathogenetic role(s).

P1865

Evaluation of γδT cells influence on the functional activity of B-1 lymphocytes

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The study of cell-cell interactions and understanding of their role in various immune processes are promising. Much attention is paid to the study mechanisms of pathological conditions of gastrointestinal tract. Many cellular and humoral immunity factors in these processes are involved. IgA is key component of the mucosal immunity. It maintains homeostasis by interacting with the intestinal microbiota. B-1 cells produce about 50% of intestinal IgA without the participation of T-helpers and respond mainly to T-independent antigens (TI-2). The IgA production may be influenced by number of humoral factors as well as macrophages and dendritic cells. γδT lymphocytes are also considered as cellular factor affecting the immunoglobulin production by B-1 cells. This assumption is based on the ability of γδT lymphocytes to participate in both innate and adaptive immunity processes, as well as on the close proximity of γδT and B-1 lymphocytes in the intestinal mucosa and the participation in the formation of the first line defense.
We investigated the effect of γδT of intestinal cells on the functional activity of B-1 cells. We have developed a model of co-incubation of these subpopulations in vitro. The production of IgM and IgA by B-1 cells was assessed using ELISPOT, the dynamic expression of the corresponding genes using PCR-RT. The data suggest the possible suppression of IgM and IgA production by B-1 cells with presence of γδT cells and TI-2. Moreover, an increase of IL-10 in cultures including γδT cells was observed. The samples were also examined by scanning electron microscopy.

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DOI: 10.1002/eji.201970400

P1866

Isolated anti-CD4 monoclonal antibodies from an HIV-1 long term non-progressor display high potency against diverse HIV-1 subtypes

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Background: 1% of HIV-positive subjects develop exquisitely potent and broad neutralizing antibody responses. In several well characterized cases of such elite neutralizers, the cross-neutralizing activities of sera were determined to be due to neutralizing antibodies that target a small number of structurally conserved regions of *Env* including the CD4bs and conserved elements of the variable loops V2 and V3, with the later specificities being glycan dependent.
Methodology: Based on surface marker expression, individual cells at different stages of human B cell development were isolated by fluorescence-activated cell sorting at the Scripps Institute, USA. For each cell, Ig heavy and corresponding Ig light chain gene transcripts were amplified by nested RT-PCR and cloned in eukaryotic expression vectors and characterized for specificity, neutralization breadth and potency in Entebbe, Uganda.
Results: We isolated and cloned 4 monoclonal antibodies with ability to neutralize HIV-1 pseudoviruses; PC63V78P2C3, PC63V78P1B9, PC63V78P2B9 and PC63V78P2C6 from Participant PC63. All 4 mAb were specific to the CD4 BS. PC63V78P2C3 had a lower neutralization breadth of 41.7%. PC63V78P2C6, PC63V78P1B9 and PC63V78P3B9 all had a neutralization breadth of 75% (12v panel). However, only PC63V78P2C3 neutralized the Murene Leukemia Virus.
Conclusion: The study isolated 4 monoclonal antibodies from the VH and Vk regions of an HIV-1 long term progressor. All 4 isolated mAb were highly specific to the CD4 binding site but only one showed high potency. We suggest that future vaccine strategies should target eliciting antibodies towards the whole CD4 binding site and not just to single epitopes for particular bNabs.

P1867

Mice with b cell-specific deletion of protein tyrosine kinase 2 beta (PTK2B or PYK2) have preserved marginal zone b cells

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Splenic marginal zone B (MZB) cells are strategically located between the circulation and lymphoid tissue, providing a first-line defense to blood-borne antigens by rapidly producing IgM and class-switched IgG antibodies. MZB cells function as innate-like lymphocytes that respond to both T cell-dependent and T cell-independent antigens. These responses are well characterized, however, much less is known about the interaction of MZB cells with other cell types during these immune responses. Protein tyrosine kinase 2 beta (PTK2B, also known as PYK2) plays a critical role in the development and function of MZB cells in mice, with whole-body deletion of PTK2B resulting in complete absence of MZB cells in the spleen. We used a recently developed mouse model in which exons 15-18 of the *Ptk2b* gene are flanked with LoxP sequences to generate a whole-body PTK2B knock-out (PKO) mouse and further study the effects of PTK2B deletion on MZB cell development and function. Using this mouse model, we replicated previous historic observations and found an absence of MZB cells in

DOI: 10.1002/eji.201970400

whole-body PTK2B deficient mice. However, surprisingly, when PTK2B was deleted only in B cells, MZB cells were preserved in these mice. This finding contradicts previous observations in a whole-body PKO mouse model claiming an intrinsic MZB cell defect. Together, our results suggest that the underlying mechanism for MZB cell loss in whole-body PTK2B deficient mice is not MZB cell intrinsic and is more complex than previously thought.

P1868

A preliminary study on AICD expression during chronic HIV infection: a role for HIV tat proteins

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Cytopathic viruses like HIV-1 have evolved mechanisms to evade antiviral protection by antibodies. In rare cases, A small group of HIV infected individuals, known as elite controllers, develop potent broadly neutralizing antibodies (bNAbs) able to neutralise a broad spectrum of HIV-1 viral variants. bNAbs is highly mutated in comparison to antibodies produced during general immunization.Activation-induced cytidine deaminase (AICD) an enzyme that belongs to the cytosine deaminase family is indispensable for somatic hypermutations (SHM). AICD is predominantly expressed in germinal center B cells and will play a pivotal role in generating the highly mutated bNAbs produce by elite HIV controllers.It was also demonstrate that the HIV Tat proteins enhance AID mediated SHM of antibody V regions through their modulation of the endogenous polymerase II (Pol II) transcriptional process. The activity levels of the AICD might be important in generating SHM as the AID gene can be regulated by miRNA binding to the 3'-UTR region. This might implies that mutations in the AID gene outside the protein coding region can have an influence on AICD expression levels. It is also argued that Post-transcription modification of AID gene through phosphorylation provides regulation of the intrinsic DNA cytidine deaminase activity of AID as such that deficiencies in signal transduction pathways may cause over phosphorylation of AID protein. Whether differential AID expression is associated with antibody neutralizing activity during chronic HIV infection is not known and was studied here.

P1869

Inositol polyphosphates promote T cell-independent humoral immunity via the regulation of Bruton´s tyrosine kinase

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T cell-independent (TI) B cell response is critical for the early protection against pathogen invasion. The regulation and activation of Bruton´s tyrosine kinase (Btk) is known as a pivotal step of B cell antigen receptor (BCR) signaling in TI humoral immunity, as observed in patients with X-linked agammaglobulinemia (XLA) experiencing a high incidence of encapsulated bacterial infections. However, key questions remain as to whether well-established canonical BCR signaling pathway is sufficient to regulate the activity of Btk. Here, we find that inositol hexakisphosphosphate ($InsP_6$) acts as a physiological regulator of Btk in BCR signaling. Absence of higher order inositol phosphates (InsPs), inositol polyphosphates, leads to an inability to mount immune response against TI antigens.

DOI: 10.1002/eji.201970400

Interestingly, the significance of $InsP_6$-mediated Btk regulation is more prominent in IgM^+ plasma cells. Hence, present study identifies higher order InsPs as principal components of B cell activation upon TI antigen stimulation and presents a novel mechanism for InsP-mediated regulation of the BCR signaling.

P1870

Interchromosomal templated mutagenesis accounts for mutation clusters in B cell non-Ig genes

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The immunoglobulin (Ig) genes of germinal center B cells (GCB) undergo somatic hypermutation in order to generate antibody diversity. During this process, non-immunoglobulin (non-Ig) genes can accumulate mutations. Although it is generally accepted that these mutations arise in an untemplated manner in humans and mice, findings from our lab suggest that some mutations in the non-Ig genes of murine GCB arise through a gene conversion-like mechanism, templated mutagenesis. Gene conversion, which is known to occur at the Ig locus of chickens and rabbits, involves the use of a template sequence to repair a DSB resulting in the template being copied at the site of repair. Using GCB from the Peyer's patches of aged mice (> 6 mo.), we amplified ~500bp sections from non-Ig genes including *CD79B, RHOH, BTG1, EBF1* and sequenced them. We found that non-Ig mutations tend to be clustered rather than diffuse. Our computational pipeline called Template Recognition via Analysis of Monte Carlo Experiments (TRACE) matches mutations clusters within an input sequence to partially homologous donor templates elsewhere in the genome. TRACE-identified donor templates are statistically likely to have been used in a templated mutagenesis event leading to the mutation of the input sequence. Through this analysis, we found that unlike gene conversion originally observed in chickens, murine templated mutagenesis occurs between sequences on different chromosomes and tend to originate in introns and intergenic regions, rather than exons. These findings have opened an avenue into the controversial study of non-Ig templated mutagenesis in murine B cells

P2548

Role of B lymphocytes in the process of autoimmune ovarian diseases

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Objective: To explore the involvement of B lymphocytes in the autoimmune ovarian disease (AOD).
Methods: The thymus-extracted BALB/C mice were established on the 3rd day of birth(D3tx). The dynamic changes of B lymphocytes in spleen and pelvic lymph nodes were observed from 1-10 weeks after thymus extracted using flow cytology. The proliferation of B lymphocytes co-culture with Treg was detected by CSFE experiment.
Results: D3tx mice showed obvious inflammatory cell infiltration and follicular atresia in the ovarian tissue till the 5th week after operation, besides the higher FSH and LH, lower E2 in serum. The activated B cells and plasma cells in spleen and pelvic lymph nodes increased from 5 weeks after surgery, maintained to 10 weeks, while no significant changes in the memorial B lymphocytes. The proliferation rate of B lymphocytes co-culture with Treg decreased obvious.
Conclusion: Significant changes occurred in B lymphocytes in AOD, while it was late than the T lymphocytes according to our previous research. The abnormal activate condition of B lymphocyte in AOD might be corrected though the Treg.

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DOI: 10.1002/eji.201970400

P2549

Clinical observation of low dose rituximab in the treatment of epstein-barr virus infection

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Objective: To investigate the clinical efficacy of low-dose Rituximab in the treatment of patients with EB virus infection.
Methods: Six patients with EBV infection after hematopoietic stem cell transplantation or ATG treatment were given Rituximab 100mg qw*2 times. The DNA markers of EBV were monitored one week, two weeks, three weeks and four weeks before and after the first use of Rituximab.
Results: Of the 6 patients, 4 were negative for EB virus DNA and 2 were ineffective. Two of the ineffective patients were screened for EB virus and the virus was found in NK/T cells.
Conclusion: Although EB virus is a B lymphocyte virus, it can also infect NK / T cells. Low dose of Rituximab is ineffective in the treatment of this kind of EB virus infection. It is suggested that EB virus should be sorted early in patients infected with EB virus, which is helpful for clinical treatment.

P2550

NEK2 promotes drug resistance through enhancing autophagy by upregulation of beclin1 in multiple myeloma cells

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Multiple myeloma (MM) is a neoplastic plasma-cell disorder that is characterized by clonal proliferation of malignant of plasma cells in the bone microenvironment, and monoclonal protein in the blood or urine. Drug resistance is one of the main causes of cancer relapse, since exploring the molecular mechanisms of drug resistance is important for improving therapeutic effect in MM. Our previous study has demonstrated NEK2 mediated drug resistance via upregulation of multidrug resistance protein. In this study, we found autophagy was involved in NEK2 induced Bortezomib(a priority drug in MM) resistance in MM cells. Firstly, immunofluorescence result showed NEK2 expression and LC3B-Ⅱ labeled autophagosome were increased in MM cells derived from relapsed MM patients compared with newly diagnosed MM patients and health donors. Moreover, overexpression of NEK2 enhanced antophagy, while downregulation of NEK2 suppressed autophagy in MM cells. Then we discovered NEK2 induces autophagy in MM cells through mediating USP7 induced deubiquitination of Beclin1, thus increasing the expression of Beclin1 in protein level and promoting the formation of the Beclin1-Vps15-Vps34 complex. We further demonstrated that downregulation of Beclin1 inhibited NEK2 induced autophagy and overcomed NEK2 mediated Bortezomib resistance in MM cells. Taken together, our findings reveal novel mechanism of drug resistance in MM cells induced by NEK2, and provided novel insight into treating relapsed MM patients.

DOI: 10.1002/eji.201970400

P2551

Abnormal expression of BAFF in systemic lupus erythematosus and preparation of recombinant human BAFF protein

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B-cell activating factor (BAFF), an autocrine cytokine predominantly produced by monocytes and dendritic cells in addition to T cells, has been proven to play a crucial role in lupus, for example, by promoting proliferation, differentiation and survival of B cells. However, the functional mechanism of BAFF in B cells has not been fully elucidated, and the expression level of BAFF in inflammatory sites has not been reported so far. The clinical efficacy of the monoclonal antibody belimumab developed for BAFF is not significant. In order to explain the above scientific problems, the expression levels of BAFF and its receptors in skin lesions, PBMCs and serum of SLE patients were detected by flow cytometry, ELISA, polychromatic pathological section system and antibody formulations and detection methods independently developed by the laboratory; the rapid construction of recombinant plasmid, the construction of prokaryotic and eukaryotic protein expression system, as well as eukaryotic protein suspension expression system were preliminarily explored, providing important theoretical and experimental basis for studying the biological structure and function of BAFF protein and revealing its unknown function and molecular mechanism in other systems; the recombinant human BAFF protein has been successfully prepared, which provides raw materials and experimental basis for the preparation of anti-BAFF monoclonal antibodies and the further development of SLE targeted therapeutic drugs.

P2552

YY1 binds to the E3′ enhancer and inhibits the expression of the immunoglobulin kappa gene via epigenetic modifications

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The rearrangement and expression of immunoglobulin (Ig) genes is regulated by enhancers and their binding transcriptional factors that activate or suppress the activities of the enhancers. The immunoglobulin kappa (Igκ) gene locus has three important enhancers, i.e. the intrinsic enhancer (Ei), 3′ enhancer (E3′), and distal enhancer (Ed). Ei and E3′ are both required for Igκ gene rearrangement during early stages of B cell development, whereas optimal expression of the rearranged Igκ gene relies on both E3′ and Ed. The transcription factor YY1 affects the expression of many genes involved in B cell development, probably by mediating interactions between their enhancers and promoters. Herein, we found that YY1 binds to the E3' enhancer and suppresses Igκ expression in B lymphoma cells by epigenetically modifying the enhancer. Knocking down YY1 enhanced Igκ expression, which was associated with increased levels of E2A (encoded by the TCF3 gene) and its binding to the E3' enhancer. Moreover, in germinal centre B cells and plasma cells, YY1 expression was reversely associated with Igκ levels, implying that YY1 might facilitate antibody affinity maturation in germinal centre B cells via the transient attenuation of Igκ expression.

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DOI: 10.1002/eji.201970400

P2553

JNK/AP1 pathway regulates MYC expression and BCR signaling through Ig enhancers in Burkitt lymphoma cells

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In Burkitt Lymphoma (BL), the chromosomal translocation by which MYC gene is juxtaposed to the immunoglobulin (Ig) gene locus has been frequently found. The translocated MYC gene is overexpressed, which is the major driver of BL tumorigenesis. Studies have shown that Ig enhancers are essential for the MYC overexpression, but the mechanisms are not fully understood. In addition, the survival of BL cells relies on the B-cell receptor (BCR) signaling that is determined by the levels of Ig molecules expressed on the cell surface. But whether MYC has any impact on Ig expression and its functional relevance in BL has not been investigated. Herein, we show that MYC upregulates Ig kappa (Igκ) expression in BL cells through two Igκ enhancers, the intronic enhancer (Ei) and 3' enhancer (E3'). Mechanistically, by activating JNK pathway, MYC induces the phosphorylation of c-Fos/c-Jun and their recruitment to the AP1 binding sites in the Igκ enhancers, leading to the enhancers' activation and subsequent Igκ upregulation. The AP1-mediated activation of the Igκ enhancers is also required for the translocated MYC gene expression, indicating positive feedback for the MYC overexpression in BL cells. Importantly, interrupting the JNK pathways inhibits both Igκ and MYC gene expression and suppresses BL cell proliferation. Our study not only reveals a novel mechanism underlying the MYC overexpression in BL but also suggests that targeting the JNK pathway may provide a unique strategy to suppress BL tumorigenesis.

P2554

Tespa1 deficiency dampens thymus-dependent B-cell activation and attenuates collagen-induced arthritis in mice

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Thymocyte-expressed, positive selection-associated 1 (Tespa1) plays an important role in both T cell receptor (TCR)-driven thymocyte development and in the FcεRI-mediated activation of mast cells. Herein, we show that lack of Tespa1 does not impair B cell development but dampens the *in vitro* activation and proliferation of B cells induced by T cell-dependent (TD) antigens, significantly reduces serum antibody concentrations *in vivo* and impairs germinal center formation in both aged and TD antigen-immunized mice. We also provide evidence that dysregulated signaling in Tespa1-deficient B cells may be linked to the CD40-induced TRAF6 degradation, and subsequent effects on 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2 (PLCγ2) phosphorylation, MAPK activation, and calcium influx. Furthermore, demonstrate that Tespa1 plays a critical role in pathogenic B cells, since Tespa1-deficient chimeric mice showed a lower incidence and clinical disease severity of collagen-induced arthritis. Overall, our study demonstrates that Tespa1 is essential for T cell-dependent B cell responses, and suggests an important role for Tespa1 during the development of autoimmune arthritis.

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DOI: 10.1002/eji.201970400

P2555

Analysis of the change of the peripheral blood B cell count in patients with acquired immunodeficiency syndrome after long-time highly active antiretroviral therapy

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Objective: To investigate the effect of the initial B cell count on the reestablishment of B cell count during HAART.
Methods: AIDS outpatients and healthy controls were collected $CD4^+$ T and B lymphocytes counts of outpatients (before HAART, 12, 24 and 36 months after the treatment) and controls. According to whether or not the $CD4^+$ T lymphocyte count increased by 100/µL after 1 year of the first HAART, patients were divided into treatment-effective group and treatment-ineffective group. The most suitable B cell count to initiate HAART was identified by mean of receiver operating characteristic (ROC) curve. According to the most suitable B cell count threshold, patients were divided into the above-the-threshold group (ATG) and the below-the-threshold group (BTG).
Result: The B cell counts of AIDS patients before treatment, 12, 24 and 36 months after treatment and healthy controls were significant differences ($F=12.53$, $P< 0.01$). The B cell counts before treatment of the treatment-effective group and treatment-ineffective group were significant difference ($t=2.43$, $p< 0.05$).The most suitable B cell count is 102.0/µL. The B cell counts of ATG significantly increased at the initiation of treatment, and stabilized at the middle and late period. **The B cell counts of BTG also significantly increased at the initiation of treatment, but decreased slightly at the middle period and decreased significantly at the late period.**
Conclusions: B cell count before treatment can obviously impact on the reestablishment of B cell count during HAART.

T Cell Development and Activation

O033

Protein phosphatase 2A has an essential role in promoting thymocyte survival during selection

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The development of thymocytes to mature T-cells in the thymus is tightly controlled by cellular selection, in which only a small fraction of thymocytes equipped with proper quality of TCRs progress to maturation . It is pivotal to protect the survival of the few T-cells which pass the selection. However, the signaling events which safeguard the cell survival in thymus are not totally understood. In this study, protein Ser/Thr phosphorylation in thymocytes undergoing positive selection is profiled by Mass Spectrometry. The results revealed large numbers of dephosphorylation changes upon TCR activation during positive selection. Subsequent substrate analysis pinpointed protein phosphatase 2A (PP2A) as the enzyme responsible for the dephosphorylation changes in developing thymocytes. PP2A catalytic subunit α (Ppp2ca) deletion in the T-cell-lineage in $Ppp2ca^{flox/flox}$-Lck-Cre mice (PP2A cKO) displayed dysregulated dephosphorylation of apoptosis related proteins in double positive (DP) cells and caused substantially decreased numbers of DP $CD4^+$ $CD8^+$ cells. Increased levels of apoptosis in PP2A cKO DP cells was found underlie aberrant thymocyte development. Finally, the defective thymocyte development in PP2A cKO mice could rescued by either Bcl2 transgene expression or by p53 knockout. In summary, our work reveals an essential role of PP2A in promoting thymocyte development through the regulation of cell survival.

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DOI: 10.1002/eji.201970400

O034

Modulation of NOD2 with Rho GTPases enhances thymic regeneration and promotes naïve T cell reconstitution after damage

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Although endogenous thymic regeneration is a crucial function that allows for renewal of immune competence following immunodepletion caused by infection or common cytoreductive cancer therapies, such as chemotherapy or radiation, the molecular mechanisms governing this process remain poorly understood. Our recent studies have identified two key pathways driving thymic regeneration; centered on secretion of BMP4 by endothelial cells (ECs) and IL-22 from innate lymphoid cells (ILCs). Here, we reveal an unexpected suppressive role for the cytoplasmic innate immune receptor NOD2 in orchestrating these distinct regenerative pathways, with *Nod2*-deficient mice exhibiting increased levels of the regeneration-associated factors (RAFs) BMP4, IL-22, and IL-23 (a key regulator of IL-22 secreted by dendritic cells, DCs) after damage, and superior thymic regeneration. Given the thymus is considered a sterile organ, it is unlikely that the canonical NOD2 ligands are mediating this process; however, several Rho GTPases have been described as non-bacterial regulators of NOD2. Consistent with this, inhibition of RhoGTPases *in vitro* augmented *Bmp4* and *Il-23* transcription by ECs and DCs, and thymic regeneration was improved by inhibiting Rho GTPase *in vivo* following total body irradiation in a NOD2-dependent manner. Importantly, this translated into superior peripheral naïve T cell reconstitution. These findings provide evidence not only of a novel master regulator of endogenous tissue regeneration, but also provide an innovative therapeutic strategy for boosting thymic regeneration and T cell reconstitution after damage such as that caused by infection or cytoreductive therapy.

O035

The transcriptional coactivator TAZ regulates reciprocal differentiation of Th17 cells and Treg cells

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Imbalance of immunosuppressive regulatory T cells (Treg cells) and the inflammatory Th17 subset of helper T cells results in the development of autoimmune disease. Here, we reported that the transcriptional coactivator TAZ, the downstream effector of the Hippo kinases Mst1 and Mst2, is substantial enriched in the Th17 cell subset and regulates the differentiation of Th17 cell and Treg cell by modulating the function of RORγt and Foxp3, the critical transcription factor for Th17 cells and Treg cells respectively. TAZ and RORC (which encodes RORγt) had higher coexpression in memory CD4+ T cells isolated from the peripheral blood of patients with rheumatoid arthritis or Sjögren's syndrome disease than in that of healthy people. In addition, TAZ enhanced the development of Th17 cell-mediated inflammatory diseases, such as colitis and EAR. Mechanistically, TAZ was a critical co-activator of the Th17-defining transcription factor RORγt. Additionally, TAZ attenuated Treg cell development by preventing Foxp3 acetylation mediated by the histone acetyltransferase Tip60, which targeted Foxp3 for proteasomal degradation. In contrast, under Treg cell-skewing conditions, TEAD was induced and sequestered TAZ away from the transcription factors RORγt and Foxp3 to promote Treg cell differentiation. Furthermore, deficiency in TAZ or overexpression of TEAD1 induced Treg cell

DOI: 10.1002/eji.201970400

differentiation, whereas expression of a transgene encoding TAZ or activation of TAZ directed Th17 cell differentiation. Our results demonstrate a pivotal role for TAZ in constituting additional mechanisms in the modulation of the differentiation of Treg cells and Th17 cells.

O036

MHCI expression level delimits naive CD8 T cell population size and quality

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The survival of naïve CD8 T cells (T_N) in the periphery is dependent on tonic interaction between the T cell receptor and MHCI, and IL-7 signalling. Both signals are essential for survival, but the extent to which tonic MHCI signals limit and control the size and quality of the T_N pool is unknown. To examine this, we modulated MHCI levels in C57BL/6 mice, which expresses two MHCI-genes: H-2K^{b}and H-2D^b. We generated "full-dose" mice that are homozygous for each gene in isolation (H-2K$^{b+/+}$D$^{b-/-}$and H-2K$^{b-/-}$D$^{b+/+}$) or "half-dose" mice, which are hemizygous at either gene (H-2K$^{b+/-}$D$^{b-/-}$and H-2K$^{b-/-}$D$^{b+/-}$). We found that expression of one MHCI-gene did not affect the other gene, and allele dosage was proportional to the amount of MHCI expressed. Full-dose mice have 50% fewer T_N compared to WT mice, highlighting that T_N are maintained in a pMHCI-specific manner, as expected. When we compared half-dose to full-dose mice, peripheral T_N numbers were further reduced by half, indicating that MHCI-levels in the periphery is a limiting factor in T_N survival. Half-dose mice had similar thymic-output to full-dose mice, implying that reduced peripheral numbers is due to reduced peripheral survival of T_N. Of note, T_N in half-dose mice do not homeostatically proliferate despite their lymphopenic status, suggesting that MHCI-expression levels play a crucial role in delimiting the number of circulating T_N. Given that humans express a diverse array of MHCI-alleles, the level of expression of individual alleles could differ across individuals and shape distinct patterns of maintenance of peripheral T_N pools.

O037

Inside-out mechanisms of CD6 signaling: cytoplasmic and extracellular interactions impact on T cell activation

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CD6 is a T cell surface glycoprotein that contains three extracellular SRCR domains. We have shown that CD6 can repress early signals upon TCR stimulation. During antigen presentation and T cell activation, CD6 is translocated to the IS established between the T cell and the APC. It has been accepted that CD6 binding to its ligand, CD166, guides the receptor-ligand pair to the IS where both molecules help to stabilize T cell-APC adhesions and to deliver regulatory signals. We have now found, however, that CD6 translocation to the IS occurs in a ligand-binding independent process, although binding to APC-expressed ligand CD166 may confer additional/complementary

DOI: 10.1002/eji.201970400

function/inhibition. Interestingly, the translocation of CD6 to the IS depends on cytoplasmic tail motifs. So far, we concluded that CD6 may contribute with apparently opposing mechanisms to the outcome of T cell activation: the extracellular domain may contribute positively to enhance T cell:APC interactions, whereas the intracellular part modulates the strength of signaling, independently of the cellular localization. Importantly, we describe that upon T cell activation, the ligand-binding domain of CD6 (SRCR domain 3, d3) can be omitted as consequence of alternative splicing regulation, resulting in the physiological interruption of the CD6-CD166 interaction. We constructed a mouse model (CD6Δd3) expressing CD6 devoid of the ligand-binding domain, and addressed the functional effect of interrupting the CD6-CD166 interaction. Remarkably, CD6Δd3 mice are significantly more susceptible to the development of EAE, indicating that the molecular interaction established by CD6 with its ligand is crucial to control the disease outcome.

O038

The role of AEBP2 in thymic epithelial cell biology

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Lysine tri-methylation at position 27 of histone 3 (H3K27me3) is an important posttranslational modification that epigenetically silences gene expression. A notable exception to this restrictive role of H3K27me3 appears to be the marking of loci that are promiscuously expressed in medullary thymic epithelial cells (TEC) to generate a molecular library of self-antigens required for effectively central T cell tolerance induction. H3K27me3 marks are placed by the Polycomb Repressive Complex 2 (PRC2) which includes the Adipocyte Enhancer-Binding Protein 2 (AEBP2), a well conserved zinc finger protein understood to serve as a targeting protein for the complex. To assess the role of AEBP2 in TEC biology in general and promiscuous gene expression (PGE) in AIRE+ medullar TEC in particular, we analyzed mice with a loss of *Aebp2* expression in all TEC. The absence of AEBP2 in these cells compromised PGE and resulted in a preferentially decrease of tissue restricted antigen (TRAs) expression dependent on AIRE. In parallel, early development, negative selection in both cortex and medulla and the post-selection maturation of thymocytes were compromised by the absence of AEBP2 expression in thymocytes. Taken together, the expression of AEBP2 is essential for the expression of a comprehensive repertoire of self antigens and an effective negative selection of developing thymocytes.

O039

Deciphering the impact of developmental timing on lymphocyte output in the embryonic mouse thymus

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During embryonic development multiple waves of hematopoietic progenitors with distinct lineage potential are differentially regulated in time and space. Consistent with that view,two waves of distinct thymic settling progenitors (TSPs) colonize the fetal thymus where they contribute to thymic organogenesis and homeostasis. TSPs of the first and second wave generate specialized lymphocyte subsets which are produced during embryogenesis and remain in tissues throughout life. It has remained unclear what is the relative contribution of each wave to thymopoiesis and tissue resident

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DOI: 10.1002/eji.201970400

innate-like lymphocytes throughout life, addressing this question highlights a critical challenge in the understanding of lymphopoiesis. We found that the first wave of TSPs are generated through a unique developmental program initiated in hematopoietic stem cells (HSCs) derived lympho-myeloid primed progenitors, which lose myeloid and B cell potential. They further migrate to the thymus to give rise to embryonic T cell receptor-invariant T cells beside differentiating into innate lymphoid cells (ILC) with bias towards the ILC3 lineage. Moreover, transcripts that define the molecular signature of ILCs and more precisely the ILC3 lineage are upregulated in these TSPs on the single-cell level. At later developmental stages, adaptive T lymphocytes are derived from lympho-myeloid progenitors that colonize the thymus and retain B-cell, ILC and myeloid potential. In addition, TSPs of both waves give rise to all ILC subsets *in vivo*.This sequence of events highlights the requirement for stratification in the establishment of immune functions that determine efficient seeding of peripheral tissues by a limited number of cells.

O040

Redox sensor NPGPx restrains ZAP70 recruitment to lipid rafts for modulating TCR responses and autoimmunity

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Excessive T cell activation can provoke inflammation and autoimmunity in mammalians. Therefore, precise elucidation of the T cell regulatory mechanism is necessitated for therapeutic treatment in autoimmune diseases. Emerging evidences demonstrated that the functional redox machineries are imperative for maintaining the intact T cell responses. The tyrosine kinase, ζ-chain-associated protein of 70 kDa (ZAP70), is essential for T cell development and activation. However, it remains elusive whether a direct redox regulation affects ZAP70 activity upon TCR stimulation. Here, we report that deficiency of non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx), a redox sensor, resulted in T cell hyperactivation and elevated autoimmune development in mice. Through unbiased proteomic approaches, ZAP70 is identified as the key interacting protein of NPGPx through disulfide bonding. NPGPx is activated by TCR-induced ROS and subsequently binds to a specific cysteine residue of ZAP70 to restrain its recruitment to membrane lipid rafts. The retention of ZAP70 in cytosol by NPGPx diminishes its kinase activity and subdues TCR responses. These results elucidate a delicate redox mechanism that NPGPx acts as a modulator to curb ZAP70 functions in maintaining T cell homeostasis and preventing autoimmunity. Moreover, we also observed that NPGPx expression was downregulated in the T cells as well as in the PBMC of SLE patients compared with healthy controls. Therefore, this novel redox modulation of ZAP70 by NPGPx casts new light on the intimate link between redox regulation and TCR responses, providing a potential therapeutic window for treating autoimmune diseases.

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DOI: 10.1002/eji.201970400

LB008

The regulation of T cell responses by cytohesins

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Cytohesins are guanine nucleotide exchange factors for adenosine diphosphate ribosylation factor proteins. In Mammalia the cytohesin protein family consists of four members; cytohesin-1, cytohesin-2, cytohesin-3 and cytohesin-4. Through their interaction with integral transmembrane proteins such as integrins, cytohesins modulate a diversity of distinct intracellular signalling pathways such as cell motility, cell adhesion, chemotaxis and rearrangement of the cytoskeleton. Because of their engagement in phagocytic processes through the WAVE regulatory complex, cytohesins are also targeted by different pathogens to modulate host cell phagocytosis. Although *in vitro* studies have identified cytohesins to interact in different intracellular events, the exact mechanisms as well as the immunological relevance *in vivo* is not understood yet.
Using acute infection models with the respiratory pathogens such as *Legionella pneumophila* and influenza A virus in cytohesin knockout mice, we aimed to define the *in vivo* function of different cytohesins in pathogenesis and the complex immune response. These data demonstrate that cytohesins appear to primarily regulate T cells within the immune responses. Remarkably, different cytohesins seem to have opposing roles in T cell biology. To further elucidate the regulation of the T cell responses by cytohesins, we plan to investigate their mechanistic involvement in T cell signalling and metabolism.

LB046

Medullary thymic epithelial cells are a dominant source of the canonical type 2 cytokine IL-13

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How the thymic medullary environment is established, subdivided, and maintained remains poorly understood as does its role in shaping thymocyte development beyond TCR selection. We recently used a novel lineage-tracing model and bulk RNA-seq to identify IL-25 expressing tuft cells as a minor population of mTECs. Because barrier cytokine production within the parenchyma of the thymus was unexpected, we used the same experimental system to examine cytokine expression broadly amongst medullary epithelial subsets. Surprisingly, mature MHCII$^+$ AIRE$^+$ mTECs were found to express considerable levels of *Il-13* transcript. Protein expression was confirmed by intracellular flow cytometry and IL-13 was readily detected in about 15% of thymic epithelial cells. IL-13 expression was AIRE-independent and did not require thymic tuft cells or tuft-derived IL-25. TEC-restricted deletion of the *Il-4/Il-13* locus in *FoxN1-Cre;Il4/13-flox* mice was found to result in decreased thymic size and considerable alterations in medullary myeloid populations. Taken together, these data reveal a non-redundant and previously undescribed role for expression of a canonical type 2 cytokine by the thymic epithelium in establishing the normal medullary microenvironment.

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DOI: 10.1002/eji.201970400

P0096

LARP4-mediated stabilization of NFkB1 and other key mRNAs promotes CD4$^+$T cell activation

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T cell activation is a well-established model for studying cellular responses to exogenous stimulation. In this study, we performed BruChase-Seq to experimentally monitor the expression dynamics of nascent transcripts in resting and activated CD4$^+$T cells, and applied computational modelingto investigate the impact of intron retention (IR) on the kinetics of mRNA degradation. As expected, intron retained transcripts are considerably less stable than spliced transcripts. In addition, the decrease in steady-state IR transcripts in activated CD4$^+$T cells is in part due to increased splicing efficiency and further stabilization of the spliced transcripts. We also found that this mRNA stabilization appears more determined by RNA binding proteins (RBPs) includingLARP4, rather than 3'-untranslated region (UTR)-isoform choice. Indeed, mRNAs in activated CD4$^+$T cells showed decreased stability for some key genes in LARP4 Knock-out (KO) mice relative to wild-type (WT) mice, including *nuclear factor kappa-light-chain-enhancer of activated B cells1（NFkB1）* and *26S proteasome non-ATPase regulatory subunit 7(PSMD7)*, resulting in their functional down-regulation. In addition, NFkB1 target genes *Interleukin 2 (IL2)* and *Interferon Gamma (IFNG)*which are critical for T cell proliferation and function, were also down-regulated in LARP4 KO mice upon CD4$^+$T cell activation. Finally, we propose that coordination between splicing regulation and mRNA stability may provide a novel paradigm to achieve spatiotemporal control of gene expression during T cell activation.

P0097

Reexamine αβ-T cell development in the thymus via single-cell sequencing

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In the thymus gland, most CD4/CD8 double negative (DN) precursors committed to *αβ-T* cell lineage and go through CD4/CD8 double positive (DP) and CD4/CD8 single positive (SP) stages to achieve maturation. Abnormal development of thymocytes lead to T cell dysfunction and result in immunodeficiency or autoimmune diseases. However, many details of *αβ-T* cell development still remain uncovered, especially the most majority population of DP cells. Here, utilizing single-cell RNA-seq, we shaped out the four main subtypes of DP stage, sequentially preDP, DPbla, DPre and DPsel. Cell cycle analysis showed that DPbla cells differentiate into DPre cell with just single round cell cycle. Meanwhile, elevated antigen presentation progress of MHC I was observed in DPsel and SP stages, suggesting selection actions between thymocytes, which was confirmed by immune synapse staining. Moreover, many marker genes of *αβ-T* cell development are autoantigens, implicating the importance of selection actions between thymocytes. Marker genes of DPsel is also high corelated with autoimmune diseases associated SNVs, reemphasized the crucial impact of thymocyte selection for T cell functions. Together, our data detailed the *αβ-T* cells development and helped to understand the association with autoimmune diseases.

DOI: 10.1002/eji.201970400

P0098

PRAK-activated autophagic clearance of mitochondria is essential for survival of recent thymic emigrants

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Limiting reactive oxygen species (ROS) by reducing mitochondria content is implied to be the mechanism for T cells to survive in the periphery but how it is regulated is largely unknown. We show here that p38-regulated/activated protein kinase (PRAK) plays a key role in the initiation of autophagic clearance of mitochondria in RTE and is essential for their survival under oxidative stress. *Prak* deficiency disrupts the developmentally programmed downregulation of mitochondrial content, leading to overproduction of ROS and poor survival of T cells in the periphery. PRAK executes the pro-survival function through inactivation of Rheb and suppression of mTORC1. The reduced mTORC1 activity in turn promotes the removal of superfluous mitochondria by autophagy. In summary, this study reveals a new physiological function of PRAK and defines a signaling pathway regulating the cellular response to oxidative stress.

P0099

Hassall's corpuscles with cellular-senescence features maintain IFNα production through neutrophils and pDC activation in the thymus

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Hassall's corpuscles (HCs) are composed of cornifying, terminally differentiated medullary thymic epithelial cells (mTECs) that are developed under the control of Aire. The function of HCs has not been elucidated. Using SASPase-GFP knocking mice, we demonstrated that HC-mTECs show features of cellular senescence and produce inflammatory cytokines and chemokines including CXCL5, thereby recruiting and activating neutrophils to produce IL-23 in the thymic medulla. We further indicated that thymic plasmacytoid dendritic cells (pDCs) expressing IL-23 receptors constitutively produced Ifna, which plays a role in single positive (SP) cell maturation, in an Il23a-dependent manner. Neutrophil depletion with anti-Ly6G antibody injection resulted in a significant decrease of Ifna expression in the thymic pDCs, suggesting that thymic neutrophil activation underlies the Ifna expression in thymic pDCs in steady state conditions. A New Zealand White mouse strain with HC hyperplasia exhibited greater numbers and activation of thymic development and SP thymocyte maturation showed significantly compromised numbers and activation of these cells. These results collectively suggested that HC-mTECs with cell-senescence features initiate a unique cell activation cascade including neutrophils and pDCs leading to the constitutive IFNα expression required for SP T-cell maturation in the thymic medulla,which implies the involvement in the central tolerance and autoimmune diseases.

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DOI: 10.1002/eji.201970400

P0100

Tsc1 controls the homeostasis of medullary thymic epithelium via preventing lysosome-mediated apoptosis in vivo

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Thymic epithelial cells (TECs) are critical for the development and generation of functional competence T cells. However, the mechanism that regulate the homeostasis of TECs are poorly understood. More and more evidence showed that *Tsc1* plays critical role in the regulation of many immune cells. To study the cell-autonomous role of *Tsc1* in TECs, we generated mice with conditional deletion of *Tsc1* in TECs. We found that *Tsc1* controls the homeostasis of medullary thymic epithelial cells (mTECs) by inhibiting lysosome-mediated apoptosis pathway. TEC-specific deletion of *Tsc1* reduced medullary TEC (mTEC) compartment at adult and embryonic stage. The defect of mTECs caused by *Tsc1* deficiency impaired thymocyte development and peripheral T cell homeostasis. *Tsc1*-dificient mTECs exhibited excessive apoptosis, which was partially reversed by decreasing mTORC1 activity via heterozygous knockout of *Raptor*. *Tsc1*-deficient mTECs exhibited increased cell metabolism and lysosome biogenesis, which would led to the increased production of ROS and dysfunction of lysosome in mTECs. The elevated ROS caused lysosome membrane permeabilization (LMP) and the release of cathepsin B and cathepsin L to cytosol, which then cleaved Bid into active tBid that subsequently induced intrinsic apoptosis. Thus, *Tsc1* is essential for the homeostasis of mTECs by inhibiting lysosome-mediated and tBid-dependent cell death pathway.

P0101

Sel1L regulates the development and differentiation of CD4 T cell

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It is well known that there are several protein quality control machineries, including ER-associated (ERAD), which is consist of Lin-12-lkie suppressor/enhancer (Sel1L) and E3 ligase, to support these secretory and transmembrane proteins synthesis and folding in the endoplasmic reticulum (ER). When CD4 T cell do maturation in thymus or activation and differentiation into different effective subsets, such as Th1 or Th2, a plethora of effector proteins including cytokines, co-stimulators and receptors should be synthesized in these cells. Unfortunately, up to now, the exact role of ERAD in the development and differentiation of CD4 T cells is still not well explored. In this study, we firstly specifically delete Sel1L gene in T cell by breeding Sel1L$^{flox/flox}$ mice with Lck-^{Cre} mice. The phenotypes and functions of CD4 T cells in thymus and peripheral immune organs were analyzed using flow cytometer. The data from thymus in KO mice showed that the percentage and the total number of DN cells were increased, but decreased in DP, CD3+CD4+ SP cells and CD4+Foxp4+ nTreg cells. Furthermore, more proliferative DN cells and less proliferative DP cells were detected in thymus. If activated peripheral T cells with anti-CD1 and anti-CD28, both the proliferation and apoptosis of T cells were increased in KO group. But the naïve Sel1L CD4+ T cells from these KO mice showed less efficiency in iTreg differentiation *in vitro*. Collectively, these data indicated that Sel1L regulates T cell development and differentiation in thymus and peripheral.

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DOI: 10.1002/eji.201970400

P0102

RNA editing enzyme ADAR1 is essential for T cell development

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Adenosine Deaminase Acting on RNA 1 (ADAR1) is an RNA-editing enzyme that plays an essential role in embryonic hematopoiesis. ADAR1 converts adenosine to inosine in RNA molecules (A to I RNA editing) through its deamination activity. A-I RNA editing therefore durably modifies the sequence of targeted RNAs and, consequently, the proteins that they encode.
We recently reported that the RNA editing activity of ADAR1 was required for adult hematopoietic progenitor cell differentiation. ADAR1 is expressed in T cells during their development in the thymus. However, little is understood about its function because of the embryonic lethality of traditional ADAR1-null mutations. In order to explore the functions of ADAR1 in T cells, we generated a mouse model in which ADAR1 was specifically deleted at different stages during T cell differentiation. We found that ADAR1 is required for T cell development at the DN stage. Loss of ADAR1 induced T cell progenitor apoptosis at the DN4 stage and prevented T cell maturation in the thymus. The population of TCRβ expressing cells diminished in the absence of ADAR1, though TCRβ loci recombination was shown to be ADAR1-independent. Additionally, ADAR1 deficiency was found to be associated with interferon (IFN) family genes up-regulation.
These findings have demonstrated an essential role for the RNA editing enzyme ADAR1 in T cell survival after pre-TCR expression, and raised the prospect of RNA editing in the regulation of immunologic homeostasis.

P0103

Smad3 and STAT3 synergistically upregulate TAZ expression in Th17 cells

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IL-6, together with TGF-b, induces the development of Th17 cells from naïve T cells, while TGF-b alone induced Treg differentiation. Recently, we found that the transcriptional coactivator of the Hippo signaling pathway, TAZ, but not YAP, was robustly induced in Th17 cells and Treg cell and underwent the greatest induction in Th17 cells. The abundance *Taz* mRNA was significantly increased by TGF-β alone, but not by IL-6 alone and this increase was further enhanced by stimulation with a combination of TGF-β and IL-6. The transcription factors Smad3 and STAT3 (the main transcription factor downstream of signaling via TGF-β or IL-6) were identified to bind to the *Taz* promoter. Further studies revealed that knockdown of Smad3 or knockout of STAT3 significantly decreased TAZ expression in naive $CD4^+$ T cells cultured under Th17-skewing conditions. Conversely, overexpression of Smad3 resulted in the induction of TAZ expression, and co-expression Smad3 with STAT3 further enhanced the TAZ expression. Consistently, Smad3 increased the activity of the *Taz* promoter, but STAT3 did not, while Smad3 together with STAT3 synergistically enhanced this activity. In addition, we identified that a Smad-binding site in the region −225 bp to −192 bp and a STAT3-binding site in the region of −282 bp to −240 bp were critical for TAZ expression. These results showed that TGF-b and IL-6 use their downstream transcription factors Smad3 and STAT3 to induce the expression of TAZ, which regulates reciprocal differentiation of Th17 cells and Treg cells.

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DOI: 10.1002/eji.201970400

P0104

Liver X receptor β is important to the survival of sngle-positive thymocytes by sustaining the IL-7Rα expression

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Cholesterol metabolism plays important roles in T cell activation and proliferation. But, the roles of liver X receptors (LXRs) in T cells, as key transcription factors in the cholesterol metabolism, remain largely unknown. In this study, we showed that LXRβ was important to the survival of single-positive (SP) thymocytes. LXRβ-deficient mice exhibited fewer peripheral naïve T cells and SP thymocytes compared with wild-type and LXRα-deficient mice, whereas the negative- and positive-selection in the transition from double-positive to SP thymocytes were similar. Moreover, LXRβ deficiency enhanced the apoptosis but not proliferation of SP thymocytes. Mechanistically, LXRβ regulates the expression of IL-7Rα in SP thymocytes and forced expression of IL-7Rα or Bcl2 restored the development of LXRβ-deficient SP thymocytes. In addition, administration of LXR agonist T091317 promoted the expression of IL-7Rα and the development of SP thymocytes. Thus, our results clarify that LXRβ functions as an important transcription factor upstream of IL-7Rα to regulate the survival of SP thymocytes by sustaining the IL-7Rα expression.

P0105

NF-KB-mediated miR-31 regulates $CD4^+CD25^+$ Tregs generation by targeting Ceacam1-S in systemic lupus erythematous

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Treg defects have been identified in SLE, which were principally characterized by a concomitant loss of CD25. Carcinoembryonic antigen-related cell adhesion molecule 1 (Ceacam1) is an immune coreceptor with critical roles in T-cell activation and function. Our previous study demonstrated Ceacam1 was up-regulated in PBMC from patients with SLE using microarray. However, its role in SLE is still unknown. In the present study, Ceacam1 mRNA level, especially Ceacam1-S, was up-regulated in PBMC from SLE patients and activated $CD4^+$T cells induced by anti-CD3/CD28/TGF-β1 in vitro. Ceacam1-S over-expression inhibited $CD4^+CD25^+$ generation, whereas Ceacam1-knockdown promoted $CD4^+CD25^+$ Tregs differentiation. Targetscan prediction and luciferase reporter gene assay indicated Ceacam1 is the target of miR-31. MiR-31 was decreased in PBMC from SLE patients and activated $CD4^+$T cells. MiR-31 mimic decreased the expression of Ceacam1 and enhanced $CD4^+CD25^+$ Tregs generation in activated $CD4^+$T cells, while Ceacam1-S overexpression turned over the expansion of $CD4^+CD25^+$ Tregs mediated by miR-31. Furthermore, the down-regulation of miR-31 in SLE was mediated by the loss of NF-KB activity, which negative regulated by TGF-β1 signal, and circulating TGF-β1 level was significantly increased in sera from compared with healthy individuals by ELISA assay. Importantly, Ceacam1, TGF-β mRNA level were decreased, while the percentage of $CD4^+CD25^+$ Tregs and miR-31 level were increased in inactive patients compared with active patients with SLE. Ceacam1 mRNA level and $CD4^+CD25^+$Treg were positive correlated while miR-31 negative correlated with SLEDAI score. In conclusion, NF-KB-mediated miR-31 reduction inhibited the generation of $CD4^+CD25^+$ Tregs via directly targeting Ceacam1 and promoted autoimmunity.

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DOI: 10.1002/eji.201970400

P0106

Endogenous galectin-9 potentiates CD4+T cell activation through promoting TCR downstream signaling

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Galectin-9, ab-galactoside-binding protein, is highly expressed in immune cells.Accumulating data indicated that galectin-9treatment induced T cell apoptosis and ameliorated autoimmune diseases in an exogenously modulated manner.However, endogenous role of galectin-9 in regulating T cells is largely unknown.We observed thatgalectin-9 is mostly expressed inside T cells and its membrane-associated and secreted forms are barely detected. Endogenous galectin-9 was quickly recruited to immune synapse upon T cell activation, suggesting its role in regulating TCR signaling. Our data revealed that proximal TCR signaling was impaired in galectin-9 deficient T cell, resulting in less proliferation through a Tim-3-independent pathway. Moreover, Th17 differentiation and B cell responses are downregulated in galectin-9 knockout mice. Finally, we demonstrated an inability of galectin-9 deficient T cells in the induction of T cell-transfer colitis and experimental autoimmune encephalomyelitis. Taken together, these findings indicate that intracellular galectin-9 is a positive regulator of T cell activation and modulates pathogenesis of autoimmune diseases.

P0107

Florescent timer protein as a molecular stop watch during embryonic thymic epithelial cell development

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The thymus is the primary lymphoid organ where T-cell lineage commitment, development, maturation, and repertoire selection take place. Thymic epithelial cells (TEC) are critical for these processes. Their differentiation and function are dependent on the transcription factor FOXN1. Although not required for the initial fate specification, the expression of FOXN1 by TEC is essential for their growth and differentiation but its target genes in early thymus organogenesis are not well defined. To elucidate these direct transcriptional targets and their precise temporal expression, we developed a novel mouse model (Foxn1-TP) where a fluorescent timer protein is transcribed under the control of the *Foxn1* locus thus precisely mirroring the temporal expression of endogenous FOXN1. Upon translation, the fluorescent timer protein first emits a blue fluorescence that gradually changes *in vivo* within 40 hours to a red emission. This dynamic change in fluorescence serves as a “molecular stop watch” that profiles in a single cell the length of time since FOXN1 has been expressed. This approach thus enables the identification of FOXN1-dependent transcriptional programmes at precise chronological resolution. We will present the correlation of distinct timer protein spectra with both TEC phenotypic and transcriptional changes at distinct embryonic stages. Moreover, our results also reveal novel dynamics in the temporal emergence of the separate TEC lineages and establish an exact hierarchy of FOXN1-dependent transcription over the course of embryonic TEC differentiation. In

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DOI: 10.1002/eji.201970400

summary, Foxn1-TP mice allow a time-based dissection of the early molecular events in thymus organogenesis.

P0108

Roles of bystander-activated memory CD8+ T cells in influenza A virus infection

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During viral infection, unrelated bystander T cells can be activated without TCR engagement. However, functional implication of bystander T cell activation varies depending on infection models. In the current study, we analyzed the cellular characteristics of adoptively transferred OT-1 memory CD8+ T cells during influenza A virus (IAV) infection. After IAV infection, adoptively transferred OT-1 memory CD8+ T cells upregulated T-cell activation markers, such as CD69 and CD25. In addition, the expression level of NKG2D increased on OT-1 memory CD8+ T cells at early time of IAV infection (as early as day 2). Moreover, the expression of granzyme B and CD107a was significantly increased, and IFN-g production was increased and rapidly decreased within one day in OT-1 memory CD8+ T cells. Importantly, we confirmed that OT-1 memory CD8+ T cells were activated independently of TCR engagement by examining endogenous Nur77 expression. Next, we compared the expression of effector molecules between adoptively transferred OT-1 memory CD8+ T cells and NK cells and found that the percentage of granzyme B or CD107a-positive cells among OT-1 memory CD8+ T cells was comparable to that among NK cells. Furthermore, at early time of IAV infection (day 2) the majority of IFN-γ-producing cells was OT-1 memory CD8+ T cells. In conclusion, adoptively transferred OT-1 memory CD8+ T cells were bystander activated independently of TCR engagement and exerted innate-like functions at early time during IAV infection.

P0109

Human KIR/NKG2A$^+$CD8$^+$ T cells exhibit distinct NK-like functions by IL-15 stimulation

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Recent studies have reported that human peripheral blood (PB) CD8$^+$ T cells co-expressing the transcription factor Eomesodermin (EOMES) and inhibitory receptors such as KIR and NKG2A are cells equivalent of murine innate/memory CD8$^+$ T cells. In the present study, we examined characteristics of human PB KIR/NKG2A$^+$CD8$^+$ T cells and their NK-like functionality. Compared to the conventional memory CD8$^+$ T cell, KIR/NKG2A$^+$CD8$^+$ T cells expressed higher levels of CD212 (IL-12Rβ2) and CD218 (IL-18Rα). In addition, they produced more IFN-g in response to stimulation with IL-12 and IL-18 as known previously. Intriguingly, KIR/NKG2A$^+$CD8$^+$ T cells expressed higher levels of CD122 (IL-2/IL-15Rβ) than conventional memory CD8$^+$ T cells, and *in vitro* IL-15 stimulation significantly increased the expression of NK cell receptors, including CD16, CD56 and NKG2D, in KIR/NKG2A$^+$CD8$^+$ T cells. Moreover, IL-15-stimulated KIR/NKG2A$^+$CD8$^+$ T cells exerted innate-like cytotoxicity against K562 cells that lack MHC class I expression. Furthermore, IL-15-stimulated KIR/NKG2A$^+$CD8$^+$ T cells killed rituximab-coated 721.221 cells by antibody-dependent cytotoxic activity. In conclusion, human PB KIR/NKG2A$^+$CD8$^+$ T cells exhibit distinct NK-like phenotypes and functional capacity particularly by IL-15 stimulation.

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DOI: 10.1002/eji.201970400

P0110

Posttranscriptional control of CD4$^+$ Foxp3$^+$ Treg function by RNA binding protein HuR

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Posttranscriptional regulation of CD4$^+$ T cell differentiation is poorly understood. The RNA-binding protein (RBP) HuR (Elavl1) controls differentiation of CD4$^+$ T cells into Th2 and Th17 helper subsets by regulating mRNA transcript stability and translatability of key genes such as *Gata3* and *Il17a*. To better understand the role of HuR in CD4$^+$ T cell development, we conditionally ablated HuR using dLck-Cre HuR$^{fl/fl}$ and Foxp3$^{YFP\text{-}Cre}$ HuR$^{fl/fl}$ mice. dLck-Cre HuR$^{fl/fl}$ mice have 5:1 skewing toward HuR sufficient Tregs, reductions in peripheral Tregs, cannot efficiently differentiation into iTregs nor upregulate Foxp3 upon activation. Since this model ablates HuR in all CD4$^+$ T cells late during thymic development, we made Foxp3$^{YFP\text{-}Cre}$ HuR$^{fl/fl}$ mice to directly target Tregs. In these mice, yellow fluorescent protein (YFP) fate maps those cells which are HuR null. Both male and female Foxp3$^{YFP\text{-}Cre}$ HuR$^{fl/fl}$ mice have significant failure to thrive (39% and 30% reduction in weight, respectively) and closely resemble scurfy mice with multi-organ and systemic autoimmunity, including lung, skin, stomach, kidney and liver. These mice also have significant reductions in peripheral Tregs and large increases in Teff cells and are deficient in *in vitro* suppression assays. We combined RNA immunoprecipitation (RIP)-Seq and RNA-Seq to identify a number of genes which are direct and indirect HuR targets. Together, these data suggest HuR posttranscriptionally critically regulates genes which are required for effective Treg function and generation of iTreg.

P0682

The role of mucosal-associated invariant T cells in HIV infection

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Mucosa-associated homeostatic T (MAIT) cells that express the canonical hVα7.2-Jα33 or mVα19-Jα33 TCR rearrangement are preferentially located in the gut lamina propria of humans and mice. MAIT cells home to mucosal sites and exert antimicrobial activity against bacteria and other microorganisms. HIV infection leads to early depletion of gut T cells and translocation of bacterial products. MAIT cells were depleted early in blood, lymph nodes and intestinal mucosa during chronic HIV infection, and residual MAIT cells are functionally impaired. Antiretroviral therapy could partly recover numbers and functions of MAIT. Residual and functionally active MAIT cells persist and may still be able to assist in controlling bacterial translocation during HIV infection. MAIT cells express higher level of PD-1 than conventional CD3+ T cells during HIV infection. Cytolytic capacity of MAIT is severely defective in HIV-1 infected patients. MAIT cells are part of a vicious circle that perpetuates exhaustion of the immune system and progression of untreated HIV infection and challenge immune reconstitution in patients on cART. Therefore, we examined MAIT in HIV patients with multiple disease states, including frequencies and functions. In vitro experiments were conducted to imitate TCR-dependent and -independent stimulation, and the functional changes of MAITs in untreated HIV-infected patients were detected. The functional changes of MAIT were observed by blocking PD-1/PD-L1 pathway. The aim is to clarify the role of MAITs in the development of HIV-infected disease, and to deepen our understanding of the pathogenesis of HIV which has important clinical and scientific significance.

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DOI: 10.1002/eji.201970400

P0683

IFN-γ inhibits the proliferation and differentiation of adult liver hematopoietic stem cells by inhibiting the activation of notch signal

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Background: The microenvironment and the regulation of bone marrow (BM) hematopoiesis have been extensively studied. However, differences between BM and liver hematopoietic stem cells and hematopoietic microenvironment remains poorly understood. And the regulations of the liver hematopoiesis remains unknown.
Methods and Results: In our present study, clone formation assay was used to confirm that the proliferation of adult mice liver hematopoietic stem and progenitor cells (HSPCs) could be inhibited by IFN-γ in vitro. And with wild-type mice and IFN-γ-deficient (GKO) mice, it was found that IFN-γ inhibited the differential process from long-term hematopoietic stem cells (LT-HSCs) to short-term hematopoietic stem cells (ST-HSCs) and multipotent progenitor (MPP) in vivo. Using a co-culture system comprised of liver HSPCs, we found that IFN-γ inhibited the development of liver Lineage$^-$Sca-1$^+$c-Kit$^+$ (LSKs) into T precursor cells and γδT cells. Furthermore, we demonstrated that IFN-γ impaired liver HSPCs proliferation and differentiation through inhibiting the activation of the notch signal.
Conclusions: IFN-γ may play indispensable role in regulation of liver hematopoiesis as it plays in microenvironment of BM, including proliferation of liver-derived HSPCs and differentiation from liver LT-HSCs to ST-HSCs and MPP and from liver LSKs to T precursor and γδT cells through inhibiting the activation of notch signal.

P0684

miR19a-3p inhibits cell apoptosis by regulating SOCS3 and FoxO1 phosphorylation in TALL-104 cells

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Increasing evidences indicate that miRNA are implicated in T cell activation, proliferation and survival. Our previous study showed a decreased level of miR-19a-3p in hyperplastic thymus accompanied with an increased expression of SOCS3, FoxO1 and its downstream molecules KLF2, CCR7, CD62L and S1P1. FoxO1 regulating S1P1 on naïve T cells through KLF2 is essential for their survival and trafficking. It was known that SOCS3, a direct target gene of miR-19a-3p negatively regulates Akt signaling which could suppress the activation of Foxo1. In this study, Flow cytometry analysis using Annexin V-FITC/PI double staining showed that transient overexpression of miR19a-3p induced by mimics transfection significantly inhibited the cell apoptosis with the up-regulated Bcl2 and down-regulated SOCS3, while had no effects on cell proliferation in TALL-104 cells. Although no significant change occurred in mRNA level of Foxo1, up-regulated miR-19a-3p led to increased phosphorylation of Foxo1 which resulted in the decreased expression of KLF2 and S1P1. The results indicated a possibility that miR-19a-3p could inhibit cell apoptosis by regulating the expression of SOCS3 and the phosphorylation of Foxo1.

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DOI: 10.1002/eji.201970400

P0685

Kupffer cells promote the differentiation of adult liver hematopoietic stem and progenitor cells into lymphocytes via ICAM-1 and LFA-1 interaction

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It has been reported that the adult liver contains hematopoietic stem and progenitor cells (HSPCs), which are associated with long-term hematopoietic reconstitution activity. Hepatic hematopoiesis plays an important role in the generation of cells involved in tumor surveillance and rejection. However, how the progenitors differentiate into functional myeloid cells and lymphocytes in the liver microenvironment remains unknown. In the present study, HSPC transplantation experiments were used to confirm that adult murine liver HSPCs differentiate into both myeloid cells and lymphocytes (preferentially T cells) compared with bone marrow HSPCs. Using a coculture system comprised of kupffer cells and HSPCs, we found that kupffer cells promote adult liver HSPCs to primarily generate T cells and B cells. We then demonstrated that kupffer cells can also promote HSPC expansion. A blockade of intercellular cell adhesion molecule-1 (ICAM-1) in a liver HSPC and kupffer cell coculture system impaired the adhesion, expansion, and differentiation of HSPCs. These results suggest a critical role of kupffer cells in the maintenance and promotion of adult mouse liver hematopoiesis. These findings provide important insight into understanding liver extramedullary hematopoiesis and its significance, particularly regarding therapy for liver tumors and immune rejection during organ transplantation.

P0686

The mechanism of CTLA-4 interacting with its ligands in autoimmune diseases

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CTLA-4 is an important immunosuppressive receptor and plays a key role in maintaining autoimmune tolerance. Both the loss of CTLA-4 in mice and mutations in human resulted in a systemic autoimmune disease with similar phenotype. However, the precise mechanisms of CTLA-4 haven't been clarified. Structural and functional analysis on the reported cases revealed that pathogenic CTLA-4 mutations occur frequently in their ligand binding and dimerization regions. These well-defined mutations provide us a unique research tool to study CTLA-4-mediated immune tolerance mechanisms. In our previous genetic mutation screening study, we have found a new CTLA-4 point mutation in a patient with recurrent autoimmune inflammation. We used steered molecular dynamics (SMD) simulation, single-molecule biophysical approaches, and functional assays to collectively demonstrate that mechanical force induces conformational changes in CTLA-4 to enhance pre-existing contacts and activates new interactions at the CTLA-4-CD80 and CD86 binding interface. The mutant CTLA-4 can't function well to deliver the inhibitory signals which makes over activation of effector T cells. The conclusions from this study will help us elucidate the immunoregulatory mechanisms of CTLA-4 and provide new strategies for more effective targeting of CTLA-4 in autoimmune diseases and tumors.

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DOI: 10.1002/eji.201970400

P0687

Correlation of the T cell PD-1 level with clinical characteristics and short-term treatment efficacy in nasopharyngeal carcinoma patients

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Objective: Our aim is to study the relationship between PD-1 level on T cells inperipheral blood and clinical characteristicsas well as short-term treatment efficacy inpatients with nasopharyngeal carcinoma (NPC).
Methods: Fifteen patients with NPC admitted to our hospital from January 2015 to April 2016 and 10 healthy subjects as control group were enrolled. PD-1 levelon T cells in the peripheral blood were determined by flow cytometry before and after chemoradiotherapy.
Results: 1.The $CD4^+$ T cell frequency in NPC was (46.49±3.43)%, lower than in controls (54.08±1.58)%($P< 0.05$) whereas the opposite trend was observed in $CD8^+$ T cells (43.11±3.62)% vs. (33.76±2.16)%, NPC vs control, ($P< 0.05$). 2.The frequency of $PD\text{-}1^+$ $CD4^+$ T cells in patients (17.71±1.18)% was significantly higher than controls (13.02±1.36)% ($P< 0.05$). The same trend can be seen in $CD8^+$ T cells(18.48±1.86)% vs. (12.22±0.51)% ($P< 0.05$). 3. In patients diagnosed as stage IV or those who were smokers, the frequency of $PD\text{-}1^+CD4^+$ T cells were significantly higher than that in stage III group and non-smokers, respectively. 4. Compared to the blood collected before treatment, the frequency of $PD\text{-}1^+CD4^+$ T cells significantly increased at the third month after the treatment ended but then decreased with time($P< 0.05$).
Conclusion: The PD-1 level on T cells in peripheral blood of NPC patients was related to some clinical characteristics, but its relationship to short-term treatment efficacy and prognosis remained to be determined.
Keywords: NPC；PD-1；T cell；Flow cytometry

P0688

Satb1 mediates interactions between super-enhancers and promoters in DP thymocytes

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Special AT-rich sequence binding protein 1 (SATB1) is a T cell-specific chromatin organizer that plays a role in T cell development. Satb1 is specifically expressed in thymocytes and Satb1 expression increases with the development of thymocytes, peaks in CD4+CD8+ double-positive (DP) cells, and then decreases, In our study, we found that Satb1 occupied at promoters of many T cell specific genes and super-enhancer regions in DP thymocytes, suggesting that Satb1 may be involved in interactions between super-enhancers and promoters of T cell specific genes. The ChIP-seq data showed the evidence for co-localization of Satb1 and other chromatin organizers like Nipbl, CTCF and cohesion which play an essential role in DNA looping between super-enhancers and promoters. To determine the epigenetic role of Satb1 in DP thymocytes, we detected chromatin accessibility of Satb1 deficient DP thymocytes by using ATAC-seq. We observed that Satb1 deficiency reduced numbers of super-enhancers and chromatin accessibility in both gene transcription start sites (TSS) and Satb1 binding sites. This result suggest that Satb1 may regulate gene expression by targeting super-enhancers and promoters in DP thymocytes.
Keywords: Satb1 Super-Enhancer chromatin conformation thymocyte

DOI: 10.1002/eji.201970400

P0689

Sclerostin domain-containing protein 1 is dispensable for the differentiation of follicular helper and follicular regulatory T cells during acute viral infection

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T follicular helper (T_{FH}) cells are important for effective humoral immunity by providing the required signals to cognate B cells and promoting germinal center (GC) formation. Many intrinsic and extrinsic factors have been reported to be involved in the multistage, multifactorial differentiation process of T_{FH} cells. By comparing gene expression between T_{FH} cells and T_H1 cells based on published GEO data, we found selective and high expression of sclerostin domain-containing protein 1 (SOSTDC1) in T_{FH} cells but not in T_H1 cells; however, it is unclear whether SOSTDC1 is crucial for the differentiation and/or function of T_{FH} cells. Utilizing a mouse model of acute lymphocytic choriomeningitis virus (LCMV) infection, we confirmed the selective expression of SOSTDC1 in T_{FH} cells compared to that in T_H1 cells, but the deletion of SOSTDC1 did not affect T_{FH} cell differentiation or effector function. Thus, our results indicate that the SOSTDC1 protein is merely a specific marker of T_{FH} cells but does not play a functional role in the differentiation of T_{FH} cells during acute viral infection.

P0690

The role of super-enhancer in transcription regulation of Ets2 gene and thymocyte development

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The transcription factor Ets2 plays a critical role in the proliferation, differentiation and apoptosis of thymocytes, but the mechanism of its transcription regulation is still unclear. Super enhancers (SE) are a class of genomic regulatory sequences that control cell type-specific gene expression. Bioinformatics analysis predicts a super enhancer spanning 160kb at the downstream of Ets2 gene, which has a strong binding signal of T cell specific chromatin organizer Satb1. The Hi-C and 4C data showed that the SE interacts with Ets2 promoter in thymocytes. To confirm the role of the SE in Ets2 expression and thymocyte development, we generated the Ets2 super-enhancer knockout mouse and observed impaired thymocyte development. The reverse transcription qPCR data showed that Ets2 expression is dramatically reduced in thymocytes of Ets2-SE deficient mice. The reduced expression of Ets2 was also observed in Satb1 deficient thymocytes. We propose that Ets2 expression in thymocytes is controlled by the SE and chromatin organizer Satb1 is involved in the chromatin interaction between the SE and the Ets2 promoter.

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DOI: 10.1002/eji.201970400

P0691

Preliminary study on the effect of low dose fractional irradiation on the expression of PD-L1 in lung adenocarcinoma

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Objective: To investigate the effect of low dose fractional irradiation on the expression of programmed death factor ligand 1 (PD-L1) in the immune microenvironment model of lung adenocarcinoma.
Methods: A549 cells were irradiated with different low dose fractions in vitro, and cell survival curves of different treatment groups were detected by flow cytometry. Flow cytometry was used to detect the expression of PD-L1 on cell surface.
Results: When the radiation dose is between 0 and 0.3 Gy, the survival fraction of cells decreases gradually, and the curve becomes smooth with the increase of dose; when the radiation dose is 0.3 Gy, the cell changes from radiation sensitivity to radiation resistance; then the radiation resistance increases slowly, reaching its maximum at 0.5 Gy; and in the dose region after 0.5 Gy, the survival fraction of cells decreases with the increase of radiation dose. The expression of PD-L1 increased first and then decreased with the increase of low dose radiation. The expression of PD-L1 was the highest at 0.5 Gy.
Conclusion: This study confirms that low dose radiation may stimulate immune response by inhibiting the expression of PD-L1 in lung adenocarcinoma cell line, which provides a theoretical basis for radiotherapy combined with anti-PD-L1 targeted therapy.
This work was supported by grants from Hainan Key Program of Science &Technology (ZDYF2019148), and Hainan Provincial Natural Science and Technology Foundation of China (20168303).

P0692

Using single cell RNAseq to understand the past, present and future of CD8+ memory T cell differentiation

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T cells are a critical component of the adaptive immune system as they are capable of directing targeted responses to infections and providing long-term immunity. Due to its importance in protective immunity against infectious diseases such as influenza, understanding the molecular mechanisms that program T cell differentiation forms the fundamental basis for therapeutic applications of T cell immunity in infection and cancer. To understand T cell lineage commitment, we therefore used single cell RNAse (scRNAseq) to trace transcriptional changes during critical stages of T cell differentiation in the transgenic OT-I mouse model, from newly dividing CD8+ T cells to fully differentiated effector and memory CD8+ T cells in response to influenza infection. Despite the long process of memory formation(~30 days), preliminary data suggests that the effector-memory cell fate decision occurs within the first 2 rounds of cell division after T cell activation. By combining both mathematic and cellular approaches, we can potentially identify gene signatures that drive the bifurcation process between effector and memory T cells, thus providing a predictive measure for vaccine efficacy against influenza.

DOI: 10.1002/eji.201970400

P0693

Keratinocytes costimulate naïve human T cells via CD2: A potential target to prevent the development of pro-inflammatory Th1 cells in the skin

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The interplay between keratinocytes and T cells plays an important role in the pathogenesis of chronic inflammatory skin diseases. During psoriasis, keratinocytes attract T cells by the release of chemokines while skin-infiltrating self-reactive T cells secrete proinflammatory cytokines (IFNγ, IL-17A), which cause the pathophysiological hallmarks of psoriasis, e.g. epidermal hyperplasia. Similarly, in chronic graft versus host disease, allogenic IFNγ-producing Th1/Tc1 and IL-17-producing Th17/Tc17 cells are attracted by keratinocyte-derived chemokines and accumulate in the skin. However, whether keratinocytes could act as non-professional antigen-presenting cells to activate naïve human T cells directly in the epidermis remained so far unknown.
We demonstrated that under pro-inflammatory conditions, primary human keratinocytes indeed activated naïve human T cells. This required cell contact formation and costimulatory signaling via CD58/CD2 and CD54/LFA-1. Naïve T cells, costimulated by keratinocytes, selectively differentiated into Th1 and Th17 cells. Especially keratinocyte-initiated Th1 differentiation was triggered by costimulatory signals through CD58/CD2. Gene expression analysis and subsequent phospho flow cytometry revealed a direct link between CD58/CD2 interaction, STAT1 activation and T cell specific IFNγ-production. The latter one initiated the keratinocyte-dependent Th1 differentiation.
Costimulation of T cells by keratinocytes resulting in Th1 and Th17 differentiation provides a so far unknown explanation for the local enrichment of Th1 and Th17 cells in skin of patients with chronic inflammatory skin diseases. Consequently, local interference with T cell-keratinocyte interactions may open up novel strategies for the treatment of Th1 and Th17-driven skin diseases.

P0694

T lymphocyte activation and immunomodulation levels: comparison between senegalene and ghanaian populations

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Objectives: Our main objective was to study the variations of CD25 activation and immunomodulationPD-1 levels of T lymphocytes isolated from two West African populations, living in areas endemic to Schistosoma sp.
Materials and methods: This was a prospective study that included a total of 339 people from two distinct geographical areas: 200 Senegalese and 139 Ghanaians. T lymphocyte activity was evaluated by flow cytometry after cell labeling with anti-CD3, anti-CD4, anti-CD8, anti-HLA DR, anti-CD25 and anti-PD1, respectively coupled to fluorochromes FITC, Alexa Fluorine, PE, PE-Cy7 and Per-CP. The search for anti-schistosoma sp antibodies was also done by ELISA.
Results: The proportion of LTCD4 PD-1 was significantly higher among Senegalese than among Ghanaians. In all age groups except the group of participants aged over 29, LTCD4 PD-1 were statistically more prevalent among Senegalese than among Ghanaians Similar trends were observed for LTCD8 PD-1. Concerning the activation of T cells, higher percentages of CD25 LTCD4 were

observed among Senegalese. In addition, expression of PD-1 by CD4 T cells was positively correlated with cell activation (CD25 LTCD4) in both Ghanaians and Ghanaians.
Conclusion: Our results, which show a notable difference in T lymphocyte activation and immunomodulation profiles between Senegalese and Ghanaian populations, seem to indicate a geographical variation of the immune response. The potential impact of these geographical and environmental differences on the immune response deserves further studies to integrate these ecological aspects into the development of vaccine strategies and future cancer immunotherapy protocols.
Keywords: Activation, Immunomodulation, PD-1 / PD-L1, CD25, Shistosomiasis

P0695

Aryl hydrocarbon receptor(AhR)-dependent CD8-like/cytotoxic reprogramming of CD4$^+$ T cells

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Mature T cells are divided into two distinct subsets: CD4 helper T (Th) cells and CD8 cytotoxic T (Tc) cells, performing either supportive role for immunity or killing function against invaders of the host. Although the fate to become a Th or Tc lineage is pre-determined during the T cell development in thymus, recent studies have revealed that mature Th cells, when losing Th lineage-determining transcription factor ThPOK, could upregulate Tc lineage-determining transcription factor Runx3, express CD8 and gain cytotoxic function. The cytotoxic reprogramming of Th cells are particularly observed in a significant population of intraepithelial lymphocytes (IELs), which are also shown to suppress intestinal inflammation. With the emerging evidence highlighting a fundamental role of gut immune homoeostasis for human health, our lab aims to identify signals that regulate the generation and function of intraepithelial CD4CD8 (IEL-CD4CD8) cells, particularly those extracellular and intracellular signals with potentials to be targeted for immunomodulation and immunotherapies. This project focused on AhR, an intracellular sensor of aryl hydrocarbons, which are enriched in the mammalian mucosal environment, as a physiological regulator for IEL-CD4CD8 cells. Our results show that certain AhR ligands induce CD8 expression on mouse CD4 T cells *ex vivo*. Correspondingly, AhR-deficient mice demonstrate a defective generation of IEL-CD4CD8 cells *in vivo*. Such finding reveals a new mechanism that regulates the gut immune homeostasis and highlights the plasticity between Th and Tc cells.

P1280

Loss of SENP1 expression triggers Treg differentiation and antitumor immunity via TGF-β signaling

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SENP1 is a member of SUMO-specific proteases (SENPs) family that is important for the protein SUMOylation status in many signaling pathways and regulating the development of early T cells. Whether SENP1 and SUMOylation play any role during the generation of TGF-β-induced Tregs (iTregs) is unknown. Here, we have discovered that SENP1 is markedly downregulated in iTregs and

DOI: 10.1002/eji.201970400

the loss of SENP1 expression is also required for optimal TGF-β signaling that promotes efficient development of iTregs. Restore of SENP1 impaired the development and function of both peripherally derived iTregs and *in vitro*-induced Tregs. Mechanistically, we identified an interaction between SENP1 and Smad4. Sumoylation protects Smad4 from ubiquitin-dependent degradation. In the absence of SENP1, SUMO-modified Smad4 accumulates in naive CD4+ T cells to sustain activation of TGF-β signaling. We also found that the downregulation of SENP1 is required for iTreg conversion in the tumor microenvironment. Our study establishes SENP1 as an important regulator of SUMOylation and stability of Smad4 to suppress TGF-β signaling and identifies SENP1's role in the inhibition of the iTregs generation. It also suggests that modulation of SENP1 is a potential therapeutic target for Treg-related immune disorders.

P1281

Targeting CD147 is beneficial for tumor therapy by promoting T to NK Lineage reprogramming

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At present, immunotherapy for tumor has shown more advantages. Normally, the immune cells release some cytokines by identifying MHC expressed on tumor cells to lyse the target cells. However, due to the shutdown of MHC-I on the surface of tumor cells, cytotoxic T cells have weakened killing effect on target cells. Nevertheless, NK cells recognize target cells without MHC, and respond quickly. Therefore, **if convert MHC-restricted T cells into non-MHC-restricted NK cells, it may be beneficial for tumor treatment.**
In this study, we found that **CD147 is a surface molecular to control the conversion of T-to-NK cells.** Using T cell-specific deletion, we showed here that in thymus CD147 is indispensable for the stable αβ T cell lineage commitment: loss of CD147 biases both multi-potent DN (double negative) and fully committed DP (double positive) cells into innate NK-like lineages. Mechanistically, CD147 deficiency results in impaired Wnt signaling and expression of BCL11b, a master transcription factor in determining T cell identity. In addition, functional blocking of CD147 by antibody phenocopies genetic deletion enrich NK-like cells in the periphery. Furthermore, using a melanoma model and orthotopic liver cancer transplants, the augmentation of NK-like cells strongly associates with resistance against tumor growth upon CD147 suppression.
In conclusion, **targeting CD147 can promote the reprogramming of T cells into NK-like cells and enhance the non-MHC-dependent anti-tumor immune response. Therefore, CD147 is an effective surface target for immune modulation in tumor therapy.**

P1282

Roles of mTORC1 and mTORC2 in controlling γδ 1 and γδ 17 differentiation and function

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Metabolism-controlled differentiation in αβ T cells have been well documented, however, the role of metabolism program in γδ T cell differentiation and function is elusive. Here, using CD2-cre; mTORC1 Raptor-f/f and mTORC2 Rictor-f/f mice (KO mice), we find that mTORC1 is required for the proliferation and survival of peripheral γδ T cells, especially Vγ4 γδ T cells, however, mTORC2 is dispensable. Moreover, mTORC1 is essential for both γδ 1 and γδ 17 differentiation, whereas

DOI: 10.1002/eji.201970400

mTORC2 is only required for γδ 17, but not γδ 1 development. We further studied the underlying molecular mechanisms and demonstrated that depletion of mTORC1 resulted in increased expression of SOCS1, which in turn suppressed the key transcription factor, Eomes, and consequentially reduced IFN-γ production, whereas the reduced glycolysis was responsible for impaired γδ 17 differentiation in Raptor KO γδ T cells. Quite differently, mTORC2 potentiated γδ 17 induction through suppressing ROS production. Consistent with their cytokine production profiling, Raptor KO γδ T cells lost anti-tumor function both in vitro and in vivo, whereas both Raptor and Rictor KO mice were resistant to IMQ (Imiquimod)-induced psoriasis-like skin pathogenesis. Overall, we discovered previously unknown functions of mTORC1 and mTORC2 in γδ T cell differentiation and their divergent roles in mediating the function of γδ T cells against tumor and autoimmunity.

P1283

USP16-mediated deubiquitination of calcineurin A controls peripheral T cell maintenance

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Calcineurin acts as a calcium-activated phosphatase which directly dephosphorylates the nuclear factors of activated T cells (NFAT) family, to trigger their nuclear translocation and transcriptional activity. However, the detailed mechanism regulating the recruitment of NFATs to calcineurin remains poorly understood. Here, we report that calcineurin A (CNA), encoded by *Ppp3cb* or *Ppp3cc*, is constitutively ubiquitinated on lysine 327, and **this polyubiquitin chain is rapidly removed by ubiquitin carboxyl-terminal hydrolase 16 (USP16)** in response to intracellular calcium stimulation. **The K29-linked ubiquitination of CNA impairs NFAT recruitment and transcription of NFAT-targeted genes.**
USP16 is a deubiquitinase that has been found to be required for chromosomal segregation in mitosis. However, the underlying mechanism through which USP16 mediates T cell hyperactivation remains unclear. Our results show that **USP16 deficiency prevents calcium-triggered deubiquitination of CNA in a manner consistent with impaired peripheral T cell maintenance and proliferation of peripheral T cells.** T cell-specific USP16 knockout mice exhibit reduced severity of T cell-mediated autoimmune diseases.
Our data reveal the mechanism by which USP16 regulates the activation of the calcineurin-NFAT axis in calcium signaling, and plays a critical role in peripheral T cell maintenance and proliferation. Notably, our findings demonstrate USP16 might serve as a novel therapeutic target in the treatment of T cell-mediated autoimmune diseases.

P1284

CD160 is essential for the development of memory CD8$^+$ T cells

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Understanding the mechanism regulating the development of memory CD8$^+$ T cells is paramount to improve the formulation of vaccine and T-cell based immunotherapies. Here we found that CD160 is vital for the development and differentiation of memory CD8$^+$ T cells. In acute lymphocytic

DOI: 10.1002/eji.201970400

choriomeningitis virus infection, the loss of CD160 skewed the differentiation of $CD8^+$ T cells towards short-lived effector T cells, leading to failure of the development of central, effective and tissue-resident memory $CD8^+$ T cells and consequently diminished capacity to protect from subsequent viral challenge. We further identified that CD160 deficiency resulted in up-regulation of Akt/mTOR signaling pathway. Importantly, the administration of rapamycin, a mTOR kinase inhibitor, was able to rescue the memory $CD8^+$ T cell development in CD160 knockout mice. Altogether, our studies discovered that CD160 is an essential regulator of memory $CD8^+$ T cell development by acting on Akt-mTOR signaling pathway.

P1285

Cbl-b negatively regulates T cell activation by targeting SHP-2

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Cbl-b, as an E3 ubiquitin ligase, has been implicated in setting the threshold of T cell activation, since Cbl-$b^{-/-}$ T cells do not require CD28 engagement for activation. However, the underlying mechanisms remains largely unknown. SHP-2 is a tyrosine phosphatase, which participates in signaling pathways via IL-2R, PD-1 and TCR. SHP-2-deficient T cells shows impaired proliferation and IL-2 production driven by TCR/CD3 or TCR/CD3 plus CD28 engagement, which suggests a positive role of SHP-2 in T cell activation. However the underlying mechanisms are also not very clear. To explore whether the negative regulation of T cell activation by Cbl-b is dependent on SHP-2, we established LckCre Cbl-$b^{-/-}$ SHP-$2^{f/f}$ mouse model. We found that T cells from double knockout mice could not bypass the requirement CD28 engagement for activation like Cbl-$b^{-/-}$ T cells, and even could not proliferate in the presence of CD28 engagement, which indicates SHP-2 is located downstream of Cbl-b in TCR signaling and (or) CD28 signaling, and negative regulation of T cell activation by Cbl-b is via SHP-2. Moreover, we found that upon TCR triggering, Cbl-b inducibly bound to SHP-2, which led to subsequent SHP-2 ubiquitination, however CD28 signaling can interrupt this interaction. Since SHP-2 protein level was increased in Cbl-$b^{-/-}$ T cells, it indicated that its ubiquitination by Cbl-b may result in degradation. Much further work is needed to fully explain how Cbl-b interact with and affect SHP-2, and how SHP-2 promotes T cell activation.

P1286

UHRF1 affects the lineage decisions of αβ and γδ T cells by epigenetically regulating the EGR1 expression

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Thymocyte differentiation is a highly complex process accompanied by epigenetic changes. Ubiquitin-like containing PHD ring finger 1 (UHRF1), a critical epigenetic regulator involved in DNA methylation, is expressed in T-cell precursors in thymus, especially at double negative (DN) stage. UHRF1 deficiency resulted in dramatically reduced thymocyte cellularity and thymus size. Through single-cell RNA-seq unsupervised clustering and pseudotime analysis, we found that UHRF1 deficiency thwarts αβ lineage development while biasing γδ lineage differentiation. Moreover, the reconstructed gene-regulatory network indicated that EGR1 expression was augmented in UHRF1 deficient thymocytes. Mechanistically, upon UHRF1 deficiency, the chromatin accessibility of EGR1 is more active, with declined binding of DMNT1 and H3K9me3 at the CpG promoter region. Our study reveals an

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DOI: 10.1002/eji.201970400

epigenetic mechanism that UHRF1 influence the expression of EGR1, which is crucial for thymocyte fate decision.

P1287

Loss of ABHD5 inhibits the infiltration of CD8+T in colorectal carcinoma via Reg3A-mediated E. coli inactivation

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It has been well known that the canonical role of ABHD5 is promotes triglyceride (TG) hydrolysis by coactivating adipose triglyceridelipase (ATGL). Our recent studies in mice with diminished levels of ABHD5 ($Apc^{Min/+}$/$Abhd5^{f/f/Cre+}$) clearly show the ATGL independent functions and the role of colorectal cancer (CRC) suppression for ABHD5, but the underlying mechanism remains elusive. Here we found ABHD5 deficiency significantly inhibits the infiltration of CRC with effector CD8+T cells and therefore facilitates cancer progression. We further demonstrated that ABHD5 deficiency-induced the inhibition of effector CD8+T cells is mediated by the increased ABHD5-binding protein, the antimicrobial protein Reg3A. Importantly, by 16S analysis, our study discovered the lack of intestinal bacteria E. coli when Reg3A overexpression, which is positively associated with the infiltration and activition of effector CD8+T cells. Mechanically, Reg3A is directly antimicrobial protein that bind its bacterial targets via interactions with peptidoglycan carbohydrate. Overall, our study further explained the mechanism responsible for the tumor suppressor role of ABHD5 in CRC, strongly suggesting the role of ABHD5 in effector CD8+T cells' infiltration.

P1288

Core fucosylation of T cell receptor (TCR) promotes activation of $CD4^+$ T cells through enhancing TCR-pMHC-II interaction in systemic lupus erythematosus

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CD4+ T cell activation promotes the pathogenic process of systemic lupus erythematosus (SLE). T cell receptor (TCR) complex are highly core fucosylated glycoproteins, which play important roles in T cell activation. In this study, we found that the core fucosylation of CD4+ T cells was significantly increased in SLE patients. Loss of core fucosyltransferase (Fut8), the sole enzyme for catalyzing the core fucosylation of N-glycan, significantly reduced CD4+ T cell activation and ameliorated the experimental autoimmune encephalomyelitis-induced syndrome in Fut8−/− mice. T cell activation with OVA323-339 loaded major histocompatibility complex II (pMHC-II) on B cell was dramatically attenuated in Fut8−/−OT-II CD4+ T cells compared with Fut8+/+OT-II CD4+ T cells. Moreover, the phosphorylation of ZAP-70 was significantly reduced in Fut8+/+OT-II CD4+ T cells by the treatment of fucosidase. Our results suggest that core fucosylation is required for efficient TCR-pMHC-II contacts in CD4+ T cell activation, and hyper core fucosylation may serve as a potential novel biomarker in the sera from SLE patients.

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DOI: 10.1002/eji.201970400

P1289

Ablation of PKA-Cβ2 subunit in mice curtails progression of breast adenocarcinoma and turns "cold" tumor "hot"

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Protein kinase A (PKA) is a member of the serine-threonine kinase superfamily that transduces cAMP signals through catalytic subunits Cα and Cβ. Several studies highlighted the role of PKA in promoting progression and aggression of breast cancer; however, its contribution to cancer immunoediting remains unexplored. The splice variant Cβ2 is exclusively expressed in immune cells and mediates anti-proliferative effect by phosphorylation of the C-terminal Src kinase (CsK). To determine the role of PKA-Cβ2 in regulating cancer progression, eight to twelve weeks old, Cβ2 knockout and wild type mice were injected with estrogen receptor (ER^+) EO771 adenocarcinoma cells and tumor growth was assessed. Preliminary data showed a delay in tumor growth in Cβ2 knockout mice associated with elevated infiltration of lymphocytes and macrophages in their primary tumors as compared with wild type counterparts. Moreover, flow cytometric analysis of spleen revealed increased numbers of Th1 ($CD4^+CD183^+$), Th9 ($CD4^+CD194^-CD196^+$), and Th17 ($CD4^+CD194^+CD196^+$) cells in Cβ2 knockout mice. These findings will lead to a better understanding of the role of Cβ2 in modulating immune responses against tumor cells and may make Cβ2 an attractive new target for cancer immunotherapy.

P1290

Role of IKK signalling during T cell activation and response to influenza infection in vivo

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Canonical NF-κB signalling has an important role for cell survival, differentiation and function. In particular, NF-κB signalling is central to an effective inflammatory immune response and is thought to have significant roles in T cell homeostasis and activation. The IKK complex, consisting of IKK1, IKK2 and NEMO, is required for the activation of NF-κB. Our lab has previously shown that defective NF-κB signalling, through loss of IKK2, leads to impaired maturation of new T cells. We sought to investigate the role of IKK signalling during T cell activation. When IKK2 deficient F5 TCR transgenic T cells were stimulated with influenza peptide *in vitro,* T cell activation was impaired and proliferation was reduced. Furthermore, deletion of IKK2 in T cells profoundly compromised their response to influenza infection *in vivo.* To further probe how IKK2 is required during T cell activation, we are currently exploring potential roles for IKK2 in regulation of the cell cycle and cell survival.

P1291

The function of Ubiquitin-modifying enzyme A20 in thymic selection and diseases

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A20, also known as TNFAIP3, is an ubiquitin - modifying enzyme, which has a key role in the negative regulation of inflammation and innate immune response. Previous studies reported that A20 is highly

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DOI: 10.1002/eji.201970400

expressed in lymphoid organs, such as thymus, spleen and lymph nodes. However, its function in T cells and diseases is still not completely clear. To assess this, we crossed A20 floxed mice with CD4 Cre Tg mice which deleted A20 from CD4+CD8+ (DP) cell stage. As a result, we found that the number of Foxp3^{+}regulatory T cells was significantly increased in thymus of A20/CD4 Cre mice (within 4 days after birth). In vitro FTOC culture, there were more Treg cells in A20 deficient fetal thymus than control. In addition, Treg cells were also increased in periphery of A20 deficient mice and exhibited the normal suppressive function as control in diseases. Moreover, we crossed A20 floxed mice with Foxp3 Cre mice. The number of Treg cells in A20/Foxp3 Cre mice was equal to those in control mice. So A20 could affect thymic Treg development at the stage of DP to Foxp3+ cells. On the other hand, we also found that the loss of A20 caused fewer of iNKT cells and nIEL precursor cells in thymus. These defects may be related with the changed TCR signaling. So, these results illustrate that A20 plays an important role in thymic selection and diseases.

P1292

Sulforaphane inhibits inflammatory responses of primary human T-cells by inducing a pro-oxidative intracellular milieu

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Sulforaphane (SFN), a compound from plants of the brassicaceae family, was reported to suppress cancer cell growth. Information about its relevance for human T-cells is limited. This is surprising as T-cells play a critical role in tumor control. We, therefore, investigated the effects of SFN on human peripheral blood T-cells. SFN did not diminish T-cell viability and interfere with early T-cell activation, i.e. the formation of a mature immune synapse. However, it affected later activation events as upregulation of CD69 and CD25. These inhibitory effects could be rescued by thiol-containing antioxidants. In line with that, SFN led to an increase of intracellular reactive oxygen species (ROS) and a marked decrease of glutathione. Consistently, increased global cysteine sulfenylation was detected. Importantly, a major target for SFN-mediated protein oxidation was STAT-3, a transcription factor involved in the regulation of T_H17-related genes. In a gene expression signature analysis, we found that T_H17-related genes were predominantly inhibited by SFN. Since IL-17 and ROS regulation are attractive targets for treating rheumatoid arthritis (RA), we tested the effect of SFN on whole blood from RA patients and found increased ROS levels in T-cells. Furthermore, costimulation-induced expression of IL-17 was markedly decreased in SFN-treated RA T-cells. Together, our study shows that SFN may act as a promising immunosuppressive substance via regulating the redox balance in T-cells. Currently, we are investigating its effects on additional T-cell functions relevant for tumor defense, namely migration and cytotoxicity. Furthermore, we compare the effects of SFN with those of other herbal compounds.

P1293

Effective *in vitro* activation, polarization and evaluation of CD4+ T cell lineages

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DOI: 10.1002/eji.201970400

CD4+ T cells are a key component of the immune system. They mediate humoral and cellular immune responses against pathogens and other diseases like cancer by inducing and activating effector cells such as B cells, cytotoxic T cells and innate cells. They are also involved in the regulation of immune responses by controlling the persistence and magnitude of the response. Activation of naïve T cells is initiated by interaction with antigen presenting cells. The downstream signal transduction and the cytokine milieu can lead to their proliferation and differentiation into different T cell subtypes. To facilitate the study of different CD4+ T cell lineages, we present a series of protocols to effectively activate, polarize and evaluate differentiated CD4+ T cells *in vitro*, including Th1, Th2, Th17 and Treg lineages. CD4+ naïve T cells are initially isolated using our MojoSort™ magnetic separation system. Canonical anti-CD3 and anti-CD28 clones are used to activate the purified cells. Different sets of bioassay-validated cytokines, including IL-12, IL-4, IL-6, IL-1β, IL-23, IL-2, and TGF-β1 are used together with neutralizing antibodies to activate and polarize naïve T cells to Th1, Th2, Th17, or Treg cells. Successfully polarized populations can be identified by surface staining of cytokine receptors and intracellular staining of key cytokines and transcription factors. Hallmark cytokine expression profile of different lineages are evaluated by LEGENDplex™ bead-based multiplex assay. In summary, we have successfully developed protocols to polarize T cells *in vitro* to Th1, Th2, Th17, or Treg lineages using high quality reagents developed in BioLegend.

P1294

Nuclear speckle-related protein 70 (NSrp70) is a novel RNA binding protein that is critical for late T cell development

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We have previously identified a novel gene named NSRP1, whose function was attributed to alternatively splice mRNA. NSrp70—a protein product of NSRP1 gene—was highly expressed in early developmental stages and knockout of NSRP1 resulted in embryonic lethality. In the present study, we found that NSrp70 is also exclusively expressed in immune-related tissues such as thymus, spleen, and lymph node as well as in T and B lymphocytes. These results led us to investigate whether NSrp70 plays a role for the development of both T cells and B cells. In thymus, the expression of NSrp70 was high in $CD4^+CD8^+$ double positive (DP) thymocytes, but low in mature $CD4^+$ and $CD8^+$ single positive (SP) thymocytes, suggesting that NSrp70 may involve in the transition of DP thymocytes into both the $CD4^+$ and $CD8^+$ SP lineages. To prove this hypothesis, we used a CD4Cre/loxP system to generate conditional deletion of NSRP1 in DP stage. $NSRP1^{f/f}$CD4Cre mice showed dramatic reduction of the number of mature thymic $CD4^+$ and $CD8^+$ SP T cells, while no significant changes were induced in double negative (DN) and DP populations. Strikingly, RNA-seq analysis revealed that knockout of NSRP1 increases the expression of gene clusters that regulate cell cycle progression. In accordance with this, DP thymocytes with NSRP1-deficiency showed significant proliferation with high expression of Ki-67 as compared to wild-type DP thymocytes. These results suggest that NSrp70 is a key regulator at the stage when cells transit from DP thymocytes to SP lineage.

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DOI: 10.1002/eji.201970400

P1871

Does CD4 T cell activation require a quorum of lymphocytes?

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Understanding how an antigen interacts differently with a lymphocyte to cause either its activation or inactivation has been the subject of intense investigation over the past 60 years, as it helps in understanding the mechanism of immunological peripheral tolerance. The commonly held "Danger model" postulates that a single T lymphocyte can be activated by an antigen presenting cell (APC) under appropriately "dangerous" conditions that instruct the APC to upregulate its expression of co-stimulatory molecules necessary for T cell activation. We propose an alternative mechanism whereby the activation of an antigen-specific lymphocyte requires the presence of a quorum of lymphocytes specific for the same antigen. In the absence of such a quorum, the antigen inactivates the lymphocyte. Using enzyme-linked immune-spot (ELISPOT) assay and flow cytometry, we investigated whether the activation of naïve, T cell receptor (TCR) transgenic CD4 T cells in vitro is quorum-dependent. Keeping all other variables constant, varying the number of CD4 T cells per culture dramatically influenced the ability of "naïve" CD4 T cells to give rise to IL-2 and/or interferon gamma producing cells. In general, relatively low numbers of CD4+ T cells did not support the generation of cytokine-producing cells, medium numbers generated IL-2 producing cells, while higher numbers supported the generation of IL-2 and interferon-gamma producing cells. Our data support our proposal that a quorum of TCR-transgenic CD4 T cells is required for the generation of effector CD4 T cells.

P1872

T cell-intrinsic differences skew the thymic selection threshold in neonates versus adults

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Although it is known that cell-intrinsic differences in mature T cells contribute to the distinct effector functions of neonatal and adult T cells, the factors involved remain unclear. Given emerging evidence that the binding strength of a T cell receptor (TCR) for self-peptide presented by MHC (self-pMHC) impacts T cell function, we sought to determine if altered thymic selection influences the self-reactivity of the TCR repertoire during ontogeny. Indeed, T cell subsets in the thymus and periphery of neonates and young mice expressed higher levels of cell-surface CD5, a surrogate marker for TCR avidity for self-pMHC, as compared to their adult counterparts. This physiologically relevant difference in self-reactivity is independent of both the germline bias of the neonatal TCR repertoire and reported defects in negative selection. Rather, thymic selection is altered in young mice such that thymocytes bearing TCRs with low affinity for self-peptide are not efficiently selected and stronger TCR signals accompany both conventional and regulatory T cell selection. Our data further suggest that thymocyte-intrinsic differences contribute to this shift in the thymic selection threshold in neonates versus adults. Overall, the relatively higher self-reactivity of the T cell pool may endow the limited neonatal T cell repertoire with the ability to bind more foreign antigens with higher affinity but may also contribute to the ineffective neonatal immune response to infection.

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DOI: 10.1002/eji.201970400

P1873

Immunological changes in monocyte subsets and their association with Foxp3+ regulatory T cells in HIV-1-infected individuals with syphilis

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The incidence of syphilis has increased dramatically in men who have sex with men (MSM), especially those with HIV-1 infection. Treponema pallidum and HIV-1 are bidirectionally synergistic, accelerating disease progression reciprocally in co-infected individuals. We have shown that monocytes have different effects on T helper cells at different stages of HIV-1 infection. However, the immunological changes in the three monocyte subsets and in regulatory T cells (Tregs), and the associations between these cell types during syphilis infection among HIV-1-infectedMSM remain unclear. Herein, we used cell staining methods to explore changes in monocyte subsets and Tregs and any associations between these cells. We found that the frequency of classical monocytes was higher in the rapid plasma reagin (RPR+) group than in the healthy controls (HCs) and the chronic HIV-1 infection (CHI) plus RPR+ (CHI&RPR+) group. The frequencies of Foxp3+CD25+CD45RA+ and Foxp3+Helios+CD45RA+ Tregs were significantly higher in the RPR+, CHI, and CHI&RPR+ groups than in HCs, whereas the frequency of CD45RA+ Tregs was lower in the CHI&RPR+ group than in CHI group. The frequencies of Foxp3+CD25+CD45RO+ and Foxp3+Helios+CD45RO+ Tregs were lower in the RPR+, CHI, and CHI&RPR+ groups than in HCs. The frequency of intermediate monocytes was inversely correlated with the frequency of CD45RA+ Tregs and positively correlated with the frequency of CD45RO+ Tregs. These results demonstrate for the first time that intermediate monocytes control the differentiation of Treg subsets in Treponema pallidum/HIV-1 co-infections. These findings provide new insights into an immunological mechanism involving monocytes/Tregs in HIV-infected individuals with syphilis.

P1874

Advances in the relationship between autoimmune regulatory genes and thymus negative selection

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T cells undergo positive and negative selection in the thymic cortex and medulla, respectively. Tissue restricted antigens (TRAs) are key to T-cell negative selection and central immune tolerance. The autoimmune regulatory factor AIRE was once considered to be the only transcription factor regulating TRA genes. However, recently it has been determined that transcription factor FEZF2 can independently and directly regulate TRA genes and promote the development of central tolerance. *Aire* and *Fezf2* are regulated by the receptor activator of nuclear factor κB (RANK)/CD40 and LTβR signaling pathways, respectively. The LTβR-Fezf2 axis may play an important role in the initiation of immune tolerance while the RANK/CD40-AIRE axis plays an auxiliary role and has some key functions that FezF2 cannot perform. In this review article, we expand on the above points and discuss the role of autoimmune regulatory genes in central tolerance and autoimmune diseases.
Keywords: Aire; Fezf2; tissue specific antigen; mTECs; negative selection

DOI: 10.1002/eji.201970400

P1875

Comparison of the prevention and treatment effects of dexamethasone and cyclosporine on donor t lymphocytes activation model and a mouse model with haploidentical graft-versus-host disease

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Haploidentical bone marrow transplantation is an important means for treating hematological malignancies and non-malignant genetic defects, but graft versus host disease (GVHD) often occurs as high as 50% to 80%. Donor-derived T lymphocytes mediate the occurrence of GVHD, so blocking T lymphocyte activation is a hot research topic for treatment.
CSA and Dexa both could significantly inhibit the proliferation of lymphocyte from donor mice in one-way MLR, and showed similar effects. The donor mice spleen lymphocyte were stimulated by PHA, $CD3^+$ T lymphocytes were selected and rates of Annexin V^+/PI^- apoptosis cells were then detected by flow cytometry. CSA and Dexa could induced the apoptosis of T lymphocyte after PHA stimulation, Dexa was much better than CSA. CSA and Dexa showed the regulation effect on cell cycles of T lymphocytes, the proportion of sub-diploid apoptosis cell was significantly increased, the rates of cells in G0/G1 phase were raised, while the proportion of S phase was decreased, Dexa show more obvious with role. CD25 and CD69 were markers of T cells response for GVHD, CSA and Dexa could decrease the expression rates of CD25 and CD69 on T cells, CSA showed much better effects than Dexa. A stable mice GVHD model after Haploidentical bone marrow transplantation was established, CSA and Dexa could reduce the severity degree of GVHD, prolong the survival, CSA was much better than Dexa.
The donor T lymphocytes activation model and stable mice GVHD model is useful for drug screening and were validated by CSA and Dexa.

P1876

Novel plate-based detection method for T cell activation/proliferation, migration, and cytotoxicity assay using image cytometry

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The field of cancer immunotherapy has grown significantly in recent years. Traditionally, ELISA or ELISpot are standard assays for detecting cytokines concentration for measuring T cell response. Functional assays are performed using flow cytometry or qualitative analysis by microscopy in general. Recently, image cytometry has been employed as a new tool to develop robust assays to monitor immune response.
In this work, we demonstrate the use of Celigo Image Cytometer to perform in vitro cell-based assays to monitor immune response for cancer vaccine research. Three novel methods are developed: label-free T cell activation and proliferation measurement, T cell migration assay, and a T cell-mediated cytotoxicity assay using direct cell counting. For T cell activation and proliferation, primary T cells are seeded into a 96-well plate and stimulated with antibodies or co-culture with cancer cells. The cells are directly counted in the well over 48 hours using the image cytometer in bright field images. To measure T cell migration, T cells are seeded in a transwell plate with chemokines in the bottom well. As the T cells migrate through the transwell and drop to the bottom of the wells, the cells can be directly counted using bright field or fluorescence imaging with Hoechst staining. Finally, T cell-mediated cytotoxicity assay is performed by co-culturing CD8 T cells with calcein- or fluorescent

DOI: 10.1002/eji.201970400

protein-labeled cancer cells. As target cancer cells are killed, the fluorescence dissipates, and by counting the changes in the number of live target cells, the cytotoxicity percentages are measured.

P1877

Immunoglobulin expression in T lymphocytes

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The traditionally concept is that the expression of Immunoglobulin(Ig)is restricted to B lineage cells.However,a great of studies had reported that many non B cells can also produce Ig.However,so far,it is remains no conclusion whether the T lymphocytes can produce Ig although the rearrangement and transcription of Immunoglobulin heavy chain gene have been found to occurred in murine T lymphomas.The aim of this study was to determine whether immunoglobulin can be produced by T lymphocytes and explore its possible biological function in T lymphocytes.Multiple RT-PCR and high-throughput sequencing were used to analyze Ig gene rearrangement in mature T cells sorted from mice.Then we sorted T cells in different stages and different activation states,and analyzed the Ig expression by Western blotting.By cell proliferation assay,we determined the activation,proliferation,and effector molecules production of $CD4^+$T and $CD8^+$T cells in the Ig heavy-chain-knockout mice ($Igh^{-/-}$mice) and the membrane Igμ-deficient mice (μMT mice).In this study, we first found Ig gene rearrangement in mature T cells sorted from wildtype mice.Furthermore,the IgG,IgA,IgM and Igκ were also found in T cells in B cell deficient mice.Our results found that the number of $CD8^+$T cells were significantly reduced,and the activation,proliferation,and effector molecules production of $CD8^+$T cells were inhibited in $Igh^{-/-}$mice,but there were no significant differences in $CD4^+$T cells,which suggested that Ig is important for differentiation and function of $CD8^+$T cells,but not $CD4^+$T cells.However,the activation and proliferation were enhanced in both $CD4^+$T cells and $CD8^+$T cells in μMT mice,which suggested that the Igμ may play a negative role in T cells activation.

P1878

Specific activation and expansion of human peripheral blood γδ T cells stimulated by B cell epitopes from *Mycobacterium tuberculosis* antigen

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The γδ T cells are unique T cell subset with distinct features, such as antigen recognition, similar to B cells. However, whether the γδ T cells can be specifically stimulated with B cell epitope of antigen is not known. In this study, we selected six B cell epitopes (BP1-BP6) from *Mycobacterium tuberculosis* (Mtb) antigen that reported in literatures, and two γδ T cell epitopes (TP14, TP15) that were screened by phage display technique in our laboratory, as the antigen peptides to stimulate human γδ T cells. The PBMCs isolated from whole blood of 18 healthy subjects and four TB patients were stained with CFSE, and then cultured with B cell and γδ T cell epitopes in IL-2 containing medium for 12 days. By using flowcytometry, the percentages of γδ T cells in culture with BP2, BP4, TP14, and TP15 increased significantly in 14 samples ($P<0.05$). After re-stimulated with same peptide, the CD69 expression and IFN-γ secretion of the expanded γδ T cells increased significantly. Using RT-PCR and CDR3 gene spectrum scanning assay, the TCRVδ-CDR3 gene fragment distribution of the γδ T cells

DOI: 10.1002/eji.201970400

stimulated with B epitopes was in oligoclonal pattern, compared to Mtb-HAg stimulated γδ T cells that showed in polyclonal distribution. In conclusion, our results showed human γδ T cells can be specifically stimulated by B cell epitope from Mtb Antigen, and B cell epitope expanded γδ T cells might be clonal specificity.
Funding: The Innovation Team Project in Scientific Research Platform of Anhui Colleges and Universities (2016-40)

P1879

MicroRNA-126 deficiency affects the development of thymic CD4$^+$ single positive cells through elevating IRS-1

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Background: MicroRNA-126 (miR-126), a distinct miRNA family member, has been reported to be involved in the development and function of immune cells. However, the potential role of miR-126 in the development of CD4$^+$ T cells remains to be elucidated.
Objectives: To investigate the potential role of miR-126 in the development of thymic CD4$^+$ T cells and explore its significance.
Methods: The relative expression of miR-126 in thymic CD4$^+$ single-positive (SP) cells was detected by Real-Time PCR assay. The total cell number of thymocytes and the expression of activation-associated molecules including CD62L, CD69, and CD44, as well as proliferation-associated nuclear antigen Ki-67, in CD4$^+$ SP cells were assessed by flow cytometry (FCM). The expression of IRS-1 and related signaling pathways including Akt and Erk were determined by FCM.
Results: Compared with that in wild-type(WT) mice, the total cell number of thymocytes in miR-126 knockdown (KD) mice increased significantly. Moreover, the percentage and absolute cell number of thymic CD4$^+$ SP cells decreased significantly in miR-126 KD mice. Further analysis showed that the frequencies of CD62L, CD69, CD44, and Ki-67 in CD4$^+$ SP cells also changed significantly, respectively. Mechanism aspect, the expression of IRS-1, a putative target of miR-126, increased significantly in CD4$^+$ SP cells in miR-126 KD mice. Moreover, the expression levels of signaling molecules phosphorylated (p)-Akt and p-Erk also changed significantly.
Conclusions: Our work is the first to reveal a previously unknown role of miR-126 in the development of thymic CD4$^+$ SP cells, which might ultimately benefit studies on thymocyte development.

P1880

Characteristics of clinical immunological response in the patients with active tuberculosis

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We have realized that tuberculosis was not only a bacterial infectious disease, but also an immune disease. **The status of immune response determines the occurrence, development and prognosis of diseases after *M. tuberculosis* infection.** The number of T lymphocyte, CD4 + T lymphocyte, CD8 + T lymphocyte, NK cell, NKT cell and B lymphocyte in peripheral blood of 444 tuberculosis patients were analyzed by flow cytometry. We found that the number of T cell and CD4+

DOI: 10.1002/eji.201970400

T cell in TB patients aged above 60 years old decreased significantly, the number of lymphacyte subsets in bacteria-positive patients aged over 60 years old was lower than that in bacteria-negative patients, CD4+ T cells in IFN-γ-positive patients was significantly higher than that in IFN-γ-negative patients, NK cells in retreated patients was significantly lower than that in newly diagnosed pulmonary tuberculosis patients, 75.3% of the patients with pulmonary tuberculosis had a lower number of lymphocyte subsets than the normal reference rang, and 44% had a lower CD4+ lymphocyte number than the normal range. In conclusion, **the most patients with tuberculosis have different degree of cellular immune dysfunction, especially CD4+ T lymphocytes**. **Analyzing lymphocyte subsets in patients with tuberculosis is helpful to determine the immune function of the patients and can provide experimental basis for clinical immune intervention.**

P1881

Investigation the roles of IL-12 family cytokines on the thymic T cell selection and the subsequent impact on peripheral T cell pool

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IL-12 family cytokines play crucial roles in T cell immune responses in the peripheral which regulate the inflammatory and tolerance. Interestingly, previous reports have demonstrated that IL-12 and IL-23 have supportive effect in the thymic negative selection and regulatory T cell generation. However, their roles in the TCR repertoire diversity and subsequent effect on the peripheral immunity is unclear. In this study, we used IL-12p35 knockout and IL-23p19 knockdown NOD mouse model to address this issue. We first found increased thymocyte numbers in IL-12p35 knockout and IL-23p19 knockdown NOD mouse. Then we used thymus transplantation and bone marrow chimera model to investigate the effects of these cytokine on TCR repertoire distribution and autoimmune phenotype. The NOD-RAG1$^{-/-}$ mice which received IL-23p19 knockdown thymus displayed increased susceptibility to autoimmunity. Interestingly, T cells derived from IL-12p35 knockout thymus showed increased IFN-g and decreased IL-17 production in peripheral and inflammatory lesion. Our data suggested that IL-12 and IL-23 have supportive role in the central tolerance and defect of these cytokines in the thymus could leads to autoimmunity.

P1882

A novel function for Rab35 in CD8$^+$ T cell function

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Rab GTPases are important in organelle trafficking and protein transport between organelles. Rabs are highly expressed in T cells, yet their functions in T cells is not well elucidated. In our studies of the Rab35 GTPase, a Rab member involved in recycling of proteins to the plasma membrane, Rab35 does not affect T-cell antigen receptor internalization nor recycling. Surprisingly, Rab35 regulates CD8 T cell activation through inhibiting Akt/mTOR activation. In turn, Rab35-deficient CD8 T cells have increased PI(3,4,5)P_3 level, Akt/mTOR signaling, and transcriptional activation of downstream effector in response to anti-CD3/28 stimulation, when compared to wild-type CD8 T cells. Following infections with *Listeria monocytogenes*, mice repopulated with *rab35*$^{-/-}$ CD8 T cells demonstrate greater T cell expansion and lower bacterial load when compared to mice reconstituted with WT CD8 T cells. Finally, our preliminary evidence suggests that Rab35 co-localizes with OCRL, an inositol polyphosphate-5-phosphatase 5F (INPP5F) that hydrolyses PIP_3 within the endosomal compartment. Together, our

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DOI: 10.1002/eji.201970400

data supports a working model where PIP_3 levels are highly regulated during T-cell activation in $CD8^+$T cells by SHIP1 (INPP5D) at the plasma membrane and by Rab35/OCRL(INPP5F) in a Rab7+ endosomal compartment. Understanding the mechanistic basis of this dual regulation will provide a better ability to specifically modulate $CD8^+$ T cell functions.

P1884

Tumor regression with CD8+ T cells in tumor micro-environment and central immune system following PD-1 mAb plus IL-2 treatment

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A key issue in cancer immunotherapy is to block the immunosuppression in the tumor microenvironment (TME) and boost the proliferation of CD8+ T cells. The immunosuppression in TME of patients is frequently induced by the programmed death-ligand 1/programmed death 1 (PD) pathway associated with immune tolerance and impaired proliferation of lymphocytes, especially CD8+ T cells. Blockade of the PD pathway overcomes the adaptive resistance of the PD pathway but less contributes to the rescue of the impaired lymphocyte proliferation. To solve the both problems, we used both PD-1 monoclonal antibody (mAb) and IL-2 mutein in which PD-1 mAb removes the suppression of the PD pathway upon CD8+ T cells whereas IL-2 mutein increases the proliferation of lymphocytes. As expected, significant regression of xenograft tumors occurred in the PD-1 mAb+IL2 mutein combotherapy group compared with PD-1 mAb alone and IL-2 mutein alone groups. CD8+ T cells in the TME, spleen and blood stream increased significantly. This increased the anti-tumor immunity in TME and reduced the tumor volume of human SPC-A2-PDL1 xenografts in NSG mice. We, therefore, concluded that this immune combotherapy ultimately killed tumor cells in animal models and normalized the immune responses in TME by rescuing the anergic and apoptotic CD8+ T cells and simultaneously increasing the proliferation of CD8+ T cells, locally and systemically.

P1885

Regulation of Rbpj expression reveals a pre-thymic role for Notch in T-cell differentiation

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T-cell specification and commitment requires Notch signaling. Although the requirements for Notch during intrathymic T-cell development have been well studied, it is still unclear whether the onset of T-cell priming can occur in a pre-thymic niche and whether RBPJ-dependent Notch signaling plays a role during this event. To address this, we established a novel Rbpj-inducible (RBPJind) system that allows for temporal and tissue-specific control of Notch responsiveness in hematopoietic cells. This allowed us to precisely control the initiation and duration of T-cell development in vivo. We show that Notch signaling is required prior to the appearance of early T-cell progenitors (ETPs) in the thymus. We also found that lymphoid-primed multipotent progenitors (LMPPs) in the bone marrow (BM) undergo active Notch signaling upon Rbpj induction, which inhibits myeloid lineage skewing in thymus-seeding progenitors (TSPs). Thus, our results indicate that the onset of T-cell differentiation occurs in a pre-thymic setting, and that Notch plays an important role during this event.

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DOI: 10.1002/eji.201970400

P2556

Antitumor activity of lipid-DNA aptamer modified T lymphocytes in pancreatic ductal adenocarcinoma

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Pancreatic adenocarcinoma (PAC) currently represents the deadliest malignancy. Antibodies or aptamers could be employed to deliver cells to the site of interest. In this study, we firstly used the aptamer XQ-2d which targets PL45 cells, mimicking native cell surface and ligand receptor interactions. Secondly, the PEG linker was applied to extend DNA from the cell surface. It is beneficial for the conformational folding of XQ-2d, which facilitates XQ-2d binding to target cells. Thirdly, two stearic acids were used to compound the diacyl lipid tail and conjugated to the 5' terminus of XQ-2d. The diacyl lipid tail with hydrophobic effects could be inserted into the cell membrane firmly. Fourthly, Anti-CD3/anti-CD28 monoclonal antibodies activate T cell proliferation and secretion of anticancer cytokines, and help T-lymphocytes regulate non MHC-restricted cytotoxicity. T cell redirection for targeting and eliminating tumor cells is promising as immunotherapeutic strategy for cancer treatment. Anti-CD3/anti-CD28 monoclonal antibodies co-activated T lymphocytes as effector cells to kill the tumor. Importantly, the Lipo-XQ-2d probe was inserted into activated T cells and redirected them to kill the target PL45 cells.
We demonstrated that the diacyl lipid tail with aptamer anchoring on T cells is simple and effective, causing no harm on the modified cells. Based on this study, it is expected to develop Lipo-aptamers, thus enhancing the anti-tumor effect of T cells to achieve a more accurate and rapid killing of tumor cells.

P2557

SET-CAN fusion gene as poor prognosis predictor in adult T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is a malignant neoplasm accounts for approximately 20% of all ALL cases. Here, we report three rare cases of T-ALL with SET-CAN fusion gene, especially two patients with a complex karyotype never reported. We describe the morphologic, cytogenetic, immunophenotypic, molecular findings, therapy and outcome of patients. We found that SET-CAN gene developed resistance to chemotherapy drugs including large dose glucocorticoids. The SET-CAN-mediated loss of regulation of histone acetylation was a potential mechanism of glucocorticoid resistance. These three patients with SET-CAN fusion gene had a poor prognosis, and the CLAG chemotherapy combined with asparaginase might be a potential treatment.

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DOI: 10.1002/eji.201970400

P2558

Dissecting the functions and mechanisms of VAX2 in the differentiation of Th17 cells in autoimmune diseases

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Th17 cells play an important role in many autoimmune diseases such as multiple sclerosis and inflammatory bowel disease. Neutralizing the formation and gathering of Th17 cells is a promising strategy in the treatment against these diseases. Here we identified VAX2 as an important transcription regulator for Th17 cells. VAX2 promoted the Th17 differentiation and IL-17 production. However, the detailed mechanism of VAX2 in the regulation of Th17 remains unknown. We constructed Vax2-deficient mice. Next we plan to study VAX2 in vivo by constructing the models of autoimmune diseases including experimental autoimmune encephalomyelitis and inflammatory bowel disease. **This project tries to figure out the pathway and regulation network of VAX2 for the differentiation and function of Th17, and to provide theoretical proof for diagnosis and therapy of autoimmune diseases.**

P2559

Transcription factors TCF-1 and LEF-1 cooperatively regulate the function of intestinal regulatory T cells

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TCF-1 and LEF-1, key effector transcription factors in the canonical Wnt pathway, play important roles in many T-cell subsets. However, the roles of TCF-1 and LEF-1 in regulatory T cells (Treg) remain largely unknown. In this study, we showed that TCF-1 and LEF-1 were required for the function of intestinal Treg cells. Only TCF-1 and LEF-1 double-deficient (DKO) mice exhibited abnormal inflammatory T cells infiltration in intestine, although the differentiation of splenic and intestinal Treg cells looked normal. Moreover, DKO mice showed more severe symptom and more T_H1 and T_H17 cells infiltration in colon during TNBS-induced chronic colitis. Mechanically, the intestinal Treg cells in DKO mice limited the suppression capacity *in vitro*, although the splenic Treg cells showed normal. In addition, the induction of DKO Treg cells *in vitro* also looked similar. Importantly, forced expression of TCF-1 restored the function of intestinal DKO Treg cells. Thus, our results clarify that TCF-1 and LEF-1 cooperatively regulate the function of intestinal Treg cells, and TCF-1 or LEF-1 are dispensable for differentiation of intestinal Treg cells and the function of splenic Treg cells.

P2560

Fibroblast is potent T cell immunity suppressor via MHC molecule

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Sustained inflammation is one of the main characteristics of autoimmune diseases. T cells are the main effector cells in inflammation, and the mechanisms in T cells regulation have not been

DOI: 10.1002/eji.201970400

completely explored. Sustained inflammation is always accompanied with fibrosis, indicating the close relation between fibroblast and inflammation. Fibroblast is one of the most important cells in tissue remodeling. However, the specific role of fibroblast in inflammation is still not very clear, studies about its effect on T cells were controversial. We speculated that fibroblast was involved in inflammation regulation by regulating T cells. In this study, we investigated the role of fibroblast on proliferation and differentiation of T cells. We found that fibroblast can prominently inhibit proliferation of T cells by contact or not. It can greatly decrease the production of IL-4 while no significant effect on IFNγproduction. Soluble factors secreted by activated T cells can induce the expression of several surface molecules including MHC and PD-L1 on fibroblast. The inhibition of MHCII molecules expression by siRNA on fibroblast can decrease the effect of negative regulation on T cell proliferation, indicating the important role of MHCII molecules on fibroblast in T cells negative regulation.

P2561

Antigen-specific CD8+ memory stem T cells generated from human peripheral blood effectively eradicate allogeneic targets in mice

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As the implantation and long-term existence of tumor-specific T cells in host are the prerequisite for adoptive immunotherapy, memory stem T cells (T_{SCM}) with self-renewal and differentiation capacity show the greatest potential to implant and long-term exhibit function in vivo, compared with other T cells of differentiation stages. Hence, tumor-specific T_{SCM} have become potential candidate for adoptive T cell therapy of cancer. Here, we reported a protocol to generate allogeneic antigen-specific CD8+ T_{SCM} cells from human PBLs. To prepare allogeneic antigen-specific CD8+ T_{SCM}, we used an LCL named E007 of defined HLA allotyping as simulator, a co-culture of E007 and allogeneic PBLs was carried out in the presence of differentiation inhibitor TWS119 for 7 days. Sorting of proliferation cells ensured the E007-specificity of the prepared T_{SCM} cells. The sorted lymphocytes underwent further expansion by cytokines IL-7 and IL-15 for further 7 days, making the E007-specific CD8 + T_{SCM} expanded in number. The stem cell and T memory cell properties of the prepared CD8+ T_{SCM} were observed in NOD-SCID mice. Our protocol began with 1×10^7 PBLs and resulted in 2×10^7 E007-specific CD8+ T_{SCM} cells in 2 weeks of preparation. The prepared T_{SCM} cells exhibited a proliferative history and rapid differentiation into effector cells upon the E007 re-stimulation. Importantly, the prepared T_{SCM} cells were able to exist long and reconstitute other T cell subsets in vivo, eradicating the E007 cells effectively after transferred into the LCL burden mice. This study provided a reference method for generation of antigen-specific T_{SCM} for T cell adoptive immunotherapy.

P2562

Effects of FASN-mediated lipogenesis in T cells differentiation

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Metabolic processes are critical for directing immune cells differentiation and function. Lipids are indispensable elements for membrane construction, energy storage/supply and participates in signaling transduction, and lipid metabolism directs the activation, differentiation and effector function of immune cells. Recently, we discovered that endogenous fatty acid synthesis, mediated by fatty acid synthase (FASN), directs the differentiation of T cell subsets. In the absence of FASN, the differentiation of Th1 and Th2 cells was dampened, produced less IFN-γ and less IL-4 respectively.

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DOI: 10.1002/eji.201970400

Besides, T cell-specific deletion of FASN retrains the development of Th17 cells and promotes Foxp3 expression under Th17-polarizing conditions, which is consistent with previous reports. Surprisingly, FASN deficiency didn't alter the differentiation of Treg cells from naïve T cells, suggesting an intrinsic divergent demands for lipogenesis in Teffs and Tregs. In conclusion, endogenous fatty acid synthesis have critical effects on T cell differentiation and cell fate decisions.

P2563

Antigen-specific CD4$^+$ T cell-derived IFNg is both necessary and sufficient for clearing *Chlamydia* from the small intestine but not the large intestine

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Objectives: We compared Chlamydia colonization in the genital tract and gastrointestinal tract and investigated whether CD4+T cell-derived IFNγ necessarily and sufficiently clears Chlamydia in gastrointestinal tract.
Methods: C57BL/6J mice and CBA/1J mice were infected with Chlamydia muridarum intravaginally and were sacrificed in different timepoint. We quantitated the live chlamydial organisms from vaginal swabs, rectal swabs and mouse organs or tissue segments. C57BL/6J mice and mice with deficiency of α/β T cells or CD4+ T cells or CD8+ T cells or IFNγ signal or IL-22 signal or developing Th1 immunity were infected with Chlamydia muridarum intragastrically, and we find which immune effectors play role in regulating Chlamydia colonization in gastrointestinal tract.
Results: Mice deficient in a/b T cells or CD4+ T cells but not CD8+ T cells permitted chlamydial persistence in the small intestine. Mice deficient in IFNg but not IL-22 signaling pathways rescued chlamydial colonization in the small intestine; Exogenous IFNg was sufficient for clearing Chlamydia from the small intestine but not the large intestine. Mice deficient in developing Chlamydia-specific Th1 immunity permitted chlamydial persistence in the small intestine.; IFNg-producing CD4+ but not CD8+ T cells from immunized donor mice were sufficient for eliminating Chlamydia from the small intestine but not the large intestine of recipient mice.
Conclusions: CD4+ T cell-derived IFNγ is responsible for clearing Chlamydia from the small intestine but not the large intestine, indicating that chlamydial colonization in different regions of the gastrointestinal tract is regulated by distinct immune mechanisms.

P2564

The effect of nicotinamide on Tfh cells differentiation

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Background: T follicular helper (Tfh) cells play an important role in the pathogenesis of systemic lupus erythematosus that is a fatal autoimmune disease. Bcl-6, the key transcription factor of Tfh cells, is inactivated by acetylation. The mammalian Sirt1 deacetylase might has interaction with the deacetylation of Bcl-6. We speculate that nicotinamide (NAM), the precursor for the synthesis of NAD (+) and also an inhibitor of SIRT1, probably increase the acetylation of Bcl-6 by SIRT1, then inactivate Bcl-6 and inhibit the differentiation Tfh cells.
Methods: We analyzed the impact of NAM on the differentiation of naïve CD4+T cells toward the Tfh phenotype using an in vitro culture system in 0 hour, 72 hours, and 120 hours by FACS between the NAM group and the control.

DOI: 10.1002/eji.201970400

Result: Compare with the control group, the differentiation of Tfh cells in the NAM group was suppressed in 72 hours.
Conclusion: The NAM can inhibit the differentiation of Tfh cells, but the mechanism is not clear.

P2565

Memory stem T cells generated by Wnt signaling from blood of human renal clear carcinoma patients

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Memory stem T cells (Tscm) are a sub-population of T_{CM} with stem cell-like behavior because of their enhanced proliferation, survival, and anti-tumor capacities, which exceed those of T_{CM} and T_{EM} cells. However, little is known about Tscm in patients with renal clear carcinoma (RCC). The Wnt/β-catenin signaling pathway regulates the progression of thymocyte development at different stages while its role in mature T cells requires further study. In the present study, we evaluated Tscm from RCC patients concerning their activation of Wnt signaling in vitro and explored the mechanism of preferential survival. Flow cytometry identified surface markers and cytokines produced from accumulated Tscm in the presence of the glycogen synthase kinase beta inhibitor TWS119. Apoptosis was evaluated after induction using tumor necrosis factor-alpha. Immunofluorescence and western blot analyses were used to investigate the activation of the nuclear factor-kappa B (NF-ΚB) pathway. RCC patients had a similar percentage of CD4 + and CD8 + Tscm as healthy donors. Activation of Wnt signaling by TWS119 could result in the accumulation of Tscm in activated T cells, but was unable to reverse the differentiated T cells back to Tscm. Preferential survival of Tscm was associated with increased anti-apoptotic ability mediated downstream of the NF-ΚB activation pathway. Our finding will help in devising new cancer therapy strategies of Tscm-based adoptive immunotherapy, such as dendritic cell-stimulated Tscm, and T cell receptor or chimeric antigen receptor-engineered Tscm.

P2566

The progress of T cell development would been blocked in these children who have hypoxia condition caused by congenital heart disease

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Objective: The congenital heart diseases (CHDs) is one of various sources of hypoxia in human body. And some of these children would have chronic hypoxia before surgery. But the effects of hypoxia on T cell development which caused by CHDs have not been reported and attracted so much focus in particular. Therefore, we focus on the T cell development progress in children who have hypoxia caused by CHDs.
Method: The blood of the patients at 0 to 2-years-old who have CHDs were collected to detect four mature signals (CD3, CD19, CD4 and CD8) on T cells and number of NK cells by Flow Cytometry before surgery. We divided them into three groups by SpO_2: control group (con, SpO_2=100), hypoxia group 1 (h1, 100>SpO_2≧90) and hypoxia group 2 (h2, SpO_2< 90).
Result: We found that two mature T cells ($CD3^+$ and $CD19^+$) but not $CD4^+$/$CD8^+$ T and NK cells ($p>0.05$) were both decreased in h1 group comparing con ($p< 0.05$). And four mature T cells ($CD3^+$,

DOI: 10.1002/eji.201970400

CD19$^+$, CD4$^+$ and CD8$^+$) were both decreased in h2 group comparing con ($p< 0.05$)
Conclusion: In this paper, we found that the ratios of four mature T cells (CD3$^+$, CD19$^+$, CD4$^+$ and CD8$^+$) both have changed between con, h1 and h2 groups. And interestingly, the ratios of four mature T cells both decreased gradually by SpO_2 (con>h1>h2). But not the NK cells. So, we considered that the progress of T cell development would been blocked in these children which have hypoxia condition caused by CHD.

P2567

The ubiquitin E3 ligase TRIM31 promotes K33-linked Raf-1 ubiquitination to control T-cell activation and proliferation

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T cells are the central players of adaptive immune responses against infections. Dysregulation of T cells activation usually leads to immunodeficiency or autoimmune diseases. Protein ubiquitination is an important mechanism that regulates T cell activation and proliferation. Here we found that Lys-33-linked polyubiquitination of the signaling kinase Raf-1 mediated by the E3 ubiquitin ligase TRIM31 promoted the dephosphorylation of Raf-1 and thereby suppressed TCR signaling. We found TRIM31 is elevated in T cells upon TCR engagement. TRIM31 deficiency promoted the proliferation of T cells, which produced significantly larger amounts of IL-2. Additionally, we found that TRIM31 deficiency enhanced expression of the cytokine receptor CD25, the activation marker CD69. After engagement of the TCR, TRIM31 deficiency did not affect the phosphorylation of upstream signaling mediators, but enhanced the phosphorylation of Raf-1 as well as the downstream mediators. Mechanistically, TRIM31interacted with Raf-1and decreased its phosphorylation. Our study suggests that TRIM31 regulated the activation and proliferation of T cell by inactivating Raf-1 and provides new mechanistic insights into the non-proteolytic regulation of TCR signaling.
Keywords: T cell activation and proliferation; TRIM31; Raf-1

P2568

Inhibition and mechanism of PM2.5 on transformation of t lymphocyte from human peripheral blood

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To investigate the effect of PM2.5 on the transformation of T lymphocyte in human peripheral blood, and explore the mechanism of the inhibitory effect from the changes of oxidative stress.
Methods: Human lymphocytes of peripheral blood were cultured with 0, 10, 30, 100 ug/ml PM2.5 containing PHA in culturing medium for 24,48 and 72 hours. Then measured inhibition rate of lymphocyte proliferation via MTT. The concentrations of IL-6, TNF-and 8-OHdG in supernatant were detected by ELISA, and ROS in cells were detected via spectroflurometer.
Results: With the prolongation of PM2.5 exposure time, the inhibition rate of lymphocyte proliferation was increased; When cultured for 48 and 72 hours, the inhibition rate of lymphocyte proliferation in 30 and 100ug/ml group was significantly enhanced than 0ug/ml($P< 0.01$), which was positively correlated with PM2.5 dosage; When cultured for 72 hours, the contents of IL-6, TNF-and 8-OHdG in supernatant with 30 and 100ug/ml PM2.5, were significantly increased than 0ug/ml($P< 0.01$), the intracellular ROS level was also significantly enhanced($P< 0.01$).

DOI: 10.1002/eji.201970400

Conclusion: PM2.5 exposure can inhibit the transformation of human peripheral blood lymphocyte induced by PHA via stimulating the production of reactive oxygen species free radicals and DNA oxidative damage.

P2569

Fas signaling-mediated TH9 cell differentiation favors bowel inflammation and antitumor functions

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Fas causes activated T cell apoptosis to maintain immune homeostasis. However, the effects of Fas-induced non-apoptotic signaling on T cells remain largely unknown. Here we find that Fas promotes TH9 cell differentiation by activating NF-κB via Ca2+-dependent PKC-β activation. PKC-β also phosphorylates p38, inactivating and extinguishing the cooperative effect of NFAT1, which results in compromised Fas-induced TH9 cell differentiation. Fas ligation exacerbates inflammatory bowel disease. Fas ligation combined with p38 inhibitor-induced TH9 cells shows potent antitumor activity. Moreover, low-dose p38 inhibitor exhibits robust TH9-dependent antitumor effects without systemic side effects. In tumor patients, higher TH9 cells indicates a better prognosis. Thus, our study reveals a mechanism for TH9 cell induction. Using low-dose p38 inhibitor is promising for cancer therapy. In addition, Fas ligation combined with a p38 inhibitor holds high potential to generate sufficient TH9 cells for the adoptive treatment of cancer.

P2570

Upregulation of Tim-3 on $CD4^+$ T cells is associated with Th1/Th2 imbalance in patients with allergic asthma

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T cell Ig and mucin domain-containing molecule-3 (Tim-3) is a negative regulator preferentially expressed on Th1 cells. Allergic asthma is a clinical syndrome well characterized by Th1/Th2 imbalance. To investigate the role of Tim-3 in the pathogenesis of asthma and its relationship with Th1/Th2 imbalance, a total of 40 patients with allergic asthma and 40 healthy controls were enrolled. Expression of Tim-3 and Th1/Th2 imbalance as well as the relationship between them was analyzed by flow cytometry and real-time PCR. Th1/Th2 cytokines in the culture supernatant were detected by enzyme linked immunosorbent assay (ELISA). The mRNA level of T-bet and IFN-γ were significantly decreased in allergic asthma patients, while GATA-3 and IL-4 were significantly increased. Expression of Tim-3 on $CD4^+$ T cells was much higher in allergic asthma patients and it was negatively correlated with T-bet/GATA-3 ratio or IFN-γ/IL-4 ratio. Blocking of Tim-3 significantly increased Th1 cytokines (TNF-α and IFN-γ) and decreased Th2 cytokines (IL-4, IL-5, IL-13) in the culture supernatant of PBMCs. Blocking of Tim-3 dramatically reduced the production of IgG and IgE in the co-culture supernatant of $CD4^+$ T cells and B cells. In conclusion, Tim-3 was up-regulated in allergic asthma patients and related with the Th1/Th2 imbalance. Blocking of Tim-3 may be of therapeutic benefit by enhancing the Th1 cytokines response, down-regulating the Th2 cytokines response, and reducing IgG/IgE production.

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DOI: 10.1002/eji.201970400

P2571

Stage-pecific regulation of TCR signaling during positive selection by Tespa1

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Double positive thymocytes undergo positive selection to become mature single positive $CD4^+$ and $CD8^+$ T-cells under the instruction of T-cell receptor signal. Unlike mature T-cells, DP cells respond to low affinity self-peptide-MHC ligands before fully upregulation of their surface TCR expression. Thus, DP thymocytes must be more sensitive to ligand than mature T-cells. A number of molecules have been found to be able to augment the TCR signal strength to facilitate positive selection. However, almost all of them are also active in mature T-cells. We have previously described the role of Tespa1 in TCR signaling and thymocyte development. Tespa1 deficiency leads to defective positive selection. We also proved that Tespa1could facilitate TCR-induced Calcium flux signal through direct binding to IP3R and the recruitment of IP3R to TCR proximal region. Besides the severe defect in thymocyte development, *Tespa1*$^{-/-}$ cells exert a substantial degree of homeostatic expansion in peripheral lymphoid organs, suggesting Tespa1 is a thymic-specific TCR signaling regulator. Here we analysis the gene expression profile in different thymocyte development stages upon Tespa1 deficiency and found that Tespa1 deficiency led to confined stage-specific gene expression changes in cells undergoing positive selection. More importantly, Tespa1 expression decreases substantially in peripheral T cells and the conditional deletion of Tespa1 in peripheral T-cells didn't affect TCR signaling and cell proliferation. In sum, we conclude that Tespa1 is a thymic-specific regulator of TCR signaling which provides an explanation for the unusual sensitivity of DP thymocytes to TCR stimulation, a property that is essential for positive selection.

Cytotoxic T Lymphocytes

O065

CXCL13-mediated recruitment of intrahepatic $CXCR5^+CD8^+$T cells benefit viral control in chronic HBV infection

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Background: Although $CD8^+$T cell exhaustion hampers viral control during chronic HBV infection, the pool of $CD8^+$T cells is phenotypically and functionally heterogeneous. Therefore, $CD8^+$T cell subpopulations with unique properties and defining functions should be further investigated. This study aims to dissect a subset of $CD8^+$T cells expressing CXCR5 in chronic HBV infection.
Methods: The frequency of $CXCR5^+CD8^+$T cells and the levels of CXCL13, a chemokine of CXCR5, were measured in patients with chronic HBV infection. C57BL/6, IL-21 receptor- or B cell-deficient mice were hydrodynamically injected with pAAV-HBV1.2 plasmids. Phenotype and functions of $CXCR5^+CD8^+$T cells were assessed.
Results: $CXCR5^+CD8^+$T cells were partially exhausted but possessed a stronger antiviral ability than the $CXCR5^-$ subset in patients with chronic HBV infection; moreover, $CXCR5^+CD8^+$T cells were associated with a favorable treatment response in chronic hepatitis B (CHB) patients. High levels of

DOI: 10.1002/eji.201970400

CXCL13 from CHB patients facilitated the recruitment of intrahepatic $CXCR5^+CD8^+$T cells, and this subpopulation produced high levels of HBV-specific IFN-γ and IL-21. Notably, PD1 blockade and exogenous IL-21 enhanced the production of IFN-γ. More strikingly, mice injected with $CXCR5^+CD8^+$T cells showed remarkably decreased expression of HBsAg. Additionally, an impaired production of HBV-specific IFN-γ from intrahepatic $CXCR5^+CD8^+$T cells was observed in IL-21 receptor- or B cell-deficient mice.
Conclusion: CXCL13 promotes the recruitment of $CXCR5^+CD8^+$T cells to the liver, and this subpopulation benefits viral control in chronic HBV infection. The identification of this unique subpopulation may contribute to a better understanding of $CD8^+$T cell functions and provide a potential immunotherapeutic target in chronic HBV infection.

O066

HLA-C restricted neoepitopes contribute significantly to the immune recognition of cancer

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Immune therapy has experienced unprecedented success in the treatment of cancer, yet there is an unmet need for tumor specific targeting of the immune response. To achieve this, mutation-derived neoepitopes represent attractive targets due to their unique tumor expression. Neoepitopes are presented at the cell surface in the context of MHC class I molecules, in humans called HLA-A, -B and -C. Some studies have reported HLA-C restricted neoepitopes, but in many studies only HLA-A and -B restricted neopeptides have been included for T cell response evaluation. This supposedly relates to; 1) a previous lack of suitable prediction algorithms and available HLA-C reagents for T cell analyses and 2) the assumption that HLA-C plays a minor role in the T cell recognition landscape, due to lower expression. Here, we present data from the evaluation of neoepitope restricted T cell recognition in three cancer cohorts. HLA-C responses were detected in all cohorts and in all 12 individual patients. The HLA-C responses covered alleles C*0304, C*0401, C*0501, C*0602, C*0701, and C*0702. We observed an enrichment of HLA-C out of total HLA-A/B/C, when comparing predicted peptides to detected responses and in two cohorts HLA-C peptides accounted for the largest fraction of responses. Thus, despite lower expression levels, responses to HLA-C seem to play a substantial or even dominating role for the generation of T cell responses towards neoantigens. HLA-C molecules should therefore be taken into account when assessing the T cell recognition of cancer, and could serve as valuable targets for antigen-directed immunotherapeutic strategies.

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DOI: 10.1002/eji.201970400

O067

The transcription factor TCF1 preserves the effector function of exhausted CD8 T cells during chronic viral infection

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During chronic viral infection, the long-term persistence of antigen exposure drives virus-specific CD8 T cell exhaustion. Yet exhausted, CD8 T cells still retain certain level of effector function, by which they can keep viral replication in check. However, the underlying mechanisms involved in regulating the effector function of exhausted CD8 T cells remain largely unknown. Using mouse models of chronic LCMV infection (CL-13), we found that the deletion of transcription factor TCF-1 in CD8 T cells resulted in the profound impairment of virus-specific CD8 T cell response, evidenced by decreased total number of antigen specific $CD8^+$T cells as well as attenuated cytokine production and degranulation. Conversely, ectopic over expression or using agonist to enhance the expression of TCF-1 promote the effector function of exhausted CD8 T cells. By using bone marrow chimera mice and Tamoxifen induced knock out mice, we identified the cell intrinsic role of TCF1, both in early phase (Day 8) and advanced stage (D20 and later) during CL-13 infection, in maintaining virus specific $CD8^+$T cell pool as well as their effector function. Mechanistically, TCF-1 fuels the functionalities of exhausted CD8 T cells by promoting the expression of an array of effector function-associated transcription regulators, including Foxo1, Zeb2, Id3 and Eomes. Above all, these results indicate that targeting TCF-1 mediated transcriptional pathway may represent a promising immunotherapy strategy against chronic infections, and even tumorigenesis, by reinvigorating the effector function of exhausted virus-specific CD8 T cells.

O068

Large-scale identification and transcriptional characterisation of auto-reactive CD8 T cells in multiple sclerosis

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An increasing number of studies detect auto-reactive T-cells in healthy donors as well as in patients with various autoimmune diseases. This is also true for multiple sclerosis (MS), which is a demyelinating autoimmune disease of the central nervous system. We use DNA barcode-labeled MHC multimers to perform a large-scale screen for auto-reactive CD8 T-cells in blood samples from 53 MS patients and 30 healthy controls. We have predicted peptides from 6 different proteins all related to myelination of neurons, with the ability to bind 4 different HLA types, HLA-A*02:01, -A*01:01, -B*07:02 and -B*08:01. Peptides containing an arginine were also included in the study in a citrullinated form, in order to dissect the role of citrullination in MS related CD8 T-cell recognition. Due to the possible link between Epstein Barr Virus (EBV) infection and MS, predicted peptides from 4 different EBV proteins were included in our screening as well. In total we are screening for CD8 T-cell recognition of 1178 potential auto- and virus-derived epitopes. In order to investigate any potential functional differences between auto-reactive CD8 T-cells present in MS patients and healthy donors, and between virus- and auto-antigen specific CD8 T-cells, we are combining the MHC multimer screening with RNA sequencing.

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DOI: 10.1002/eji.201970400

Our initial findings suggest the presence of CD8 T-cells with specificity towards MS related proteins in both MS patients and healthy donors. Furthermore, they indicate that such antigen specific CD8 T-cells have a predominantly memory-like phenotype in MS patients, whereas they are largely naïve in the healthy donors.

LB083

Adenomatous polyposis coli modulates actin and microtubule cytoskeleton at the immunological synapse to tune cytotoxic T cell functions

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Adenomatous polyposis coli (Apc) is a tumor suppressor gene and a cell polarity regulator. Its mutations are associated with intestinal epithelium dysregulations such as familial adenomatous polyposis and colorectal cancer. However, Apc involvement in T lymphocyte functions and anti-tumor immunity remains poorly understood.
Using Apc-depleted human $CD8^+$ T cells and T cells from *Apc*$^{Min/+}$ mutant mice, we found that Apc modulates actin polymerization and microtubule network remodeling at the immunological synapse. Apc-deficient $CD8^+$ T cells exert reduced forces to maintain their interaction with antigen-presenting cells, leading to defects in T cell polarization and early TCR signaling.
Furthermore, Apc tunes cytotoxic T cell activity required for tumor cell killing. Cytotoxic granules comportment and docking at the synapse, but not release, could be impaired in Apc-deficient cells. These, together with previous data on Apc function in regulatory T cells indicate that Apc mutations may cause a dual damage, first unbalancing epithelial cell differentiation and growth driving epithelial neoplasms, and, second, impairing T cell-mediated anti-tumor immunity in various manners.

P0111

5-aza-2'-deoxycytidine (DAC) treatment induces the tumor antigen MAGE-A10 expression and improve the cytotoxicityof MAGE-A10-specific CTLs in lung cancer cells

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Background: Epigenetic-modifying drugs could enhance the expression of tumor antigen and improve the cytotoxicity of antigen-specific T cells. 5-aza-2'-deoxycytidine (DAC), a DNA methyltransferase inhibitors (DNMTis) considered as an epigenetic-modifying drug, could enhance the expression of MAGE-A10 in cancer cells.
Methods: 5-aza-2'-deoxycytidine to induce the expression level of MAGE-A10 in tumor cells. MAGE-A10 antigenic peptide (sequence: SLLKFLAKV) were prepared to induce differentiation of MAGE-A10-specific cytotoxic T lymphocytes (CTLs). Interferon-γ released ELISA assay was used to detect the capacity of MAGE-A10 peptide to induce CTLs. Cell Counting Kit-8 (CCK-8) analysis was performed to detect the cytotoxicity of MAGE-A10-specific CTLs. Immunohistochemistry was performed to detect the expression in cancer and adjacent normal tissue. Kaplan-Meier plotter online database was used to analyze the overall survival (OS), post-progression survival (PPS) and first progression (FP).
Result: The lysis rate of MAGE-A10-special CTLs in L419 and H1975 were detected in 65.9 %and 80.5%, respectively. Both of L419 and H1975 showed significantly higher lysis rate in group 1 than those in group 3 (6.7, 26.7%) and group 2(0, 0%) and group 4(0, 0%) (P=0.0003, P=< 0.0001, P=<

0.0001, respectively). In online data mining of Kaplan-Meier plotter, the results suggest that the high expression of MAGE-A10 has significantly negative association with OS (Plogrank=2.1e-05) and PPS (Plogrank=0.0057)and FP (Plogrank=3.2e-12）
Conclusion: Thus we hypothesize that high-level expression of MAGE-A10 in vivo may inhibit the formation of differentiation of MAGE-A10-specific CTLs.

P0112

IL-23 enhances T lymphocyte function adjuvant by exosomes derived from Rab27a-overexpressing HCC cells

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Aim: Tumor-derived exosomes (TEX) carry tumor antigens which are potentially immunogenic, contribute to their promising utility as tumor vaccines. This research was going to explore the effect of TEX combined IL-23 on T cell to induce anti-tumor activities.
Methods: In this study, A Rab27a-overexpressing line was established via transfection of a pRab27a/G418 vector into the HepG2 cells. Exosomes derived from Rab27a-overexpressing HepG2 cells were isolated and then confirmed by Transmission electron microscopy (TEM) ,Nanoparticle Tracking Analysis (NTA) and western blotting. Internalization of TEX in DC cells was detected by Confocal microscope.The IL-23(IL-12p40) release assay by ELISA. Subsequently, naive T cells were stimulated with DCs loaded with TEX or TEX combined with IL-23 (TEX/IL-23) to investigate the specific T cell-mediated immune responses against HCC in vitro and in vivo.
Results: Our findings showed that exosomes from Rab27a-overexpressing HepG2 cells with diameters of approximately 50-100 nm ,harbor typical exosomal protein and tumor antigens, which were demonstrated to be capable of inducing DC maturation but inhibit the secretion of IL-12p40. In cytotoxicity assays and animal experiments, Tex/IL-23- loaded DCs induced an antitumor cytotoxic T-lymphocyte immune response more effectively than did TEX,TEX/Apilimod (small molecular inhibitor of IL-12/IL-23) and nonloaded DCs ($P<0.05$) and could induce more memory cells.
Conclusions: Collecting more exosomes from overexpressed Rab27a tumor cells to combine with cytokine IL-23 can produce stronger and more durable anti-tumor immune effects, which may sheds light on the development of efficient exosome-based cancer vaccines.

P0696

CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of umbilical cord blood -derived T cells engineered to target NY-ESO-1

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Background: Despite the clear role of PD-1/PD-L1 in regulating endogenous anti-tumor responses, the impact of inhibitory receptors on TCR-T cell function remains largely unexplored. Here, in this study, we wanted to test whether ablating PD-1 specifically in NY-ESO-1/TCR-T cells prepared from UCB-derived T cells would allow for generation of tumor-specific cells with enhanced anti-tumor functionality.
Methods: The CRISPR/Cas9 system has recently emerged as a simple and efficient way for multiplex genome engineering. In this study, we employed the techniques of genome editing and examined the effects of combination of Sleeping Beauty Transposon-based NY-ESO-1 knock-in and CRISPR-Cas9

DOI: 10.1002/eji.201970400

mediated knock-out of PD-1 on UCB-derived T cells. We compared the expansion ability, phenotypic characteristics, immune response and cytotoxicity of PD-1 disruption NY-ESO-1/TCR-T cells prepared fromUCB-derived T cells.
Results: In this study we found that PD-L1 expression on tumor cells impaired NY-ESO-1/TCR-T cell immune response and cytotoxicity. These defects could be mitigated by Cas9-mediated PD-1 disruption within the NY-ESO-1/TCR-T cells. Function of these PD-1-disrupted NY-ESO-1/TCR-T cells prepared from UCB-derived T cells was characterized by upregulated cytokine production and enhanced cytotoxicity in vitro. These results suggest that the PD-1 disruption by CRISPR-Cas9 could provide a new strategy to improve the efficacy of NY-ESO-1/TCR-T in future clinical studies.
Conclusions: This is the first report that generating PD-1-disrupted NY-ESO-1/TCR-T prepared from UCB-derived T cells by multiplex genome simultaneous editing. This universal, good manufacturing practice-compliant, “off-the-shelf” PD-1-disrupted TCR-T cells prepared from UCB-derived T cells might become available in the near future, enabling wider access to cellular immunotherapy.

P0697

Protective role of follicular CXCR5$^+$CD8$^+$ T cells against dengue virus 2 infection

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Follicular CXCR5$^+$CD8$^+$ T cells have antiviral effects in chronic virus infection, but the roles of these cells during dengue virus 2 (DENV2) infection remain poorly understood.
Objective: This study was conducted to analyzed in detail the dynamic changes and functional properties of circulating follicular CXCR5$^+$CD8$^+$ T cells to explore their effects on DENV2 infection.
Methods: Circulating follicular CXCR5$^+$CD8$^+$ T cells and cytokines were analyzed by flow cytometry in DENV2 patients at difference days after DENV2 infection. CD8$^+$ T cells were isolated and purified from DENV2 patients, then were stimulated with NS1 peptides and TCR stimulant. After cultivation, multiple parameters were tested.
Results: 1) CXCR5$^+$CD8$^+$ T cells emerged after DENV2 infection, with high PD-1 expression, and were correlated with the reduction in DENV2 RNA viral loads. 2) PD-1$^+$CXCR5$^+$CD8$^+$ T cells were negatively associated with disease progression. 3) Serum IFN-g, IL-6 and IL-10 levels were increased late in the course of DENV2 infection. 4) CXCR5$^+$CD8$^+$ T cells from DENV2 patients exhibited increased cytotoxicity and IFN-γ and IL-10 secretion.
Conclusion: CXCR5$^+$CD8$^+$ T cells could play a protective role in dengue pathogenesis and may be a novel strategy for controlling DENV2 infection and vaccine development.

P0698

IL-15 enhances CCR5 expression and CCR5-mediated gene expression in memory CD8$^+$ T cells

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IL-15 is a homeostatic cytokine that induces proliferation of NK cells and memory CD8$^+$ T cells. In addition, IL-15 can induce TCR-independent bystander activation of memory CD8$^+$ T cells to play a pathological role during viral infection. In the present study, we examined how IL-15 regulates migration of TCR-independently activated memory CD8$^+$ T cells and chemokine receptor-mediated gene expression. First, we performed gene expression analysis by RNA-seq and found that mRNA levels of CCR5 were significantly upregulated by IL-15 in the absence of TCR stimulation in human memory CD8$^+$ T cells. We confirmed that IL-15 upregulated CCR5 expression in human memory CD8$^+$

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DOI: 10.1002/eji.201970400

T cells by FACS analysis and that IL-15 increased CCL5-induced chemotactic activity of memory $CD8^+$ T cells in *in vitro* migration assays. Moreover, CCL5 treatment of IL-15-stimulated memory $CD8^+$ T cells increased the expression of Ki-67 and granulysin, indicating that the proliferation and function of IL-15-stimulated memory $CD8^+$ T cells are further changed by CCR5 ligation. Finally, we found that CCR5 was upregulated in peripheral blood and liver-infiltrating $CD8^+$ T cells in patients with hepatitis A virus infection, which is known to induce IL-15 over-expression. These findings indicate that IL-15 stimulation of memory $CD8^+$ T cells upregulates the expression of CCR5 which contributes to not only tissue migration but also CCL5-CCR5-mediated gene expression of memory CD8+ T cells during viral infection.

P1295

Changes of CD8+ T cell subsets before and after treatment of nasopharyngeal carcinoma

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Development of innovative immunotherapy is imperative to improve the poor survival of the nasopharyngeal carcinoma (NPC) patients. While $CD8^+$ T cells play an important role in host immunity. In this study, we used flow cytometry to investigate the percentage of $CD8^+$ T lymphocyte subsets in peripheral blood of 12 healthy donors and 11 patients with NPC before and after radio-therapy treatment, analyzed the dynamic change of differential $CD8^+$ T-cell subsets and its association with EBV-DNA viral load. We found that the frequency of naive T-cells subset (CD45RA+CCR7+) in the NPC patients was lower than that in healthy donors ($P< 0.05$); the higher frequency of central memory T-cells subset (CD45RA-CCR7+) and effector memory T-cells subgroups (CD45RA-CCR7-) were observed compared with healthy donors ($P< 0.05$). After treatment of NPC, the frequency of naive T-cells subset and central memory T-cells subset were lower than that before treatment ($P< 0.05$); the terminally differentiated effector T-cells subset (CD45RA+CCR7-) was significantly higher than before ($P< 0.05$). The frequency of effector memory T-cells in the EBV-DNA low viral load group was lower than the high viral load group ($P< 0.05$). In conclusion, our study found a significant change in the frequency of differential $CD8^+$ T-cell subsets between NPC patients and healthy donors, the changes may associate with the EBV-DNA viral load level. However, after radio-therapy treatment, the frequency of $CD8^+$ lymphocytic subsets gradually returned to initial level that observed before treatment.
Keywords: nasopharyngeal carcinoma; EBV-DNA; $CD8^+$ T lymphocytes

P1296

Vγ9Vδ2 γδ T cell anti-tumor cytotoxicity could be potentiated through selenium nanoparticle-based nanotechnology

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Immune cell therapy has now been considered as a new hope of malignant tumor patients, particularly after great success of CD19 CAR-T therapeutics. Among several candidates that could be used as tumor immune cell therapy, Vγ9Vδ2 γδ T cells, a subset of peripheral γδ T cells, has been shown to be an alternative for tumor therapy and have promising anti-tumor activity. Currently, researches on how

DOI: 10.1002/eji.201970400

to further improve anti-tumor activity of cytotoxicity T cells including Vγ9Vδ2 γδ T cells are under continuous development. Our present work is to develop new methodology, selenium nanoparticles (SeNPs) based strategy, to strengthen anti-tumor cytotoxicity of Vγ9Vδ2 T cells. We found SeNPs could induce significant upregulation of killing molecules NKG2D and IFN-γ and downregulation of PD-1. We further revealed that SeNPs could potentiate anti-tumor cytotoxicity of Vγ9Vδ2 T cells through promoting tubulin acetylation modification. In brief, SeNPs could significantly potentiate cancer killing and tumor growth inhibition efficacy of Vγ9Vδ2 T cells, and both killing related molecules and tubulin acetylation are involved in fine-tuning of cytotoxicity. Here, we presented a new strategy for further enhancing anti-tumor cytotoxicity of Vγ9Vδ2 T cells through SeNPs-based nanotechnology rather than gene modification, providing an alternative methodology for cytotoxic T cell modulation.

P1297

A new subset of CD8+ cytotoxic T lymphocyte curtails chronic viral infection

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Around 50 years ago, CD8+ cytotoxic T lymphocyte (CTL) was defined as the key effector cell to eliminate virus or to kill tumor cells. During chronic process of inflammation, antigen -specific CD8+ T cells become exhausted, exhibit poor effector function and lose memory potential. Nevertheless, exhausted CD8+ T cells can still contain viral replication in chronic infections or tumor progression , although the mechanism is largely unknown. Here we show that a new subset of exhausted CD8+ T cells expressing the chemokine receptor CXCR5 plays a critical role in the control of viral replication in mice that were chronically infected with lymphocytic choriomeningitis virus. These CXCR5+CD8+T cells were preferentially localized in B-cell follicles (defined as Tfc), expressed lower levels of inhibitory receptors and exhibited more potent cytotoxicity than the CXCR5- subset. Furthermore, we identified the ID2/E2A axis as an important regulator for the generation of this subset. In HIV,HBV patients, we also identified a virus specific CXCR5+CD8+T cells subset, and its number was inversely correlated with viral load. The CXCR5+ subset showed greater therapeutic potential than CXCR5- subset when adoptively transferred to chronically infected mice and exhibited the synergistic effect on reducing viral load when combined with anti-PD-L1 treatment. Our work defines a unique subset of exhausted CD8+ T cells that plays a pivotal role n the control of viral replication during post-exposure.

P1298

High glucose enhances cytotoxicity-mediated by cytotoxic T lymphocytes

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High glucose, also termed hyperglycemia, is a typical symptom of diabetes. Uncontrolled high glucose in blood in most cases can lead to severe associated complications such as heart attack, diabetic nephropathy, nerve damage and higher risk for infection and slow healing. Cytotoxic T lymphocytes (CTLs) are the main players in adaptive immune system that eliminate their target cells mainly by two killing pathways: Lytic granules and Fas/FasL pathway. Although it is known that CTLs play a pivotal role in initiation and progression of diabetes, the impact of high glucose on effector functions of CTLs still remains elusive. We used 5.6 mM (normal) and 25 mM (high) glucose to mimic healthy and diabetic condition, respectively. Using real-time killing assay, we found that high glucose increases cytotoxicity-mediated by CTLs. To identify the underlying mechanisms, we analyzed proliferation, differentiation and glucose uptake. Analysis of flow cytometry shows that the differentiation and

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DOI: 10.1002/eji.201970400

proliferation of CTLs are not affected by high glucose. There is no significant difference in glucose uptake between CTLs cultured in normal or high glucose. Analysis of the expression of cytotoxic proteins, including perforin, granzyme B and FasL, shows no difference in normal or high glucose. Using degranulation assay, we examined the release of lytic granules and found no change by high glucose. Finally, we identified that TNF-related apoptosis inducing ligand (TRAIL) in CTLs was significantly up-regulated by high glucose. Our findings suggest that high glucose enhances cytotoxicity-mediated by cytotoxic T lymphocytes, most likely in an unspecific manner.

P1300

Defining the specific role of Th1 cells in control of chronic infection

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Upon viral infection, the host immune system elicits the type I immune response, in which the cytokine IFN-γ plays pivotal roles. IFN-γ acts on diverse cell subsets to orchestrate antiviral immunity, including induction of genes mediating cytotoxic activity and class switching of antibodies. While Th1 and CD8 T cells are the major producer of IFN-γ, it remains unknown whether IFN-γ derived from distinct subsets, including Th1 cells, plays non-redundant roles in antiviral responses. To define the role of Th1 cells in response to chronic LCMV infection, we have developed a new strain of Cre knock-in mice, in which Cre expression is under the control of the Cd40lg locus and thus deletes a gene of interest specifically in CD4 T cells. To restrict Th1-derived IFN-γ, we are now breeding *Cd40lg*-cre to *Tbx21* (encoding T-bet)-flox mice and infected the mice either acutely or chronically with LCMV. Our preliminary data in acute LCMV infection show that IFN-γ production was completely inhibited in LCMVarm-specific CD4 T cells lacking T-bet but not in LCMVarm-specific CD8 T cells. In chronic LCMV infection, deletion of Tbx21 in CD4 T cells resulted in decrease of LCMV-specific CD8 T cells despite their initial expansion, and defective control of viremia compared to control mice. We are currently conducting detailed and mechanistic analysis of the phenotype.

P1886

Efficient control of chronic LCMV infection by a CD4 T cell epitope-based heterologous prime-boost vaccination in a murine model

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CD4+ T cells are essential for sustaining CD8+ T cell responses during a chronic infection. The adoptive transfer of virus-specific CD4+ T cells has been shown to efficiently rescue exhausted CD8+ T cells. However, the question of whether endogenous virus-specific CD4+ T cell responses can be enhanced by certain vaccination strategies and subsequently reinvigorate exhausted CD8+ T cells remains unexplored. In this study, we developed a CD4+ T cell epitope-based heterologous prime-boost immunization strategy and examined the efficacy of this strategy using a mouse model of chronic lymphocytic choriomeningitis virus (LCMV) infection. We primed chronically LCMV-infected mice with a Listeria monocytogenes vector that expressed the LCMV glycoprotein-specific I-Ab-restricted CD4+ T cell epitope GP61-80 (LM-GP61) and subsequently boosted the primed mice with an influenza virus A (PR8 strain) vector that expressed the same CD4+ T cell epitope (IAV-GP61). This heterologous prime-boost vaccination strategy elicited strong anti-viral CD4+ T cell responses, which further improved both the quantity and quality of the virus- specific CD8+ T cells and led to

DOI: 10.1002/eji.201970400

better control of the viral loads. The combination of this strategy and the blockade of the programmed cell death-1 (PD-1) inhibitory pathway further enhanced the anti-viral CD8+ T cell responses and viral clearance. Thus, a heterologous prime-boost immunization that selectively induces virus-specific CD4+ T cell responses in conjunction with blockade of the inhibitory pathway may represent a promising therapeutic approach to treating patients with chronic viral infections.

P1887

$CD8^+CXCR5^+$ T cells in hepatocellular carcinoma produce IL-21 to support the B cell response

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$CD8^+$ T cells are considered the main cytotoxic lymphocytes to play an antitumor role. However, few studies have focused on the humoral immunity roles of $CD8^+$ T cells, and the role of $CD8^+$ T cell-B cell interactions in hepatocellular carcinoma (HCC) is largely unknown. Here, the frequency of IL-21-producing $CD8^+CXCR5^+$ T cells in the HCC tumor tissue was higher than that in the paired peritumoral tissue, paired peripheral blood and healthy donor blood. Moreover, $CD8^+CXCR5^+$ T cells migrated in response to supernatants from primary HCC cells (HCC-SN), and HCC-SN powerfully induced CXCR5 expression in $CD8^+$ T cells and IL-21 expression in $CD8^+CXCR5^+$ T cells. $CD8^+CXCR5^+$ T cells from HCC patients, but not those from healthy individuals, were able to stimulate $CD19^+$ B cells to differentiate into IgG-producing plasmablasts. Our study revealed that $CD8^+CXCR5^+$ T cells strongly infiltrated HCC tumors, and their infiltration predicted a better prognosis. Moreover, $CD8^+CXCR5^+$ T cells surprisingly produced IL-21 to induce B cells to differentiate into IgG-producing plasmablasts to play a role in humoral immunity in HCC.

P1888

The blockage of fibrinogen-like protein 2 inhibiting hepatoma growth via activating the cytotoxic lymphocytes and dendritic cells

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Introduction: Fibrinogen-like Protein 2*, a kind of* prothrombinase, has been reported as an immunological inhibitory molecule damping T cells and dendritic cells (DCs) *in vitro*. The immunological mechanism of FGL2 promoting tumor is still not completely illustrated.
Objectives: The aim of the research was to illustrate the regulatory role of *FGL2* in hepatoma microenvironment.
Methods: 8×10^6 BNL and Hepa1-6 cells were subcutaneously injected in BALB/c and C57BL/6 mice, respectively. Tumor volume was measured and mice were treated with 100μg FGL2 antibody every 3 days when the volume is over 100 mm^3. After 14 days mice were sacrificed. The phenotype and proportion of immunocytes in tumor were examined.
Results: The tumor volume of FGL2 antibody treated group was significantly smaller than that of control groups. In BALB/c mice, the volume of the treated group(n=24) was (793.8±57.54) mm^3 and that of the control group(n=26) was (1407±102.1) mm^3 after 14 days of inoculation($P< 0.0001$). The similar result was also observed in C57BL/6 mice. With FGL2 depletion, the number and the expression of Perforin and Granzyme B of $CD8^+$ T cells were upregulated, and meanwhile the expression of B7-H4 and CD31 on DCs was downregulated in tumor of BALB/c mice.
Conclusions: Blockage of FGL2 remodeled the microenvironment of hepatoma by elevating $CD8^+$ T

DOI: 10.1002/eji.201970400

cells and activating DCs, which demonstrated the immunological regulatory role of FGL2 in hepatoma progression. These data also indicated that FGL2 might be a potential target of hepatoma treatment.

P1889

Distribution of memory T cell subsets within the HER2-specific cytotoxic T lymphocytes

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Effector CD8+ T cells, also commonly known as cytotoxic T lymphocytes (CTLs), play a key role in antitumor cellular immunity and, when properly activated, are able to effectively destroy tumor cells. The viability and proliferation of T cells directly correlate with the antitumor activity of adoptive T-cell therapy. In this regard, the presence of memory T-cells in the transferred T-cells is of particular importance.
The aims of this study were to obtain CD8+ CTLs specific for the HER2/neu epitopes E75 and E88 and to assess effector functions and the composition of these cells in terms of the distribution of memory T-cell subsets.
Here we investigated the content and ratio of all commonly determinable circulating T-cell subsets within particular populations of antitumor antigen-specific T cells using multicolor flow cytometry in combination with the technology of HLA-streptamer staining. We analysed such subsets as naive T cells (TN), central memory T cells (TCM), effector memory T cells (TEM), stem cell-like memory T cells (TSCM) and terminally-differentiated T cells (TEMRA) via eight-color flow cytometry. HER2-specific CTLs were largely (~40-50%) represented by TSCM cells, a population capable of mounting pronounced antitumor immune responses due to a combination of effector function and self-maintenance.
We also showed the presence of HER2-specific CTLs in healthy individuals and increase in them in HER2-positive breast cancer patients. Collectively, the resulting data complement the current understanding of the content and distribution of circulating peripheral blood $CD8^+$ T cells in terms of their effector/memory phenotype.

P1890

Molecular mechanisms of melanoma cell resistance to perforin-mediated cytotoxicity at the lytic synapse: role of calcium signaling

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It is well established that Ca^{2+} signaling plays a key role in lytic granule secretion by cytotoxic immune cells at the lytic synapse. Yet, whether this signaling pathway also plays a role on the target cell side of the lytic synapse, remains to be elucidated. In the present study, we provide several lines of evidence indicating that Ca^{2+}pathway elicits melanoma cell resistance to CTL attack. High-speed live single cell imaging combined with FACS analysis-based measurements of cell membrane reparation show that: i) a Ca^{2+}-dependent reparative membrane turnover is triggered in melanoma cells by perforin-mediated plasma membrane damage; ii) Ca^{2+} microdomains are rapidly formed in melanoma cells upon perforin-mediated pore formation; iii) pre-treatment with calcium chelators or silencing the Ca^{2+} sensor synaptotagmin VII in melanoma cells reduces their resistance to CTL attack; iv) Ca^{2+} microdomain-dependent reparative responses at the lytic synapse are extraordinarily rapid and can

DOI: 10.1002/eji.201970400

even precede CTL lytic machinery repolarization.
Our results show that melanoma cells adapted Ca^{2+}-dependent membrane repair mechanisms to promptly resist to perforin-mediated cytotoxicity. They reveal an unpredicted role of the Ca^{2+} signaling pathway in target cells that counteracts Ca^{2+}-dependent killer cell activation.

P1891

Multiplex single-cell profiling of signalling pathways reveals how stimulation strength coordinates T cell responses

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Cytotoxic T lymphocytes (CTLs) play a key role in the immune response against virally-infected and tumourigenic cells. T cell receptor (TCR) recognition of foreign peptides presented by Class I MHC molecules stimulates naïve $CD8^+$ T cell differentiation to effector cells and triggers their cytolytic activity. Signal transduction downstream of the TCR is a highly diverse and coordinated network of post-translational protein modifications that ultimately drive transcriptional, translational, metabolic and cytoskeletal changes in the cell. How cytotoxic T cells coordinate their signalling machinery in response to strong versus weak stimuli remains unknown. In naïve T cells, we previously showed that ligand strength controls the frequency and the rate of transcriptional activation of CTLs, however all activated cells achieved cytolytic capacity. Here we studied how ligand affinity affects the coordination of proximal $CD8^+$ T cell signalling responses.
We utilised the ability of mass cytometry to simultaneously study multiple signalling events at a single-cell resolution. We designed a custom panel of metal-conjugated antibodies to profile naïve transgenic $CD8^+$ T cells stimulated with ligands of differing potencies. Our data revealed that stimulation strength exerted a differential effect on the rate with which cells commenced activation of key signalling molecules within the first 4 hours of stimulation. However, after 4 hours, cells converged to the same activatory phenotype with similar proportions exhibiting this phenotype, regardless of the initial stimulation strength. Hence, stimulation strength does not regulate the sequence of signalling events or the activatory phenotype, but does affect the initial rate of coordinated signalling events.

P2572

Autophagy negatively regulates the activation and function of CD8+ T cells

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Autophagy is a physiological process to maintain cellular homeostasis, and play a key role in many clinical diseases. However, the effect of autophagy on the activation and function of $CD8^+$ T cells remain unknown, which may be important to anti-virus and anti-tumor immune response. Here, we utilized autophagy-related gene (Atg3) conditional knock-out mice and a inducible Cre (ER-Cre) to delete Atg3 in mature T cells. We found that Atg3-deficient $CD8^+$ T cells exhibited stronger cytotoxicity both *in vitro* and *in vivo* compared with wild type $CD8^+$ T cells. To exclude Atg3 specific effect, Atg7-deficient $CD8^+$ T cells also showed high cytolytic activity, suggesting the key role of autophagy in the regulation of $CD8^+$ T cell function. Therefore, autophagy-deficient CTL possess stronger anti-tumor effect in vivo, and prolonged the survival of tumor-bearing mice. Furthermore, autophagy-deficient $CD8^+$ T cells is susceptible to be activated, evidence as higher level of activation markers and proliferation activity. T cell activation signals analysis revealed the phosphorylation of ZAP-70, PLCγ1 and ERK, as well as intracellular calcium flux all increased in autophagy-deficient $CD8^+$ T cells

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DOI: 10.1002/eji.201970400

compared to wild type CD8$^+$ T cells. The nuclear translocation of kay transcription factors, T-bet and NF-kB, also be enhanced. However, the expression level of TCRβ on the surface of autophagy-deficient CD8$^+$ T cells did not show significant change. These data suggested that autophagy may negatively regulate the activation and function of CD8$^+$ T cells through controlling the early step signals in T cell activation pathway.

P2573

CD8+CD103+ tumor-infiltrating lymphocytes are tissue-resident memory T cells and a prognostic factor for survival in oral squamous cell carcinoma patients

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Background: Current strategies for immune monitoring focus on the quantification of the density of tissue-resident memory T cells (TRM) in promoting intratumoral CTL response. However, little is known about its expression or prognostic implications in OSCC. In this study, we investigated the clinical significance of CD8$^+$CD103$^+$ Tumor-Infiltrating Lymphocytes in OSCC.
Methods: In our study, 50 formalin-fixed, paraffin-embedded tumor tissue sample from a cohort of OSCC patients were collected. The expression of CD103, CD8, CD25, Ki67 was examined by multiplexed quantitative immunofluorescence. The prognostic significance of CD8$^+$CD103$^+$ TILs was evaluated by Kaplan-Meier analysis.
Result: Tumor infiltration by CD103$^+$ TILs was strongly associated with patient survival in OSCC. High numbers of CD8$^+$CD103$^+$ TILs were correlated with improved survival in patients with early stage OSCC. Enhanced CD8$^+$CD103$^+$ TILs correlates with increased active effector phenotype (CD25$^+$, Ki67$^+$).
Conclusion: Our study indicated that a greater density of CD8$^+$CD103$^+$ tissue-resident memory T cells was predictive of a better survival outcome in OSCC.

P2574

Id2 protein regulates CD8 T cell exhaustion in chronic viral infection

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Chronic viral infection threat people's health world widely. Due to continuous T cell stimulation combining with upregulated negative regulatory signals, the effector CD8 T cells generated in chronic viral infection could not get rest and finally step into exhaustion rather than form functional memory pool. However, the exhausted CD8 T cells are not inert. Despite the decreased functionality, exhausted CD8 T cells still retain crucial functions that limit viral replication and disease progression, yet the underline mechanism is still unknown. In acute viral infection, Id2 modulate the effector function and differentiation of CD8 T cells via E protein E2A. However, the regulatory function of Id2 in CD8 T cell exhaustion in chronic viral infection is still undefined. Therefore, based on previous results, we suppose that Id2may play an essential role in the modulation of CD8 T cell exhaustion in chronic viral infection. To confirm this, we will use mice LCMV-Cl13 model to explore the regulatory function and mechanism of Id2 in CD8 T cell exhaustion in chronically infected mice. This research will help us to get a more comprehensive understanding of the regulatory mechanism of CD8 T cell exhaustion and lay solid ground for the development of future immunotherapies to cure chronic viral infection.

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DOI: 10.1002/eji.201970400

P2575

Enhancement of proliferation and anti-lung-tumor activity of human peripheral blood lymphocytes by low-intensity pulsed ultrasound

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Tumor immunotherapy is a hot topic in the field of basic medicine at present. However, how to improve the anti-tumor effect is still an unconquered problem in the clinical medicine. In this study, we investigated the effects of low intensity pulsed ultrasound (LIPUS) on the proliferation and anti-lung-tumor effect of human peripheral blood lymphocytes (HPBL). The proliferation of HPBL was stimulated by the intensity of LIUPS between 0 and 80mW/cm^2. The proliferation activity of the cells was measured by the CCK-8 assay. The expressions of anti-tumor factors (INF- γ, IL-2, TNF- α and GM-CSF) in the supernatant of culture medium were detected by the ELISA assay. The antitumor activity was detected by co-culture of HPBLs and human lung giant cell carcinoma cells (95D). Our study shows that, compared to the control group (0 mW/cm^2), the proliferation of HPBL cells was enhanced ($P < 0.01$) after 2 days of stimulation. The intensity of LIPUS is 30 mW/cm^2 and duration is 10 minutes a day. The anti-tumor factors are INF- γ, IL-2, TNF- α and GM-CSF were higher than the control group, respectively; Co-culture experiment showed that the killing rate of tumor was 14.9% ±0.03 in the stimulation group and 8.6% ±0.01 in the control group. The proliferation activity and killing rate of HPBLs were increased after LIPUS stimulation. The results may have great therapeutic potential and basis for the use of LIPUS combined with lymphocytes in the treatment of lung tumor.

P2576

Expression of cytotoxic molecules leads to a dismal prognosis of peripheral of T-cell lymphomas

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Objective: Cytotoxic molecules (CM) are apoptosis-inducing molecules present in the azurophilic cytoplasmic granules of cytotoxic cells, including T-cell intracellular antigen-1 (TIA-1), perforin and granzymes. We reviewed 94 patients' medical records to study the relationship between the expression of CM and the prognosis of patients diagnosed with peripheral T-cell lymphoma (PTCL).
Patients and methods: We investigated 94 patients pathologically diagnosed with PTCL who tested by immunohistochemistry either positive or negative for CM expression of their tumor tissues. We studied the clinical features and the survival outcomes of these patients. Univariate and multivariate analyses were used to analyze the data.
Results: The complete remission (CR) rate of all patients was 35.1%, the partial remission (PR) rate was 30.9%, and the objective response rate (ORR) was 66.0%. The ORR of the CM positive expression group was 48.8%, and that of the CM negative expression group was 79.2%, *P*=0.004. By multivariate analyses, three parameters independently associated with a worse overall survival (OS) rate, namely, high IPI scores(HP, 1.870; 95% CI, 1.269--2.756; *P*=0.002), bone marrow involvement (HP, 9.559; 95% CI, 2.202--41.495; *P*=0.003), and positive CM expression (HP, 3.765; 95% CI, 1.409--10.056; *P*=0.008). Positive CM expression with a 3-year OS rate of 48.3%, and a negative CM expression associated with a 3-year OS of 76.7%.
Conclusion: CM expression had an independent effect on the ORR and OS rate of patients diagnosed with PTCL. However, it had no effect on the PFS.

DOI: 10.1002/eji.201970400

P2577

Application of small molecule fluorescent probes in early activation imaging of CD8 + T lymphocytes

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As a subset of T lymphocytes, CD8+ T lymphocytes play a vital role in the body´s immune system. CD8+ T lymphocytes are responsible for the clearance of target cells through direct killing, such as cells infected with viruses, malignant transformed cells and allogeneic cells. And, it is also involved in the occurrence of transplant rejection. Activated CD8 + T lymphocyte-mediated granulocytosis is considered to be the most significant pathway for killing "alien cells". The granzyme of the serine protease family member is the main granule component that enters the target cell after immunological recognition. Among them, Granzyme B (GrB) is extremely fast and effective and rich in content, which can induce cell death in multiple pathways.
Fluorescence spectrometry has been widely used in analytical chemistry, especially in biological analysis because of its high sensitivity and selectivity. In recent years, with the development of molecular imaging, fluorescence imaging has become one of the most important method to monitor biomolecules and biological processes in living cells and living organisms, and fluorescent probes with excellent performance are the material basis for imaging analysis.
This study designed a novel near-infrared small molecular probe that can be specifically recognized and cleaved by granzyme B, using fluorescence microscope, which is the main effector of cytotoxic CD8+ T lymphocytes— Granzyme B is imaged in living cell.

P2578

PD-1/PD-L1 axis, rather than high-mobility group alarmins or CD8+ TIL, is associated with survival in HNSCC patients who received surgical resection

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In the present study, we used immunohistochemistry to investigate the expression and significance of high mobility group box 1 (HMGB1), HMG nucleosome-binding protein 1 (HMGN1), as well as tumor CD3+, CD8+, FOXP3+ lymphocyte infiltration, the receptor programmed cell death 1 (PD-1) and its ligand programmed cell death ligand 1 (PD-L1) in head and neck squamous cell carcinoma (HNSCC). Furthermore, we assessed the prognostic value of HMG proteins, TILs and PD-1/PD-L1 in postoperative patients. Tumor tissue sections were collected from 81 cases of patients with resectable HNSCC. Results showed that the number of CD3+ T cells in HMGB1 cytoplasmic expression group (339.39 ± 230.76) was more than that with negative HMGB1 cytoplasmic expression (233.30 ± 230.91, $P< 0.05$). The number of CD3+, CD8+ and FOXP3+ T cells in HMGN1 cytoplasmic expression group (400.74 ± 224.04, 158.10 ± 112.10, 36.00(15.00, 69.00)) was more than that in group without HMGN1-cytoplasmic expression (222.84 ± 217.78, $P< 0.01$; 105.10 ± 108.25, $P< 0.05$; 13.00(6.75, 32.25), $P< 0.01$). The positive rates of PD-1 and PD-L1 in tumor tissues were 29.6% and 67.9%, respectively. Multivariate analysis suggested tumor expression of PD-L1 was an independent prognostic factor and PD-L1 overexpression indicated a poor overall survival and disease-free survival. We concluded that HMGB1 and HMGN1 secreted by cancer cells may relate to recruitment of tumor infiltrating lymphocytes (TILs) in HNSCC. PD-1/PD-L1 axis, rather than HMG proteins or CD8+ TILs, has a critical role in tumor immune microenvironment and could predict the outcome of HNSCC patients who received surgical resection.

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DOI: 10.1002/eji.201970400

P2579

Prognostic values of CD38$^+$CD101$^+$PD1$^+$CD8$^+$ T cells in pancreatic cancer

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Programmed death-1 (PD-1), a key immune checkpoint molecule, has been developed as an oncotherapy target for various carcinomas. However, treatment with anti-PD-1 elicited only a minimal effect in pancreatic ductal adenocarcinoma (PDAC). Subsequent studies revealed the existence of a subset of PD-1$^+$ T cells coexpressing CD38 and CD101, representing a fixed dysfunctional subpopulation that are not able to be rescued by anti-PD-1 immunotherapy. However, whether this subpopulation of PD-1 expressing CD8$^+$ T cells could be useful in predicting PDAC stage or prognosing survival is unknown. In this study, we used flow cytometry and immunofluorescence assay to analyze the expression of CD38 and CD101 in 183 clinical PDAC samples, including 84 of peripheral blood and 99 of surgical tissues. High coexpression of CD38/CD101 on peripheral PD-1$^+$CD8$^+$ T cells or tumor-infiltrating lymphocytes (TILs) was found to be most significantly correlated with Tumor/Node/Metastasis (T/N/M) classification and clinical stage, in contrast, PD-1$^+$CD8$^+$ T cells could not correlate with T classification. CD38/CD101 co-repression on TILs also correlated with the poor survival in these PDAC patient samples. Our data suggest that CD38/CD101 might represent a more helpful biomarker than PD-1 alone for diagnosis and prognosis of PDAC.

P2580

The role of cytokine-induced killer (CIK) cells therapy in modulating tregs in patients with non-small cell lung cancer

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Targeting Tregs has the capability to augment cancer vaccine approaches. We therefore designed to evaluate the role of cytokine-induced killer (CIK)cells infusion in regulating Tregs in patients with non-small cell lung cancer (NSCLC). Fifteen patients with advanced NSCLCs were treated by autologous CIK cells. By FACS and Liquid Chip, subsets of T cells and NK cells in peripheral blood, plasma cytokine profiles in the treated patients were analyzed two and four weeks post the infusion of CIK cells. Cytotoxicity of PBMCs and NK cells and progression-free survival (PFS) and overall survival (OS) from NSCLC patients were also assessed. We found an increase in NK cells with enhanced NKG2D expression concomitant with a decrease in Tregs two weeks after CIK cells infused. Two weeks after CIK cells therapy, the cytotoxicity of PBMCs and NK cells were significantly increased. Correspondingly, plasma cytokine profiles showed increasing levels of IFN-γ, IP-10, TNF-α, GM-CSF, MCP-3 and IL-21 and decreasing levels of TGF-β1. We found that Tregs still maintained at a very low level in PBMCs four weeks post CIK infusion. The median PFS and OS in CIK cells treatment group was significantly longer than that in the control group. During the whole treatment, CIK cells therapy can suppress Tregs and enhance the anti-tumor immunity of NK cells with improved PFS and OS in

DOI: 10.1002/eji.201970400

advanced NSCLC patients. This therefore may present a promising therapeutic treatment which can be combined with chemotherapy and radiotherapy for those patients with advanced NSCLCs.

P2581

A tumor specific super-enhancer drives immune evasion by guiding synchronous expression of PD-L1 and PD-L2

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Immune checkpoint blockade targeting PD-1/PD-L1(PD-L2) has groundbreaking improvements in clinical response for certain cancer patients. But the responsive patients also suffer immune-related adverse-events (irAEs) including fatal adverse effect such as cardiovascular toxicities. Dissecting the regulation of PD-L1 and PD-L2, two known ligands for PD-1, will be greatly helpful to design more specific strategies to block tumor-associated immune checkpoint function while leave normal immune function intact. Here, we found that the expression of PD-L1 and PD-L2 is almost always co-expressed in cancer cell lines and tissues. The co-expression of PD-L1 and PD-L2 could be greatly reduced by inhibitors of super-enhancers. Bioinformatic analysis identified a potential super-enhancer (PD-L1L2-SE) that located between PD-L1 and PD-L2 encoding genes, CD274 and PDCD1LG2, respectively. Genetic deletion of PD-L1L2-SE profoundly reduced the expression of PD-L1 and PD-L2. PD-L1L2-SE deficient cancer cells failed to generate immune invasion and were sensitive to T cell killing. Specifically epigenetic activation of such region is correlated with the expression of PD-L1 and PD-L2. Taken together, we identified a novel super-enhancer (PD-L1L2-SE) that responsible for the over-expression of PD-L1 and PD-L2 as well as immune invasion in cancer. Targeting PD-L1L2-SE and its regulatory mechanism might provide a more tumor-specific immune checkpoint blockade strategies to reduce adverse effect. The status of PD-L1L2-SE might also be useful to predict the response to immune checkpoint blockade.

P2582

Prognostic significance of tissue resident $CD103^+CD8^+$T cells in human colorectal cancer

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Herein, we aimed to characterize the resident $CD8^+$T cells in human colorectal cancer tissues by using double staining of CD103 and CD8. We further evaluated the prognostic significance and clinical implications of the numbers of resident $CD8^+$T cells. Based on the con-focal scanning of $CD103^+CD8^+$T cells in human colorectal cancer tissues, we found that the OS rate of the colorectal cancer patients with higher infiltration of $CD8^+$T cells, or with higher numbers of resident $CD103^+CD8^+$T cells, or with higher ratio of $CD103^+CD8^+$T cells over total $CD8^+$T cells in cancer tissues was significantly better than that of the patients with lower infiltration of $CD8^+$T cells, or with lower numbers of resident $CD103^+CD8^+$T cells, or with higher ratio of $CD103^+CD8^+$T cells over total $CD8^+$T cells in cancer tissues respectively. Moreover, higher infiltration of $CD8^+$T cells in colorectal cancer tissues was significantly and inversely correlated with advanced TNM stage. Higher numbers of resident $CD103^+CD8^+$T cells in colorectal cancer tissues were significantly and inversely correlated with distant metastasis status. Higher ratio of $CD103^+CD8^+$T cells over total $CD8^+$T cells in colorectal cancer tissues was significantly and inversely correlated with age status. The COX model analysis demonstrated that higher infiltration of $CD8^+$T cells, higher numbers of resident $CD103^+CD8^+$T cells, or

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DOI: 10.1002/eji.201970400

higher ratio of $CD103^+CD8^+$T cells over total $CD8^+$T cells in colorectal cancer tissues, could serve as independent prognostic predictors for colorectal cancer patients. Thus, our present study demonstrated resident $CD103^+CD8^+$T cells in colorectal tissues could be used as an important prognostic predictor for this malignancy.

P2583

The role of $CD8^+$ $CD38^{low}PDL1^{hi}$exhausted T cells in lung cancer microenvironment

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This study used CyTOF to detect and analyze the distribution and functional differences of immune cell subsets in lung cancer micro-environment, suggesting that CD38, PDL1 may be associated with exhausted T cells. In vitro experiments showed that the expression of CD38 in $CD8^+$T was decreased from the early stage of exhaustion to the end, and PDL1 was highly expressed at the final stage and co-expressed with PD1. $CD8^+CD38^{low}PDL1^{hi}$ cells affect the ability to exhausted $CD8^+$ T cell proliferation, apoptosis, and secrete cytokines. Thus, $CD38^{low}PDL1^{hi}$ expression may be one of the markers of the end stage of exhausted T cells. This population of cells can affect the proliferation of non-exhausted T cells and the secretion of cytokines to increase the proportion of exhausted T cells and affect the effects of T cells in the tumor microenvironment.

Helper T Cells and Subsets

O129

Understanding the role of T helper cells during asthmatic inflammation

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T helper (Th) cells are critical to the development of asthma. These cells are known to acquire specific functions that can cause pathological inflammation in the lung, leading to asthma. How many functions those Th cells can acquire during the development of asthmatic disease remains unclear. This study aimed to understand the heterogeneity of the Th cell response in a well-defined mouse model of airway inflammation using House dust mite (HDM) allergens and subsequently identify those genes vital to pathogenic subsets. Single cell RNA sequencing was performed on Th cells isolated from the airways (Bronchial Alveolar Lavage). We successfully identified both known and potentially novel asthmatic Th cell subsets based on single cell transcriptomes. Previously implicated subpopulations such as T helper-2 (Th2) and regulatory Th cells (Tregs) formed discrete clusters based on differential gene expression. A novel population responding to type I interferons was also detected. In the Th2 cell population, IL-4, IL-13 and IL-5 were highly enriched, but many genes with as yet unknown function were also upregulated by this cell cluster. Further work has confirmed novel target genes previously not implicated in asthmatic T cells using flow cytometric analysis. Airway Th2 cells were enriched for genes associated with lipid metabolism and experiments with blockers of metabolic pathways supported roles for glucose and lipid metabolism in Th2 cell differentiation. Thus, we have identified

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DOI: 10.1002/eji.201970400

novel genes and pathways important for Th2 function and development in the setting of airway inflammation.

O130

Differentiation of innate-like T-bet^{high} memory-phenotype CD4^{+} T lymphocytes is promoted by homeostatic IL-12 derived from CD8α^{+} dendritic cells in steady state

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CD4^{+} T lymphocytes consist of naïve, conventional antigen-specific memory, and memory-phenotype (MP) cell compartments in steady state. We recently showed that MP cells exert significant, innate-like effector function in host defense against pathogens controlled by Th1-type immunity. However, the mechanisms determining the differentiation of MP cells under homeostatic conditions remain to be defined. In this study, we first characterize MP lymphocytes as consisting of T-bet^{high}, T-bet^{int}, and T-bet^{low} subsets, with innate Th1-like effector activity exclusively associated with T-bet^{high} cells. We further show that differentiation of T-bet^{high} MP cells is promoted by IL-12 homeostatically produced by CD8α^{+} dendritic cells (DCs). Finally, we present evidence arguing that this tonic IL-12 production depends on microbial-independent TLR-MyD88 signaling and is further enhanced by CD40-CD40L interactions with MP CD4^{+} T lymphocytes. The latter enhancing function is associated with the TCR affinity of these cells to self. Taken together, our findings reveal that the optimal differentiation of host-protective T-bet^{high} MP lymphocytes in steady state requires IL-12 tonically produced by CD8α^{+} DCs in a MyD88 and CD40 signaling-dependent fashion.
This research was supported by the Intramural Research Program of the NIAID, NIH.

O131

TH2 subset differentiation follows a Bcl6 independent pathway that develops into distinct non-overlapping Th2 functional subtypes in the skin

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We used IL-4/IL-13 dual reporter “4C13R” mice to characterise the functional subsets of CD4+ T cells that arise in the lymph node and skin following priming with House Dust Mite (HDM) allergen. In the draining lymph node (dLN) only IL-4 single producing (SP) cells were detected while in the allergen exposed skin three subsets of CD4 Th2 cells were detected, IL-4 single producers (SP), IL-13 SP and dual IL-4/ IL-13 double producers (DP). The appearance of the three subsets in the skin was not dependent on Bcl6 transcription factor expression but the IL-4 SP was dependent on IL-4, while the IL-13 SP was independent of IL-4. This indicates that there is an alternative signal driving IL-13 expression at the site of the allergen challenge. To characterise unique transcriptional programmes between the Th2 subsets we performed RNA-sequencing to bring some of the complexities of the Th2

DOI: 10.1002/eji.201970400

system to light. Analysis of this data has suggested that the IL-13 SP subset is very distinct and expresses a “pro-inflammatory” signature in comparison with IL-4 SP subset. With this data we have been able to address the degree of plasticity within the Th2 differentiation process. Taken together our results suggest the Th2 subset differentiation can occur via multiple unique developmental pathways with the potential to lead to diverse functional disease outcomes.

O132

The transcription factor T-bet is required for optimal type I follicular helper T cell maintenance during acute viral infection

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Follicular helper T cells (TFH cells), known as the primary “helpers” of the germinal center (GC) reaction, promote the humoral immune response to defend against various pathogens. Under conditions of infection by different types of pathogens, many shared transcription factors (TFs), such as Bcl-6, TCF-1, and Maf, are selectively enriched in pathogen-specific TFH cells, orchestrating TFH cell differentiation and function. In addition, TFH cells also coexpress environmentally associated TFs as their conventional T cell counterparts (such as T-bet, GATA-3, or ROR-γt, which are expressed in Th1, Th2, or Th17 cells, respectively). These features likely indicate both the lineage-specificity and environmental adaption of the TFH cell responses. However, the extent to which the TFH cell response relies on these environmentally specific TFs is not completely understood. Here, we found that T-bet was specifically expressed in Type I TFH cells but not Type II TFH cells. While dispensable for the early fate commitment of TFH cells, T-bet was essential for the maintenance of differentiated TFH cells, promoting their proliferation, and inhibiting their apoptosis during acute viral infection. Microarray analysis showed both similarities and differences in transcriptome dependency on T-bet in TFH and TH1 cells, suggesting the distinctive role of T-bet in TFH cells. Collectively, our findings reveal an important and specific supporting role for T-bet in type I TFH cell response, which can help us gain a deeper understanding of TFH cell subsets.

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DOI: 10.1002/eji.201970400

O133

Influenza vaccine specific human blood CXCR5+PD-1+ cells reflect germinal center origin clonally and phenotypically

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cT_{FH} (circulating follicular helper T) is a subset of CD4+ memory T cells identified in the peripheral blood constitutively expressing follicular homing chemokine receptor CXCR5, a major character of *bona fide* T_{FH}. Subsets of cT_{FH} are reported to be capable of promoting B cells producing antibody thus reflecting germinal center T_{FH} in terms of function. However, the direct evidence that cT_{FH} is the descendent of germinal center T_{FH} still lacks, especially in human studies. On the other hand, how cT_{FH} relates with CXCR5- memory T cells counterpart (non-cT_{FH}) is also unclear. We seek to study these questions from the perspective of clonal relationship, in the context of antigen specific response. Firstly, we found out influenza vaccine specific response showed low level of clonal type overlapping between cT_{FH} and non-cT_{FH}, as a comparison *C.Albicans* specific cT_{FH} were more clonally related with non-cT_{FH}. This led us to investigate the possible relation of Influenza specific cT_{FH} with GC T_{FH}. PD-1 expression level on cT_{FH} has been reported to correlate with higher resemblance with GC T_{FH}. *Ex-vivo* stimulation demonstrated influenza specific cells were enriched in $PD1^{hi}$ cT_{FH} fraction. Moreover, influenza specific $PD\text{-}1^{hi}$ cT_{FH} cells displayed a distinct TCR repertoire pool compared with $PD\text{-}1^{lo}$ and non-cT_{FH}. Besides, T_{FH} related genes were preferentially expressed by $PD\text{-}1^{hi}$ cT_{FH} rather than $PD\text{-}1^{lo}$, notably CXCL13, the production of which is the major attribute of *bona fide* T_{FH}. Our study has identified a small population of human cT_{FH} with influenza specificity that reflects germinal center origin clonally and phenotypically.

O134

Deciphering the molecular pathway of Th9 cells differentiation

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IL-9 producing T helper 9 (Th9) cells play a critical role in inducing anti-tumour immunity as well as in allergic inflammation, however, the generation of these cells are still not completely understood. To understand the molecular pathways that are important for the development and differentiation of Th9 cells, we applied approaches of transcriptomics, proteomics and metabolomics. Transcriptomics analysis revealed the involvement of EGFR pathway in differentiation of Th9 cells. Further analysis demonstrated that amphiregulin (Areg), a ligand of EGFR is required for the induction of IL-9 in Th9 cells through the upregulation of EGFR as in $Areg^{-/-}$ mice, both EGFR and IL-9 expression was attenuated. Moreover, EGFR signaling upregulates hypoxia-inducible factor 1α (HIF1α) which is essential to promote the differentiation of Th9 cells. Mechanistically, we have identified that HIF1α binds and transactivates IL-9 in Th9 cells. Furthermore, inhibition of EGFR and HIF1α pathway suppressed IL-9 production in both mouse and human Th9 cells, and substantially promoted the tumor development. Since HIF1α is a metabolic regulator so we performed mass spectrometry-based metabolomics analysis in mouse Th9 cells which revealed that Th9 cells from wild-type mice has elevated total levels of glycolytic, pentose phosphate pathway (PPP), and TCA cycle intermediates as compared to Th9 cells from $HIF1\alpha^{-/-}$ mice suggesting that HIF1α is essential for the metabolism of Th9 cells. Our findings thus identify a critical role of EGFR-HIF1α module in development and functions of Th9 cells which can be targeted for successful cancer immunotherapy.

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DOI: 10.1002/eji.201970400

O135

Epigenetic initiation of the Th17 differentiation programme is promoted by Cxxc finger protein 1

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IL-6/STAT3 signalling is known to initiate the Th17 differentiation programme, but the upstream regulatory mechanisms remain minimally explored. Here we generated mice with conditional deletion of Cxxc1, an important epigenetic regulator functioning mainly through regulating H3K4me3. We found that Cxxc finger protein 1 (Cxxc1) promoted the generation and stability of Th17 cells as an epigenetic regulator and prevented their differentiation into Treg cells. Mice with a T cell-specific deletion of Cxxc1 were protected from experimental autoimmune encephalomyelitis and were more susceptible to *Citrobacter rodentium* infection. Using RNA-seq technology, we demonstrated downregulated genes were associated with Th17 cells differentiation and *Cxxc1*-deficient T cells acquired a Treg cell-biased programme that dominantly suppressed Th17 cell generation via IL-6Rα production. Cxxc1 deficiency decreased IL-6-induced p-stat3 activation, whereas overexpression of Cxxc1 could reverse the expression of IL-6Rα. And overexpression of IL-6Rα could reverse the differentiation and stability defects in *Cxxc1*-deficient Th17 cells in vitro and in vivo. Using ChIP-seq technology, Genome-wide occupancy analysis revealed that Cxxc1 bound to *Il6rα* gene loci in the Th17 programme by maintaining the appropriate H3K4me3 modification of its promoter. Therefore, these data highlight that Cxxc1 as a key regulator governs the balance between Th17 and Treg cells by controlling the expression of IL-6Rα, which subsequently affects IL-6/STAT3 signalling.

O136

IL-27 increases the plasticity of differentiated Th17 cells by inducing IL-12R beta 2 expression

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The role of Th17 cells in inducing tissue inflammation in autoimmune diseases is well established. IL-23-IL-23R-induced pathogenic T_H17 cells are critical in inducing tissue inflammation in experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis. Interleukin (IL-) 27, an IL-12 family cytokine, has been shown to suppress tissue inflammation in EAE partly by inhibiting differentiation of T_H17 cells. In addition to inhibition the differentiation and functions of Th17 cells, IL-27 also found induce the differentiation of IL-10 producing type regulatory T cells (Tr1), which are found to play an essential role in inhibiting effector T cells function in EAE. Although the anti-inflammatory role of IL-27 was established, the precise mechanism by which IL-27 suppresses the effector functions of differentiated T_H17 cells is not well understood. Using the global gene profile data, we now show that IL-27-induced expression of IL-12Rb2 plays a critical role in regulating the generation and functions of T_H17 cells in EAE. Moreover, IL-27-induced IL-12Rb2 make differentiated Th17 cells responsive IL-12-mediated conversion into IFN-gamma producing T cells. IL-27 receptor deficiency leads to the inhibition of IL-12Rb2 while enhancing the expression of IL-23R on Th17 cells, and therefore increases the IL-23 responsiveness of Th17 cells. Moreover, IL-27 is unable to inhibit T_H17 cells and development of experimental autoimmune encephalomyelitis (EAE) in *Il12rb2*$^{-/-}$ mice. Indeed, IL-27-induced expression of IL-12Rb2 increased responsiveness of T_H17 cells to IL-12. This provides a

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DOI: 10.1002/eji.201970400

novel mechanism by which IL-27 inhibits T_H17 cells by increasing their plasticity and responsiveness to immunosuppression.

P0114

Increased circulating CD4$^+$CXCR5$^+$ T cells, Tfh cells and the ratio of Tfh and Tfr cell correlate with disease severity in patients with chronic spontaneous urticaria

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Background: Accumulating evidence demonstrated that circulating CD4$^+$CXCR5$^+$, Tfh, Tfr or Tfh/Tfr maintain immune homeostasis and humoral immune response. The dysregulation is involved in the occurrence and development of certain diseases.
Objectives: To investigate the status of CD4$^+$CXCR5$^+$, Tfh, Tfr and Tfh/Tfr in CSU and their correlation with the disease activity.
Methods: Frequencies of CD4$^+$CXCR5$^+$, Tfh and Tfr cells in blood were detected by flow cytometry with Tfh/Tfr calculated. The expression of CXCR5 were evaluated using qPCR and western blot. The levels of IL-4, IL-6, IL-21 and total IgE in plasma were measured by ELISA. Correlation between CD4$^+$CXCR5$^+$, Tfh, Tfr and Tfh/Tfr and urticaria activity score (UAS) were analyzed, with their diagnostic value by ROC curve.
Results: As compared with healthy donors, frequencies of circulating CD4$^+$CXCR5$^+$, Tfh and Tfh/Tfr, but not Tfr, were elevated in CSU. Significant differences were not observed of CD4$^+$CXCR5$^+$, Tfh, Tfr and Tfh/Tfr among different CSU subgroups. The expression of CXCR5 was higher in patients, both at mRNA and protein levels. Higher levels of IL-4, IL-6 and total IgE were observed in patients, with positive correlation between IL-4/IL-6 and total IgE. The IL-21 was lower, negatively correlated with total IgE. The UAS was positively correlated with CD4$^+$CXCR5$^+$, Tfh and Tfh/Tfr, but not with Tfr. Their area under the curves (AUCs) were all greater than 0.7, with statistical significance.
Conclusions: Our findings indicated that abnormalities of CD4$^+$CXCR5$^+$, Tfh and Tfh/Tfr might involve in the immunopathogenesis of CSU. The specificity of these reactions in CSU remains to be determined.

P0115

Transcription factor Tox2 drives T follicular helper (Tfh) cell development via regulating chromatin accessibility

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T Follicular helper (Tfh) cell is an effector CD4+ T cell subset specialized in helping B cells in germinal centers (GC) reactions. Although Bcl6 was identified as a Tfh-specific transcription factor essential for their development, the molecular mechanisms underlying Bcl6 regulation and Tfh cell commitment remain unclear. Here, we report that Tox2 transcription factor is highly expressed in Tfh cells, regulated by Bcl6 and STAT3. Forced expression of Tox2 drives Bcl6 expression and Tfh development. Mechanistically, Tox2 directly binds to Tfh-associated genes, including Bcl6, and functions to promote their chromatin accessibility and modulate the activities of other Tfh-regulating factors. Conversely, genetic deletion of Tox2 results in defective Tfh differentiation, and inhibiting both Tox and Tox2 in T cells abolishes Tfh differentiation and GC response. Thus, **our results**

DOI: 10.1002/eji.201970400

demonstrate that Tox2 is a key transcription factor that regulates Bcl6 expression and Tfh development and suggest a Tox2-Bcl6 axis in feed-forward regulation of Tfh program.

- **Tox2 is highly expressed in Tfh cells and regulated by Bcl6 and STAT3**
- **Forced expression of Tox2 drives Tfh development**
- **Tox2 directly binds Tfh-associated genes and promotes their chromatin accessibility**
- **Tox2 and Tox are necessary for Tfh development**

P0116

Foxo3 promotes the differentiation and function of follicular helper T cell

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Follicular helper T (Tfh) cells and their secretion of IL-21 are essential for germinal center formation, germinal center B cell proliferation and maturation, and the development of high-affinity antibodies. However, the intrinsic mechanisms that regulate Tfh cell differentiation and IL-21 production are largely unknown. Here, we demonstrated that together with increased Tfh population, Foxo3 was significantly upregulated in CD4 T cells from mesenteric lymph node and spleen of mice which was intraperitoneally immunized with 3 doses of OVA. The frequency of Tfh cells and GC B cells, the production of IL-21 and OVA specific antibodies markedly decreased in Foxo3-/- mice under OVA immunization. The model of OVA immunization on lymphocyte-repopulated Rag1-/- mice further proved that WT T cells, but not Foxo3-deficient T cells, provoked WT GC B cell and plasma cell maturation and antibody production. ICOS signaling is essential for Tfh-cell differentiation, however, deficiency of Foxo3, ICOS stimulation failed to promote Tfh cell differentiation and IL-21 production. The chromatin immunoprecipitation assay strongly suggested that Foxo3 was able to bind to the IL-21 promoter and was involved in the regulation of IL-21 secretion of CD4 T cells. In conclusion, our study unveiled the critical role of Foxo3 in the regulation of Tfh cell differentiation and IL-21 production. Modulating the activity of Foxo3 may provide benefit in the protection of humoral immunity related autoimmune diseases and transplant rejection.

P0117

IL-33 induce immunosuppression of brucellosis by promotes Treg function

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Brucellosis is a worldwide zoonotic disease, and patients are prone to chronic disease. CD4+Foxp3+ regulatory T (Treg) cells play an important role in immunosuppression in patients with brucellosis . The key factors driving the Treg cell function remain largely unknown。 We investigated 39 patients with brucellosis（BRU） and 42 health control people（HC）. We detected the IL-33 by ELISA and

DOI: 10.1002/eji.201970400

measured Treg by Flow cytometry. Compare with the HC group we found that IL-33 (165.8±31.90, *P*< 0.001) and Treg (9.20±1.61, *P*< 0.001) increased significantly in brucellosis. It was related to the increased proportion of Treg (r=0.632, *P*< 0.05). PBMCs from BRU group and HC group were co-cultured with IL-33 stimulation and anti-IL-33 neutralization, respectively. The expression and gene changes of FOXP3 and ST2 on Treg were observed. We found that IL-33 can promote the expression of FOXP3 and ST2 genes in Treg cells, especially in patients with brucellosis. Compare with the ST2- Treg, ST2+Treg releases more IL-10 and TGF-β in patients with burcellosis. We have uncovered a relationship between IL-33 and Treg. These finding highlights IL-33 exerts immunosuppressive function by up-regulating the expression of FOXP3 and ST2 , and increasing the production of IL-10 and TGF-β by promoting ST2+Treg in patients with brucellosis, IL-33 may be harnessed for therapeutic benefit for chronic brucellosis. This study funded by NSFC (81860375).
Keywords: IL-33; Treg; brucellosis; ST2 ; FOXP3

P0118

Glycogen synthase kinase 3 controls the maintenance and function of regulatory T cells

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Regulatory T cells (Treg) regulate immune homeostasis and implicated in autoimmunity, anti-tumor immune response and organ transplant rejection. Glycogen synthase kinase 3 (GSK3) has been demonstrated that regulates Th1, Th17 cell differentiation and $CD8^+$ cytotoxic T cell function. However, whether GSK3 controls Treg cell differentiation and function are totally unknown. Our results showed that Treg-cell specific deletion of Gsk3a and Gsk3b resulted in scurfy-like phenotype in mice with fatal autoimmunity. Percentage and cell number of Treg cells were decreased and that of effector/memory type of $CD4^+$ and $CD8^+$ T cells were largely increased in the peripheral lymphoid organs from GSK3-deficient (DKO) mice. Effector $CD4^+$ T cells produced increased amounts of IFN-g, IL-4 and IL-17, and IFN-g secretion by effector $CD8^+$ T cells was drastically increased in DKO mice. DKO Treg cells also produced IL-17 and failed to maintain their Foxp3 expression during lymphopenia-induced proliferation. In addition, DKO Treg cells had impaired suppressive function of anti-tumor immunity in a B16 melanoma model and failed to inhibit disease progression in a T-cell transfer colitis model. These results suggested that GSK3 regulated Treg cell stability and function. To uncover the molecular mechanism of Treg cell maintenance and function regulated by GSK3, we combined transcriptomic, proteomic and metabolomic analysis and developed an *in vitro* screening system to identify and validate several candidate genes that involved in GSK3-mediated regulation Foxp3 stability and/or Treg cell function. Our findings clarified the functions of GSK3 in Treg cells and provided potential therapeutic targets of autoimmune diseases and cancer.

P0119

The role of the transcription factor interferon-regulatory factor 4 in regulation of Th17 cells

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The transcription factor Interferon Regulatory Factor 4 (IRF4) is an essential regulator of $CD4^+$ T cell maturation to helper T cell lineages. As a consequence, IRF4 is essential for effective T cell responses against various pathogens.
We aim to characterize the role of IRF4 in Th17 cell differentiation, as well as in the maintenance of the differentiation status and function of these cells. $CD4^+$ T cells from *Irf4*$^{+/+}$, *Irf4*$^{+/-}$and *Irf4*$^{-/-}$ mice as

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DOI: 10.1002/eji.201970400

well as *Irf4*$^{fl/-}$ × CreERT2 mice were stimulated under Th17-inducing conditions and cultured for up to three weeks. Furthermore, we adoptively transferred *Irf4*$^{fl/+}$ × CreERT2 and *Irf4*$^{fl/-}$ × CreERT2 T cells into *Rag1*$^{-/-}$ mice which were infected with *Citrobacter rodentium*. In all experiments, the *Irf4*^{fl} allele was subsequently deleted by CreERT2 activation with tamoxifen. At different time points, the expression of RORγt and cytokines was determined by intracellular mAb staining and FACS analysis. First results indicated that induction of Th17 cells in culture strictly depended on IRF4 during T cell activation. In contrast, when the functional *Irf4* allele was deleted in CD4$^+$ T cells from *Irf4*$^{fl/-}$ × CreERT2 mice after stimulation, cells retained the capacity to produce IL-17A. *In vivo,* IRF4-heterocygous T cells showed reduced Th17 differentiation and accumulation in the colon. However, after deletion of the remaining functional allele, induced *Irf4*$^{-/-}$ CD4$^+$ T cells retained some Th17 function. So far, our results suggest that maintenance of Th17 cells is less dependent on IRF4 than the induction of these cells.

P0120

Tec tyrosine kinase regulates Th17 cell differentiation by quenching IL-6 sensing

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Th17 cells are intensively studied, as they are involved in controlling infections mediated by extracellular bacteria and fungi, but are also triggering auto-immune responses. Two independent studies involved the cytoplasmic non-receptor protein tyrosine kinase Tec in the differentiation and function of Th17 cells. However, it was not known how Tec regulates Th17 cells on a mechanistic level. Here we found that Tec was specifically expressed in Th17 cells in contrast to other Th subsets. In addition we could further implicate Tec in the IL-6 sensing by differentiating CD4$^+$ T cells. Thus our results identified Tec as a fine-tuning cytokine sensor specifically implicated in the control of Th17 cell differentiation *in vivo* and *in vitro*.

P0121

Unique futures of IL-6 mediated neonatal germinal center reaction

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Most pediatric vaccines are given at 2 months of age and repeated two or three more times in order to elicit protective antibodies. The inability of neonates to develop CD4+CXCR5+PD-1+ T follicular helper (Tfh) cells contributes to their weak vaccine responses. The poor Tfh response is accompanied by a disproportionate expansion of the inhibitory CD4+CXCR5+PD-1+Foxp3+ regulatory Tfh cells (Tfr) in immunized neonatal mice. We have recently shown that this enhanced Tfr cell-response is due to elevated IL-6R on Tfr cells and preferential activity of IL-6 on Tfr cells over Tfh cells. Here, we sought to improve neonatal vaccine responses by injecting an anti-CD25 antibody and removing CD25+Foxp3+ regulatory T (Treg) cells, the precursors of Tfr cells. However, immunization of neonatal mice with a pneumococcal conjugate vaccine (PCV) after the removal of Treg cells did not improve vaccine responses, most likely because of the persistence of CD25-Tfr cells, the Tfr cell subset shown to be most relevant in the germinal center. In contrast, co-injection of IL-21 or CpG with PCV increased IgG and IgA responses in neonatal mice. The improvement in antibody development was accompanied by an increase in Tfh and a decrease in Tfr cell populations. Supporting our

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DOI: 10.1002/eji.201970400

hypothesis on the role of IL-6R on Tfr cells, we found decreased IL-6R expression on Tfr cells from neonatal mice injected with IL-21 or CpG. These findings reveal a mechanism for the ablated T_{FH} development in immunized neonatal mice and provide insights into adjuvant mediated immune response-improvements in neonatal age group.

P0699

Sox5-mediated interaction between a novel enhancer and RORγt gene promoter promotes the RORγt gene expression and Th17 differentiation

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Th17 cells play a critical role in the pathogenesis of inflammatory and autoimmune diseases, as well as in host protection against pathogens. The retinoid-related orphan receptor-γt (RORγt) is the specific transcription factor which is necessary for Th17-cell lineage differentiation and function. However, the molecular mechanisms underlying the expression of RORγt are still poorly understood, especially about the long range cis-regulatory elements such as enhancers and silencers of this gene. In this study, we identified a potential active enhancer, named as RORCE2, in the Th17 cells through the epigenetic markers of active enhancer including H3K4me2, H3K4me1 and H3K27ac. RORCE2 has a significant enhancer activity for RORγt promoter but not for RORγ promoter both in 293T and EL4 cells using the luciferase reporter assay. Furthermore, RORCE2 deficiency significantly suppresses the expression of RORγt gene expression and differentiation of Th17 cells, which leads to the decreased severity of EAE. According to the mechanism of an enhancer, we also find that the RORCE2 can interact with the promoter of RORγt gene in Th17 cells, and the interaction depend on the binding of transcriptional factor Sox5 to the RORCE2. The deficiency of Sox5 binding site in RORCE2 also result in the decreased severity of EAE. These results will firstly provide not only a new molecular mechanism of the regulation of RORγt expression but also some new potential targets for the clinical prevention and treatment of Th17-related diseases.

P0700

Decreased circulating Treg/pTfh in active pSS patients

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Objective: To explore the significance of Treg/Tfh cell subsets in patients with Sjogren´s syndrome.
Methods: We designed a cross-sectional control study including 56 Sjogren´s syndrome (SS) patients and 20 healthy controls. Flow cytometry was used to detect the relative number of Treg and Tfh cell subsets (Total Tfh, Tfh1, Tfh2, Tfh17) in all patients and controls. ESSDAI scores, ESSPRI scores, immunoglobulins, and related cytokines were evaluated. The Mann-Whitney U Test was used to test the difference between different groups. The correlation between the two indicators was tested by Spearman correlation analysis.
Results: 1. Compared with healthy controls, the number of Tfh cells in peripheral blood of SS patients increased [24.1 (21.55, 26.3) vs. 14.45 (12.35, 16.5), $P < 0.001$], and the ratio of Treg/Tfh decreased [0.30 (0.24, 0.45) vs. 0.39 (0.29, 0.51), $P < 0.001$]. 2. The ratio of Treg/Tfh decreased in patients with low disease activity (ESSPRI≤4) group compared with that in the high disease activity (ESSPRI>4) group [0.29 (0.23, 0.42) vs. 0.39 (0.250.46), $P< 0.1$]. 3. Correlation analysis indicated:1) The ratio of Treg/Tfh17 was negatively correlated with ESSPRI scores (ρ=-0.237, $P< 0.05$). 2) The number of Tfh1 cells was positively correlated with serum IgG concentration (ρ=0.325, $P< 0.05$).

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DOI: 10.1002/eji.201970400

Conclusion: There is an increase in the number of Tfh cells and decreased ratio of Treg/Tfh in patients with SS. Tfh cell subsets are associated with disease activity scores and inflammatory cytokines, and may play an important role in pSS disease activity.

P0702

JQ1 suppress Th17 effectors by blocking p300-mediated acetylation of RORγt

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Aim: JQ1, a bromodomain (BRD) inhibitor was reported to suppress the expression of Th17 effectors. However, the underlying mechanisms remain unclear. Here, we investigated the effect of JQ1 on Th17 cells and the underlying mechanisms.
Methods: The effect of JQ1 was investigated on human Th17 and HEK293T. The involvement of p300 in the acetylation of RORγt was investigated by co-infection p300 and RORγt plasmids. Immunoprecipitation and Western blot analysis was used to determine protein interaction and ROR acetylation. qPCR was used to evaluate mRNA expression of Th17 cell effectors. In vivo inhibition of Th17 function by JQ1 was investigated using *Schistosoma japonicum* infected mouse model, in which Th17 cells are immunopathogenic. The recombinant BRD-containing and BRD-lacking p300 fragment were expressed respectively. Acetyltransferase activity of p300 fragment was detected with/without JQ1.
Results: JQ1-induced p300 inhibition reduced the Th17 effectors expression. JQ1 reduces RORγt acetylation by p300 in both human Th17 and HEK293T cells. Notably, JQ1 treatment inhibits acetyltransferase activity of p300, and this inhibition requires interaction with bromodomain. In vivo, JQ1 attenuated egg-induced liver pathology accompanied by down-regulation of Th17 associated genes in mice model.
Conclusion: JQ1 alleviates Th17 mediated immunopathology by dampening RORγt acetylation by p300, thereby reduced the RORγt mediated genes transcription. In addition, JQ1 inhibits the acetyltransferase activity of p300, by interacting with BRD. Strategies targeting p300 may provide a new therapeutic approach for controlling inflammatory-related diseases.
(Funding: Natural Science Foundation of China (81471982) , Natural Science Foundation of Anhui Province (1808085MH269),Foundation of Education Department of Anhui province (KJ2018A0168).

P0703

The role of CD4+ T cell exosomes in helper T cell mediated B cell responses

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Introduction: T cells secrete bioactive exosomes, but the potential biological effects of CD4+ T cell exosomes are not clear. The main purpose of this study was to investigate the effects of CD4+ T cell exosomes on B cell responses and explore possible mechanisms.
Materials and Methods: In this study, the exosome release of CD4+ T cells was inhibited by nSMase2 inhibitor GW4869. In addition, anti-CD63 microbeads were also used to remove most exosomes in supernatant of CD4+ T cells by immunomagnetic separation. For the study on the functions of CD4+ T cell exosmes, exosomes derived from activated CD4+ T cells and EL-4 cell were prepared.
Results: CD4+ T cell exosomes were involved in the T-B cell intercellular interaction and played an

DOI: 10.1002/eji.201970400

important role in B cell responses in vitro, which dose-dependently promote B cell activation, proliferation and antibody production. Moveover, antigen-specific exosomes were found to be more efficient than control exosomes in enhancing B cell responses. Furthermore, using mouse T cell line EL-4, we showed that CD40L was involved in T cell exosome-mediated B cell responses.
Conclusions: Our results have demonstrated that CD4+ T cell exosomes can enhance B cell responses by releasing exosomes. Mechanically, exosome-derived CD40L was found to be involved in this process.

P0704

Lysosome-related organelle tethering controls directionally distinct cytokine transport in T cells

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Cytokines are small proteins playing a key role in orchestrating activation and responses of immune cells. It is of markedly importance to target cytokines to the desired destination. In CD4+ T cells, in particular, cytokines are delivered in two distinct modes: either exclusively to the immunological synapse (IS) (eg. IL-2) or transported multi-directionally (eg. TNFα). However, the molecular mechanisms responsible for these distinct transport patterns remain elusive. Here we show that in primary human CD4+ T cells both TNFα+ and IL-2+ vesicles tether with lysosome-related organelles (LROs), mediated by the SNARE protein Vti1b. Only LRO-tethered but not untethered cytokine vesicles are preferentially transported to the desired secretion sites, namely LRO-tethered IL-2+ to the IS and LRO-tethered TNFα+ multi-directionally. LRO tethering enhances the recruitment of kinesin and dynein to TNFα+ vesicles, favoring the transport to both directions along microtubules. In contrast, the recruitment of dynein but not kinesin is selectively enhanced in LRO-tethered IL-2+ vesicles, promoting minus-end transport to the MTOC, which localizes at the IS. Our findings suggest that LRO tethering serves as a novel mechanism to regulate directionally distinct cytokine transport, especially in CD4+ T cells.

P0705

Mechanism of IgE production induced by dysfunction of regulatory T cells

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The germinal center (GC) reaction, which requires interactions between germinal center B (GCB) cells and follicular helper T (Tfh) cells, is critical for the production of high-affinity antibodies. It has been known that Foxp3^{+} regulatory T (Treg) cells control this reaction. Our group developed Foxp3 time-specific knockout mice (*Foxp3* ^{flox} *R26* ^{CreERT2}: cKO) in which the *Foxp3* gene was inducibly deleted by tamoxifen administration. After oral administration of tamoxifen, the proportion of Tfh cells and GCB cells and titer of IgE in cKO were increased compared with those in controls. These results suggested that high IgE production was induced by the excessive GC reaction. However, it was unclear whether IgE class switch recombination induced by the dysfunction of Treg cells required the GC reaction. To clarify the requirement of GC reaction for IgE production induced by dysfunction of Treg cells, we developed *Foxp3* ^{flox} *Bcl6* ^{flox} *R26* ^{CreERT2} (cDKO) mice in which the *Bcl6* gene, the master transcription factor of Tfh cell and GCB cell, and the *Foxp3* gene were inducibly deleted by administration of tamoxifen. In unimmunized cDKO mice, IgE production was comparable with that in cKO mice. In contrast, antigen-specific IgE production in immunized cDKO mice was significantly decreased compared with that in cKO mice. These results suggested that dysfunction of Treg cells enhanced

DOI: 10.1002/eji.201970400

spontaneous and antigen-specific IgE production in a GC-independent and -dependent manner, respectively.

P0706

Plasticity of splenic CD4+ T cell subsets in lymphoma/leukemia patients

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Disturbed immune balance represents a central guide of pathogenic mechanisms in hematological malignancies. T lymphocytes play a cardinal role to mediate the functions of other immune cells. Therefore, investigations including T lymphocytes are critical for elucidating the pathogenesis of such diseases and for elaborating targeted therapeutic approaches. Splenectomy represents the therapeutic intervention in certain types of lymphoma/leukemia. Our retrospective study aims to explore the effector and suppressive CD4+ T lymphocyte subsets in the spleen and blood of patients with lymphoma/leukemia.
Mononuclear cells from peripheral blood and dissociated spleen tissue of patients with lymphoma/leukemia are purified and the expressions of the specific transcription factors (Tbet, RORγt and FoxP3) are quantified within the CD4+ compartment of T lymphocytes. Data are acquired on a FacsCalibur flow cytometer and analyzed using FlowJo® v10 software. Correlative analyses of Th1/Treg, Th1/Th17 and Th17/Treg ratios are established between splenic and circulating lymphocytes.
Our data suggest that in lymphoma/leukemia patients the frequency of total splenic CD4+ T lymphocytes is significantly high compared to their circulating counterparts. However, percentages of CD4+Tbet+, CD4+RORγt+ and CD4+FoxP3+ cells corresponding to Th1, Th17 and Treg subsets respectively, are comparable between splenic and blood samples. Of importance, correlative analyses of effector to suppressor cells revealed that the ratios of Th1/Treg, Th1/Th17 and Th17/Treg are shifted towards immunosuppressive cells and it is more evident in spleen compared to blood. Splenic T cell subsets can be considered as good biomarkers to assess disease pathogenesis and, respectively, prospective therapeutic targets in patients with lymphoma/leukemia undergoing therapeutic splenectomy.

P1301

EGCG and quercetin protect mouse liver against triptolide-induced hepatic injury by restoring Th17/Treg balance through Tim-3 and TLR4-MyD88-NF-κB pathway

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Triptolide (TP) is a diterpene triepoxide with various biological activities, but its clinical applications have been limited by potential hepatotoxicity. EGCG and Quercetin have been reported to have many benfits and medicinal properties, including hepatoprotective activity against TP-induced liver injury. The present study was designed to explore the protective effects and the mechanisms of EGCG and Quercetin against TP-induced liver injury. Treatment with EGCG and Quercetin prior to TP administration restored TP-induced alterations indicating that EGCG and Quercetin were able to inhibit TP induced liver injury. One mechanism underlying this effect was the shifting balance in Th17 and

DOI: 10.1002/eji.201970400

Treg cells from Th17 dominance to Treg dominance. EGCG and Quercetin markedly decreased the expression level of the Th17 related pro-inflammatory cytokines interleukin IL-17 and IL-6, as well as the Th17 transcription factor ROR-γt. TP induced downregulation in the expression of anti-inflammatory cytokine IL-10, but the expression of Treg transcription factor Foxp3 was restored by EGCG and Quercetin. We found that EGCG and Quercetin significantly reduced both protein and mRNA expression of TLR4, which in turn not only inactivated MyD88, NF-κB, but also simultaneously increased the levels of Tim-3. Furthermore, blocking of TLR4 enhanced the effect of EGCG and Quercetin in regulating the Th17/Treg imbalance. In summary, this report has demonstrated for the first time that the protection afforded by EGCG and Quercetin against TP-induced liver injury was associated with a shift in the balance of Th17 and Treg cells to Treg dominance, which was regulated by Tim-3 and TLR4-MyD88-NF-κB signaling pathway.

P1302

Dysfunction of follicular T helper cell was related to immune disruption of B cells in chronic hepatitis B virus infection associated liver cirrhosis

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Hepatitis B virus associated cirrhosis (HBC) leads to B cell disruption which was characterized as impaired responses to vaccines. Follicular T helper cell (Tfh) cells were considered as the specialized cell subset which was responsible to B cell regulation, but its role in HBC was not clear by now. In this study, we determined the changes of peripheral B cells of those patients and healthy controls (HCs). The results showed that the frequency of memory B cells (MBs) decreased and naïve B cells (NBs) increased significantly in HBC patients as compared with those indicators of healthy controls (HCs). Plasma B cells (PLCs) were observed to increase significantly in decompensate-HBC (d-HBC) patients compared to HCs and compensated-HBC (c-HBC) patients. Our results also showed that the frequency of Tfh increased significantly in patients with d-HBC compared to those with c-HBC. Then, Tfh2 subset was comparable between different patient groups, but the profound downregulation of Tfh17 of HBC patients was observed which was paralleled with Child-Pugh classification and negatively correlated with PLCs frequency in HBC patients. IL-21+Tfh was also downregulated in HBC patients compared to HCs. Although IL-21+ Tfh was not associated with Child-Pugh classification of patients, it was positively correlated with frequencies of MBs but not PLCs. Our results suggested that the function of Tfh was disrupted significantly and then exert its influences on the function of B cells during the development of HBC. Tfh cell might be the therapeutic target to improve B cell function of patients with HBC.

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DOI: 10.1002/eji.201970400

P1303

CD147-mediated chemotaxis of CD4⁺CD161⁺ T cells may contribute to local inflammation in Rheumatoid Arthritis

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CD161 is used as a surrogate marker for Th17 cells, which are implicated in the pathogenesis of rheumatoid arthritis (RA). In this study, we evaluated the percentage, clinical significance, and CD98 and CD147 expression of CD4+CD161+ T cells. The potential role of CD147 and CD98 in cyclophilin A-induced chemotaxis of CD4+ CD161+ Tcells was analyzed. Thirty-seven RA patients,15 paired synovial fluid (SF) of RA, and 22 healthy controls were recruited. The cell populations and surface expression of CD98 and CD147 were analyzed by flow cytometry. Spearman's rank correlation coefficient and multiple linear regression were applied to calculate the correlations. Chemotaxis assay was used to investigate CD4+CD161+ T cell migration. We found that the percentage of CD4+CD161+ Tcells and their expression of CD147 and CD98 in SF were higher than in the peripheral blood of RA patients. Percentage of SF CD4+CD161+ T cells was positively correlated with 28 Joint Disease Activity Score (DAS28). CD147 monoclonal antibody (HAb18) attenuated the chemotactic ability of CD4+CD161+ T cells. An increased CD4+CD161+ Tcell percentage and expression of CD147 and CD98 were shown in RA SF. Percentage of SF CD4+CD161+ T cells can be used as a predictive marker of disease activity in RA. CD147 block significantly decreased the chemotactic index of CD4+CD161+ cells induced by cyclophilin A (CypA). These results imply that the accumulation of CD4+CD161+ T cells in SF and their high expression of CD147 may be associated with CypA-mediated chemotaxis and contribute to local inflammation in RA.

P1304

Immunomodulatory effects of eriodictyol on experimental autoimmune encephalomyelitis by inhibiting Th17 cell differentiation

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T helper cells that express Interleukin-17 (IL-17) (Th17 cells) have pathological roles in inflammatory and autoimmune diseases through producing inflammatory cytokines IL-17, IL-17F, and IL-22. The differentiation of Th17 cells and the induction of IL-17 transcription are distinguished by the nuclear receptors retinoic acid receptor-related orphan receptors γt (RORγt). Here we present Eriodictyol (EDT), a natural small molecule flavonoids derived from food-borne plants, that specifically enter the active center of RORγt to change its conformation and inhibit its activity, thereby supressing the differentiation of Th17 cells and the expression of IL-17. Additionally, EDT treatment effectively suppressed the clinical severity of experimental autoimmune encephalomyelitis (EAE) and reduced CNS inflammatory cells infiltration and demyelination. It also obviously reduced Th17 cell subset both in spleen and CNS, and the production of inflammatory cytokines such as IL-17 and GM-CSF in EAE mice. The results suggest the feasibility of targeting the RORγt to specifically inhibit Th17 cell differentiation and function and indicate that EDT has potential utility in the treatment of Th17-mediated autoimmune diseases.

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DOI: 10.1002/eji.201970400

P1305

Development of Tfh-Th1-like cells through epigenetic modification by IL-12-mediated co-activation of STAT1 and STAT4

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Objectives: Follicular helper T (Tfh) cells play a key role in the pathogenesis of systemic lupus erythematosus (SLE). However, the characteristics and mechanisms of differentiation of Tfh cells in human are still undefined. To assess the mechanisms of development of Tfh cells, we probed the phenotypes of T helper cells in patients with SLE and underlying epigenetic modifications by cytokine-induced signal transducer and activators of transcription (STAT) family factors.
Methods: Naive $CD4^+$ T cells and memory $CD4^+$ T cells were isolated and stimulated by various cytokines and T cell receptor (TCR) in vitro. Expression of characteristic markers of Tfh-Th1-cells and phosphorylation of STATs were analyzed by flow cytometry and qPCR. Histone modifications were evaluated by chromatin immunoprecipitation.
Results: Differentiation of $CXCR5^+CXCR3^+Bcl\text{-}6^+T\text{-}bet^+IL\text{-}21^+IFN\text{-}\gamma^+$ Tfh-Th1-like cells was induced by IL-12. Among STAT family, STAT1 and STAT4 were phosphorylated simultaneously by IL-12 independent of IFN-γ and directly bind on *BCL6* and *TBX21* gene loci accompanied by suppression of trimethylated histone 3 lysine 27. Responsiveness of activation of STAT1 and STAT4 by IL-12 and proportion of activated Tfh-Th1-like cells were increased in patients with SLE.
Conclusions: Our findings suggest that IL-12-mediated co-activation of STAT1 and STAT4 alter histone modification, resulting in development of Tfh-Th1-like cells that are characteristically expanded in patients with SLE. These findings could be one of underlying pathogenesis of SLE and potentially helpful towards development of cell-specific treatment.

P1306

RNA-binding protein Quaking deficiency in mouse $CD4^+$ T helper cells impairs the immune-homeostasis and contributes to the auto-immune and tumor diseases

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$CD4^+$ T helper cells play pivotal roles in host immune-homeostasis, an imbalance in T helper cell subsets contributes to the autoimmune disorders such as inflammatory bowel disease and psoriasis. Here, we show that RNA-binding protein Quaking (QKI), whose function is previously unknown in $CD4^+$ T helper cells, coordinates Th differentiation in vitro and in vivo. We first confirmed that the expression of QKI in various immune cells both in mouse and human and found that QKI was relatively higher in regulatory T cells (Treg cells) than naïve $CD4^+$ T cells and effector/memory T cells in mouse. In vitro differentiation of naïve $CD4^+$ T cells sorted from $CD4^{cre}$ QKI $^{fl/fl}$ mouse showed that deletion of QKI in $CD4^+$ T cells lead to more Th1 cells other than Th2 cells and Treg cells. $CD4^+Foxp3^+$ Treg cells populations were also decreased in knock out mice. Then we constructed $Foxp3^{cre}$ QKI $^{fl/fl}$ mouse and performed experimental model of ulcerative colitis. Treg cells specific knock out of QKI mice showed aggravated inflammatory phenotypes including lower body weight, sever hematochezia and histological scores, higher levels of inflammatory cytokines production in splenocytes, mesenteric lymphocytes and lamia propria lymphocytes. Tumor implant models showed a relatively higher amount of $IFNr^+$ $CD8^+$ T cell and lower Treg cells in tumor infiltrating immune-cells. At last, we found QKI expression were lower in $CD4^+$ T cells in psoriasis patients compared to healthy individuals.

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DOI: 10.1002/eji.201970400

Underlying mechanism are undergoing. Taken together, our results identified QKI as a new T helper cell modulator in diseases.

P1307

Splenic vs. circulating CD4+CD39+ T cell subsets in patients with immune thrombocytopenia (ITP) and lymphoma/leukemia

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Investigations precluding T lymphocyte counts and functions are critical in autoimmune disorders and cancer for elucidating the pathogenesis of such diseases and for elaborating targeted therapeutic approaches in patients resistant to standard treatment options. Splenectomy represents the therapeutic intervention in many types of hematological disorders. Splenic T cell subpopulations, especially relatively newly described subsets, are yet unexplored in such disorders. Recent findings suggest that the expression of ectoenzyme CD39 in CD4+ T lymphocytes indicates on their suppressive nature. Our study aims to explore the differential expressions of CD39 and FoxP3 in splenic and circulating CD4+ T cells of patients with immune thrombocytopenia (ITP) and lymphoma/leukemia. Individuals undergoing splenectomy due to other reason than autoimmune disease or cancer are used as controls.
Our data suggest that the frequency of total circulating and splenic CD4+ T lymphocytes are comparable between study groups: ITP patients, lymphoma/leukemia and controls. However, CD39 expression is diminished in CD4+ T cells in patients with immune thrombocytopenia compared to controls and patients with lymphoma/leukemia. Importantly, the differential expression of CD39 resembles FoxP3 levels in both groups of patients and is more evident in spleen compared to blood. Therefore, splenic CD4+CD39+ T cells might be considered as prospective therapeutic targets in patients with hematological disorders.

P1308

Silencing RORγt in human CD4$^+$ T cells with CD30 aptamer-RORγt shRNA chimera

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Targeting specific T cell subtypes and intervening in their function are emerging a critical strategy for treatment of autoimmune diseases. Here we report that an RNA CD30 aptamer was utilized to deliver short hairpin RNA (shRNA) to CD30$^+$ T cells to target retinoic acid receptor-related orphan receptor gamma t (RORγt), leading to impaired expression of RORγt and suppression of IL-17A and IL-17F. A DNA template consisting of CD30 aptamer and RORγt shRNA sequences was synthesized and was transcribed into CD30 aptamer-RORγt shRNA chimera (CD30-AshR-RORγt). Insertion of 2'-F-dCTP and 2'-FdUTP was incorporated during CD30-AshR-RORγt transcription to increase its resistance to RNase. CD30-AshR-RORγt was specifically up-taken by CD30$^+$ Karpas 299 cells, but not by Jurkat cells which lack CD30. It was also up-taken by activated, CD30 expressing human CD4$^+$T cells, but not by resting CD4$^+$ T cells. The RORγt shRNA moiety of CD30-AshR-RORγt chimera was cleaved

DOI: 10.1002/eji.201970400

and released by Dicers. Then, CD30-AshR-RORγt suppressed RORγt gene expression in Karpas 299 cells and activated human CD4$^+$ T cells. Consistently, silence of Th17 cell differentiation and IL-17A and IL-17F synthesis with CD30-AshR-RORγt was demonstrated in activated human CD4$^+$ T cells from healthy donors and RA patients. CD30-AshR-negative control chimera and prostate specific membrane antigen (PSMA)-AshR-RORγt had no significant impact on the expression of RORγt or IL-17A and IL-17F. These data present a novel strategy for shRNA delivery using CD30 RNA aptamers to down-regulate CD30$^+$ Th17 cells and can be developed as a targeted therapy for treating Th17 cell mediated conditions.

P1309

Transcription factor T-bet is required for pathogenicity of "GM-CSF-only" Th cells in experimental autoimmune encephalomyelitis

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Myelin-specific Th1 and Th17 cells play a pivotal role in the pathogenesis of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). However, recent descriptions of a new Th subset, tentatively called "GM-CSF-only" Th cells, or ThG cells, raised the possibility that these cells also contribute to the pathogenesis of MS and other autoimmune diseases. ThG cells express GM-CSF, but lack expression of master transcription factors (TF) and hallmark cytokines of established Th cell lineages. Previous studies showed that Th17 cells switch to Th1 phenotype by upregulating T-bet, which is required for their encephalitogenicity. To test whether pathogenicity of ThG cells is also dependent on T-bet, we evaluated the role of T-bet expression by ThG cells in EAE. T-bet was not required for development of ThG cells in vitro or in vivo. Adoptively transferred ThG cells upregulated T-bet expression in the CNS of EAE mice, while they had low or undetectable levels of RORgt, GATA3 and Foxp3. Next, we adoptively transferred ThG cells that developed from WT and T-bet-deficient 2D2 mice to RAG-/- recipients. WT ThG cells were highly encephalitogenic, whereas T-bet-deficient ThG cells failed to induce disease. Taken as a whole, our data show that ThG cells, similar to Th17 cells, have plastic phenotype, upregulate T-bet and IFN-g in the CNS of EAE mice, and that their encephalitogenicity is dependent on T-bet.

P1892

The kinase mTORC1 promotes the generation and suppressive function of follicular regulatory T cells

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Follicular regulatory T (Tfr) cells differentiate from conventional regulatory T (Treg) cells and suppress excessive germinal center (GC) responses by acting on both GC B cells and T follicular helper (Tfh) cells. Here, we examined the impact of mTOR, a serine/ threonine protein kinase that senses and integrates diverse environmental cues, on the differentiation and functional competency of Tfr cells in response to protein immunization or viral infection. By geneti- cally deleting Rptor or Rictor, essential components for mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), respectively, we found that mTORC1 but not mTORC2 is essential for Tfr differentia- tion. Mechanistically, mTORC1-

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DOI: 10.1002/eji.201970400

mediated phosphor- ylation of the transcription factor STAT3 induced the expression of the transcription factor TCF-1 by promoting STAT3 binding to the Tcf7 50-regulatory region. Subsequently, TCF-1 bound to the Bcl6 pro- moter to induce Bcl6 expression, which launched the Tfr cell differentiation program. Thus, mTORC1 initiates Tfr cell differentiation by activating the TCF-1-Bcl-6 axis during immunization or infection.

P1893

Changes of Tfh subsets in Sjögren's syndrome after taking influenza vaccination

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Objective:Investigating the safety of influenza vaccine for pSS patients.
Method: Collecting and detecting after the vaccinating, pSS patients and healthy controls the general information, clinical symptoms, laboratory test,IgG, autoantibody (ANA, anti-SSA/SSB, α-Fodrin), Tfh cells (Tfh1、Tfh2,、Tfh17，CCR7- PD-1 high) ratio and flu neutralizing antibody level.
Results: 13 patients with pSS met the diagnostic criteria of pSS in 2002, with an average age of 45.50（±12.20） years old.Compared with the control group, 1 week after influenza vaccine injection, pSS patients showed no significant aggravation of symptoms such as dry mouth and eyes, fatigue, muscle pain, joint pain, cough, shortness of breath after activity and fever, and no significant increase in fatigue (1/13) and fever (1/13) compared with the control group.
The proportion of Tfh cells at 1 week before and after influenza vaccination (pd-1 High, CCR7-) was (56.55±6.81532) and (46.675±7.092), P=3.027.The proportions of Tfh1, Tfh2, and Tfh17 cells at 1 week before and after influenza vaccination were (12.7725±5.55109vs26.4625%±2.55507, 76.3125±11.14573vs53.025±11.14665, 9.1987±7.02281vs16.3625±8.45745) P>0.05, respectively.There was no significant difference in anti-SSA, anti-SSB, ANA,α-Fodrin,WBC, NEU%, HGB, PLT and protein electrophoresis between the two groups (P>0.05).
Conclusion: Influenza vaccine for pSS patients is safe, but longer clinical follow-up and larger sample size are needed.

P1894

The histone methyltransferase EZH2 primes the early differentiation of follicular helper T cells during acute viral infection

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Epigenetic modifications to histones dictate the differentiation of naïve $CD4^+$ T cells into different subsets of effector T helper (T_H) cells. The role of histone methyltransferase enhancer of zeste homolog 2 (EZH2) has been implicated in the differentiation of T_H1, T_H2 and regulatory T (T_{reg}) cells. However, whether and how EZH2 regulates follicular helper T (T_{FH}) cell differentiation remains unknown. Using a mouse model of acute lymphocytic choriomeningitis virus (LCMV) infection, we observed an abundant EZH2 expression and associated H3K27me3 modification preferentially in the early committed virus-specific T_{FH} cells compared to T_H1 cells. Ablation of EZH2 in LCMV-specific $CD4^+$ T cells led to a selective impairment of early T_{FH} cell fate commitment but not late T_{FH} differentiation or memory T_{FH} maintenance. Mechanistically, our data showed that EZH2 specifically stabilizes the chromatin accessibility of a cluster of genes that are important for T_{FH} fate commitment, especially *B cell lymphoma 6* (*Bcl6)*, and thus directs T_{FH} cell commitment. Therefore, we identified the

DOI: 10.1002/eji.201970400

chromatin-modifying enzyme EZH2 as a novel regulator of early T_{FH} differentiation during acute viral infection.

P1895

HSP90α on tumor-released autophagosomes induces CD4⁺T cell-mediated immunosuppression via a TLR2-IL-6 cascade

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$CD4^+$ T cells are critical effectors of anti-tumor immunity, but how tumor cells influence $CD4^+$ T cell effector function is not fully understood. Autophagosomes are a major type of immunomodulatory vesicle released by tumor cells. Here, we found that heat shock protein 90α (HSP90α) on the surface of tumor-released autophagosomes (TRAPs) from malignant effusions of cancer patients and tumor cell lines stimulated $CD4^+$ T cell production of IL-6 via TLR2. TRAPs-induced autocrine IL-6 further promoted $CD4^+$ T cells secretion of IL-10 and IL-21. TRAPs-elicited $CD4^+$ T cells inhibited $CD4^+$ and $CD8^+$ effector T cell function in an IL-6- and IL-10-dependent manner and induced IL-10-producing regulatory B cells (Bregs) via IL-6, IL-10 and IL-21, thereby promoting tumor growth and metastasis. Inhibition of tumor autophagosome formation or IL-6 secretion by $CD4^+$ T cells markedly retarded tumor growth. Our findings reveal a vital mechanism by which tumors regulate $CD4^+$ T cell differentiation and define TRAPs or their membrane-bound HSP90α as targets for immunotherapy.

P1896

The changes of Th9 cells in peripheral blood of patients with alloimmune recurrent spontaneous abortion

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Objective: Application fluid cytology technique to explore changes of Th9 cells in peripheral blood of patients with alloimmune recurrent spontaneous abortion.
Methods: Selected 50 cases of patients with alloimmune recurrent spontaneous abortion as the research object, in which there are 31 cases of patients with two times miscarriages , 10 cases with three times miscarriages and 9 cases with greater than or equal to four times miscarriages. And then choosing another 50 cases of health women as the control group. Application fluid cytology technique to test TH9 cells percentage and explore the changes of Th9 cells in peripheral blood of patients with alloimmune recurrent spontaneous abortion.
Results: TH9 cells percentage in peripheral blood of patients with alloimmune recurrent spontaneous abortion were significantly increased than the control group（$P< 0.05$）. Th9 cells percentage in peripheral blood in patients with different numbers of recurrent miscarriage abortion has no statistically significant difference（$P>0.05$）.
Conclusion: TH9 cells percentage in peripheral blood of patients with alloimmune recurrent spontaneous abortion were significantly increased than the control group. We hypothesized that Th9 cells may be associated with the occurrence of recurrent spontaneous abortion and not with the number of abortions. Key words: Recurrent spontaneous abortion; Alloimmune; TH9 cell; IL-9

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DOI: 10.1002/eji.201970400

P1897

The effects of Th9, Th17, CD4+CD25+FoxP3+ regulatory T cells in adult primary immune thrombocytopenia

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Objective: The aim of this work was to investigate the presence and significance of Th9, Thl7, $CD4^+CD25^+FoxP3^+$ regulatory T(Treg) cells and the related cytokines (IL-9、IL-17 、TGF-β) in peripheral blood of patients with adult primary immune thrombocytopenia.
Methods: Peripheral blood samples were obtained from 49 patients with ITP and 41 age- and sex-matched healthy controls. The percentages of Th9、Th17 and Treg cells in peripheral blood were detected with Flow cytometry. Furthermore, the serum level of IL-9、IL-17 and TGF-β were detected with the use of enzyme linked immune sorbent assay(ELISA) .
Results: Compared with healthy subjects, the percentages of Th9、Thl7 and concentrations of IL-9、IL-17 were all significantly increased in patients with ITP[(1.29±0.33)% vs. (0.75±0.24)%, P < 0.05; (2.07±0.41)% vs. (0.98±0.31)%, P < 0.05; (26.55±7.49)% vs. (16.19±5.25)%, P < 0.05; (10.92±3.91)% vs. (7.18±2.71%), P < 0.05]；the percentages of $CD4^+CD25^+FoxP3^+$ cells and concentrations of TGF-β were lower in patients [(4.64±0.81)% vs. (7.19±1.95)%, *P* < 0.05; (3.79±1.22)% vs. (6.45±1.81)%, *P* < 0.05]. Moreover, the percentages of Th9、Thl7 and concentrations of IL-9、IL-17 were all negatively correlated PLT accounts (γs=-0. 345, p=0.039；γs=-0. 391, p=0.032；γs=-0. 432, p=0.015；γs=-0.405, p=0.028) of patients with ITP; the percentages of $CD4^+CD25^+FoxP3^+$ cells and concentrations of TGF-β were positively correlated with PLT accounts (γs=0.413, p=0.026；γs=0. 409, p=0.028) of patients with ITP.
Conclusions: The abnormality Th9、Thl7 and Treg cells and their related cytokines （IL-9, IL-17 and TGF-β） changes in patients with ITP ,which may be involved in immunological pathogenesis of ITP.
Keywords: primary immune thrombocytopenia;Th9 cells;Th17 cells; $CD4^+CD25^+FoxP3^+$Regulatory T cells; IL-9; IL-17; TGF-β

P1898

PI3K-Akt pathway enhances the differentiation of interleukin 27-induced type 1 regulatory T cells

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Interleukin 27 (IL-27) has been identified as a potent cytokine in the differentiation of type 1 regulatory T (Tr1) cells through interactions with several key elements, including transcription factors such as aryl hydrocarbon receptor and IL-21. Autocrine production of IL-21 is known to be important for maintaining IL-10 expression by Tr1 cells. Although previous studies have shown that the phosphoinositide 3-kinase (PI3K) -Akt axis contributes to the differentiation of helper T cell subsets, the role of the PI3K pathway on Tr1 cell differentiation remains to be elucidated. In this time we demonstrate that suppression of the PI3K-Akt pathway results in impairment of IL-27-induced Tr1 (IL-27-Tr1) cell differentiation in vitro and in vivo . Furthermore, this suppression down-regulates IL-21 receptor expression by Tr1 cells, followed by suppression of IL-10 expression by IL-27-Tr1 cells.

DOI: 10.1002/eji.201970400

These results suggest that the PI3K pathway enhances IL-10 expression by IL-27-Tr1 cells through up-regulation of IL-21 receptors.

P1899

The Swi/Snf chromatin remodeling complex promotes TH17 differentiation by coordinating RORγt-driven epigenetic program

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T_H17 cells play a critical role in mucosal immunity and also in multiple autoimmune diseases. The differentiation of T_H17 cells is dictated by a master transcription factor, RORγt; however, the mechanism by which RORγt controls target genes is poorly understood. Here we identify the Swi/Snf complex as an essential regulator of T_H17 cell differentiation. Loss of the expression of SRG3/mBAF155, a core subunit of the Swi/Snf complex, results in the specific downregulation of RORγt target genes such as IL-17A, IL-17F and IL-23R. Importantly, the comparative transcriptional analysis reveals a high similarity between RORγt and the Swi/Snf complex in the control of T_H17-related gene expression. Mechanistically, the Swi/Snf complex partners with RORγt and coordinates a variety of RORγt-mediated epigenetic modifications, including both permissive and repressive ones. These results provide a basis for the understanding of how a master transcription factor RORγt cooperates with the Swi/Snf complex to govern T_H17 cell differentiation.

P1900

Repertoire sharing between CD4$^+$ T follicular helper cells in peripheral blood and tonsil

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T follicular helper (Tfh) cells are specialized CD4$^+$ T cells, primarily localized to germinal centers in secondary lymphoid organs and play a fundamental role in B cell selection and antibody maturation. Circulating Tfh-like (cTfh) cells have been found in peripheral blood, but the relationship between Tfh cells in lymph nodes and cTfh as well as their distinction from non-Tfh CD4$^+$ cells, which represent the majority of CD4$^+$ T cells in the blood, remain unclear. Using donor-matched blood and tonsils, we investigated the relationships between peripheral cTfh and tonsillar Tfh cells and between Tfh and non-Tfh cell populations. Whether antigen-specific cTfh cell clones were present within tonsillar Tfh populations was also addressed.
T cell receptor (TCR) sequencing of blood and tonsil populations showed a considerable repertoire overlap between peripheral cTfh and tonsillar Tfh subsets as well as a clear distinction between Tfh and non-Tfh cells. These findings were confirmed by comparing the specificity and TCRs of haemagglutinin-responsive Tfh and non-Tfh clones and their match to the tonsil repertoire.
Studies of cTfh cells, which are readily accessible, can therefore be used to gain insights into Tfh

DOI: 10.1002/eji.201970400

responses in lymphoid tissues, which it will facilitate the design and evaluation of vaccination strategies.
This study was funded by CHAVI (NIH UM AI 00645), MRC (K012037/1), Wellcome Trust (100326/Z/12/Z). DMC is supported by grant of the Ministry of Education and Science of the Russian Federation (14.W03.31.0005).

P1901

Quantification of populations of Th17 lymphocytes in peripheral blood of patients with generalized chronic periodontitis: Stimulation protocol

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Th17 lymphocytes have been linked to the pathogenesis of periodontal disease. There is a relationship between periodontal pathology and systemic involvement associated with the possible mechanisms of bacterial migration from the mouth into the bloodstream. The description of different populations of Th17 lymphocytes: Teff17, Treg17 and Th17 / Th1 with antagonistic functions may explain a different function to that attributed as clearly pro-inflammatory.
Objective: To compare the number of lymphocytes Teff17, Treg17 and Th17 / Th1 in peripheral blood of systemically healthy patients with: generalized chronic periodontitis, with respect to patients with healthy periodontium or gingivitis associated with biofilm.
Methodology: Two groups of patients: 10 patients with chronic periodontitis and 10 with gingivitis. Sampling of peripheral blood and obtaining mononuclear cells. For the description of the populations, the production of intracellular cytokines was evaluated by means of specific monoclonal antibodies for each cytokine, with different fluorochromes: α-IFN-γ PE-Cy7, α-IL-10 BV421, α-IL-17A BV510, α-IL-17F V450, α-IL-21 BV421 by flow cytometry, following the protocol of the Golgi Plug kit.
Results: Between the two groups there was a significant difference in the production of all the pro-inflammatory cytokines, finding a greater number in patients with periodontitis. The IL-10 did not have significant differences between the two groups.
Conclusions: IL-10, which is important due to its immunoregulatory capacity, appears to be reduced in the peripheral blood of the two groups. This would explain the relationship of periodontal disease with systemic inflammatory diseases.

P2584

The histone methyltransferase EZH2 regulates the mTOR signaling pathway of follicular helper T cells during the early stage of acute viral infection

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Epigenetic modification of histones has been demonstrated to be involved in the regulation of T cell differentiation. The enhancer of zeste homolog 2 (EZH2) is a catalytic subunit of polycomb repressive complex 2, which can regulate the differentiation of follicular helper (T_{FH}) cell through Tcf7-Bcl6 or H3K27me3-Cdkn2a-p19Arf axis. However, as an epigenetic regulator, EZH2 can regulate many downstream targets, so there may be other potential mechanisms by which EZH2 regulates T_{FH} cell differentiation. Using a mouse model of lymphocytic choriomeningitis acute viral infection, we found that loss of EZH2 resulted in a significant down-regulation of the mTORC1 signaling pathway in T_{FH} cell, and this regulation mainly occurs in the early stage of T_{FH} cell differentiation. While in the later stage of T_{FH} cell, using conditioned knockout mice to knock out the EZH2 gene of T_{FH} cell has no

DOI: 10.1002/eji.201970400

obvious effect on the mTORC1 signaling. This suggests that EZH2 may regulate the differentiation of T_{FH} cell by regulating the mTOR pathway in the early stage of infection. After all, previous studies have revealed that mTOR signaling pathway is crucial for the development and function of T_{FH} cell. Of course, we are still in the process of further revealing this phenotype, hoping to find more and meaningful mechanisms to regulate the T_{FH} cell differentiation at the epigenetic level.

P2585

Prohibitin 1 interacts with signal transducer and activator of transcription 3 in T-helper 17cells

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T-helper 17 (Th17) cells are involved in the occurrence and development of several inflammatory-associated diseases. Interleukin(IL)-17, the main cytokine secreted by differentiated Th17 cells, mediates immunoreactions and plays important roles in immunological diseases, including psoriasis, rheumatic arthritis, and inflammatory bowel disease. The maturation and stabilization of the differentiated Th17 cell phenotype is associated with the expression of IL-17A, which is induced by activation of signal transducer and activator of transcription 3 (STAT3). Prohibitin1 (PHB1) also plays an essential role in T-cell activation. However, the molecular mechanisms underpinning Th17 cell differentiation and the role of PHB1 are not yet fully understood, and this understanding would greatly facilitate the development of therapeutic strategies to prevent or treat immune-associated diseases. Here, we found that STAT3 expression was correlated with PHB1 mRNA expression during Th17 cell differentiation. Double-labeling immunofluorescence assay results showed that exogenous PHB1 and STAT3 proteins were located primarily in the nucleus but also in the cytoplasm of human (HEK293T) cells. Co-immunoprecipitationassays of Th17 cells showed that PHB1 interacted with STAT3 and with activated STAT3 phosphorylated at Ser727 but not at Tyr705. Knocking down PHB1 with specific short hairpin RNAs attenuated both STAT3 and IL-17 expression levels as well as IL-17 secretion in Th17 cells. These results indicate that PHB1 and STAT3 interact to affect IL-17 secretion in Th17 cells and provide important insights for modulating Th17-mediated pathogenic immune responses.
The work was supported by the Natural Science Foundation of China (No.81471982).

P2586

Increased PD-1 are assiciated with the suppressive immunophenotype of T cell in patients with brucellosis

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Background: human brucellosis is caused by Brucella species,which can multiply within phagocytic cells with human beings. Infection in humans occurs mainly by consumption of contaminated milk and meat products, contact with fuids of infected animals and inhalation of infectious aerosols. After infecting the host, the pathogen becomes sequestered within cells. The mechanisms by which brucella enters cells and evades intracellular killing and the host immune system are still unclear. The purpose of this study was to determine the potential mechanisms of brucella-induced immunophenotyping of circulating T lymphocytes from patients with brucella bloodstream infection.
Methods: blood was obtained from 41 patients with brucella blood culture positive and 30 health controls. The expression of co-stimulatory molecular on T cells and interferon gamma(INF-γ) as well as interleukin-2(IL-2) production were analyzed by analyzed by flow cytometry.

DOI: 10.1002/eji.201970400

Results: compared to health control, PD-1 expressed on CD4 T and CD8 T cells from patients with brucellosis was increased while CD4 T cells had decreased expression of positive co-stimulatory molecule CD28. The INF-γ and IL-2 were decreased as well.
Conclusion: Increased PD-1 is assiciated with the suppressive immunophenotype of T cell in patients with brucellosis. These finding may help explain why brucella could evades intracellular killing and the host immune system. Development of immunoadjuvants that reverse - T cell exhaustion and boost immunity may offer new way to cure the Brucellosis.
The work was supported by National Natural Science Foundation of China (81560322), Postdoctoral Foundation of China (2017M613261)

P2587

Mechanistic study of de novo long non-coding RNA Lnc-Bcl6 in mediating Tfh differentiation

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Tfh-cell-controlled germinal center response is the source of humoral immunity. Currently, extensive efforts have been paid to study protein regulators in controlling Tfh cells differentiation and functions, whether long non-coding RNA (LncRNA) is involved in the regulation of Tfh cells remains unknown. By using Bcl6-RFP reporter gene mice combined with RNA-sequencing technique, we found that Tfh cells have specific lncRNA expression profiles. Remarkably, we identified a de novo lncRNA expressed from the promoter region of *Bcl6* gene, and named it as Lnc-Bcl6. We found that the expression level of Lnc-Bcl6 is negatively correlated with Bcl6. Knocking down of Lnc-Bcl6 promoted Bcl6 expression and Tfh cells differentiation, and overexpression of Lnc-Bcl6 can inhibit Tfh cells differentiation.

P2588

HBV exposure in utero influences the function of specific $CD4^+$ T cells

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Background: Hepatitis B virus (HBV) surface antigen (HBsAg) in cord blood is thought to induce immunological defects, which might be the reason why those individuals have higher risk of developing chronic HBV infection in adulthood. We analyzed function of $CD4^+$ T cells from neonates born to mothers with different HBsAg-carrying status.
Methods: We determined maternal HBsAg and HBV DNA before delivery, then cord blood was obtained from neonates born to HBsAg-positive mothers with serum HBV DNA $>10^5$ IU/mL, HBV DNA $< 10^5$ IU/mL, HBV DNA-negative. Cord blood mononuclear cells were isolated and stimulated with recombinant HBsAg. We detected the cytokines of HBsAg-specific $CD4^+$ T cells by ELISPOT.
Results: HBsAg level was 2.5-fold increased among mothers of HBV DNA $>10^5$ IU/mL compared with the HBV DNA $< 10^5$ IU/mL. The number of HBsAg-specific IL-21-producing follicular helper T cells (T_{FH} cells) were 4.9-fold decreased in the HBV DNA $< 10^5$ IU/mL versus HBV DNA-negative, and those cells were often undetectable in the HBV DNA $>10^5$ IU/mL. The number of IL-4-producing Th2-cells decreased 15.1-fold in the HBV DNA $< 10^5$ IU/mL, and 306.4-fold in the HBV DNA $>10^5$ IU/mL compared with HBV DNA-negative. The number of IFN-γ-producing Th1-cells in HBV DNA $< 10^5$ IU/mL decreased 7.4-fold compared with the HBV DNA-negative, but increased 3.5-fold compared with HBV DNA $>10^5$ IU/mL.

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DOI: 10.1002/eji.201970400

Conclusions: HBV exposure in utero decreased cytokines of HBsAg-specific $CD4^+$ T cells which might lead to much lower B cell responses and HBV vaccination failure.
Keywords: HBV; In utero; Cord blood; Cytokines

P2589

Restoring ovarian function with human Adipose tissue derived stromal cells in autoimmune-induced premature ovarian failure mice mediated by Tim-$3^+$$CD4^+$$CD25^+$Treg cells

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Objective: Human Adipose tissue derived stromal cells (hADSC) transplantation has been demonstrated to be an effective way of recovering ovarian function in mice with autoimmune induced premature ovarian failure (POF), while the exact function of the Tim-$3^+$$CD4^+$$CD25^+$Treg cells in the transplantation still needs to be further investigated.
Methods: The ZP3 peptide was used to establish the POF mice model, and hADSCs were isolated and cultured in our previous study. 30 Balb/c mice aged 6-8 weeks were divided randomly into three groups (n=10 each): control group,POF group and POF+hADSC group. All mice were killed two weeks after hADSC transplantation.Flow cytometry was used to detect the expression of Tim-3 and propotion of Treg Cells in peripheral blood and spleen; ELISA was used for detecting the level of Tim-3, TGF-βand IL-10 in serum; RT-qPCR was used to explore the expression of Tim-3 and Treg cells related factors Foxp3 in peripheral blood.
Results: Flow cytometry results found that the percentages Tim-$3^+$$CD4^+$$CD25^+$Treg Cells was obviously higher in control group and POF+hADSC group compared with POF group ($P< 0.05$); ELISA result showed that concentration of Tim-3,TGF-βand IL-10 were higher in control group and POF+hADSC group compared with POF group ($P< 0.05$); qRT-PCR results show that mRNA levels of Tim-3 and Foxp3 were were higher in control group and POF+hADSC group compared with POF group ($P< 0.05$).
Conclusion: Our data suggest that the recovery of ovarian function in POF mice is mediated via the regulation of Tim-$3^+$$CD4^+$$CD25^+$Treg cells and production of associated cytokines following hPMSCs transplantation.

Innate Lymphoid Cells

O069

Autophagy is critical for type 2 innate lymphoid cell metabolic homeostasis and effector function

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Allergic asthma is a chronic inflammatory disorder that is characterized with airway hyperreactivity (AHR) and driven by Th2 cytokine production. Type 2 innate lymphoid cells (ILC2s) secrete high amount of Th2 cytokines and contribute to the development of AHR. Autophagy is a cellular degradation pathway that recycles cytoplasmic content. However, the role of autophagy in ILC2s remains to be fully elucidated. Here, we demonstrate that autophagy is extensively used by activated

DOI: 10.1002/eji.201970400

ILC2s to maintain their homeostasis and effector functions. Deletion of the critical autophagy gene Atg5 resulted in decreased cytokine secretion, increased apoptosis, and repressed NF-κB signaling. On the other hand, induction of autophagy among *WT* ILC2s was associated with increased cell survival and cytokine secretion. Moreover, lack of autophagy among ILC2s impaired their ability to utilize fatty acid oxidation and strikingly promotes glycolysis as evidenced by our transcriptomic and metabolite analyses. This shift of fuel dependency led to impaired homeostasis and Th2 cytokine production, thus inhibiting the development of ILC2-mediated AHR. Notably, this metabolic reprogramming was also associated with an accumulation of dysfunctional mitochondria producing excessive reactive oxygen species. Interestingly, pyruvate treatment to promote mitochondrial respiration rescued these effector functions and restored glucose metabolism among ATG5 deficient ILC2s. Together our findings provide new insight into the metabolic profile of ILC2s and suggest that modulation of fuel dependency by autophagy is a potentially new therapeutic approach to targeting ILC2 dependent inflammation.

O070

Group 2 Innate lymphoid cells regulates the susceptibility to allergic inflammation via IL-4-dependent innate amplification circuit

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The discovery of group 2 innate lymphoid cells (ILC2) has shed new light on a variety of type 2 immune responses. For example, dust mites and pollens induce epithelial cells to secrete IL-25 and IL-33 via protease activities, leading to ILC2-dependent allergic lung inflammation. However, the cause of drug-induced allergies such as non-steroidal anti-inflammatory drug (NSAIDs)-exacerbated respiratory diseases (NERD) remains poorly understood. We demonstrate that these drugs induce the production of cysteinyl leukotriene (CysLT), which stimulates ILC2 to produce IL-4 via Ca^{2+} signaling. This IL-4 production requires IL-33-induced *Irf4* expression and IL-2-induced CysLT receptor expression in ILC2. Remarkably, injection of mice with IL-2 plus IL-33 induced CysLT-mediated IL-4 production in ILC2 and resulted in antigen-independent 'innate' IgE production by B1 cells. B1 cell-derived innate IgE supports the survival and expansion of $Fc\varepsilon R^+$ cells thereby regulating susceptibility to allergic disorders as demonstrated by the finding that injection of innate IgE into asthma model mice accelerates ILC2-mediated allergic responses. CysLT inhibition blocks innate IgE-dependent allergic responses, suggesting that ILC2, B1 cells and $Fc\varepsilon R^+$ cells generate an amplification circuit that defines allergic susceptibility. Finally, we verified our findings in an additional asthma model using indomethacin. These results further elucidate our understanding of allergic disease susceptibility and drug-induced allergic responses.

DOI: 10.1002/eji.201970400

O071

Neuropeptide receptors expressed on type 2 innate lymphoid cells regulate airway inflammation

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Type 2 innate lymphoid cells (ILC2s) are critical for maintaining tissue homeostasis, yet they are also important drivers of pathologic type 2 immune responses in allergy and asthma. Recent work has highlighted the importance of neuropeptides in regulating ILC2 responses, in addition to other tissue-derived signals, such as alarmins and lipid mediators. We undertook a massively parallel single-cell RNA sequencing (scRNA-seq) analysis of 24,187 lung-resident ILCs isolated from mice challenged with PBS or the alarmin cytokines (IL-25 or IL-33) and found significant heterogeneity within the transcriptional profiles of activated ILCs. ILC2s expressed a number of neuropeptide receptors including Neuromedin U receptor 1 (*Nmur1*) and *Ramp1* and *Calcrl*, which encode the receptor for the neuropeptide CGRP (Calcitonin Gene-Related Peptide). IL-25 and Neuromedin U (NMU), the ligand for NMUR1, activate ILC2s *in vitro* and induce significantly more severe airway inflammation in an ILC-specific manner than either IL-25 or NMU alone. In contrast, the neuropeptide CGRP negatively regulates proliferation and type 2 cytokine production of lung ILC2s in response to alarmins *in vitro* and inhibits both cellular and physiologic readouts of airway inflammation. We define a gene expression signature based on genes regulated by CGRP and show that a distinct subset of ILC2s isolated from alarmin-treated mice scores highly for this signature, indicating that ILC2s respond to endogenous CGRP *in vivo*. Our work therefore identifies the neuropeptides NMU and CGRP as novel regulators of ILC2 responses and airway inflammation.
A.W., P.R.B. and S.J.R. contributed equally to this work.

O072

Allergen-derived protease activates GSDMD and controls IL-33 release in airway inflammation

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Interleukin-33 (IL-33), an epithelial-derived cytokine that respond rapidly to environmental insult, has critical role in initiating allergic inflammation. However factors that control IL-33 secretion following the allergen exposure is not clear. Here we demonstrate that Gasdermin D (GSDMD) is highly expressed in the airway epithelial cells in patients with asthma and it has an important function in regulating IL-33 release. Thus, we hypothesize that GSDMD may play an important role in allergic response of IL-33 secretion to initiate airway inflammation.
Methods and conclusion: Protease allergen can induce IL-33 release in airway epithelial cells. We stimulate human airway epithelial cell A549 in vitro with allergen protease from plant, fungal and

DOI: 10.1002/eji.201970400

bacteria, resulting in functional GSDMD cleavage (N-terminal GSDMD) in a short time. This was confirmed in accordance with elevated IL-33 protein level in the culture supernatant. Inhibition of allergen protease activity with specific protease inhibitor reduces GSDMD cleavage in this process. The purified full- length flag-GSDMD protein could also be cleaved into its functional N-terminal GSDMD form when it is treated with these allergen proteases. This GSDMD cleavage relies on protease activity that is different from classical GSDMD activation by inflammasome. GSDMD deficiency in mice leads to an impairment of IL-33 secretion in the Broncho alveolar lavage, and consequently dampens ILC2 activation, eosinophils infiltration and inhibit airway inflammation after airway exposure with protease allergen. Our finding uncovers a route for IL-33 release from airway epithelial cells under allergen exposure condition, demonstrating a central role of GSDMD in initiating type 2 immunity.

P0122

KIR+NKG2A+Eomes-CD8+ T cells have a similar distribution as Eomes+ subset but with lower differentiation degree

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KIR+NKG2A+Eomes+CD8+ T cells which are preferential with a T_{EMRA} (CD45RA+CCR7-) phenotype, while having the capacity to rapidly produce IFN-γ in response to innate stimulation (IL-12 and IL-18) have been demonstrated existed in human cord blood as well as in adult's circulation. It is termed as NK like CD8+ T cells due to their innate immune response features which like that found in mice, however, KIR+NKG2A+Eomes-CD8+ T cells also represent a small proportion of unconventional T cells which never been defined. Here we compared the memory phenotype subsets distribution and senescence markers in these two T cell populations by flow cytometry in 102 healthy individuals range from 6 to 84 years old. We found that both of Eomes- and Eomes+ KIR+NKG2A+CD8+ T cell populations mainly have a T_{EMRA} phenotype, but Eomes+ population seems to have a higher differentiation degree than Eomes- population which contains fewer T_{EMRA} but more naïve and T_{CM} subsets, besides, Eomes- population showed linearly decreasing with aging. In addition, Eomes+ population possess more CD57+ and CD28- T cells. Overall, KIR+NKG2A+Eomes- CD8+ T cell population have the similar T cell subset distribution as their Eomes+ population, owning less differentiated degree, lower senescence marker expression, and decreasing with aging. Thus, we suspect that KIR+NKG2A+ Eomes-CD8+ T cells may represent the “memory” subset of KIR+NKG2A+ Eomes+ CD8+ T cell population.

P0123

Kinetics of accumulation of group 2 innate lymphoid cells in IL-33 and IL-25 induced murine surrogates of asthma: potential role of the chemokine CXCL16

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DOI: 10.1002/eji.201970400

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ILC2s are implicated in asthma pathogenesis but little is known about the mechanisms underlying their accumulation in airways. We investigated the time courses of ILC2 accumulation in different tissues in murine surrogates of asthma induced by a serial per-nasal challenge with ovalbumin (OVA), IL-25 and IL-33 and explored the potential roles of ILC2-attracting chemokines in this phenomenon. Flow cytometry was used to enumerate ILC2s at various time points. The effects of cytokines and chemokines on ILC2 migration were measured *in vitro* using a chemotaxis assay and *in vivo* using small animal imaging. Compared with saline and OVA challenge, both IL-25 and IL-33 challenge alone induced significant accumulation of ILC2s in mediastinal lymph nodes, lung tissue and bronchoalveolar lavage fluid of challenged animals, but with distinct potency and kinetics. *In vitro,* IL-33 and CXCL16, but not IL-25 or CCL25 directly induced ILC2 migration. Small animal *in vivo* imaging further confirmed that a single intranasal provocation with IL-33 or CXCL16 was sufficient to induce accumulation of ILC2s injected via the tail vein into the lungs. Moreover, IL-33-induced ILC2 migration involved the activation of ERK1/2, p38, Akt, JNK and NF-κB while CXCL16-induced ILC2 migration involved the activation of ERK1/2, p38 and Akt. These data support the hypothesis that epithelium-derived IL-25 and IL-33 induce lung accumulation of ILC2s, while IL-33 exerts a direct chemotactic effect in this process. Although ILC2s express CXCR6 and CCR9, only CXCL16, the ligand of CXCR6, exhibited a direct chemoattractant effect.
Key words: ILC2s, asthma, accumulation, IL-33, IL-25, chemokine

P0124

CD8$^+$ T cells promote maturation of liver-resident NK cells *via* the CD70-CD27 axis

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Liver-resident natural killer (LrNK) cells are a unique subset of NK cells that are distinct from conventional NK cells. However, little is known about the mechanisms by which LrNK cells mature. In this study, we discovered that LrNK cells exhibit a relatively immature phenotype and impaired cytotoxic capacity in the absence of CD8$^+$ T cells. The provision of CD8$^+$ T cells to *Cd8*$^{-/-}$ or *Rag1*$^{-/-}$ mice led to the restoration of LrNK cell maturation. Furthermore, co-culture with CD8$^+$ T cells induced immature CD27$^+$ LrNK cells to convert to mature CD27$^-$ LrNK cells, whereas blocking the interaction of CD70 and CD27 abrogated the ability of CD8$^+$ T cells to promote the maturation of LrNK cells. Thus, our findings indicate that CD8$^+$ T cells promote the maturation of LrNK cells via the CD70-CD27 axis, and therefore highlight a previously unknown mechanism responsible for the mediation of LrNK cell maturation

P0125

RAGE-dependent group 2 innate lymphoid cell expansion causes type 2 immune dysfunction in hemorrhagic shock

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DOI: 10.1002/eji.201970400

Type 2 immune dysfunction contributes to acute lung injury and lethality following hemorrhagic shock (HS) and trauma. Group 2 innate lymphoid cells (ILC2s) play a significant role in the regulation of type 2 immune responses. However, the role of ILC2 in post-HS acute lung injury and the underlying mechanism have not yet been elucidated. We investigated the regulatory role of ILC2s in HS-induced acute lung injury and the underlying mechanism in patients and animal model. Circulating markers of type 2 immune responses in HS patients and healthy controls were characterized. Using a murine model of HS, the role of HMGB1-RAGE signaling in regulation of ILC2 proliferation, survival, and function was determined. And the role of ILC2 in inducing type 2 immune dysfunction was assessed as well. We found that the number of ILC2 was significantly increased in the circulation of HS patients that was correlated with the increase in the markers of type 2 immune responses in the patients. Animal studies showed that HMGB1 acted via RAGE to induce ILC2 accumulation in the lungs by promoting ILC2 proliferation and decreasing ILC2 death. The expansion of ILC2s resulted in type 2 cytokines secretion and eosinophil infiltration in the lungs, both of which contributed to lung injury after HS. These results indicate that HMGB1-RAGE signaling plays a critical role in regulating ILC2 biological function that aggravates type 2 lung inflammation following HS.

P0126

Metformin inhibits the initiation of allergic asthma through LKB1-AMPK-mTOR pathway in lung ILC2s

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Metformin is a first-line drug for the treatment of type 2 diabetes, which work through activating the LKB1/AMPK pathway. The latest research found that it can alleviate the inflammation of allergic asthma, but the mechanism is not clear. ILC2s have similar functions to Th2 and could rapidly secrete type 2 cytokines, which are also critical for promoting the occurrence of allergic asthma. We found that metformin could significantly reduce the number of ILC2s in lung and eosinophils in BALF and lung, thus inhibits the inflammation of allergic asthma. Then, we found the mTOR signaling pathway inhibitor rapamycin had similar functions to metformin. As LKB1 is an important signaling molecule upstream of AMPK, we used a RORa^{cre}LKB1$^{fl/fl}$ mouse model, in which LKB1 can be selected depleted in ILC2s, to explore the role of LKB1 in ILC2s in the initiation of allergic asthma. We found that after papain treatment, compared to WT mice, the number of ILC2s in lung and eosinophils in BALF and lung from RORa^{cre}LKB1$^{fl/fl}$ mice was significantly increased, and the inflammation of allergic asthma was also remarkably exacerbated in RORa^{cre}LKB1$^{fl/fl}$ mice. However, there was no significant change in the number of ILC2s in lungs and the inflammation of allergic asthma in RORa^{cre}LKB1$^{fl/fl}$ mice after treated with papain plus metformin. Thus, these results suggested that metformin might inhibit the initiation of allergic asthma through LKB1-AMPK-mTOR pathway in lung ILC2s.

This work was supported by grants from 2015CB943203, NSFC31300730, ZR2018LC009, 2017WS426, and 201810439028.

DOI: 10.1002/eji.201970400

P0127

Long-chain saturated fatty acids in breast milk are associated with the pathogenesis of atopic dermatitis via induction of inflammatory ILC3s

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Breastfeeding influences the immune system development in infants and may even affect various immunological responses later in life. Breastmilk provides a rich source of early nutrition for infant growth and development. However, the presence of certain compounds in breastmilk, related to an unhealthy lifestyle or diet of lactating mothers, may negatively impact infants. Based on a cohort study, we found that the composition of mother's milk containing high amounts of long-chain saturated fatty acids (LCSFAs) was related to a higher incidence of atopic dermatitis (AD) in children. Similarly, a mouse model in which breastfed offspring were fed milk high in LCSFAs also showed AD onset later in life. We showed that LCSFAs are a type of damage-associated molecular patterns, which initiate a series of inflammatory events in the gut involving type 3 innate lymphoid cells. A remarkable increase in inflammatory ILC3s was observed in the gut, and the migration of these ILC3s to the skin contributed to the pathogenesis of AD. Gene expression analysis of ILC3s isolated from the gut revealed upregulation of genes that increase ILC3s and chemokines, which may play a role in ILC migration to the skin. Even in the absence of adaptive immunity, Rag1 knockout mice fed a high-LCSFA milk diet developed eczema. Here, we propose that early exposure to LCSFAs in infants may affect the balance of intestinal innate immunity, inducing a highly inflammatory environment with the proliferation of ILC3s and production of interleukin-17 and interleukin-22, which are attributed to the pathogenesis of AD.

P0707

ILC3 regulates type 2 immunity through controlling the retention of RORγt$^+$ pTreg cells via intergrin αvβ8

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Emerging data suggested that the transcription factor RAR-related orphan-like γt（RORγt）expressing immune cells play an essential role in maintenance of intestinal homeostasis and tolerance. Transforming growth factor-β (TGF-β) is essential for negatively regulating adaptive immunity, and is secreted as an inactive precursor that needs to be activated by integrins such as αvβ8 to exert biological effects. As αvβ8 is expressed by RORγt+ cells in the gut, we want to know whether RORγt+ cells-mediated TGF-β activation via αvβ8 plays a key role in maintenance of intestinal homeostasis. Here, we show conditional depletion of αvβ8 on RORγt+ cells causes severe inflammatory bowel disease, type 2 allergic inflammation and plasmacytic enteritis. Then, we showed this phenotype was largely due to lack of αvβ8 on group 3 innate lymphoid cells (ILC3s), but not on other RORγt+ cells such as TH17, Treg or γδT cells. Furthermore, we found the mice that ILC3s lack αvβ8 have reduced proportions of RORγt+ pTreg cells, which control type 2 immunity, in the gut. Moreover, we observed the tissue residency of RORγt+ pTreg cells might be impaired in mice that ILC3s lack αvβ8 due to loss expression of CD103 and CD69. Finally, we demonstrated ILC3s could

DOI: 10.1002/eji.201970400

maintain the expression of CD103 and CD69 on RORγt+ pTreg by αvβ8-mediated TGF-β activation in vitro. Altogether, these results suggested αvβ8-mediated TGF-β activation by ILC3s is essential for RORγt+ pTreg cells retention in the gut. This study highlights the center role of ILC3s in maintenance of intestinal homeostasis.
Supported by 2015CB943203, 31600738 and ZR2016CQ12.

P0708

Effects of myeloid and plasmacytoid dendritic cells on ILC2s in patients with allergic rhinitis

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Background: Group 2 innate lymphoid cells (ILC2s) were recently reported to serve a critical role in allergic diseases. Myeloid dendritic cells (mDCs) and plasmacytoid DCs (pDCs) play significant roles in allergic immune response. However, effects of DCs on ILC2s in allergic diseases, especially for patients with allergic rhinitis (AR), remain unclear.
Objective: We aimed to address the roles of mDCs and pDCs in regulating ILC2 function in AR.
Methods: The presence of ILC2s, mDCs, and pDCs was determined in the nasal mucosa of patients with AR. mDCs and pDCs were co-cultured with human PBMCs or ILC2s isolated from patients with AR to measure soluble Th2 cytokines, intracellular cytokines, transcription factors, signaling pathways and the following mechanisms were further investigated. The levels of peripheral $ST2^+$mDCs and $ST2^+$pDCs were studied in patients with AR under an inhaled allergen challenge.
Results: mDCs were found to activate ILC2s isolated from patients with AR to produce Th2 cytokines and increase the levels of GATA-3 and STAT signaling pathways, in which IL-33-producing mDCs exerted the major role. pDCs, in contrast, inhibited the cytokine production of ILC2s isolated from patients with AR by secretion of IL-6. We further identified high levels of ST2 (IL-33 receptor)$^+$mDCs and $ST2^+$pDCs in patients with AR during the antigen inhalation.
Conclusions: While mDCs promote ILC2 function by the IL-33/ST2 pathway and activation of pDCs suppresses ILC2 function through IL-6 in patients with AR. Our findings provide new understanding of the interplay between DCs and ILC2s in the pathology of allergic diseases.

P0709

Characerization of IL18R1-expressing ILC precursors

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Innate lymphoid cells (ILCs) are important players in host defense, tiusse homeostasis and immune regulation, particulary at mucosal barriers. Despite the complexity of ILC subsets and their tissue specific phenotypes and functions, all ILCs are derived from a common lymphoid progenitors in the bone marrow in adult. The development of ILCs rely on the extracellular signals from the microenvironment and the subsequnt induction of lineage specific transcriptional regulatory network that directs the differentiation of ILC precursors. Several growth factors, cytokines and ligands have been demonstrated to play essential role during ILC differentiation, including IL-7 and Notch, however it is still uncelar whether there are other factors that contribute to this process. We found a subset of ILC precursors in the BM that express IL18R1 which belongs to the family of IL-1 recpetors. IL-18 is a pleiotropic cytokine that can modulate innate and adaptive immune responses. Here we characterized these IL18R1-expressing BM ILC precursors and investigate the role of IL-18 in the development of ILCs.

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DOI: 10.1002/eji.201970400

P0710

Role of costimulatory molecule CD137 in the antiviral activity of human γδ T cells against influenza virus

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Statement of the Problem: Influenza virus continues to threaten global human health with significant morbidity and mortality. Human γδ T cells, as the innate-like T lymphocytes, play an indispensable role in the host immune defense systems against virus infection. CD137 is a costimulatory molecule expressed on T cells. Although the role and function of CD137 in αβ T cells have been well studied, its role in γδ T cells has not been explored clearly. The purpose of this study is to determine the role of CD137 in the antiviral activity of human γδ T cells.
Method: CD137 agonist and blocking antibody were utilized during human γδ T cells expansion and activation to study the function of CD137 signal in vitro. Rag2-/-γc-/- mice infected by influenza were used to determine the role of CD137 signal in the antiviral activity of human γδ T cells in vivo.
Findings: Blocking CD137 signal could decrease the proliferation and activation of γδ T cells. Adoptive transfer of pamidronate-expanded CD137 positive γδ T cells showed more remarkable effect against influenza infection than CD137 negative in Rag2-/-γc-/- mice.
Conclusion & Significance: CD137 signal can stimulate the activation and expansion of human γδ T cells, and enhance their antiviral activity against influenza virus. This study provides a novel strategy for treatment of influenza virus infection by targeting CD137 to improve the antiviral activity of human γδ T cells, and further increase the efficacy of γδ T cell-based immunotherapy by using the combination of phosphoantigen with anti-CD137 agonist.

P0711

Stomach ILC2s are regulated by commensal bacteria and can be activated in response to the Helicobacter pylori

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Innate lymphoid cells (ILCs) are implicated as key initiators of immune responses at many organs including mucosal sites through their production of cytokines. Recent studies revealed that cytokines derived from ILC subsets can be triggered for the induction of diseases or disrupting homeostasis. The gut microbiota has a well-documented impact on immune development and regulation of immune responses, whereas the importance of microbiota for the ILCs-regulation in the mucosal tissues has not been well understood. Especially, very little is known about host microbial interactions, including immunological regulation and responses, in the stomach. We therefore compared two mucosal organs, small intestine (SI) and stomach, by focusing on ILC subsets from SPF and germ free (GF) mice. Different from the SI having distinct populations of ILC1s, ILC2s and ILC3s, the stomach had a large proportion of ILC2s with few ILC1s and virtually no ILC3s in SPF mice. These ILC2s are markedly decreased in GF compared to SPF mice. We found that microbes can elicit IL-7 and IL-33 production in the stomach, which in turn triggers ILC2s. We further detected that stomach ILC2s are also rapidly induced following infection with *Helicobacter pylori* (*H. pylori*), which in turn induced IgA

DOI: 10.1002/eji.201970400

production through IL-5 secretion. ILC2s in the stomach were still left unexamined especially in terms of relationship with microbes and innate immune responses. Our study thus identified a novel ILC2-dependent IgA response that is regulated by commensal and pathogenic bacteria, and implicated in barrier function in the stomach.

P1310

Identification of new antigen recognized by γδT cells in Hepatocellular carcinoma

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The immune protection initiated by γδT cells plays an important role in defensing Hepatocellular carcinoma. Some evidences suggested protein antigens activated γδT cells could elicit effectively protective immune responses. To date, only a few distinct Hepatocellular carcinomas related protein antigens have been identified. In the present study, we screened protein antigens recognized by γδT cells using cells transfected with the predominant Hepatocellular carcinoma γδ TCR CDR3 sequence. We identified new protein antigen which not only bind to the Hepatocellular carcinoma predominant γδ TCR but also effectively activate γδT cells isolated from Hepatocellular carcinoma patients. Moreover, they could stimulate γδT cells from Hepatocellular carcinoma patients to secrete IFN-γ and TNF-β, by which play important roles in mediating cytotoxicity to Hepatocellular carcinoma. In conclusion, our study identified new protein antigens recognized by γδ TCR, which could be potential candidates for vaccines or adjuvant development as well as biomarkers of Hepatocellular carcinoma.

P1311

Interferon regulatory factor 7 is a critical regulator of type 2 innate lymphoid cells in allergic airway inflammation

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Allergic asthma is a highly prevalent airway disease triggered by hyperresponsiveness to inhaled allergens. Similar to T_H2 cells, type 2 innate lymphoid cells (ILC2s) have also been demonstrated to play a critical role in promoting airway inflammation by secreting effector cytokines. However, the mechanisms underlying the functions of lung ILC2s remain unclear. Interferon regulatory factor 7 (IRF7) has been shown to be highly expressed in the nasal aspirates in asthma children. The role of IRF7 in ILC2-mediated innate immunity has never been investigated. Here, we demonstrated that IRF7 was highly expressed in murine lung ILC2s and was induced by papain and IL-33 stimulation. ILC2s obtained from patients with asthma also showed higher expression of IRF7 than those from healthy donors. IRF7 deficiency impaired the expansion and function of lung ILC2s in mouse models of allergic asthma. Regulation of lung ILC2 by IRF7 was cell-intrinsic and mediated by the transcription factor Bcl11b. Our findings identified IRF7 as a novel regulator of lung ILC2s, and might provide useful insight into allergic asthma. IRF7 could therefore be a promising immunotherapeutic target.

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DOI: 10.1002/eji.201970400

P1312

A TRIM family member protein controls the development and function of group 3 innate lymphoid cells

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Group 3 innate lymphoid cells (ILC3s) majorly reside at intestinal mucosal surface and contribute to the local defense and homeostasis. As a subset of ILC3s, the function of lymphoid tissue-inducer (LTi) cells during peripheral lymphoid tissue formation was early identified. Here we have investigated the role of TrimX, a member of the tripartite motif (TRIM)-containing protein family, in LTi cell development and function. We found that mice with TrimX deficiency specifically in hematopoietic system manifested less and smaller Peyer's patches. $CD4^+$ LTi cells are dramatically decreased in the intestine, and adoptive bone marrow transfer experiment demonstrates a cell-intrinsic role of TrimX in $CD4^+$ LTi cell development, but not that of other ILC subsets. We further found that total LTi cells are decreased in the fetal intestine in TrimX deficient mice, and TrimX deficient fetal liver $\alpha4\beta7^+$ lymphoid progenitors (αLPs) show impaired capacity to differentiate into LTi cells *in vitro*. Whole-transcriptome analysis indicates that, Id2 was dramatically down-regulated in TrimX deficient αLPs in the fetal liver, but not in TrimX deficient common helper ILC progenitors (ChILPs) in the adult bone marrow. Id2 is essential for all the ILC development, consistent with the variable regulation of Id2 expression, almost all the ILC subsets are decreased in the fetal TrimX deficient mice, but ILCs, expect for CD4+ LTi cells, show no significant developmental impairment in the adult TrimX deficient mice. TrimX may regulate Id2 expression during fetal LTi cell development, and the detailed molecular mechanism of these phenotypes is currently under investigation.

P1314

Regulation of ILCs by tissue microenvironment during inflammation

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Innate lymphoid cells (ILCs) lack T and B cell antigen specific receptors and are abundantly present in the mucosal tissues. ILCs are essentially involved in the initiation, progression and resolution of inflammation. We found factors from the microenvironment determined the fate and features of ILCs during inflammation. In the lung, we characterized a previously unappreciated ST2+ILC2s that produced IL-17, which exacerbated IL-33-mediated lung inflammation. Interesting, aryl hydrocarbon receptor (AHR) but not RORgt facilitated IL-17 production by ST2+ILC2s. Mechanistically, we found IL-33 and leukotriene-NFAT signaling synergistically promoted IL-17 expression by ST2+ILC2s in a cell-intrinsic manner. In the intestine, we found ILC3s served as a main source for $OX40L^{high}$ cells in the intestine. Pro-inflammatory factors, including TL1A derived from myeloid cells and Poly(I:C) representing double-stranded viral signal, enhanced OX40L expression by ILC3s. Under the steady state, ILC3-derived OX40L was important for homeostasis of intestinal regulatory T cells (Tregs) by maintaining cell survival. However, when DR3 (receptor for TL1A) signaling was activated by agonistic antibody (a-DR3), we observed a reduction of ILC3s accompanied by accumulation of pro-inflammatory myeloid cells. As a result, a-DR3 exacerbated DSS-induced colitis independently of the adaptive immune system, whereas blockade of DR3 signaling by DR3-Fc (soluble DR3) ameliorated the disease. Our findings suggest functional features of ILCs could be reshaped by environmental factors, which determines differential roles of ILCs in mucosal inflammatory diseases.

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DOI: 10.1002/eji.201970400

P1315

GITR co-signaling controls group 2 innate lymphoid cells in allergic lung inflammation

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Group2 innate lymphoid cells (ILC2) directly home to mucosal sites where they are rapidly activated by alamins released from epithelial cells. ILC2 are known to produce large amounts of type 2 effector cytokines, IL-5 and IL-13 that cause allergic inflammation in the lung. Glucocorticoid-induced TNFR-related protein (GITR) is a member of the tumor necrosis factor receptor superfamily and provides co-stimulatory signals during T cell activation. We found that lung ILC2 highly express GITR and that *Gitr*$^{-/-}$ mice displayed reduced ILC2 activation upon administration of papain or IL-33, which accompanied by impaired production of IL-5 and IL-13 and diminished lung eosinophilia. *Rag2*$^{-/-}$*Gitr*$^{-/-}$ mice, which lack T cells but have ILC2, also displayed reduced ILC2 responses compared to *Rag2*$^{-/-}$ mice after IL-33 administration. *In vitro* stimulation of GITR with IL-33 to ILC2 synergistically activated NF-κB, p38, and Erk pathways via STAT5 activation. The GITR co-stimulation with IL-33 induced more IL-9 production than IL-33 stimulation alone. Accordingly, *in vivo* co-administration of IL-9 with IL-33 restored the defective ILC2 responses in *Gitr*$^{-/-}$ mice. We confirmed that GITR co-signaling also enhanced proliferation and cytokine production in human ILC2. Furthermore, we found that IL-33 administration induced GITR-L expression in ILC2. So, cell-cell interaction between ILC2 may contribute to ILC2 activation during lung inflammation.
Our findings highlight a novel role for GITR signaling in the regulation of ILC2 activation. Thus, controlling ILC2 activation by targeting GITR would be appealing as a new therapeutic strategy for ILC2-dependent airway inflammation.

P1902

Tissue residency of innate lymphoid cells and plasmacytoid DCs altered in microenvironment of colon cancer

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DOI: 10.1002/eji.201970400

Innate lymphoid cells (ILCs), composed of type 1, 2 and 3 ILCs (ILC1s, 2s and 3s), are important tissue-resident innate immune cells that play important roles in inflammation response, tissue repair, and maintenance of epithelial integrity. At present, the biological mechanism of tissue-resident ILC3s involved in the development of colon cancer is still unclear. Hence, we performed immune cells (ILCs in particular) profiling in the tumor microenvironment in a cohort of 58 patients diagnosed colon cancer, finding that the percentage of ILC3s in the tumor was lower than that in distal regions (0.1%, vs 0.3%, p=0.0001), and that the number of ILC3s, especially NKP44^{+}ILC3s negatively correlated with cancer pathological grade (R^2= 0.2039, p=0.0199). On the other hand, the percentage of plasmacytoid dendritic cells (pDCs) increased in the tumor (0.4%, vs 0.1%, p=0.0001), and the number of pDCs positively correlated with pathological grade (R^2=0.3601, p=0.0001), with elevated ILC3s levels correlating with lower pDC levels (R^2=0.2906, p=0.027). Moreover, RNA-seq results supported the correlation between ILC3s and pDCs in colon cancer tissues and showed thousands of differentially expressed genes critical for tumor development and inhibition.These findings revealed the important roles of ILC3s and pDCs in the processes of tumor progression and inhibition in colon cancer, which might help to develop new strategies for colon cancer.

P1903

Demonstration of innate lymphoid cell in peripheral blood in patients with systemic lupus erythematosus

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To explore the function and role of innate lymphoid cells in the pathogenesis of systemic lupus erythematosus at different disease activity levels.
Methods: 40 patients with SLE were included, according to the SLEDAI, patients were divided into active group(n=20) and remission group(n=20), 15 age-matched healthy non-immune-related diseases controls. The frequency of ILCs, B cells, CD4+T and CD8+T cells from PBMCs was detected by flow cytometry. Analysis the subsets of ILCs in each group which compared with B cells and T cell subsets respectively and correlation with clinical serologic markers. Analyze the level of IL-4, IL-33 and IFN-γ in each group by ELISA. Locate the ILC2 under the Confocal Laser Scanning Microscopy.
Results: Compared with the control group, ILC1 percentage (P=0.0181) was increased but ILC3 was decreased(P=0.0013) in SLE active group; ILC2 was decreased in both remission(P< 0.0001) and active groups (P< 0.0001);The frequency of ILCs in all patients positively correlated with SLEDAI score(P=0.0172). The frequency of ILC2 in active group was negatively correlated with CD4+T cells(P=0.0308), and the serum IgG in patients was negatively correlated with ILC2 of all patients (P=0.0138). Compared with either control group or the remission group, the levels of IFN-γ (P=0.0001) and IL-4 (P=0.0047) in active group were Significantly higher. IL-33 was reduced in active group(P=0.0027). Confocal Microscopy showed the ILC2 in skin of patient with SLE.
Conclusion: The frequency of ILCs is related to disease activity, and ILCs play a "double-edged" role in the pathogenesis of SLE. Its function and mechanism are worth further exploration.

DOI: 10.1002/eji.201970400

P1904

Type-2 innate lymphoid cells are dominant ILCs in the rat intestine

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Innate lymphoid cells (ILCs) represent the innate counterpart of adaptive T lymphocytes engaged in host defense, tissue repair, homeostasis, and inflammatory diseases. ILCs are divided into three groups based on their requirement for particular transcription factors and patterns of secreted cytokines. While ILC subsets have been the subject of extensive characterization in human and mouse tissues, no study has addressed ILC distribution and function in the rat. Recent evidence suggested that significant differences existed between rat and mouse microbiota composition that was associated with distinct cardinal features of their respective immune system, but whether this could include ILC was not assessed. Here, we characterized ILCs in the intestine and secondary lymphoid organs from rats through analysis of transcription factors expression and cytokine production. Our results indicate that ILCs in rats exhibit high similarity with mice and human ILCs in terms of phenotype and cytokine production. We observed however that the frequencies of ILCs varied between rat strains. As reported in mice, ILC3 were predominant in rat mesenteric lymph nodes. Surprisingly, ILC2 were however largely dominant in both small and large intestine, contrasting with previous data showing a specific enrichment for ILC3 in the same tissues in mice and human. This observation was made regardless the rat strain, sex, animal housing facilities or genetic background. In conclusion, our study indicates that the identity and functions ILC subsets are conserved in rats as compared to mice and human but suggests that tissue compartmentalization of ILC2 at mucosal sites can vary between species.

P1905

Synergistic effect of zoledronic acid on hMSH2- mediated cytotoxicity of Vγ9δ2 T cells against human lung squamous cell carcinoma

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The distinct mechanisms underlying the hMSH2-mediated recognition/cytotoxicity of Vγ9δ2 T cells against lung cancer cells remain unclear. Here, we confirmed the unusual ectopic surface expression of hMSH2 on NCI-H520 lung squamous cell carcinoma (SCC) cells and elucidated the ectopic hMSH2-mediated recognition and cytolysis of SCC targets by Vγ9δ2 T cells via siRNA-*hMSH2*-gene-knockdown and/or Fugw-hMSH2-overexpression NCI-H520 cell models. The hMSH2-induced cytotoxicity of Vγ9δ2 T cells was characterized by the elevated CD69 expression, altered IFN-γ/TNF-a expression and a dose-dependent synergy of zoledronic acid (ZOL) with a feature of supernatant proinflammatory cytokines storms. Interestingly, Fas/FasL, Perforin and Granzyme A/B, the classic cytotoxic cytokines secreted by antigen-stimulated Vγ9δ2 T cells in conventional anti-tumor responses, were not markedly influenced by hMSH2 overexpression. Our findings provide robust evidence suggesting the participation of hMSH2 in Vγ9δ2 T cell-mediated SCC immune surveillance. The cooperative stimulatory effects of ZOL and hMSH2 on Vγ9δ2 T cell activation provides potential strategies for developing hMSH2-based lung cancer immunotherapies.

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DOI: 10.1002/eji.201970400

P1906

Reduced hematopoesis and stem cells differentiation in term low birth weight newborns with compromised innate immunity

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The high rate of morbidity and mortality of low birth weight (LBW) newborns (birth weight < 2.5 kg) is of serious concern. Due to compromised immune system LBW newborns are highly susceptible to life threatening microbial infections and sepsis. Around 3.9 million newborns die globally each year with low birth weight as the leading cause. Our studies have established under expressed immune pathway genes and inefficient neutrophil extracellular trap formation in LBW newborns. The compromised immune system in the LBW newborns can be strongly attributed to the observed reduced number of circulating granulocytes/monocytes. This prompted us to evaluate hematopoiesis and differentiation of hematopoietic stem cell (HSCs) in these newborns in comparison to normal birth weight (NBW) newborns.
Briefly, uncommitted HSCs (HSCs) ($CD34^+CD45^-$) from cord blood of NBW and LBW newborns were cultured in presence of SCF, GM-CSF, IL-3, IL-7, IL-2 to induce differentiation and colony formation. Flow cytometry analysis was done to assess the differentiation of HSCs into myeloid lineages CMP (CD135+), neutrophils (CD16b+), monocytes (CD14+) and lymphoid lineage cells CLP (CD10+), early T cells (CD3+), B cells (CD19+) in NBW and LBW newborns. We report significantly reduced hematopoiesis and differentiation of the cord blood HSCs in LBW newborns as compared to NBW. Lineage specific genes (C/EBPα, SPI1, GFI-1 and EGR-2) were downregulated in LBW as compared to NBW. Our findings suggest that both hematopoiesis and differentiation is deficient in the LBW newborns which could be potentially responsible for the observed reduction in circulating immune cells.

P1908

A disintegrin and metalloproteinase 17 is selectively required for ILC2 responses to IL-33

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Group 2 innate lymphoid cells (ILC2s) have been shown to play an important role in the initiation of type-2 immune responses. Numerous targets have been shown to activate or repress ILC2 function, though very few activation-induced negative regulatory pathways have been discovered to be operative in ILC2s. We show that loss of ADAM17 in ILC2s results in a selective defect in responsiveness to IL-33 and not IL-25. IL1R2 is found to be significantly upregulated at both the transcript and protein level in IL-33 activated ILC2s. This work is the first to show ADAM17 regulation of IL1R2 levels on ILC2s in both a constitutive and activation induced manner. We also demonstrate that $IL1R2^+$ ILC2s have decreased *Il5* and *Il13* transcripts, following IL-33 stimulation, suggesting that the expression of IL1R2 may act as an activation-induced negative regulatory mechanism to decrease ILC2 responsiveness to IL-33.

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DOI: 10.1002/eji.201970400

P2590

Modified Glasgow prognostic score and neutrophil/lymphocyte, platelet/lymphocyt ratios in different stages of silicosis

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Objective: To evaluate modified glasgow prognostic score (mGPS), neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) for predicting the prognosis of patients with silicosis.
Methods: 148 cases silicosis patients were collected in Hunan Prevention and Treatment Center for Occupational Diseases (HPTCOD) from January 2018 to December 2018. The value of PLR, CAR, NLR and mGPS for prediting the prognosis of silicosis were evaluated by ROC and the relationship between the PLR, NLR, mGPS and pulmonary function of silicosis patients were analyzed.
Results: Silicosis patients exhibited higher serum leukocyte (WBC), neutrophils(N), platelets (PLT), erythrocyte sedimentation rate (ESR), PLR, CAR, NLR, mGPS and lower lymphocytes(L) concentrations compared with the control groups (P< 0.05). However, The CAR levels was no significant difference between silicosis groups and control groups (P >0.05). the PLR, CAR, NLR, mGPS were positively correlated with CRP, ESR and were negatively correlated with FVC, In addition,the NLR of silicosis groups were negatively correlated with FEV1/FVC .The areas under the ROC curves of NLR and PLR were 0.866 (95% CI. 0.811 -0.9223, P = 0.000) and 0.731 (95% CI. 0.663- 0.799, P = 0.000).
Conclusion: PLR, NLR and mGPS can be used as indicators of inflammatory state and severity in clinical prognosis of patients with silicosis. NLR is more sensitive to assessing disease activity compared with PLR.
Keywords: Silicosis; PLR; CAR; NLR; mGPS

P2591

ILC2s regulated Tfh cell functions via PD-L1 check point control in Echinococcus granulosus

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Type 2 immune responses are critical for protective immunity against infections of extracellular parasites. Group 2 innate lymphoid cells (ILC2s) are important effector cells driving the initiation of type 2 immune responses. Here we show that ILC2s dynamically express the checkpoint inhibitor molecule PD-L1 during infection with *Echinococcus granulosus*. PD-L1 was highly up-regulated on all ILC2s during the course of infection. A time course analysis of liver-resident ILC2s revealed the highest expression of PD-L1 8d after infection, coincident with the peak of ILC2 activity and PD-1 expression on Tfh cell in this model. ILC2s stimulated increased production of IL-4 by Tfh cells both in vitro and in vivo. Conditional deletion of PD-L1 on ILC2s impaired early Th2 polarization and cytokine production, leading to delayed worm expulsion during infection with the helminth *Echinococcus granulosus*. Our results identify a novel PD-L1-controlled mechanism for Tfh cell functions, with ILC2s mediating an innate checkpoint to control adaptive T helper responses, which has important implications for the treatment of *Echinococcus granulosus* infection. This report was supported by the NSFC (81660343, 81460307).

DOI: 10.1002/eji.201970400

P2592

Potentiating tissue-resident type 2 innate lymphoid cells by IL-33 to prevent renal ischemia-reperfusion injury

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The IL-33-type 2 innate lymphoid cell (ILC2) axis has an important role in tissue homeostasis, inflammation, and wound healing. However, the relative importance of this innate immune pathway for immunotherapy against inflammation and tissue damage remains unclear. Here, we show that treatment with recombinantmouse IL-33 prevented renal structural and functional injury and reduced mortality in mice subjected to ischemia-reperfusion injury (IRI).Comparedwith control-treated IRImice, IL-33-treated IRImice had increasedlevelsof IL-4 and IL-13 in serum and kidney andmore ILC2, regulatory T cells (Tregs), and anti-inflammatory (M2) macrophages. Depletion of ILC2, but not Tregs, substantially abolished the protective effect of IL-33 on renal IRI. Adoptive transfer of ex vivo-expanded ILC2 prevented renal injury in mice subjected to IRI. This protective effect associated with induction ofM2macrophages in kidney and required ILC2 production of amphiregulin. Treatment of mice with IL- 33 or ILC2 after IRI was also renoprotective. Furthermore, in a humanized mouse model of renal IRI, treatment with human IL-33 or transfer of ex vivo-expanded human ILC2 ameliorated renal IRI. This study has uncovered a major protective role of the IL-33-ILC2 axis in renal IRI that could be potentiated as a therapeutic strategy.

P2593

ILC3 cells promote the proliferation and invasion of pancreatic cancer cells through IL-22/AKT signaling

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Type 3 innate lymphocytes (ILC3s) are reported to be involved in lung cancer, possibly by producing interleukin-22 (IL-22). However, whether ILC3s and their secreted IL-22 molecules contribute to the pathogenesis of pancreatic cancer (PC) remains unclear. To this end, in this study, we investigated the effects and possible mechanisms of ILC3s on PC pathogenesis. The IL-22 and IL-22R levels and the ILC3s frequency in cancer tissues from PC patients and in peripheral blood from PC patients and healthy controls were analyzed by flow cytometry, immunochemistry, or immunofluorescence. The effects of IL-22-induced AKT signaling on the proliferation, invasion and migration of PC cells were examined by co-culturing PC cell lines with ILC3s isolated from PC tissues, with or without the addition of neutralizing IL-22 antibody, IL-22R antibody or AKT inhibitor. Results showed that IL-22 and ILC3s were significantly upregulated in the PBMCs and cancer tissues of PC patients, and the IL-22R level was increased in PC cells. The increased frequency of ILC3s was positively correlated with the clinical features of PC patients. Co-culture experiments indicated that ILC3s promoted the proliferation, invasion and migration of PC cell lines by secreting IL-22 to activate AKT signaling because IL-22/IL-22R or AKT blockage markedly counteracted such effects on PC cells. Our data demonstrated that ILC3s may promote PC pathogenesis through IL-22/IL-22R-AKT signaling, suggesting a potential intervention target for PC treatment in the future.

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DOI: 10.1002/eji.201970400

P2594

Hepatocellular carcinoma-infiltrating eosinophils suppress CD8 T cell proliferation

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Eosinophils are granulocytes which developed from bone marrow hematopoietic stem cells. The eosinophils have been traditionally considered as terminal differentiated effector cells that play an important role in the host protection against parasites by releasing intracellular granules and play a pathogenic role in some allergic diseases such as asthma. It is worth noting that a variety of solid tumors have been observed having eosinophils infiltration clinically, and eosinophils infiltration can be an important prognostic indicator in some tumor treatment. However, the immune-regulatory functions of eosinophils in the hepatocellular carcinoma remain unclear. In order to study the effect of different types of pathological conditions on the immune microenvironment of the liver, we established the immunosuppressive liver pathological model of orthotopic hepatocellular carcinoma. And we analyzed the regulatory function of tumor-infiltrating eosinophils in the induction of immune escape and tumour metastasis. Further investigation showed that eosinophils infiltrated in hepatocellular carcinoma have a strong apoptosis-inducing effect on CD8 T cells. Preliminary mechanism studies have shown that this apoptosis-inducing function is mediated by tumor-infiltrating eosinophils through Fas and FasL pathway. Our study revealed that the tumor-infiltrating eosinophils play an important role in the tumor-induced immunosuppressive microenvironment via induction of CD8 T cell apoptosis. The data deepened our understanding of liver immunosuppressive microenvironment under pathological status, and laid a new theoretical foundation for the clinical prevention of hepatocellular carcinoma.

P2595

ILC2s induces adaptive Th2-type immunity in acute exacerbation of chronic obstructive pulmonary disease

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To investigate the effect of ILC2 cells on T Helper 2 (Th2) adaptive immunity during the acute exacerbation of chronic obstructive pulmonary disease (AECOPD). The study enrolled healthy people, stable COPD, and AECOPD patients. Th1, Th2 and ILC2 in the peripheral blood and CD80, CD86, OX40L and MHC II levels on ILC2 were deteced by Flow cytometry. QRT-PCR was used to detected GATA3, RORαand CRTH2 mRNA levels of ILC2s. Moreover, we used the *in vitro* co-culture system $CD4^+$ T cells and ILC2s from AECOPD patients. Cytokine levels and the *in vitro* coculture supernatants were measured by ELISA. The Th2 in AECOPD group increased significantly, the Th2/Th1 ratio in the AECOPD was increased. the level of IL-4 in AECOPD increased too. We also found that in AECOPD the levels of GATA3, RORα and CRTH2 mRNA also increased. CD80 and MHC II on ILC2 were significantly up-regulated and the proportion of ILC2 MHC II+ cells was significantly positively correlated with the proportion of Th2 cells in AECOPD patients. Highly expressed CD86 and OX40 were not detected in each group. We co-cultured ILC2 with $CD4^+$ T *in vitro*, which also showed a significant increase of Th2 ratio as well as Th2-associated cytokines IL-4, IL-5 and IL-13. However, we found this effect of ILC2s on Th2 cells could be inhibited by addition of anti-MHC II. During AECOPD,

DOI: 10.1002/eji.201970400

the Th2/Th1 balance is converted to Th2. ILC2 cells can up-regulate MHCII to act as APC cells and regulate adaptive immunity in AECOPD to a Th2 response.

P2596

ICAM-1 controls development and function of ILC2

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Group 2 innate lymphoid cells (ILC2s) are emerging as key players in the pathogenesis of allergic airway inflammation. The mechanisms regulating ILC2, however, are not fully understood. Here, we found that **ICAM-1 is required for the development and function of ILC2.** ICAM-1-deficient (*ICAM-1*$^{-/-}$) mice displayed significantly lower levels of ILC2s in the bone marrow and peripheral tissues than wild-type controls. CLP transfer and in vitro culture assays revealed that the regulation of ILC2 by ICAM-1 is cell intrinsic. Furthermore, ILC2s from *ICAM-1*$^{-/-}$ mice were functionally impaired, as indicated by the diminished production of type-2 cytokines in response to IL-33 challenge. The reduction in lung ILC2s caused a clear remission of airway inflammation in *ICAM-1*$^{-/-}$ mice after administration of papain or *Alternaria alternata*. We further demonstrate that ILC2 defects caused by ICAM-1 deficiency are due to ERK signaling-dependent down-regulation of GATA3 protein. Collectively, these observations identify **ICAM-1 as a novel regulator of ILC2.**

P2597

Prostaglandin e2 inhibits group 2 innate lymphoid cell activation and allergic airway inflammation Through e-Prostanoid 4-cyclic adenosine Monophosphate signaling

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Evidence is accumulating that group 2 innate lymphoid cells (ILC2) play an important role in allergic airway inflammation by producing a large amount of type 2 cytokines. But it remains poorly understood how its activities are properly controlled in vivo. Here, we demonstrated that prostaglandin E2 (PGE2) had a profound inhibitory effect on IL-33-induced ILC2 expansion and IL-5 and IL-13 production in vitro. This effect was mimicked by PGE1-alcohol but attenuated by ONO-AE3-208, indicating a selective action through the E-prostanoid 4 (EP4) receptor. In the IL-33-induced asthma model, coadministration of PGE2 or PGE1-alcohol resulted in diminished IL-5 and IL-13 production, reduced eosinophilia and alleviated lung pathology. In contrast, EP4-defcient mice displayed an exacerbated inflammatory response in another ILC2-mediated asthma model induced by Alternaria extract. Mechanistic studies demonstrated that the PGE2-mediated inhibition of ILC2 was dependent on cyclic adenosine monophosphate (cAMP) production. Further downstream, PGE2-EP4-cAMP signaling led to suppression of GATA3 and ST2 expression, which is known to be critical for ILC2 activation. These fndings reveal a novel function of PGE2 as a negative regulator of ILC2 activation and highlight an endogenous counter-regulatory mechanism for the control of innate allergic inflammatory responses.

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DOI: 10.1002/eji.201970400

P2598

Roles of γδ T cells in the lung of *Schistosoma japonicum*-infected C56BL/6 mice

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Many kinds of lymphocytes are involved in *Schistosoma japonicum* (*S. japonicum*) infection-induced disease. γδ T cells comprise a small number of innate lymphocytes that quickly respond to foreign materials. In this study, the role of γδ T cells in the lung of *Schistosoma japonicum*-infected C56BL/6 mice was investigated. The results demonstrated that *S. japonicum* infection induces γδ T cell accumulation in the lung, expressing higher levels of CD25, MHC II, CD80 and PDL1 and lower levels of CD127 and CD62L ($P< 0.05$). The intracellular cytokine staining results illustrated higher percentages of IL-4-, IL-10-, IL-21- and IL-6-producing γδ T cells and lower percentages of IFN-γ-expressing γδ T cells in the lung of infected mice ($P< 0.05$). Moreover, the granuloma size in lung tissue was significantly increased in $V\delta^{-/-}$ mice ($P< 0.05$). In the lung of *S. japonicum*-infected $V\delta^{-/-}$ mice, both Th1 and Th2 response was decreased, significantly ($P< 0.05$). The expression of CD80 and MHC = 2 * ROMAN II on B cell was decreased significantly ($P< 0.05$). In addition, SEA-specific antibody was markedly decreased ($P< 0.05$) in the blood of infected $V\delta^{-/-}$ mice. In conclusion, this study indicates that γδ T cells modulate the Th2 dominant immune response in the lung of *S. japonicum*-infected mice.

NK Cells and Invariant T Cells

O289

Tim-3 blockade promotes iNKT cell function to inhibit HBV replication

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Invariant natural killer T (iNKT) cells play a key role in chronic hepatitis B virus (HBV) infection. Improving iNKT cell function is a feasible strategy against HBV infection. Tim-3 is a powerful regulatory molecule involved in multiple diseases, but little is known about the role of Tim-3 on iNKT cells in chronic HBV infection. In order to address that, hepatitis was induced in HBV/HBs transgenic (Tg) mice by α- galactosylceramide (α-Galcer) with or without Tim-3 blocking antibody. Flow cytometry analysis showed increased Tim-3 expression on hepatic iNKT cells in HBV-Tg mice or iNKT cells stimulating with α-Galcer. Tim-3 level was positively associated with a surface marker, CD69, indicating a relationship between Tim-3 and activation of iNKT cells. In accordance, Tim-3+iNKT cells produced more IFN-γ, IL-4 and CD107a.Surprisingly, blocking Tim-3 pathway in vivo or in vitro significantly enhanced the expression of IFN-γ, TNF-α, IL-4 and CD107a in iNKT cells, which suggested that Tim-3 negatively regulated iNKT cells function. The inhibitory effect of Tim-3 on iNKT cells was further confirmed in Tim-3 knockout mice. More importantly, Tim-3 blockade promoted α-Galcer triggered inhibition of HBV replication, displaying as the decreased HBV-DNA and HBsAg level in serum, and downregulated pregenomic RNA (pgRNA) expression in liver tissues. Our data, for the first time, demonstrated the enhancement of iNKT cell functions by Tim-3 blockade, which further inhibited HBV replication in HBV-Tg mice. Therefore, aiming to Tim-3 might be a promising approach in chronic hepatitis B therapy.

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DOI: 10.1002/eji.201970400

O290

Trained or impaired - NK cell immunity in cured HCV patients

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Immune responses of natural killer (NK) cells are apparently impaired in chronically infected HCV patients, which contribute to HCV-related diseases including hepatocellular carcinoma (HCC). Since 2014, direct acting antivirals (DAAs) such as Harvoni®, were approved for therapy of HCV patients. While DAA application allowed a complete viral clearance in HCV patients, the effect on the NK cell compartment remained widely undefined.
Here, we now aim to clarify the molecular phenotype of NK cells in cured HCV patients. We collected primary NK cells from six Harvoni®-treated HCV patients before and one year after therapy and analyzed them in comparison to healthy control samples by using quantitative proteomics. In total, mass spectrometry identified 4867 proteins of $CD56^{dim/bright}$ NK cells and could reveal immune functions including interferon-stimulated genes that are responding along viral clearance. Furthermore and surprisingly, we found on average 5% of the total NK proteome still deregulated one year after therapy. Enrichment and network analyses indicated long-term effects on selected immune processes. Among those, we selected ADAP and characterized its importance in NK cell responses. We generated ADAP knock-out NK cells and performed *in vitro* assays that determined its role in cytotoxicity, degranulation and cytokine production.

O291

Exosomes derived from human Vγ9Vδ2-T cells exert therapeutic effects on Epstein-Barr virus-induced B cell lymphoproliferative disease

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Exosomes are nanovesicles of endosomal origin and carry bioactive macromolecules to mediate intercellular communication. Some hematopoietic cells derived exosomes have been demonstrated to regulate tumorigenesis. However, the characteristics and functions of exosomes derived from human Vγ9Vδ2-T cells (Vγ9Vδ2-T-Exos) remain unclear. Previously we have demonstrated that Vγ9Vδ2-T cells can be largely expanded in vitro and control Epstein-Barr virus-induced lymphoproliferative disease (EBV-LPD). However, this therapeutic effect could be counteracted by the immunosuppressive factors and acidity in tumor microenvironment. On the contrary, exosomes are resistant to immunosuppression and their accumulation and delivery can be enhanced by tumor acidity. As cell-free bioactive vesicles with good feasibility and safety, exosomes attract more and more attention in the field of cancer immunotherapy. In this study, we characterized Vγ9Vδ2-T-Exos and explored their anti-tumor effects against EBV-LPD. The Vγ9Vδ2-T-Exos were isolated by differential ultracentrifugation and identified with typical cup-shaped morphology, expression of exosomal markers CD63 and CD81. Vγ9Vδ2-T-Exos were found to carry killer proteins and display significant cytotoxic activity against EBV-transformed lymphoblastoid B cell lines (EBV-LCL) in vitro, which was partially through the expression of FasL and TRAIL. Lethal EBV-LPD tumor model was established by subcutaneous inoculation of EBV-LCL in $Rag2^{-/-}\gamma c^{-/-}$ mice. Administration of Vγ9Vδ2-T-Exos significantly decreased the tumor incidences and inhibited tumor growth of EBV-LPD in $Rag2^{-/-}$

DOI: 10.1002/eji.201970400

γc$^{-/-}$ mice. Moreover, the survival of EBV-LPD-bearing Rag2$^{-/-}$γc$^{-/-}$ mice were significantly enhanced after the treatment with Vγ9Vδ2-T-Exos. This study firstly demonstrated that Vγ9Vδ2-T-Exos could exert anti-tumor effects and thus warrant further development as a potential immunotherapeutic strategy against cancer.

O292

The role of BAFF in the biology of NK cells

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B-cell activating factor of the tumour necrosis factor family (BAFF) is, as indicated by name, a cytokine belonging to the TNF superfamily. There are three receptors which interact with BAFF called BAFF-R, TACI and BCMA. Natural killer (NK) cells are large granular innate lymphocytes that serve as a vital first line of defence against tumour cells and intracellular pathogens. The discovery that BAFF promotes the survival of B cells, and therefore can lead to autoimmune diseases if present in excess has led to the development of an anti-BAFF antibody called Belimumab which is a new drug used to treat patients with systemic lupus erythematosus (SLE). The development of an anti-TACI antibody as a more specific treatment for lupus is also underway. It is essential to study the effect of BAFF on NK cells to be aware of possible off-target effects of these drugs. Using various genetically modified mice (GM) of the BAFF system, we clarified the role of BAFF in NK cell numbers and maturation. We also assessed the effect of BAFF on NK cell activity by measuring intracellular granzyme B expression, performing chromium release assay, and creating the B16-F10 melanoma pulmonary metastasis model. To determine whether BAFF has a direct effect on NK cells, we evaluated the expression of BAFF-binding receptors at both protein and mRNA levels. This study is the first detailed investigation of the participation of BAFF in NK cell homeostasis and functions.

O293

Hematopoietic-specific deletion of Foxo1 promotes NK cell specification and proliferation

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We previously reported that deletion of Foxo1, *via Ncr1*-iCre mice from the expression of NKp46 onward, led to enhanced natural killer (NK) cell maturation and effector function. In this model, however, the role of Foxo1 in regulating NK cell specification and early development remains exclusive. Herein, we utilized a murine model of hematopoietic-specific deletion of Foxo1 before lymphoid specification, by crossing mice carrying floxed *Foxo1* alleles (*Foxo1*$^{fl/fl}$) with *Vav1*-iCre mice, to revisit the role of Foxo1 on NK cell specification and early development. The data showed that hematopoietic-specific deletion of Foxo1 resulted in increased proportion and numbers of common lymphoid progenitors (CLP) (Lin$^-$CD127$^+$c-kit$^+$Sca-1$^+$), pre-pro NK b cells (Lin$^-$Sca-1$^+$c-kit$^-$CD135$^-$CD127$^+$), as well as committed Lin$^-$CD122$^+$ cells and CD3$^-$CD19$^-$NKp46$^+$ NK cells in bone marrow. Hematopoietic-specific deletion of Foxo1 also promoted NK cells proliferation in a cell-intrinsic manner, indicated by increased Ki-67 expression and more expansion of NK cell after *ex vivo* stimulation with IL-15. The reason for Foxo1 suppressing NK cell proliferation might be its direct transcription of the cell-cycle inhibitory genes, such as *p21*^{cip1}, *p27*^{kip1}, *p130*, *Gadd45a* and *Ccng2* (*cyclin G2*) in NK cells. Furthermore, hematopoietic-specific deletion of Foxo1 resulted in increased

DOI: 10.1002/eji.201970400

ratio of mature NK subsets, such as CD11b$^+$CD27$^-$ and CD43$^+$KLRG1$^+$ NK cells, consistent with the findings in the murine model of *Ncr1*-iCre mediated Foxo1 deletion. Conclusively, Foxo1 not only acts as a negative checkpoint on NK cell maturation, but also represses NK cell specification and proliferation.

O294

IL-23 co-stimulation drives antigen-specific MAIT cell activation and enables vaccination against bacterial infection

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MAIT cells are activated in a TCR-dependent manner by antigens derived from the riboflavin synthesis pathway, including 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil, bound to MHC- related protein-1 (MR1). However, MAIT cell activation *in vivo* has not been studied in detail. Here, we have characterised the molecular signals required for activation and expansion of MAIT cells following pulmonary Legionella or Salmonella infection in mice. We show that either bone marrow-derived APCs or non-bone marrow-derived cells activate MAIT cells *in vivo* depending upon the pathogen. Moreover, optimal MAIT cell activation *in vivo* requires signalling through the inducible co-stimulator of T cells (ICOS), which is highly expressed on MAIT cells. Subsequent expansion and maintenance of MAIT-17/1-type responses is dependent on IL-23 and unlike conventional Th17 cells was independent of IL-6. Vaccination with IL-23 plus 5-OP-RU augments MAIT cell-mediated clearance of Legionella infection. These findings reveal cellular and molecular targets for manipulating MAIT cell function under physiological conditions.

O295

Neutrophils driving unconventional T cells are essential for resistance to sarcomas

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DOI: 10.1002/eji.201970400

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Neutrophils are a component of the tumor microenvironment and have been shown to exert both tumor-promoting and tumor-suppressive effects *in vivo*. Antibody-mediated cell depletion is often used to evaluate the role of neutrophils in animal models, though this approach suffers from limitations, including duration, specificity and perturbation of the system.
We therefore used a genetic approach to investigate the role of neutrophils in primary 3-methylcholanthrene (3-MCA)-induced sarcomagenesis. Neutrophils played an essential role in resistance against carcinogenesis by driving an IL-12/IFN-γ dependent type 1 immune response. Neutrophils selectively induced type 1 polarization of TCRβ+ unconventional T cells ($UTC_{\alpha\beta}$) in tumor microenvironment. Adoptive transfer of neutrophils into sarcoma-bearing neutropenic mice was sufficient to slow down tumor progression, to rescue IFNγ production and type 1 $UTC_{\alpha\beta}$polarization. Multiparametric flow cytometry and single cell RNAseq revealed the heterogeneity of sarcoma-infiltrating $UTC_{\alpha\beta}$. We then assessed the relevance of neutrophil-dependent tumor resistance in a human setting. Neutrophils were associated with better outcome and with type 1 immune responsein a subtype of human Soft Tissue Sarcomas (STS) from TCGA database, and the correlation between high neutrophil infiltrate and better survival was confirmed in a different STS cohort.
Using an *in vivo*model of sarcomagenesis and data obtained from human STS patients, we demonstrated that neutrophils mediate resistance against sarcomas, by driving a type 1 immune response and polarization of $UTC_{\alpha\beta}$.

O296

Perinatal cytomegalovirus infection drives NK cell hyporesponsiveness characterized by downregulation of T-box transcription factor

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Congenital human cytomegalovirus (HCMV) infection is the most common viral cause of long-term neurodevelopmental sequelae, including mental retardation, microcephaly and sensorineural hearing loss. NK cells have been shown to play an important role in containing cytomegalovirus (CMV) infection and various adaptive features of NK cells in response to CMV infection are recently being increasingly studied. Despite an increasing body of knowledge the involvement of NK-cell mediated immunity in congenital CMV infection is so far largely unknown. Since HCMV is species-specific, we have used a mouse model in which newborn mice are infected with the mouse cytomegalovirus (MCMV). Here we show that perinatal MCMV infection leads to a persistent alteration of transcriptional activity and strongly affects the maturation and function of NK cells. NK cell expression of T-box transcription factor Eomes, critical for NK cell development, was dramatically impaired after infection. The downregulation of Eomes correlated with major changes in the NK cell phenotype, most notably NK cell exhaustion characterized by an impaired NK cell response to different stimuli. This population of NK cells persisted for several months in infected mice indicating that congenital CMV infection shapes NK cell response over long-term period. Altogether, our data indicate that NK cells also strongly affected by the congenital CMV infection.

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DOI: 10.1002/eji.201970400

LB009

Potent tumor immunity by high salt interventions

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Dietary factors such as salts are known to alter immune haemostasis thereby influencing inflammation and autoimmunity. Recent studies suggests that high salt induced proinflammatory state could have a protective effect against tumor in mouse models. However, the effector immune cell population as well as the mechanism underlying the ameliorative effect of high salt in tumor is poorly understood. In the present study we used mouse solid melanoma, lung cancer and colon cancer model to study the mechanism involved in tumor immunity by high salt interventions. Our immunophenotyping data revealed that the protective effect of high salt is largely independent of T cells, which was further validated by using RAG1 knockout mice. Importantly, high salt interventions in tumor induced mice was found to significantly upregulate NK cells population. The up-regulation in NK cell frequency directly co-related with the ameliorative effect of high salt on tumor as neutralizing NK cell population by anti-mouse NK1.1 neutralizing antibody abrogated protective effects. Further, the protection was found to be mediated by IFNγ secreted by salt induced activated NK cells as neutralizing IFNγ masked the anti tumor effect of high salt. Taken together, our present study shows potent tumor immunity by high salt interventions which was equally robust for solid melanomas, lung cancer, metastasis and post-surgical tumor removal. Moreover, we demonstrate that the protective effect of high salt could be attributed to IFNγ secreted by NK cells. These findings could have great therapeutic potentials against tumors of human origin.

P0128

The role of HLA-E in the inhibition of NK cell cytotoxicity against trophoblast cell line JEG-3 by progesterone

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Objectives: In our preliminary study, progesterone was found to inhibit the NK cell cytotoxicity against trophoblast cell JEG-3. This study was performed to investigate if HLA-E plays a role in the regulation of NK cell cytotoxicity against trophoblasts by pogesterone.
Methods: The expression silence of HLA-E in JEG-3 cells was induced through transfection with the lentivirus carrying siRNA targeting HLA-E. JEG-3 cells were divided into 5 groups: the blank control, progesterone treatment, transfection of lentivirus carrying siRNA targeting HLA-E before progesterone treatment, transfection of lentivirus carrying siRNA targeting HLA-E, and transfection of the negative control siRNA. JEG-3 cells were collected as target cells 48h after treatments. The NK cells were obtained from peripheral blood mononuclear cells of non-pregnant healthy women. The cyotoxicity of NK cells toward JEG-3 was evaluated using LDH assay.
Results: In this study, we found that progesterone decreased NK cell cytotoxicity against JEG-3. The NK cell cytotoxicity against JEG-3 transfected with lentivirus carrying siRNA targeting HLA-E was highly enhanced compared to the controls. Further, Silence of HLA-E expression before progesterone treatment diminished the inhibitory effect of progersterone on NK cell cytotoxicity toward JEG-3.
Conclusion: HLA-E was involved in the inhibition of NK cell cytotoxicity against JEG-3 by progesterone.

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DOI: 10.1002/eji.201970400

P0129

Spatial distribution of IL4 in response to lipid antigen variants controls iNKT-DC crosstalk

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Spatiotemporal distribution of cytokines orchestrates immune responses in vivo, yet the underlying mechanisms remain to be explored. We showed here that spatial distribution of IL4 in iNKT cells regulated crosstalk between iNKT cells and DCs, and controlled iNKT cell-mediated Th1 responses. The secretory routes of IL4 were determined by type of lipid antigens. Strong lipid antigens, i.e., a-galactosylceramide (aGC), maintained polarization of microtubule organization center (MTOC) toward immunological synapses (IS), and thus ensured polarized secretion of IL4. Persistent polarization caused accumulation of IL4 at IS, which promoted activation of IL4R-STAT6 pathway and IL12 production in DCs, and therefor favored IFNg production in iNKT cell. Conversely, Th2 lipid antigens with short or unsaturated chain caused non-polarized secretion of IL4 and shifted immune response to Th2 type. We further revealed that Th2 lipid antigens caused degradation of Cdc42 in iNKT cells, which was required for polarization of MTOC and hence IL4. Together, we demonstrate that iNKT cell-mediated immune responses are shaped by the position where IL4 is released, and that is fine-tuned by different type of lipid antigens.

P0130

Non-clonal chromosome aberrations in ex vivo expanded natural killer cells for immunotherapy

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Background: The aim of this study was to investigate the significance of non-clonal chromosome aberrations (NCCAs) in natural killer (NK) cells induced by large-scale proliferation.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from patients with tumours as well as from healthy control subjects. K562 cells with membrane-bound IL-21 were used to stimulate NK cell proliferation. On culture day 7, the cells were collected for phenotype identification via flow cytometry and for cytogenetic testing by using the conventional G-banding technique. In all, 50~100 metaphases were analysed per sample.
Results: NCCAs were identified in 6 of 7 samples from the tumour group and in 6of 12 samples from the control group (85.71% vs 50%, respectively; P=0.173). In the tumour group, 17 metaphases containing NCCAs were identified of 481 metaphases, whereas only 7 metaphases containing NCCAs were detected out of 785 metaphases in the control group (3.53% vs 0.89%, respectively; P=0.028). Four patients with a history of chemoradiotherapy showed higher NCCA frequencies than both tumour patients and healthy donors without the same history (5.68% vs 0.898%, P=0.001).
Discussion: NCCAs commonly exist in *in vitro* expanded NK cells, with a higher frequency observed in NK cells originating from tumour patients (especially those with a history of chemoradiotherapy). Whether NK cells with a high frequency of NCCAs are suitable for adoptive immunotherapy is questionable, and the significance of cytogenetic examination in the quality control of NK cell products requires further evaluation.

DOI: 10.1002/eji.201970400

P0131

Hepatic NK cells attenuate fibrosis progression of non-alcoholic steatohepatitis by recruitment through CXCL10

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Background: Nonalcoholic steatohepatitis (NASH) is a major cause of chronic liver disease. The precise role of NK cells in NASH progression is unclear.
Methods: By using methionine-choline deficient diets (MCD)-induced NASH model, the role of NK cells was identified in WT mice compared with Nfil3$^{-/-}$ mice.
Results: After 8 weeks of MCD-treatment, NASH was induced as shown by the significant macrovesicular steatosis, necro-inflammation and fibrosis in the liver of WT B6 mice. In MCD-treated mice, the number of NK cells was markedly increased in the liver, but decreased in the spleen. The intrahepatic NK cells were highly activated with expression of CD107a and cytokine production such as IFN-γ, TGF-β and IL-10. Lower levels of Ki67 expression indicated the decreased proliferation of intrahepatic NK cells after MCD-treatment. Increased expression of CXCL10 in the liver tissue early after MCD treatment recruited more CXCR3^{+}NK cells into the liver. Further, in MCD-treated Nfil3$^{-/-}$ mice in which conventional NK cells were deficient, similar TG levels and macrovesicular steatosis, but more inflammatory infiltration and increased collagen deposition were detected in the liver compared with WT mice. When NK cells were depleted in WT mice by anti-asGM1 treatment, significantly increased immune cell infiltration was observed in the liver, further demonstrating NK cells inhibited hepatic inflammation during MCD-induced NASH.
Conclusion: NK cells recruited through CXCL10-CXCR3 interaction showed an inhibitive role in fibrosis progression of NASH, which will help to better understand the immunopathogenesis of NASH.

P0132

Immune-regulatory roles of liver-resident NK cells during viral infection

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Natural killer (NK) cells are known as effectors of innate immunity, and they also have immune-regulatory functions in adaptive immune responses. Our recent studies reveal that a unique NK cell subset comprises almost half of total hepatic NK cells, termed the liver-resident NK (LrNK) cell and characterized by CD49a^{+}CD49b^{-} phenotype. However, its functional properties, particularly its roles in liver diseases, are poorly understood. Considering that the liver is an immunologically tolerant organ with a predisposition to persistent viral infection, we wonder whether LrNK cells can regulate adaptive immune responses during viral infection. Here, we found that LrNK cells were enriched in genes involved in pathways of negative regulation of immune system process. Moreover, LrNK cells could inhibit hepatic T cell antiviral responses in several mouse models of hepatotopic viral infection. Conversely, cNK cells had a promoting effect on antiviral T cell responses. Further analysis revealed that LrNK cells directly inhibited T cell function and proliferation in a PD-L1-dependent manner. Thus, these findings uncover a previously unknown function of LrNK cells in shaping adaptive immune responses, providing new insight into the complex immune regulatory mechanisms that underlie hepatic tolerance and immunity.

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DOI: 10.1002/eji.201970400

P0133

Human CD96 correlates to NK cell exhaustion and predicts the prognosis of human hepatocellular carcinoma

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Immune checkpoint blockade has become a promising therapeutic approach to reverse immune cell exhaustion. Co-inhibitory CD96 and TIGIT, together with co-stimulatory CD226, bind to common ligand CD155. The balancing between three receptors fine tunes the immune responses against tumors. In this study, the cumulative percentage, absolute count, and MFI of $CD96^+$ NK cells were significantly increased in the intratumoral tissues of HCC, and broke the balance between three receptors. Human $CD96^+$ NK cells were functionally exhausted with impaired IFN-γ and TNF-α production, high gene expression of IL-10 and TGF-β1, and low gene expression of T-bet, IL-15, perforin and granzyme B. In addition, blocking CD96-CD155 interaction specifically increased lysis of HepG2 cells by NK cells. HCC patients with high level of CD96 or CD155 expression within tumor were strongly associated with deteriorating disease condition, shorter disease-free survival (DFS) and overall survival (OS) times. Patients with higher cumulative percentage of $CD96^+$ NK cells within tumor also exhibited shorter DFS. High plasma level of TGF-β1 in HCC patients up-regulated CD96 expression and dynamically shifted the balance between CD96, TIGIT and CD226 in NK cells. Blocking TGF-β1 specifically restored normal CD96 expression and reversed the dysfunction of NK cells.
Conclusion: These findings indicate that human intratumoral $CD96^+$ NK cells are functionally exhausted and patients with higher intratumoral CD96 expression exhibit poorer clinical outcomes. Blocking CD96-CD155 interaction or TGF-β1 restores NK cell immunity against tumors by reversing NK cell exhaustion, suggesting a possible therapeutic role of CD96 in fighting liver cancer.

P0134

TNF-α-induced Tim-3 expression marks the dysfunction of infiltrating natural killer cells in human esophageal cancer

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Impairment of natural killer (NK) cell activity is an important mechanism of tumor immunoevasion. T-cell immunoglobulin domain and mucin domain-3 (Tim-3) is an activation-induced inhibitory molecule, inducing effector lymphocyte exhaustion in chronic viral infection and cancers. However, its function in NK cells in human esophageal cancer remains unclear. Here, we observed up-regulation of Tim-3 expression on NK cells from esophageal cancer patients, especially at the tumor site. Furthermore, NK cells with high Tim-3 expression exhibited a phenotype with enhanced dysfunction. In vitro, Tim-3 expression on NK cells can be induced by recombinant tumor necrosis factor-α (TNF-α) via NF-κB pathway. In both animal models and patients, the Tim-3 level was positively correlated with TNF-α expression in esophageal cancer tissues. Finally, higher Tim-3 level on tumor infiltrating NK cells is correlated with tumor invasion, nodal status and poor stage in patients with esophageal cancer. Taken together, Tim-3 may play a crucial role to induce NK cell dysfunction in tumor microenvironment and could serve as a potential biomarker for prognosis of esophageal cancer.
Keywords: Tumor microenvironment; NK cells; Tim-3; TNF-α; esophageal cancer

DOI: 10.1002/eji.201970400

P0135

Liver-resident NK cells suppress autoimmune cholangitis and limit the proliferation of CD4+ T cells

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Liver-resident NK cells are distinct from conventional NK cells and play an important role in the maintenance of liver homeostasis. How liver-resident NK cells participate in autoimmune cholangitis remains unclear. Here, we extensively investigated the impact of NK cells in the pathogenesis of autoimmune cholangitis utilizing the well-established dnTGFβRII cholangitis model, NK cell-deficient (Nfil3$^{-/-}$) mice, adoptive transfer and in vivo antibody-mediated NK cell depletion. Our data demonstrated that disease progression was associated with a significantly reduced frequency of hepatic NK cells. Depletion of NK cells resulted in exacerbated autoimmune cholangitis in dnTGFβRII mice. We further confirmed that the DX5^{-}CD11c^{hi} liver-resident NK cell subset colocalized with CD4^{+} T cells and inhibited CD4^{+} T cell proliferation. Gene expression microarray analysis demonstrated that liver-resident NK cells had a distinct gene expression pattern consisting of the increased expression of genes involved in negative regulatory functions in the context of the inflammatory microenvironment

P0136

Viral infection-induced generation of hepatic CD49a^{+}CD49b^{+} NK cells

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Natural killer (NK) cells are important innate effectors that play a pivotal role in defense against tumors and infections. As an organ with predominant innate immunity, the liver is enriched with heterogeneous NK cells, among which two transcriptionally and phenotypically distinct NK cell subsets have been identified: CD49a^{-}CD49b^{+} conventional NK (cNK) cells and CD49a^{+}CD49b^{-} liver-resident NK (LrNK) cells. Previous studies have focused more on bulk NK cells, however, the dynamic composition and respective roles of the different hepatic NK cell subsets during infection remain unclear. Here, using a mouse model of lymphocytic choriomeningitis virus infection, we observed that CD49a^{+}CD49b^{+} NK (DP NK) cells emerged and underwent vigorous expansion in the liver early after infection, but then decreased progressively. These viral infection-induced DP NK cells were highly activated with high expression of the activation marker CD69 and activating receptors CD226, NKG2D and NKp46, and mainly composed of CD11b^{hi}CD27^{hi} mature NK cells. Moreover, compared with LrNK and cNK cells, DP NK cells are more functionally competent, as evidenced by higher amounts of IFN-γ production and stronger cytotoxic capabilities during viral infection. These DP NK cells were Eomes^{int}T-bet^{hi}, and their generation was independent on T-bet. Adoptive transfer experiments revealed that cNK cells could convert into DP NK cells after infection. Collectively, these results suggest that viral infection-induced DP NK cells represent a cNK cell-derived and functional competent subset.

DOI: 10.1002/eji.201970400

P0137

Function and mechanism investigation of BCL3 in regulating NK cell activity via interaction with STAT3

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It is very important for the body health to activate and timely terminate NK function. B-cell CLL/lymphoma 3 (BCL3) is a transcriptional coregulator, but its function in NK cell activation is unclear. Our current study data shown that BCL3 was highly expressed in NK cells from mice infected with cytomegalovirus (CMV). Overexpression of BCL3 inhibited the cytotoxic activity of NK-92 cell, NK cells derived from BCL3 deficient mice secreted more Perforin and INF-γ, and it was shown that BCL3 interacted with STAT3, and inhibition of STAT3 can suppress excessive activation of NK induced by BCL3 deficient. All the data suggested that BCL3 may regulate NK activity through STAT3. Next, we will establish NK cell specific deletion of BCL3 mice to analyze the role and mechanism of BCL3 interacting with STAT3. This study reveals a new mechanism of NK cells activity regulation, but also provide potential drug targets for treatment to the disease associated with excessive activation of NK cells.

P0138

Identification of novel peripheral NK cells sub-population

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NK cells are innate lymphoid cells that play role in cytotoxic killing of immune system. Several surface molecules have been used to describe NK cell sub-populations. In this study, the new sub-population of peripheral circulating NK cells was discovered using our produced monoclonal antibody (mAb), named FE-1H10. The molecules recognized by mAb FE-1H10 were expressed on a sub-population of $CD3^{-}CD56^{dim}$ NK cells. The mAb FE-1H10 bound to a glycan epitope which was different from CD57. Using our mAb FE-1H10, $CD57^{+}$ NK cells could be separated into two sub-populations. The cytotoxicity function of $FE\text{-}1H10^{-}$ NK cells was greater than $FE\text{-}1H10^{+}$ NK cells as demonstrated by increasing the number of LAMP-1 expressing cells and granzyme B releasing during K562 stimulation. In addition, the $FE\text{-}1H10^{-}$ NK cells were able to produce IFN-γ and TNF-α better than $FE\text{-}1H10^{+}$ NK cells. In summary, our studies showed that mAb FE-1H10 defines $CD3^{-}CD56^{dim}$ NK cells into two sub-populations, the $FE\text{-}1H10^{+}$ and $FE\text{-}1H10^{-}$ populations. These two sub-populations differ in their cytotoxic activity and cytokine production. Although the function of this molecule remains unclear, mAb FE-1H10 might be a valuable tool for identifying functionally competent NK cells.

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DOI: 10.1002/eji.201970400

P0139

Innate-like activation of mucosal-associated invariant T cells

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Conventional T cells display a delayed response to the initial priming of peptide antigens presented by major histocompatibility complex (MHC) proteins. Unlike conventional T cells, mucosal-associated invariant T (MAIT) cells quickly respond to non-peptidic metabolite antigens presented by MHC-related protein 1 (MR1). However, the conserved mechanisms contributing to the quick MAIT cell activation and response remain elusive. We hypothesize that both antigen presentation and MAIT cell responses employ highly conserved molecules and pathways. As a result, the functional domains of MR1 sequences are highly homologous in mammalian species and are identical between human and chimpanzee, facilitating a cognate MR1-antigen-T cell receptor interaction for MAIT cell activation. Second, xenoreactive responses of human and mouse MAIT cells further support an innate-like activation mechanism. Third, upon the stimulation with mycobacterial-infected antigen-presenting cells, human MAIT cell-enriched population (Va7.2^{+}CD161^{+}CD4^{-}CD8^{+}) quickly develop an activated phenotype labeled by CD69^{+}CD26^{++} coexpression through MR1-dependent antigen presentation. Functionally, these activated CD8^{+}MAIT cells enhanced the production of the cytokine TNFa, cytolytic molecule granulysin, and transcription factor Tbet, sharing effector molecules and surface markers with NK cells and conventional CD8^{+} T cells. Moreover, transcriptomes of the activated CD8^{+}MAIT cells also highly overlap with multiple activation and function pathways of NK and CD8^{+} T cells. Together, data strongly support that MAIT cells utilize an innate-like activation mechanism to respond to bacterial infection rapidly in a donor-unrestricted manner.

P0712

Intrahepatic development of liver-resident NK cells through interferon-γ signaling

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Recent studies have revealed a unique natural killer (NK) cell subset, referred to as liver-resident NK (LrNK) cells, that is enriched in the adult mouse liver. However, the mechanisms underlying its origin remain largely unknown. Here, we found that the adult mouse liver contained fetal liver-derived hematopoietic progenitors that were characterized by the CD45^{+}Lin^{-}Sca-1^{+}Mac-1^{+} (LSM) phenotype. These LSM cells could develop to CD49a^{+} NK precursors (CD49a^{+} NKPs), which could differentiate into LrNK cells, but not conventional NK cells. Furthermore, the absence of interferon (IFN)-γ signaling led to the impaired proliferation of liver LSM cells and CD49a^{+} NKPs, and IFN-γ overexpression conversely increased the numbers of these progenitors, thereby influencing the production of LrNK cells. Thus, our findings suggest a novel intrahepatic development pathway for LrNK cells, highlighting the contribution of extramedullary hematopoiesis to a unique regional immune composition.

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DOI: 10.1002/eji.201970400

P0713

Peripheral and liver resident mucosal-associated invariant T cell in HBV-related hepatocellular carcinoma

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Background and aims: Mucosal-associated invariant T (MAIT) cells are important innate T cells with antimicrobial and immunoregulatory activity, recently found to be associated with varies cancer. However, little is known on the role of MAIT cells in HBV-related hepatocellular carcinoma (HCC).
Methods: Peripheral blood samples were obtained from patients with HBV infected HCC (n=28) and matching healthy controls (n=49). Liver-associated mononuclear cells (LMCs) were collected from donors perfusate (n=28) or explanted livers (n=16) of HCC patients with HBV infection undergoing liver surgery. Blood and liver perfusate were analysed with flow cytometry for MAIT cell frequency, phenotype and their functionality.
Results: Peripheral and liver MAIT cells in HBV infected HCC patients were reduced together with a reduction in IFN-γ production. Peripheral blood MAIT cells up-regulate inhibitory molecule PD-1, while the level of PD1 remains unchanged in intra-hepatic MAIT cells. MAIT cells were significantly enriched in liver compared to peripheral blood in HBV infected HCC patients. High level of IL-6 and IL-1β was observed in LMC of HBV infected HCC patients, but was not found in peripheral blood.
Conclusions: Our study demonstrates that MAIT cells are associated with HBV infected HCC. Further to that, distinct function of MAIT cells were observed in peripheral and liver of HBV infection HCC patients, which may suggest the therapeutic potential for HCC patients in future.

P0714

A novel spleen-resident immature NK cell subset and maturation in a T-bet-dependent manner

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NK cells are thought to develop primarily in the bone marrow during adult life. However, increasing evidence shows that NK cell developmental intermediates can be found in different peripheral tissues with unique characteristics. Here, we identified a unique NK cell subset with the $CD49a^{-}CD49b^{-}$ phenotype in the spleen. These cells displayed an immature phenotype and could develop into mature conventional NK (cNK) cells. Parabiosis experiments revealed that these cells maintained tissue-resident properties in the spleen. Moreover, T-bet deficiency intrinsically impaired the ability of these cells to develop into mature cNK cells. Thus, our study identified a **spleen-resident immature NK cell subset that could undergo extramedullary maturation in a T-bet dependent manner**.

DOI: 10.1002/eji.201970400

P0715

Formation and maintenance of memory IL-7Rα$^+$ ILC1s via a lymph node-liver axis

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Natural killer (NK) cells have recently been described as exhibiting adaptive features of immunological memory. CD49a$^+$ liver-resident NK cells, also referred to as liver ILC1s, were defined as the subset that confer hapten-specific memory responses; however, little is known about memory ILC1 formation and maintenance. Here, we show that memory ILC1s, distinguished by IL-7Rα expression, are generated in lymph nodes (LNs) and maintain long-term survival in the liver through IL-7R signaling. Hapten sensitization initiates recruitment of IL-7Rα$^+$ ILC1s into skin-draining LNs, in a CXCR3-dependent fashion, where they are activated in response to haptens, generating memory ILC1s. Memory IL-7Rα$^+$ ILC1s then exit draining LNs and preferentially reside in the liver. Moreover, IL-7R signaling is required for the longevity of memory IL-7Rα$^+$ ILC1s. Thus, these results identify a memory IL-7Rα$^+$ ILC1 population in LNs and the liver, and reveal that a LN-liver axis is essential for memory ILC1 formation and long-term homeostasis.

P0716

Dysfunction of natural killer cells by FBP1-induced inhibition of glycolysis during lung cancer progression

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Natural killer (NK) cells are effector lymphocytes with pivotal roles in the resistance against various tumors; dysfunction of NK cells often results in advanced tumor progression. Tumors develop in three stages comprising initiation, promotion, and progression, but little is known about the interrelationships between NK cells and tumor cells at different stages of tumor development. Here, we demonstrated that NK cells prevented tumor initiation potently but did not prevent tumor promotion or tumor progression in Kras-driven lung cancer. Moreover, loss of the antitumor effect in NK cells was closely associated with their dysfunctional state during tumor promotion and progression. Mechanistically, aberrant fructose-1,6-bisphosphatase (FBP1) expression in NK cells elicited their dysfunction by inhibiting glycolysis and impairing viability. Thus, **our results show dynamic alterations of NK cells during tumor development and uncover a novel mechanism involved in NK cell dysfunction, suggesting potential directions for NK cell-based cancer immunotherapy involving FBP1 targeting.**

P0717

ATRA promotes tumor resistence to NK cells by downregulating tumor B7-H6 expression

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DOI: 10.1002/eji.201970400

The interaction between the activating receptor NKp30 and its major tumor ligand B7-H6 is important for NK cell mediated tumor lysis. However, the regulation of B7-H6 in tumor cells remains largely unknown. In this study, we investigated the regulation of B7-H6 by all-trans Retinoic acid, a terminal differentiation inducer of tumor cells utilizied in tumor therapy. We found that atRA treatment dampened U937 lysis by NK92 and NKG cells, while NK92 and NKG cells mediated U937 cell killing was mainly dependent on the NKp30-B7-H6 axis. In consistent with that, we found that B7-H6 expression on tumor cell suface was downregulated upon atRA treatment. We further found that atRA downregulated B7-H6 at the mRNA level. Epgenetic modifications such as DNA methylation and HDAC was not involved as inhibitors of these pathways did not reverse the B7-H6 downregulation induced by atRA. Instead, atRA downregulated c-myc, the transcriptional factor driving B7-H6 expression in tumor cells, and led to the transcriptional suppression of B7-H6. Together, our data showed that atRA treatment promoted tumor cell resistence to NK cells by dampening the NKp30-B7-H6 recognition.

P0718

Suppression of the protein quality control system by TRIM30a sensitizes tumor to NK cell immune surveillance

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Tumorigenesis involves overcoming intrinsic cell biology checkpoints and escaping extrinsic immune surveillance. Tumor cells display enhanced capacity to clear misfolded proteins to promote tumorigenesis intrinsically. However, the effect of tumor cells' ability to clear misfolded proteins on tumor surveillance by the immune system remains unknown. Here, we found that overexpression of tripartite motif-containing protein 30a (TRIM30a) sensitized tumor cells to Natural killer (NK) cell cytolysis both in vitro and in vivo. Although such overexpression didn't affect proliferation or apoptosis of tumor cells in vitro, tumor cells with TRIM30a overexpression displayed slower growth in vivo in immune competent mice, but not in NK -depleted mice. Transcriptome analysis revealed that expression of lysosome pathway -related gene sets was downregulated in TRIM30a -overexpressing tumor cells. We found that TRIM30a overexpression resulted in decreased clearance of misfolded protein induced by MG132 treatment, indicating that protein quality contrl system in these tumor cells was compromised upon TRIM30a overexpression. In consistence with this, accumulated misfolded protein induced by MG132 pretreatment rescued the difference in in vivo growth between TRIM30a-overexpressing tumor cells and control tumor cells. Importantly, misfolded protein induced by MG132 treatment sensitized tumor cells to NK cell cytolysis in vitro. Our study demonstrate that TRIM30a sensitizes tumor cells to NK surveillance by compromising the protein quality control system of tumor cells, and we propose that the protein quality control system of tumor cells is an immune escape mechanism of tumorigenesis.

DOI: 10.1002/eji.201970400

P0719

Reduced CD160 expression contributes to impaired NK-cell function and poor clinical outcomes in patients with HCC

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We previously reported that deficiencies in natural killer (NK)-cell number and function play an important role in the progression of hepatocellular carcinoma (HCC). However, the mechanisms underlying this phenomenon remain obscure.
In this study, we analyzed the expression of CD160 on intrahepatic NK cells by evaluating peritumoral and intratumoral tissues of 279 patients with HCC and 20 healthy livers. We observed reduced expression of CD160 on intratumoral NK cells, and patients with lower CD160 cell densities within tumors exhibited worse disease and a higher recurrence rate. High-resolution microarray and gene set enrichment analysis of flow cytometry-sorted primary intrahepatic $CD160^+$ and $CD160^-$ NK cells of healthy livers indicated that human $CD160^+$ NK cells exhibited functional activation, high IFN-g production, and NK-mediated immunity. In addition, global transcriptomic analysis of sorted peritumoral and intratumoral $CD160^+$ NK cells revealed that intratumoral $CD160^+$ NK cells aremore exhausted than peritumoral $CD160^+$ NK cells and produce less IFN-g. High levels of TGFβ1 interfered with production of IFN-g by $CD160^+$ NK cells, blocking of which specifically restored IFN-g production in $CD160^+$ NK cells to normal levels.
These findings indicate that reduced numbers of $CD160^+$ NK cells, together with the functional impairment of $CD160^+$ NK cells by TGFβ1, contribute to tumor immune escape. In addition, restoring the expression of CD160 and blocking TGFβ1 appear a promising therapeutic strategy against liver cancer.

P0720

Study of the relationship between NK cells reconstruction as well as chimerism and acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

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Background: Our study was to investigate the NK cells in patients with allogeneic hematopoietic stem cell transplantation, then provide clues for evaluation of acute graft-versus host disease and improve prognosis.
Methods: Peripheral blood or bone marrow samples from 57 patients were collected at 14 ,30, 60 ,90 days after allo-PBSCT. We measured NK cells chimerism and the expression of subgroups and receptors of NK cells.
Results: Among the 57 patients, 26 patients suffered aGVHD while 31 patients were free from aGVHD. NK cells implanted early. The percent of CD56dimNK and NKG2A-NK in aGVHD group was low at 30 days after allo-HSCT. At this time, there was difference in the occurrence of aGVHD between the group with CD56dim/CD56brigh≤0.2 and CD56dim/CD56brigh> 0.2.We found the same result between the group with NKG2A-/ NKG2A+≤0.35 and NKG2A-/ NKG2A+> 0.35.The expression of NKG2D in aGVHD group was low at 14 days,30 days after allo-HSCT. There was difference in the occurrence of aGVHD between the group with NKG2D MFI ≤1900 and MFI > 1900 at 14 days after allo-HSCT and between the group with NKG2D MFI≤1800 and MFI > 1800 at 30 days after allo-HSCT.

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DOI: 10.1002/eji.201970400

Conclusion: aGVHD developed when the percentage of CD56dim and NKG2A-NK was low at 30 days and the expression of NKG2D was low at 14 days,30 days after allo-HSCT. CD56dim, NKG2A-NK cell subgroup and NKG2D played a part in aGVHD, which can be used as biomarkers of aGVHD and guiding treatment.

P0721

Tumor cell oxidative stress promotes immune surveillance by NK cells

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Tumor cells usually display elevated levels of reactive oxygen species (ROS), potentially increasing their susceptibility to oxidative stress upon breaking down the balance of the intracellular redox system. However, the impact of oxidative stress in tumor cells on tumor surveillance by the immune system remains poorly understood. Here, we found that both tumor suppressor TXNIP overexpression -induced, and chemically induced ROS sensitized tumor cell to NK cell cytolysis, without significant affecting the proliferation and apoptosis of these tumor cells in vitro. Importantly, TXNIP suppressed tumor growth in vivo in immune competent mice in an NK-dependent manner. Our data demonstrate that oxidative stress in tumor cells facilitates immune surveillance by NK cells, suggesting that therapeutically increasing oxidative stress in tumor cells might provide additional benefits to NK -based immunotherapies against tumors.

P0722

Evaluation of the expression of T lymphocytes and Natural Killer T cells (NKT) related genes in tissue and blood of patients with tonsillar hypertrophy

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Introduction: Tonsillectomy is one of the most common surgeries which occurs in children. Palatines are secondary lymphoid organs and the major barrier protecting the digestive and respiratory tracts from potential invasive microorganisms. CD1d-restricted natural killer T (NKT) cells are a distinct subset of T cells that rapidly produce different types of cytokines upon activation and play a critical role in regulating various immune responses. The current study aimed to evaluate the expression of NKT related genes in patients with tonsillar hypertrophy and compare to healthy people.
Materials and methods: Nineteen patients who performed tonsillectomy because of tonsillar hypertrophy and fifteen healthy volunteers participated in this study. Tissue specimens from patients and peripheral blood mononuclear cells (PBMCs) from patients and healthy controls were collected and RNA extracted for gene expression analysis. Relative expression of TCR beta and Va24 genes as the representative of CD3 and NKT cells was evaluated by real time PCR method.
Results: The expression of TCR beta and Va24 genes was higher in patients´ tissues compared with their matched PBMCs (P-value < 0.05). In addition Va24 was expressed at significantly higher level in the blood of patients than healthy group (P-value< 0.05). There was no correlation between the expression of Va24 in tissue and blood samples.
Conclusion: The results of this study showed higher expression of CD3 and NKT cells in tissue and peripheral blood of patients with tonsillar hypertrophy which may support the role of NKT cells in the pathogenesis of tonsillar hypertrophy

DOI: 10.1002/eji.201970400

P0723

Altered expression of LLT1 and CS1 in pediatric Acute Lymphoblastic Leukemia (ALL)

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer in the developed world with peak incidence between the ages of 2 and 5 years. Despite current treatment protocols achieving significant improvement in survival of ALL patients, serious acute and late complications are frequent and resistance to chemotherapy often develops. Although NK cells have long been known to mediate antigen independent tumor killing, the therapeutic potential of NK cell-based immunotherapy has yet to be realized. NK cells have the capacity to kill virus-infected or tumor-transformed cells and they express several surface molecules that regulate NK cell function both positively and negatively. We have previously identified 2B4 (CD244), CS1 (CRACC) and LLT1 (CLEC2D) to play a major role in NK cell activation. In this study, we evaluated the cell surface and mRNA expression of NK cell receptors in 42 ALL subjects and 20 healthy subjects both male and female in the age range of 2 - 20 yrs from different racial/ethnic groups. The peripheral blood samples were collected and analyzed by flow cytometry and real-time PCR for expression. A significant increase in the expression of LLT1 in the T and NK cell populations and CS1 in the CD14+ monocytes was observed in ALL subjects as compared to healthy subjects. NK cells from 3 ALL subjects also showed impaired cytotoxic function as they demonstrated lower killing of K562 cells compared to a healthy subject. These results indicate that LLT1 and CS1 may play a role in NK-mediated surveillance of childhood ALL.

P1316

Efficacy of gemcitabine, pegaspargase, cisplatin and dexamethasone (DDGP) in the advanced stage extranodal NK/T-cell lymphoma, and its correlation with epstein-barr virus transformation

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To evaluate the clinical efficacy and safety of the DDGP regime in treating advanced extranodal NK/T-cell lymphoma(ENKTL) and investigate the relationship between the dynamic variation in copy number of EBV-DNA and clinical prognosis during the treatment of ENKTL.64 patients with advanced ENKTL received DDGP regime-based chemotherapy between March 2011 and April 2018.It resulted in 79.69% of response rate that included 39 cases of CR (60.94%), 12 PR (18.75%), 2 SD (3.13%) and 11 PD (17.18%). The 3-year PFS was 62.00%and 3-year OS was 74.90%.Haemocytopenia was the predominant adverse effect.No significant difference in ORR or DCR was observed between the EBV-DNA positive group and the EBV-DNA negative group (78.57% versus 80.56%, *P*=0.845 for ORR; 78.57% versus 86.11%*P*=0.428 for DCR).In the EBV-DNA positive group, the ORR and DCR of the copy number of EBV-DNA decreased within a normal range were higher than who remained positive group (93.33% versus 61.53%, *P*=0.041 for ORR; 93.33% versus 61.53%, *P*=0.041 for DCR). No significant difference was noted in PFS between the EBV-DNA-positive group and EBV-DNA-negative group(*P*=0.812).A significant difference was noted in OS between the two groups (*P*=0.046).Among the 28 patients with positive EBV-DNA,The PFS and OS of the copy number of EBV-DNA decreased within a normal range were higher than who remained positive group(*P*= 0.003,*P*=0.017).In

DOI: 10.1002/eji.201970400

conclusion,DDGP regime can significantly improve the clinical prognosis of NK/T-cell lymphoma patients with tolerable adverse reactions. The EBV-DNA variation is correlated with clinical efficacy and prognosis.

P1317

The dynamic changes of proportion and function of MAIT cells in chronic HBV-infected patients

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MAIT (Mucosal-associated invariant T) cells are innate-like T cell subset that recognize microbial metabolites. Although they are abundant in human liver, the role of MAIT cells in chronic hepatitis B remains unclear. Here, we found that MAIT cells showed elevated killing effect against HBV plasmid-transfected hepatocytes, indicating they played a role in the control of viral replication. However, a significant reduction of circulating MAIT cells were observed in chronic HBV-infected patients, especially in acute-on-chronic liver failure (ACLF). In addition, MAIT cells in HBV-infected non-neoplastic tissue were significantly lower than those in normal HBV-negative hepatic tissue. The defect in MAIT cell frequency was not related to the degree of liver injury or HBV viral load, while the proportion of hepatic MAIT cells was negatively correlated with inflammation and fibrosis level in liver tissue. This suggested inflammation rather than HBV infection was responsible for the decrease of MAIT cells in the patients. The elevated apoptotic level and upregulation of CD69, HLA-DR, Fas Land PD-1 were observed in the patients, suggesting the activation induced cell death (AICD) of MAIT cells. Functionally, the IFN-γ producing capacity of MAIT cells were remained in CHB patients but defected in ACLF patients. Meanwhile, patients with higher level of liver damage showed lower responsiveness to specific TCR stimulation. Collectively, the divergent defects in frequency and function of MAIT cells are found in chronic HBV-infected patients in different disease progressions, which is related to the inflammation and liver injury followed by HBV infection.

P1318

Low dose of ursolic acid promotes anti-tumor effect in NKT cell-dependent manner

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The invariant NKT (iNKT) cells with relatively constant TCR composition are the main concern of NKT cell population in the current research, which is thought to play anti-tumor effects. Ursolic acid is a natural class of pentacyclic triterpenoids with anti-tumor effect in high concentration (50mg/kg/day, intravenous) in nude mice. However, whether low-dose ursolic acid could enhance anti-tumor immunity based on NKT cells is unclear. In this study, C57 BL/6J wild-type and CD1dKO mice (lacking NKT cells in vivo) mice were subcutaneously inoculated with B16 melanoma cells. Tumor growth and iNKT cell responses in PBS- or UA-treated mice was monitored. We found that UA treatment (15mg/kg/2days, intragastric gavage) remarkably reduced tumor growth rate and final tumor volume in C57 BL/6J wild-type mice, revealing the anti-tumor effect of low dose of UA. However, there was no

difference in tumor growth between PBS- and UA-treated tumor-bearing CD1dKO mice. These results revealed that NKT cells contributed to the anti-tumor activity of UA. Analysis of tumor-infiltrating cells showed that UA treatment increased iNKT cell ratio in tumor local. Moreover, UA-treated C57 BL/6J wild-type mice had significantly higher proportion of IFN-γ-producing iNKT cells and conventional T cells in tumor site. Notably, the upregulation of IFN-γ-producing T cells was not found in UA-treated CD1dKO tumor-bearing mice, indicating the augmented anti-tumor effects of conventional T-cell in UA-treated mice were NKT cell dependent. Collectively, our findings reveal that low-dose of ursolic acid has an NKT cell-dependent anti-tumor effect.
Keywords: iNKT cells, Ursolic Acid (UA), anti-tumor effects, tumor-bearing model

P1319

Cytokine-based generation of CD49a+Eomes-/+ natural killer cell subsets

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Recent studies have identified CD49a+Eomes- and CD49a+Eomes+ subsets of tissue-resident NK (trNK) cells in different organs of the mouse. However, the characteristics of CD49a+Eomes-/+ NK cell development and the regulation of Eomes expression in NK cells remain unclear. Here, we established an in vitro cytokine-based feeder-free system in which bone marrow progenitor cells differentiate into CD49a+ NK cells. IL-15 was identified as being the key cytokine in this system that supported the development and maintenance of CD49a+ NK cells. The CD49a+ NK cells generated were Eomes-CD49b- and shared the same phenotype as hepatic trNK cells. IL-4 induced the expression of Eomes in generated NK cells and converted them into CD49a+Eomes+ cells, which were phenotypically and functionally similar to uterine trNK cells. Moreover, the IL-4/STAT6 axis was identified as being important in the generation of CD49a+Eomes+ induced NK cells. Collectively, these studies describe an approach to generate CD49a+Eomes-/+ subsets of NK cells and demonstrate important roles for IL-15 and IL-4 in the differentiation of these cells. These findings have potential for developmental research underlying the generation of different subsets of NK cells and the application of adoptive NK cell transfer therapies.

P1320

N-acyl homoserine lactone hampers human NK cell viability via suppressing MAPK signalling pathway

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N-acyl homoserine lactones (AHLs) mediated Quorum sensing (QS) is a crucial phenomenon in Gram$^-$ bacteria that involved in human infection, during which progress the interaction of AHLs with host immune system (T cells, B cells, macrophages, DCs included) indicated their effective regulation towards bacterial invasion. We have found 3-oxodecanoly-homoserine lactone (ODHL), a specific type of AHL molecule, to be dominant in *Vibrio* species in our previous study, which boosted bacterial physiological functions. However, there's few relevant researches have focused on its regulation to human NK cells. This study revealed that ODHL could significantly suppress NK cell proliferation and induce cell apoptosis evidently *in vitro*. By further analyzing the expression and the phosphorylation level of major proteins in the ERK/MAPK and JNK/MAPK pathways after ODHL treatment, the total ERK1/2, ERK5 and JNK expression were suppressed, and the phosphorylation level of ERK2, JNK3

DOI: 10.1002/eji.201970400

and CREB were down-regulated with the inhibition of CREB being most remarkable. Our study demonstrated that *Vibrio*-derived ODHL could directly inhibit NK cell proliferation by blocking the initiation of ERK2 transcription, which might be partially regulated by the decline of phosphor-JNK induced apoptosis of human NK cells, and resulted in the impairment of NK cells viability. In addition, the results of this present study provide a novel potential treatment direction for the human infection of *Vibrio*.

P1321

Crosstalks between mTORC1 and mTORC2 variagate cytokine signaling to control NK maturation and effector function

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Natural killer (NK) cells are a critical component of innate immunity, and represent the first line of defense against tumor cells and viral infections. Figuring out the factors involved in their development and effector functions is of great importance. Recent studies reveal mTOR, which has a highly background-specific role in immunity, regulates the development and functions of NK cells, but the exact underlying mechanisms remain unclear. Here we show, via conditional deletion of Raptor (mTORC1) or Rictor (mTORC2), that mTORC1 and mTORC2 promote NK cell maturation in a cooperative and non-redundant manner, mainly by controlling the expression of Tbx21 and Eomes. Intriguingly, mTORC1 and mTORC2 regulate cytolytic function in an opposing way, exhibiting promoting and inhibitory effects on the anti-tumour ability and metabolism, respectively. mTORC1 sustains mTORC2 activity by maintaining CD122-mediated IL-15 signaling, whereas mTORC2 represses mTORC1-modulated NK cell effector functions by restraining STAT5-mediated SLC7A5 expression. These positive and negative crosstalks between mTORC1 and mTORC2 signalling thus variegate the magnitudes and kinetics of NK cell activation, and help define a paradigm for the modulation of NK maturation and effector functions. We consider our findings have the potential to provide a new strategy for targeting NK cells in antitumor therapy and for potentially improving NK-mediated viral clearance by repressing mTORC2 activation.This work has been published on *Nature Communications* recently (PMID: 30451838).

P1322

Ezh2 regulates differentiation and function of natural killer cells through histone methyltransferase activity

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DOI: 10.1002/eji.201970400

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Changes of histone modification status at critical lineage-specifying gene loci in multipotent precursors can influence cell fate commitment. The contribution of these epigenetic mechanisms to natural killer (NK) cell lineage determination from common lymphoid precursors is not understood. Here we investigate the impact of histone methylation repressive marks (H3 Lys27 trimethylation; H3K27^{me3}) on early NK cell differentiation. We demonstrate that selective loss of the histone-lysine *N*-methyltransferase Ezh2 (enhancer of zeste homolog 2) or inhibition of its enzymatic activity with small molecules unexpectedly increased generation of the IL-15 receptor (IL-15R) CD122^{+} NK precursors and mature NK progeny from both mouse and human hematopoietic stem and progenitor cells. Mechanistic studies revealed that enhanced NK cell expansion and cytotoxicity against tumor cells were associated with up-regulation of CD122 and the C-type lectin receptor NKG2D. Moreover, NKG2D deficiency diminished the positive effects of Ezh2 inhibitors on NK cell commitment. Identification of the contribution of Ezh2 to NK lineage specification and function reveals an epigenetic-based mechanism that regulates NK cell development and provides insight into the clinical application of Ezh2 inhibitors in NK-based cancer immunotherapies.

P1323

TRAF3IP3 at the trans-Golgi network regulates NKT2 maturation via MEK/ERK signaling pathway

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Thymic natural killer T (NKT)2 cells are a subset of invariant NKT cells with PLZF^{hi}GATA3^{hi}IL-4^{+}. The differentiation of NKT2 cells in the thymus is not fully understood. We report in the present study an important role of TRAF3-interacting protein 3 (TRAF3IP3) in the functional maturation and expansion of committed NKT2 cells in thymic medulla. Mice with T cell-specific deletion of *Traf3ip3* had decreased thymic NKT2 cells, decreased IL-4 producing peripheral iNKTs, and defects in response to α-galactosylceramide. Positive selection, early phase proliferation, high Egr2/PLZF expression in CD24^{+}CD44^{lo}, CD24^{-}CCR7^{+}CD44^{lo}, and CCR7^{-}CD44^{lo} immature iNKTs, and NKT1/NKT17 cell differentiation were not affected. Only CCR7^{-}CD44^{+}NK1.1^{-} iNKTs in *Traf3ip3* cKO mice showed reduced expression of Egr2, PLZF, IL-17RB, GATA3, SLAMF6, decreased proliferation, and decreased IL-4 production upon stimulation. Such Egr2 and IL-4 expression were augmented by MEK/ERK activation in iNKTs. TRAF3IP3 at the trans-Golgi network recruited MEK and facilitated ERK phosphorylation and nuclear translocation. LTβR-regulated bone marrow-derived nonlymphoid cells in medullary thymic microenvironment were required for this MEK/ERK activation and NKT2 maturation. These data demonstrate an important functional maturation process in thymic NKT2 differentiation that is regulated by MEK/ERK signaling at the trans-Golgi network.

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DOI: 10.1002/eji.201970400

P1324

Lipid phagocytosis by invariant natural killer T cells in murine non-alcoholic steatohepatitis models

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Invariant natural killer T (iNKT) cells recognize lipid antigens and represent a major hepatic population of innate immune cells. The roles for iNKT cells in nonalcoholic steatohepatitis (NASH) are unclear. Taking advantage of Cxcr6$^{Gfp/+}$ transgenic mice, we performed intravital confocal microscopy and investigated the mechanisms of iNKT cells in maintaining lipid balance in an animal model of methionine-choline-deficient (MCD) diet-induced NASH. We performed flow cytometry to examine the recruitment of and cytokine expression levels of iNKT cells. We also isolated GFP-positive iNKT cells from the livers of Cxcr6$^{Gfp/+}$ mice by FACS sorting and performed RNA-sequencing analysis. CXCR6^{+} iNKT cells were recruited to the liver and aggregated into small clusters within 3 days after MCD treatment. These iNKT cells lost their active migratory phenotype and interacted dynamically with Kupffer cells. Most aggregated iNKT cells engulfed lipids and displayed a distinct mRNA-transcription pattern. The recruitment and lipid-processing capabilities of iNKT cells were blocked by anti-CD1d antibodies. Hepatic triacylglycerol concentrations increased in iNKT-deficient *Cd1d*$^{-/-}$ mice. Depleting Kupffer cells with clodronate liposomes (CLLs) abolished iNKT cell recruitment and significantly enhanced hepatic TG levels in MCD-treated mice. Upon changing the MCD diet to a normal diet, the migration capacity of iNKT cells gradually recovered as hepatic triglyceride accumulation diminished. These results support that hepatic iNKT cells underwent changes in migratory behavior and gained phagocytic functions in NASH. Activated iNKT cells can phagocytose lipids and help clear them from the liver during early NASH.

P1325

NK cell maturation is controlled in a sequential way by Eomes and T-bet

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NK cells are innate lymphocytes that contribute to the early defense against intracellular pathogens and to the immunosurveillance of tumors. T-bet and Eomes are T-box transcription factors involved in the determination of NK cell lineage. Here, we show that ablating either T-bet or Eomes in mice impairs NK cell development at different maturation stages. The transition from the CD27+CD11b- to the CD27+CD11b+ stage is impaired in Eomes cKO mice, while the terminal maturation from the CD27+CD11b+ to the CD27-CD11b+ is compromised in T-bet KO mice. **Eomes and T-bet restrict proliferation and metabolic activity but maintain viability of NK cells at the corresponding stages**. This sequential role correlates with the pattern of nuclear Eomes and T-bet expression in NK cells, as measured by image cytometry. Moreover, T**-bet and Eomes repress each other's expression.** We performed ChIP-seq analysis of T-bet and Eomes DNA binding using novel strains of Eomes and T-bet tagged mice. This analysis showed a broad binding of both TF on open chromatin sites in NK cells, suggesting a competition of both factors for T-box motifs. **Collectively, our analysis suggests that Eomes and T-bet shape NK cell maturation in a sequential and mutually exclusive manner, rather than in a cooperative way.**

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DOI: 10.1002/eji.201970400

P1326

Chronic inflammation and bowel cancer; are your MAITs involved?

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Mucosal associated invariant T cells (MAIT cells) are a recently described innate-like T cell subset which comprise up to 5% of the circulating T cells within humans. These MR1 restricted T cells recognise non-peptide, small molecule antigens derived from microbial vitamin B synthesis. They are also enriched within the mucosal tissues in the body such as the gastro-intestinal tract. Upon activation, MAIT cells can rapidly produce cytokines and are believed to play a role in regulating the local immune milieu.
Cancers of the large bowel have the second highest mortally rates within Australia and these cancers respond poorly to conventional immune based therapy. Bowel cancers arise in a background of chronic inflammation, and the regulation of the immune milieu at the site of these mucosal tumours is currently poorly understood.
Whilst investigating the chronically stimulated MAIT cells potentially seen in bowel cancer we have identified a cytokine response rich in interleukin 13 (IL-13). This IL-13 MAIT cell response has not been previously described and has the potential to skew the local immune milieu away from anti-tumour inflammation and promote tumour growth and spread. This IL-13 response not only has the potential to impact cancer research, but also alter the research of a number of human inflammatory disorders.

P1909

The induction mechanisms of NK cells exhaustion in acute exacerbation of chronic obstructive pulmonary disease

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NK cells exhibit an exhausted status, displaying poor effector function and altered phenotype, under the settings of tumors and chronic infections. COPD is a chronic inflammatory lung syndrome. A candidate for this disease promulgation is a dysregulated innate and adaptive immune system, including natural killer (NK) cell function. The exhaustion of NK cells in acute exacerbation of COPD may lead to the progression and deterioration of the disease, but the mechanism is unclear. Therefore, this study aimed to analyze the characteristics and performances of NK cells exhaustion in COPD patients and the cellular and molecular mechanisms inducing NK cells exhaustion. Here, we showed that the number of NK cells in the peripheral blood with acute exacerbation of COPD was decreased. The expression of activating receptors (NKp30, NKG2D) on NK cells of AECOPD patients decreased and the inhibitory receptor (NKG2A) increased compared with NK cells of health control. Furthermore, NK cells of AECOPD patients produced decreased effector function molecular IFN-γ and TNF-α. Collectively, these results suggested that under the setting of AECOPD, NK cells exhibit an exhaustion status, displaying exhausted effector phenotypes and exhausted functions. Moreover, we will explore the inducing factors and reversal strategies of NK cells exhaustion in vivo and in vitro, which may provide theoretical basis for clinical screening of new drug targets blocking AECOPD.

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DOI: 10.1002/eji.201970400

P1910

The transcription factor PBX1 drives natural killer cells to promote fetal development

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The natural killer (NK) cell promotes fetal development by secreting growth-promoting factors (GPFs) at the maternal-fetal interface during early pregnancy. However, the functional subsets and transcriptional regulation mechanisms of NK cells expressing GPFs are unclear. Here, we have identified CD49a^{+}PBX1^{+} NK cells as a unique subset in which the transcription factor PBX1 upregulated the expression of the GPFs, pleiotrophin and osteoglycin. After *Pbx1* is knocked down, decidual NK cells shown a serious decline in the ability of expressing these GPFs. The results of ChIP experiments with anti-PBX1 antibody in decidual NK cells shown that PBX1 regulates the transcription of *GPFs* genes *via* direct binding. The results of exome sequencing of decidual NK cells from patients with unexplained recurrent spontaneous abortion (URSA), revealed decreased expression of PBX1 or *PBX1^{G21S}* mutation correlated with fetal growth restriction and pregnancy failure, suggesting that the impaired *PBX1* gene in decidual NK cells is a potential biomarker for URSA. Further, conditional inactivation of *Pbx1* mice by *Vav1-Cre* and *Ncr1-Cre* were employed to demonstrate the role of PBX1 in NK cells promoting fetal development. Inactivation of *Pbx1* in mouse NK cells caused impaired fetal development due to a significantly decrease in the expression of GPFs. Our study revealed that the transcriptional regulation mechanism in NK cells at the maternal-fetal interface, identified the physiological functions of CD49a^{+}PBX1^{+} NK cells, and suggested that impaired CD49a^{+}PBX1^{+} NK cells contribute to the etiology of URSA, and provided the basis for improved approaches to NK cell-based immunotherapy for pregnancy related diseases.

P1911

Potential contribution of the peripheral blood Natural Killer cells to the Immunopathogenesis of Crohn disease

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Background: Natural Killer (NK) cells have the potential to protect the host from cancer and viral infections as well as to kill body's own cells and cause tissue destruction and promote inflammation. Their potential role in the immunopathogenesis of Crohn disease, a chronic inflammatory disease affecting gastro-intestinal tract in children and adolescents, remains unknown.
Hypothesis: NK cells from CD patients are more activated and cytotoxic as compared with their age-matched healthy controls.
Methods: The percentages and numbers of NK cells and their subsets, their expression of KIR and non-KIR receptors, activation markers and gut-homing receptors as well as their cytotoxic potential were compared between treatment-naïve, under-treatment CD patients and healthy controls using multicolor flow cytometry.
Results: NK cells from CD patients expressed higher levels of activating KIR and other non-KIR activating receptors vis-à-vis healthy controls whereas the expression of several inhibitory, KIR and non-KIR, receptors tended to decrease. The cells from the patients expressed higher levels of CD69, CX3CR1, NKG2D and IL-23R. They also expressed increased levels of different gut-homing integrins (CD103, VLA-4 and β-7). Furthermore, the cells also showed a higher expression of CD107a constitutively as well as after stimulation with K562 and higher cytotoxic activity as well. Furthermore, treatment of the patients with different drugs and biologics tended to normalize the expression of these biomarkers.

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DOI: 10.1002/eji.201970400

Conclusion: Peripheral blood NK cells from Crohn disease patients are highly activated and show enhanced cytotoxic potential. They may serve as targets for developing novel drugs.
Supported from Crohn & Colitis of Canada

P1912

Commitment to natural killer cells requires transcription factor PBX1 to up-regulating NFIL3

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Objective: The transcription factor Nfil3 (also known as E4BP4) is essential for common lymphoid progenitors (CLPs) differentiation into NK cell precursors. It has been reported that Nfil3 promotes NK cell development by upregulating Eomes and Id2. However, the upstream transcriptional regulation mechanism of Nfil3 is unclear.
Result: Here, we identify a transcription factor PBX1 (Pre-B-cell leukemia transcription factor 1) that can directly promote the expression of Nfil3 gene. Consistent with Nfil3-/- mice, the proportion and number of NK cell precursors decreased significantly in the BM of Pbx1f/f;Vav1cre mice. Furthermore, expression of Nfil3 and key downstream genes (Eomes and Ifn-γ) were reduced in spleen and BM NK cells of PBX1 conditional knockout mice. We demonstrated that PBX1 directly binds to the promoter of Nfil3 gene, promoting the transcription. In addition, we show that when lacking the homeobox domain, PBX1 localized in the cytoplasm instead of the nucleus. Single amino acid substitution experiments revealed that the PBX1 binding to the promoter region of Nfil3 gene is dependent on three key amino acid sites.
Conclusion: These findings not only identify PBX1 as a key transcription factor that regulates Nfil3, but also enrich our understanding of the transcriptional regulatory mechanisms that regulate NK cell commitment and contribute to programed differentiated NK cells in vitro.

P1913

Synergistic effect of IL-15 and IL-27 on the cytotoxity of NK-92 cells

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Advances in NK cell immunotherapy has led to several promising options for cancer patients. To date, it is unclear how IL-15 and IL-27 regulates the antitumor function of NK-92 cells. Our data showed the appropriate concentration of IL-15 and IL-27 could significantly enhance the cytotoxity of NK-92 cells to target cells. So, NK-92 cells were modified with HIL-15 by lentivirus infection. Meanwhile, IL-27 promoted the expression of NKG2D, NKp30 and NKp46 on HIL-15NK92 cells, improved the secretion of perforin by HIL-15NK-92 cells, which correlated with the up-regulation of STAT1, STAT3 and STAT5 phosphorylation levels in JAK-STAT pathway. We also identified that IL-27 enhanced the secretion of IL-15 by HIL-15NK92 cells, promoted their migration and inhibited their proliferation and apoptosis. When IL-27 and HIL-15NK92 cells were transferred into human prostate cancer xenograft models, the survival time of mice could significantly prolong, and volume of tumor was decreased. These results demonstrate that IL-15 and IL-27 enhance NK92 cell killing to human prostate cancer cells in vivo and in vitro and thus provided the basis for combination with IL-15 and IL-27 as a novel strategy for enhancing the therapeutic efficacy of NK-92 cells for solid tumor.
Keywords: IL-15, IL-27, NK-92 cells, JAK-STAT, Anti-tumor

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DOI: 10.1002/eji.201970400

P1914

STAT3 directly regulates NKp46 transcription in NK cells of HBeAg-negative CHB patients

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Background: NK cells play an important role in early control of HBV infection. The function of NK cells is inhibited in chronic hepatitis B virus (CHB) infection, although the underlying mechanism remains unknown.
Aims: To clarify the role of intrinsic STAT3 in the NK cells of CHB patients.
Methods: NK cells from HBeAg$^-$CHB patients were analyzed by FACS. STAT3 was interfered by shRNA or overexpression vector. ChIP and sequence were performed to detect the binding of STAT3 on the promoter of NKp46.
Results: The expression of STAT3 decreased in peripheral NK cells of CHB patients, and was associated with low levels of degranulation and IFN-γ secretion. In addition, STAT3 levels were positively correlated with cytolysis-associated molecules and antiviral cytokines, such as CD107a, granzyme B, perforin and IFN-γ. HBsAg directly inhibited the expression and activation of STAT3 in NK cells, and knocking down STAT3 expression in NK cells inhibited proliferation, decreased cyclin d1 levels, and suppressed responsiveness to IL-21 stimulation. Furthermore, STAT3 directly bound to the promoter of NKp46, an important activating receptor of NK cells, to regulate its transcription and expression.
Conclusions: Our findings indicate that STAT3 is an important positive regulator of NK cells, and provide a new mechanism of NK cell dysfunction in CHB.
Key words: CHB; natural killer cells; STAT3; NKp46

P1915

Stochastic neighbor embedding analysis of immune checkpoint molecules in circulating lymphocytes in cervical cancer: rare populations of PD-1$^+$CD56^{bright} NK cells and PD-1^{high}Tim-3$^+$TIGIT$^+$ T cells found

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Compared with other cancers, relatively few studies have assessed immune checkpoint expression in cervical cancer. Here, we analyzed the expression of checkpoint molecules PD-1, Tim-3 and TIGIT in NK and T cell populations in the blood of patients with cervical cancer and healthy controls. We found these molecules expressed on peripheral blood T cells as well as CD56^{dim} and CD56^{bright} NK cells in both groups analyzed. Using a stochastic neighbor embedding analysis of 8 color cytometry, we observed interesting populations co-expressing the checkpoint molecules, most notably: PD-1$^+$Tim-3$^+$TIGIT$^+$ T cells, PD-1$^+$NKG2D$^-$ T cells and PD-1$^+$TIGIT$^+$Tim-3$^+$CD56^{bright} NK cells. These cells with an apparently "exhausted" phenotype were significantly augmented in cervical cancer patients. Furthermore, we saw different PD-1 expression levels (PD1^{int}, PD-1^{high}) on T cells. The percentages of these populations and their subsets co-expressing TIGIT and Tim-3 were also significantly higher in

DOI: 10.1002/eji.201970400

cervical cancer patients. In conclusions, these results collectively suggest that the presence of T cells and CD56^{bright} NK cells co-expressing different exhaustion or ex-cytotoxic markers might explain immune escape in cervical cancer. Although it is necessary to perform functional experiments and investigate the expression of these molecules in the tumor infiltrating NK and T cells, our current findings might provide a global overview of populations important in these tumors, and potential therapeutic targets.

P1916

The effect of tuberculosis (TB), HIV and HIV-TB on mucosal-associated invariant T (MAIT) cells in humans

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MAIT cells are non-classical T lymphocytes that recognize and rapidly respond to microbial vitamin B metabolites and have the capacity to kill bacteria-infected cells. Circulating MAIT cell numbers decrease in patients with active TB and HIV, but previous findings regarding functional changes have been conflicting.
We conducted a cross sectional study to assess the effect of HIV, TB and HIV-associated TB (HIV-TB) on MAIT cell numbers, activation, inhibitory and functional profile in a high TB endemic setting in South Africa. Blood was collected from healthy controls (**HC, n=26**), individuals with active TB only (**aTB, n=30**), with HIV infection only (**HIV, n=30**) and with **HIV-TB (n=26)**. All TB participants were newly diagnosed with TB and sampled prior to treatment. Peripheral blood mononuclear cells (PBMC) were isolated and stimulated with BCG expressing GFP (BCG-GFP) and heat-killed (HK) *Mycobacterium tuberculosis (Mtb)* and analyzed using flow cytometry.
Compared to HC, there were lower frequencies of MAIT cells in individuals with HIV, and aTB. There were higher proportions of activated MAIT cells (measured by HLA-DR expression) in aTB and HIV-TB in response to BCG and HK-*M.tb* stimulation. aTB and HIV-TB were associated with both lower MAIT cell degranulation (CD107a expression) and IFN-g expression. MAIT cells from those with aTB had upregulated PD-1 expression.
Our data show that compared to healthy controls and individuals with HIV infection only, aTB and HIV-TB result in a decrease in circulating MAIT cells, a significant increase in activation and inhibitory status, and an impaired mycobacteria-induced functional profile.

P1917

Association of KIR2DL5 in chronic HBV infection

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Hepatitis B virus (HBV) infection is still a major cause of developing cirrhosis, HCC and liver failure. A large number of studies showed that HBV infection can lead to the dysfunction of immune cells, in particular, NK cells. As one of the important cell types involved in antiviral infection, NK function is mediated by their inhibitory and activating receptors i.e. NKG and KIRs. At the same time, there are also research found that the expression of KIR in NK cells is related to some viral infectious diseases. We compared the expression frequency and the functionality of NKGs and KIRs of PBMC and liver perfusate in HBV infected and uninfected liver transplant patients. Our study demonstrates that the NK cells with high expression NKG2A,2D,2C are rich in the liver. Further to that, we found the frequency

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DOI: 10.1002/eji.201970400

of inhibitory receptor KIR2DL5 is significantly increased in HBV+ patients compared with HBV-controls (79.2 vs 52.1%; odds ratio (OR) = 2.50; 95% CI 1.06, 5.91; P = 0.034), this indicates the association between 2DL5 and risks of HBV infection. Interestingly, the carrier frequency of KIR2DL5 was also significantly increased in hepatocellular carcinoma (HCC) patients compared with controls (82.4 vs 62.3%; odds ratio (OR) = 2.82; 95% CI 1.04, 7.6; P = 0.036). However, as HBV infection is a main cause of HCC, the correlations between KIR2DL5 and risks of HCC may be related to HBV. Our study suggest KIR2DL5 may play a potential role in the pathogenesis of HBV infection.

P1918

B cell leukemia/lymphoma 10 (BCL-10) promotes angiogenesis in an experimental corneal neovascularization model

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Purpose: Corneal neovascularization (CrNV) arises from many causes including corneal inflammatory, infectious, or traumatic insult and frequently leads to impaired vision. It is necessary to develop effective measures to prevent and treat CrNV based on understanding of its pathogenesis at the cellular and molecular levels. This study seeks to determine the role of B cell leukemia/lymphoma 10 (BCL-10) in the development of experimental CrNV.
Methods: Corneas from BCL-10 knockout (KO) mice and wild-type (WT) mice were burned by sodium hydroxide (NaOH) to create the CrNV model and neovascular formation in the corneas was assessed 2 weeks later. Intracorneal macrophage accumulation and the expression of angiogenic factors were quantified by flow cytometric analysis (FCM) and real-time PCR, respectively.
Results: The number of CrNV was determined 2 weeks after alkali burn. Compared to WT mice, the number of CrNV in BCL-10 KO mice was significantly decreased. FCM revealed that F4/80-positive macrophages were markedly decreased in BCL-10 KO mice compared with WT mice. RT-PCR showed that the mRNA expression levels of intracorneal vascular endothelial growth factor-A (VEGF-A), basic fibroblast growth factor (bFGF) and monocyte chemotactic protein 1 were also reduced obviously in BCL-10 KO mice compared with WT mice.
Conclusion: BCL-10 KO mice exhibited reduced alkali-induced CrNV by suppressing intracorneal macrophage infiltration, which subsequently led to decreased VEGF-A and bFGF expression, suggesting that BCL-10 may be an important target of anti-angiogenic intervention for CrNV in clinical settings.

DOI: 10.1002/eji.201970400

P1919

NKG2C$^+$ NK cells demonstrate better activation and proliferative response to *in vitro* stimulation

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Expansion of CD57$^+$NKG2C$^+$ NK cells in peripheral blood was observed during cytomegalovirus infection. This HCMV-associated subset of memory-like NK cells is not typical for innate immune cells. In this study we characterized NKG2C$^+$ NK cells on distinct stages of the differentiation by measuring the HLA-DR expression, proliferative activity and lifespan.
CD57$^{-/+}$NKG2C$^{-/+}$ NK cell subpopulations and single cells were stimulated IL-2 and K562 feeder cells expressing membrane-bound IL-21 (K562-mbIL21). The phenotype and proliferative potential of the cells were assessed using flow cytometry. Higher levels of proliferative activity measured using the vital CFSE dye were shown for less differentiated CD57$^-$ cells compared to CD57$^+$ NK cells. In bulk cultures and in NK cell clones, NKG2C$^+$ cells proliferated more actively and had a longer lifespan compared to cells from NKG2C$^-$ subset. In addition, we evaluated the surface expression of HLA-DR, known as a marker of NK cell activation. During cultivation, both NK cell subpopulations and clones derived from NKG2C$^+$ NK cells had a statistically higher levels of HLA-DR compared to NKG2C$^-$ ones. Thus, NKG2C expression in NK cells is associated with the increased level of cellular activation, higher proliferative capacity and longer lifespan.

The work was supported by Russian Science Foundation, grant #19-15-00439.

P2599

CD49a regulates the function of human decidual natural killer cells

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CD49a, a member of an integrin family, plays important roles in innate and adaptive immune-mediated responses as well as in inflammation, regulation of growth, differentiation, and liver cirrhosis. However, the role of CD49a on human natural killer (NK) cells is largely unknown. Here, we report that decidual NK（dNK）cells from human patients who underwent recurrent spontaneous abortions had lower levels of CD49a and increased perforin, granzyme B, and IFN-γ expression, when compared to dNK cells from age-matched healthy controls. Perforin, granzyme B, and IFN-γ expression levels in dNK cells were upregulated, while the migration and adhesion of dNK cells were downregulated by CD49a-neutralizing antibody. By the ^{51}Cr release assay, the killing activity of dNK cells also increased with CD49a neutralizing antibody. Further, lnc-49a, a newly identified long non-coding RNA, was shown to be a positive regulator of CD49a in primary human NK cells. Taken together, our data suggested that CD49a is involved in the regulation of human NK cells functions, including cytotoxic activity, migration, and adhesion. Further, lnc-49a is a positive regulator of CD49a in human primary dNK cells.

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DOI: 10.1002/eji.201970400

P2600

Herceptin enhances the antitumor effect of natural killer cells on breast cancer cells expressing human epidermal growth factor receptor-2

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NK cells are important effectors of the innate immune system and have the unique ability to directly lyse transformed or virally infected cells without pre-sensitization or MHC-like restriction, they are considered potential candidates for cancer immunotherapy. It is suitable as a tool for optimal ACT. However, NK cell activity is significantly reduced in all clinical stages of breast cancer patients. Herceptin is an anti-human epidermal growth factor receptor 2 monoclonal antibody (anti-her2) for the treatment of Her2+ breast cancer, but with a certain risk of cardiotoxicity. Since Herceptin can increase the cytotoxicity of lymphocytes, studying Herceptin may increase the cytotoxicity of NK cells to Her2+ breast cancer cells. Our results indicated that binding of NK cells to Herceptin can affect NK cell activity under IL-2 and IL-15 stimulation. Herceptin promotes NK cell proliferation and enhances the toxicity of NK cells to Her2+ breast cancer cells, and its degranulation effect is enhanced, and the amount of IFN-γ secretion is also significantly increased. At the same time, Herceptin promotes the migration and adhesion of NK cells to Her2+ breast cancer cells, but does not affect the expression of surface receptors such as NKP30, NKG2D and DNAM-1 on NK cells. Clinical studies have demonstrated that Herceptin-treated NK cells shrink metastatic lung nodules in Her2+ breast cancer patients who are unable to tolerate Herceptin cardiotoxic side effects, supporting its therapeutic potential in patients with Her2+ and Herceptin intolerance to breast cancer.

P2601

Hyperactivated peripheral invariant natural killer T cells correlate with the progression of HBV-relative liver cirrhosis

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Invariant NKT (iNKT) cells express markers of both T and NK cells, and may produce various cytokines to regulate liver immunity. However, the role of iNKT cells in the progression of HBV-relative liver cirrhosis (HBV-LC) is incompletely understood. Here, we investigated the impact of peripheral iNKT cells on a cohort of patients with HBV-LC. The frequency, number, activation status, apoptosis, and proliferation ability of peripheral iNKT cells were detected with flow cytometry. The impact of peripheral iNKT cells on the proliferation of hepatocyte and activation of hepatic stellate cell was detected with flow cytometry and PCR. In HBV-LC patients, the frequency and absolute number of peripheral iNKT cells significantly reduced, but the expression levels of CD25, interleukin (IL)-4, IL-13, and interferon (IFN)-γ increased. No difference was observed in the proliferation and apoptosis of circulating iNKT cells between patients and healthy controls (HCs). CXCR6 (CD186), known to be closely associated with iNKT cells migration from the periphery to the liver, was highly expressed on peripheral iNKT cells in HBV-LC patients. Furthermore, peripheral iNKT cells had a profound impact on hepatocyte proliferation and hepatic stellate cell activation through IL-4 or IL-13. Our data suggest that in HBV-LC patients, highly activated peripheral iNKT cells may migrate to the liver and affect hepatocyte proliferation and hepatic stellate cell activation through the expression of type 2 cytokines, which may result in excessive healing and contributing to the progression of fibrosis toward cirrhosis in liver.

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DOI: 10.1002/eji.201970400

P2602

Etoposide-induced protein 2.4 regulates the development and IFN-γ production of iNKT cells

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Invariant natural killer T (iNKT) cells are innate-like T cells that recognize lipid antigens presented by CD1d molecule. It has been reported that iNKT cells play important roles in regulating progression of multiple diseases. However, the molecular mechanisms that regulate iNKT cell development and functions are not fully understood. Here, we report that Etoposide-induced protein 2.4 (Ei24) is an important regulator for the development and function of iNKT cells. Using Cre/loxP system to delete Ei24 in T cells, we found that Ei24 was required for terminal maturation of iNKT cells and their IFNg production.

P2603

Study on different characteristics between CD161+NKT cells and CD161-NKT cells

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To investigate the differences in frequency, subpopulation, phenotype and expression of cytokines between CD161+ NKT cells and CD161- NKT cells in normal blood, so as to further understand the role of NKT cells in the immune response, we isolated peripheral blood mononuclear cells from normal person, and detected expressions of CD4, CD8, CD45RO, CD62L and CCR7 on surface of NKT cells. After stimulation with polyclonal stimulus, productions of IFN-γ and IL-4 by different NKT subpopulations were determined. Our datas uncovered that the average frequency of CD161+ NKT cells was 31.8%, CD161+ NKT cells and CD161- NKT cells were both mainly divided into three subgroups: CD4+, CD8+ and CD4-CD8-. The frequency of CD8+ NKT cells in CD161+ NKT cells was lower than that in CD161-NKT cells, and the frequency of CD4-CD8- NKT cells in CD161+ NKT cells was much higher than that in CD161- NKT cells, much more CD161+ NKT cells than CD161- NKT cells expressed memory molecule CD45RO. CD161+ NKT cells produced significantly higher amounts of IFN-γ and IL-4 than CD161- NKT cells, and IFN-γ and IL-4 cells were mainly produced by memory NKT cells. In conclusion, CD161 defines different NKT cell subsets, and it is speculated that CD161+ NKT cells than CD161- NKT cells play different biological functions in the process of immune response.
Keywords: CD161, NKT cells, Subpopulation, Phenotype, Cytokine.

DOI: 10.1002/eji.201970400

P2604

Stage-specific requirement of mTOR signaling pathway for NK cells development and activation

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The mammalian target of rapamycin (mTOR) signaling pathway is the central checkpoint molecule in the regulation of cell metabolism. It is needed to investigate how mTOR signaling pathway spatiotemporally orchestrates NK cells development and effector function. In this study, we used genetic models to delete mTOR signaling pathway molecules, including PDK1, TSC1, Raptor, or Rictor at respective developmental stages. We found that hematopoietic-specific deletion of PDK1 or TSC1 [TB1] caused a marked decrease in the number of NK cells and compromises rejection of "missing-self" hematopoietic tumors. PDK1-dependent metabolic signaling functions as a key regulator for IL-15 signaling to stimulate E4BP4 expression, which in turn regulates CD122 expression and confers IL-15 responsiveness. Thus, we identify a positive feedback loop explaining how IL-15 maintains its responsiveness during early NK cell differentiation by triggering metabolic signaling. Terminal-specific deletion of PDK1 decreased amounts of the cytokine IFN-g, and reduced degranulation in response to tumor cells. By individual or combined deletion of the Raptor or Rictor at various stages of NK cell differentiation, we revealed that mTOR is essential for the early NK cell development. Moreover, dissociation of mTORC1 severely compromised NK cell IFN-g production and degranulation likely by disrupting NK cell conjugate formation. Intriguingly, mTORC2 was inhibitory for NK cell function in the absence of raptor. Taken together, our data provide genetic evidence systematically dissecting the distinct roles of mTOR signaling pathway for NK cell development and effector function.

P2605

Prevalence of epstein barr virus infection and characteristics of lymphocyte subsets in newly onset juvenile dermatomyositis

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The underlying etiology of Juvenile dermatomyositis (JDM) is unknown. T cell deficiency as well as EBV infection has been suspected to be involvement in the pathogenesis, but it has been poorly evaluated in JDM patients. This study describes the traits of lymphocyte subpopulation in newly onset JDM patients and compares the incidence of EBV infection in JDM patients with match controls. Newly developed JDM patients from 2014-2018 were collected. Lymphocytes (CD3、CD4、CD8、CD19 and CD56) were tested with flow cytometry in the first admission or after 6 months of treatment. Statistical analysis was conducted to compare the EBV infection in the group of JDM patients and controls.Results showed thatJDM patients had higher positive rate of EBNA-IgG ($p<0.0001$) as well as EBVCA- IgG ($p<0.05$) than normal controls. CD3-CD16+CD56+ lymphocyte was found extremely low in early stage of JDM patients, but increased after 6 months of treatment ($p=0.0091$). The percentage of CD8+ T lymphocytes in early onset JDM patients (26.02±7.54%) were found in normal range according to the reference value. In conclusion, level of CD3-CD16+CD56+ cells may associate with the clinical course of JDM. Early stage JDM patients had higher incidence of EBV infection than normal controls which suggested that EBV may play a role in the pathogenesis of JDM.

DOI: 10.1002/eji.201970400

P2606

Interleukin-33 activates and recruits natural killer cells to inhibit cancer pulmonary metastasis

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Increasing evidence suggests that IL -33 plays an important role in regulating tumor development. Higher expression of IL33 gene indicates longer overall survival of breast cancer patients, however, the mechanisms by which IL-33 modulates tumor metastasis remain unclear. Here, we report that IL-33 administration can effectively inhibit the pulmonary metastasis in a 4T1 breast cancer model. In our model, on the one hand, IL-33 promotes the production of tumor necrosis factor (TNF) -α by macrophages, which increases IL-33 specific receptor (ST2) expression on natural killer (NK) cells and is pivotal in IL-33-induced NK cell activation. On the other hand, IL-33 treatment facilitates the production of CCL5 by eosinophils and CD8+ T cells in the lung, which mediates the recruitment of NK cells to the tumor microenvironment. Thus, IL-33 induces systemic activation and local recruitment of NK cells that results in potent tumor rejection in the lung. Our study reports a novel mechanism for the IL-33-meditated suppression of metastatic cancer and provides potential therapeutic strategies for targeting tumor metastasis.

Macrophages and Dendritic Cells

O081

Integration of immune stimuli in macrophages expands the range of activation states and identifies targets for functional reprogramming in cancer

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Macrophages adopt a range of activation states in response to environmental signals. However, it remains unclear how complex combinations of stimuli, often with opposing biological properties, are computed into specific transcriptional outputs. Dissecting the underlying principles may provide therapeutic strategies to manipulate macrophage functions in diseases such as cancer.
We show that macrophages can integrate immune stimulatory and suppressive signals at the single-cell level, in fact acquiring emerging functional states. These programs entail selective transcriptional cross-antagonisms, leading to defective expression of key inflammatory genes in co-stimulated macrophages. We identified specific features of genes vulnerable to antagonism: they had non-permissive promoters and enhancers, were poorly expressed in untreated cells, were induced with slow kinetics upon stimulation and required chromatin remodeling by Brd4 and CBP/p300. Accordingly, antagonized genes were targeted by anti-inflammatory signals able to interfere with inducible deposition of histone acetylation at these loci. Our data identify a novel mechanism of action of PGE_2, a potent suppressor of mouse and human macrophage activation. Manipulation of PGE_2-cAMP signaling in vivo displayed therapeutic potential and favored immune-mediated control in a model of pancreatic cancer.
These unpublished data indicate that differences in basal chromatin organization link transcriptional dynamics of inflammatory genes to resistance or vulnerability to antagonistic signals, and that selective inhibition of chromatin remodeling is a general mechanism of immune regulation. Our study

DOI: 10.1002/eji.201970400

may inform novel cell/gene therapy approaches for functional reprogramming of tumor-associated macrophages.

Ostuni et al., ***Cell 2013***
Piccolo et al. ***Nat. Immunol 2017***
Ostuni, Natoli ***Nat. Immunol 2019***

O082

YAP aggravates IBD via regulating M1/M2 macrophage polarization and gut microbial homeostasis

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Inflammation, epithelial cell regeneration, macrophage polarization, as well as gut microbial homeostasis are critical for the pathological process of inflammatory bowel disease (IBD). YAP is a key component in the Hippo pathway, which was recently suggested to promote epithelial cell regeneration for IBD recovery. However, it is unclear how YAP regulates macrophage polarization, inflammation, and gut microbial homeostasis. Different from the promotion of epithelial regeneration by YAP and benefits for IBD, our findings have revealed that a new function of YAP in macrophages is to aggravate IBD. Moreover, we have identified that YAP impaired IL-4/IL-13-induced M2 macrophage polarization but also promoted LPS/IFNγ-triggered M1 macrophage activation for IL-6 production. Furthermore, we demonstrate that YAP expression was increased in mouse models and that there was a positive correlation between YAP expression and IL-6 production in IBD and colorectal cancer patients. In addition, YAP expression was differently regulated during the induction of M2 vs. M1 macrophages, which was correlated with the production of antimicrobial peptides as well as gut microbial homeostasis. Therefore, fully understanding the multiple functions of YAP in different types of cells is crucial for IBD therapy.

O083

Beta2-integrins restrict dendritic cell migratory phenotype through MRTFA/SRF signaling and a mechanically regulated epigenetic mechanism

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Dendritic cells (DCs) are the classic antigen presenting cells of the immune system. DCs switch from an adhesive, phagocytic phenotype to a migratory phenotype in response to stimuli such as LPS [1] but also through unknown stimuli, resulting in migration to lymph nodes. We have found that beta2-integrins restrict the migratory phenotype of DCs and their ability to induce Th1 immune responses [2]. We used a beta2-integrin knock-in mouse model, which lacks the beta2-integrin-kindlin-3 interaction (TTT/AAA-beta2-integrin KI mice) and thus has non-functional beta2-integrins, to investigate how

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DOI: 10.1002/eji.201970400

beta2-integrins restrict DC function. Interestingly, we found that beta2-integrin KI BMDCs displayed reduced adhesion and traction force generation but increased 3D migration speed *in vitro*. We show that RhoA activation and F-actin polymerization is abolished in beta2-integrin KI DCs, which leads to a failure of MRTF-A transcription factor to localize to the cell nucleus to activate genes together with SRF. The integrin/RhoA/MRTFA/SRF pathway regulates DC gene expression, adhesion and traction forces, but not 3D cell migration.
KI DCs also display a global increase in histone methylation (H3K4me3, characteristic of active genes and H3K27me3, characteristic of repressed genes). Histone methylation is regulated by beta2-integrins through an actin- and lamin A/C-dependent mechanism, and targeting this mechanical link leads to increased DC histone methylation, maturation and migration. Furthermore, utilizing B16-OVA and B16-F10 melanoma models, we show that DCs expressing dysfunctional integrins induced increased tumour rejection *in vivo*. Thus, beta2-integrin-mediated adhesion restricts DC migration and DC-mediated tumor rejection through MRTFA/SRF signalling and a mechanically regulated epigenetic mechanism.

O084

The role and mechanism of Cbl-b/c-Cbl in regulation of inflammation in alveolar macrophages

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E3 ubiquitin ligases play important roles in innate and adaptive immune regulation. The Cbl family of E3 ubiquitin ligases have three main members in mammals: c-Cbl, Cbl-b and Cbl-c, especially c-Cbl and Cbl-b are principally expressed in the hematopoietic system. So far lots of evidence has been proved that the two molecules play important roles in lymphocyte development and activation. Macrophages are the most malleable immune cells in the hematopoietic system, they exist in all tissues with extremely strong functional diversity. They play important roles in body development, self-stabilization, damage repair and immune response. In order to explore whether the Cbl-b and c-Cbl are involved in regulating macrophage function, we established LysMCre Cbl-b-/-c-Cblf/f (dKO) mouse model. Interestingly, two weeks old dKO mice began to grow slowly, and all dKO mice died around five weeks. H&E staining showed that the lung structure was fateful damaged in dKO mice, accompanied by a large number of inflammatory cells invasion. Careful studies show that c-Cbl inhibits the differentiation and proliferation of macrophages by directly down-regulating the protein expression of M-CSFR and ubiquitin modification of PU.1 to further inhibit the mRNA expression of M-CSFR. And c-Cbl and Cbl-b both regulate the inflammation of macrophages through ubiquitination of Lyn, and lung inflammation can be reduced by Lyn inhibitor (AZD0530) in vivo. These results enrich the molecular mechanism of macrophages differentiation and development, and provide new clues for the treatment of autoimmune diseases caused by the abnormal increase of macrophages.

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DOI: 10.1002/eji.201970400

O085

Regulation of lung dendritic cell homeostasis by Epstein-Barr virus induced gene 2 (EBI2)

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Epstein-Barr virus-induced gene 2 (EBI2), is a chemotactic receptor, highly expressed by immune cells. Upon activation by its ligand, 7α,25-dihydroxycholesterol (7α,25-OHC), EBI2 activates downstream signals inducing a wide range of functional responses, including inflammation, localization and proliferation. For dendritic cells, earlier work shows that EBI2 is critical for the generation and correct positioning of splenic DCs and the initiation of subsequent immune responses. Whether EBI2 has a role in localization and function of DCs in the murine lung has not been investigated. To understand its role for lung DC homeostasis and development we analyzed the phenotypic differences in lung dendritic cell populations in WT and EBI2 KO animals using a novel high dimensional phenotyping strategy. We found that absence of EBI2 resulted in dramatically decreased percentage and number in lung resident $CD11b^+$ DCs due to decreased *in situ* proliferation as measured by Ki67 expression. In contrast, no effect loss of EBI2 was seen on bone marrow resident DC progenitors. These findings demonstrated that loss of EBI2 disrupts lung DC homeostasis and reveals EBI2 as a crucial regulator of peripheral organ resident DC homeostasis.

O086

Tumor cells induce LAMP2a expression in tumor-associated macrophage for cancer progression

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Tumor cells benefit from tumor-associated macrophages (TAMs) promoting tumor growth and modulating functions of other cells in tumor microenvironment (TME). However, how tumor cells regulate the property of TAMs during tumor invasion remains to be defined. Here, we report that lysosome associated membrane protein type 2A (LAMP2a) is upregulated in TAMs by TME factors and important for tumor progression. Overexpression of LAMP2a in TAMs, but not in tumor cells, is associated with poor prognosis in breast cancer. LAMP2a-inactivation induced by either shRNA or CRISPR/Cas9 prevents TAM activation and tumor growth *in vivo*. Further we show that LAMP2a specifically targets PRDX1 (peroxiredoxin 1) and CRTC1 (CREB-regulated transcription coactivator 1) through lysosomal degradation to activate macrophage pro-tumorigenic polarization. Thus, our study suggests that tumor cells modulate TAMs activation and function to promote tumor progression through LAMP2a-PRDX1/CRTC1 axis, which partly illustrate the molecular mechanism of TAMs' multi-functional and bi-directional activation.

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DOI: 10.1002/eji.201970400

O087

Involvement of reticulon 1A in cutaneous immunity

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Reticulon 1A (RTN1A) is an endoplasmic reticulum-associated protein and besides its expression in neuronal tissues, a marker for cells of the dendritic lineage, including epidermal Langerhans cells (LCs) and dermal dendritic cells (DCs) in humans. Expression of RTN1A before the appearance of LC markers during skin development suggests that it may play a role in the LC differentiation/migration process. To obtain prenatal human skin is challenging, therefore studies were continued in wild-type C57BL/6 mice to unravel the role of RTN1A in cutaneous physiology. Intriguingly, CD207$^+$MHC class II$^+$ LCs but not dermal DCs expressed RTN1A in adult murine skin. RTN1A$^+$ cells were also identified in MHC class II$^+$ cells of the thymus, spleen and lymph node. Ontogeny studies revealed that expression of RTN1A protein preceded expression of LC markers such as MHC class II and CD207 on CD45$^+$ LC precursors in fetal and postnatal murine skin. Neither dermal DCs nor T cell precursors in fetal and postnatal murine skin expressed RTN1A. Further, immunostaining showed co-localisation of RTN1A with the neurofilament M/H found in neurons in prenatal but not in adult murine dermis. Our findings imply an involvement of RTN1A in the differentiation of murine LCs and will serve as a starting point for the elucidation of the functional roles of RTN1A in both the immune and the nervous system of the skin. The established RTN1A staining pattern in mice will next be compared with RTN1A knock out mice and additionally analysed in functional assays.

O088

Bone marrow-derived Ly6C$^-$ macrophages promote ischemia-induced chronic kidney disease

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Macrophages play an important role in renal injury and repair after acute kidney injury (AKI) and the subsequent chronic kidney disease (CKD) that often results. However, as macrophages have a high degree of plasticity and heterogeneity, the function(s) of macrophage subtypes in AKI-to-CKD progression are not fully understood. Here, we focused on Ly6C$^-$ macrophages, which are derived from the embryonic yolk sac and post-development become resident in the kidneys. We found that CCR2 deficiency, which blocks the migration of Ly6C$^+$ macrophages from the bone marrow to the sites of injury, alleviated ischemia-induced AKI. Unexpectedly, though, CCR2 deficiency worsened the subsequent renal fibrosis, which was marked by notable intrarenal infiltration of Ly6C$^-$ macrophages. These Ly6C$^-$ macrophages were greater in number in both the acute and chronic phases after ischemia reperfusion (I/R), and we showed them to be derived from the bone marrow by bone marrow chimerism. Clodronate Liposomes-mediated depletion of Ly6C$^-$ macrophages alleviated the renal injury and fibrosis. On the contrary, adoptive transfer of Ly6C$^-$ macrophages from injured kidneys of WT mice into immune-deficient mice was sufficient to induce renal injury and fibrosis. Transcriptome sequencing of Ly6C$^-$ macrophages from injured kidneys revealed that they secreted various cytokines and growth factors, which were associated with the transdifferentiation of fibroblasts into myofibroblasts. This transdifferentiation effect was further supported by in vitro studies showing that Ly6C$^-$ macrophages induced the secretion of extracellular matrix proteins from co-cultured fibroblasts. **In conclusion, the presence of bone marrow-derived Ly6C$^-$ macrophages after ischemia induces AKI and worsens subsequent CKD.**

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DOI: 10.1002/eji.201970400

O393

lnc-Dpf3 impairs CCR7-mediated dendritic cell migration by inhibiting glycolysis

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CCR7-mediated dendritic cell (DC) migration is critical for the protective immunity as well as maintenance of peripheral tolerance. The molecular mechanisms that regulate CCR7-mediated DC migration remains poorly understood. Here we reported that a novel long non-coding RNA lnc-Dpf3 restrains DC migration via suppressing HIF1α-dependent glycolysis. CCR7 stimulation mediates m6A demethylation on lnc-Dpf3 to prevent its RNA degradation, and thus stabilizing lnc-Dpf3. CCR7 signals also rapidly activates Akt/HIF-1a pathway in DC, leading to metabolic reprogramming toward glycolysis. lnc-Dpf3 directly binds to HIF1a via a hypoxia response element (HRE) motif and suppresses HIF1α-dependent transcription of glycolytic genes Ldha, thus inhibiting glycolytic metabolism and consequently the migratory capacity of DC. In sum, we demonstrated a critical role for lnc-Dpf3 in negatively regulating DC-driven innate immune response and uncovered a new mechanism for the crosstalk between epigenetic and metabolic pathways to control DC migration and inflammatory responses.

O394

Lymph node mesenchymal and endothelial stromal cells cooperate via the RANK-RANKL cytokine axis to shape the sinusoidal macrophage niche

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Macrophages play a key role in immunity, yet the cellular and molecular elements that make up their niches are incompletely understood. Here we found that deficiency of the cytokine RANKL in lymphoid tissue organizers and marginal reticular stromal cells of lymph nodes resulted in the loss of the $CD169^+$ sinusoidal macrophages (SMs) comprising the subcapsular and the medullary subtypes. Subcapsular SM differentiation was impaired in mice with targeted RANK deficiency in the SMs. Further, temporally controlled RANK-removal in lymphatic endothelial cells (LECs) revealed that lymphatic RANK activation during embryogenesis and shortly after birth was required for the differentiation of both SM subtypes. Moreover, RANK expression by LECs was necessary for SM restoration following inflammation-induced cell loss. Thus, cooperation between mesenchymal cells and LECs shapes a niche environment that supports SM differentiation and reconstitution after inflammation.

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DOI: 10.1002/eji.201970400

O395

The ER-localised Hrd1 ubiquitinates and inactivates Usp15 to promote TLR4-induced inflammation during bacterial infection

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The special organelle-located MAVS, STING and TLR3 are important for clearing viral infections. Although TLR4 triggers NF-κB activation to produce proinflammatory cytokines for bacteria clearance, effectors with special organelle localisation have not been identified. Here, we screened over 280 E3 ubiquitin ligases and discovered that the endoplasmic reticulum-located Hrd1 regulated TLR4-induced inflammation during bacterial infection. Hrd1 directly interacted with the deubiquitinating enzyme (DUB) Usp15. Unlike the classical function of Hrd1 in ER-associated degradation, Usp15 was not degraded but lost its DUB activity for IκBα deubiquitination, resulting in excessive NF-κB activation. Importantly, Hrd1 deficiency in macrophages protected mice against LPS-induced septic shock, and knock-down of Usp15 in Hrd1 KO macrophages restored the reduced IL-6 production. This study has proposed the crosstalk between Hrd1 and TLR4 linking the ER-plasma membrane function during bacterial infection.

O396

A positive feedback mechanism between basal-like breast cancer cells and monocytes through STAT3/IL-1β/fibronectin can support chronic inflammation in the tumor microenvironment

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Introduction: Myeloid cell infiltration is high in basal-like breast cancer (BLBC). Extracellular matrix components such fibronectin, can support myeloid cells' maturation. Immune supressive character of macrophages is provided by STAT3. EDA fragment released by elastase-2-mediated cleavage of FN can stimulate NF-kB. Here, the inflammatory role of STAT3,IL-1β and fibronectin in BLBC was investigated.
Methods: IL-1β and elastase-2 production (ELISA) in human peripheral blood monocytes incubated with BLBC cells' supernatants were evaluated following STAT3 activation. BLBC cells (MDA-MB-468, MDA-MB-231 and HCC38) were stimulated with IL-1β. Total fibronectin and FN-EDA expression were evaluated by Western-Blot and ELISA. Monocytes were cultured with BLBC supernatants with or without recombinant human (rh) FN isoforms (plasma FN, FN-EDA$^-$, FN-EDA$^+$) and IL-1β production were determined. Effect of FN-EDA and BLBC supernatants on NF-kB activity was assessed by SEAP reporter assay.
Results: STAT3 activation in monocytes was stimulated by BLBC supernatants. IL-1β elastase-2 secretion increased in monocytes with high STAT3 activity. Total fibronectin and FN-EDA isoform were abundant in BLBC and increased following IL-1β. BLBC supernatants and rhFN isoforms alone couldn't induce NF-kB activity. BLBC supernatants and FN-EDA were synergistically promoted IL-1β production from monocytes and stimulated NF-kB activity. Effect on IL-1β production and NF-kB activity were associated with STAT3 activation. Either secreted by BLBC cells or recombinant FN-EDA, with elastase-2, didn't stimulate NF-kB activity.
Conclusion: Positive feedback mechanism between BLBC and monocytes through STAT3/IL-1β/fibronectin supports inflammation in tumor. Targeting STAT3, IL-1β and FN may modulate inflammatory microenvironment in favour of cancer therapy.
This study is supported by TÜBİTAK (Project no: 116Z997)

DOI: 10.1002/eji.201970400

O397

Twist1 expression by dendritic cells plays an important role in anti-tumor immune responses

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Dendritic cells (DCs) are pivotal to the induction of adaptive T-cell-mediated cancer immunity. Twist1 plays an essential role in tumor initiation, stemness, angiogenesis, metastasis, chemo-resistance and senescence. But whether and how DC-derived Twist1 regulates anti-tumor immune responses remains elusive. Here we investigate the role of Twist1 in DC-T cell mediated anti-tumor immune responses and the potential molecular mechanisms by using a mouse line with Twist1 specifically deleted in CD11c^{+} DCs (*CD11c*^{Cre}*Twist1*$^{f/f}$ mice). A mouse melanoma model is established by subcutaneous injection of B16 cell line. In immunohomeostasis, the deletion of Twist1 has no significant effect on the proportion and function of DCs, CD4^{+} T cells, CD8^{+} T cells and myeloid cells. In B16 tumor model, the proportion of DCs, the activation of CD4^{+} and CD8^{+} T cells and the secretion of IFN-r by CD8^{+} T cells are reduced in *CD11c*^{Cre}*Twist1*$^{f/f}$ mice compared with *CD11c*^{Cre} mice. The transcriptome sequencing results show that ROS induced apoptosis of DCs is significantly increased in *CD11c*^{Cre}*Twist1*$^{f/f}$ mice compared with *CD11c*^{Cre} mice, in B16 tumor model. Tumor volume in Twist1-deficient mice is significantly larger than that in control mice. In conclusion, the deletion of Twist1 increases the apoptosis of DCs, weakens the ability of CD8^{+} T to secrete IFN-r, and ultimately promotes the growth of B16 tumor cells in mice.

O398

Regulation of dendritic cell function improves survival in experimental sepsis through immune chaperone

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Dendritic cells (DCs) are professional antigen-presenting cells that play a critical role in both innate and adaptive immune responses. DCs recognize and respond to bacteria through multiple pattern-recognition receptors, including Toll-like receptors (TLRs). Heat shock protein gp96, also known as grp94, is a master essential chaperone for TLRs in the endoplasmic reticulum and plays critical roles in innate immunity. We generated DC-specific gp96-knockout (KO) mice and showed that gp96 KO DCs were unable to respond to multiple TLR ligands. TLR-mediated hyper-inflammatory response can lead to sepsis. However, the roles of neither DC nor the DC-intrinsic gp96 in the process are completely understood. In the LPS-induced sepsis model, we hereby found that deletion of gp96 in DCs significantly reduced serum TNF-α levels and improved survival. Furthermore, using the well-defined polymicrobial sepsis model of cecal ligation and puncture, we found that DC-specific ablation of gp96 improved survival with significantly attenuated liver and renal injuries, decreased circulating inflammatory cytokines, altered DC maturation and activation, and increased serum immunoglobulin. Collectively, we demonstrate that deletion of gp96 in DCs is beneficial in protecting mice against sepsis induced by both endotoxemia and polymicrobial infections. Our study indicates that targeting gp96 in DCs may provide a potential novel approach for reducing the morbidity and mortality of sepsis.

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DOI: 10.1002/eji.201970400

O399

The role of biomarkers of macrophage polarization as etiologic causes and activity of intrinsic acute kidney injuries

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Background and objectives: Macrophage polarization is a major contributing factor in acute kidney injury (AKI). We aim to determine the value of urinary sCD163 and urine sediment macrophage subtypes in differentiating etiologic causes of various intrinsic renal AKI in a large cohort.
Methods: A total of 205 patients with renal intrinsic acute kidney injuries verified by renal biopsy were enrolled. Urinary sCD163 was quantified using enzyme-linked immunosorbent assay, and macrophage subtypes in urine sediment and in renal biopsy samples were determined by immunostaining.
Results: Compared to healthy controls (0 pg/µmol) and AKI due to interstitial or tubular injuries (0, 0 - 16 pg/µmol), the level of urinary sCD163 was markedly higher in glomerulopathy, especially in diffuse proliferative glomerulonephritis (275.5, 121.1-631.7 pg/µmol, $P< 0.001$) and significantly correlated with the proportion of cellular crescent formation ($P=0.04$). Urine sediment analysis of the macrophage M1/M2 ratio could differentiate acute tubulointerstitial nephritis (M1/M2>2.35, with sensitivity of 98% and specificity of 100%) from crescentic glomerulonephritis (M1/M2< 0.27, with sensitivity of 100% and specificity of 69%). Urinary sCD163 levels and urine M2 subtype positively correlated with infiltrated M2 macrophages in the glomeruli, whereas urine M1 subtype positively correlated with infiltrated M1 macrophages in the interstitium.
Conclusions: Markers of macrophage polarization associate with etiologic causes and activity of intrinsic AKI, which could be viewed as the noninvasive “liquid biopsy” in the presence of various intrinsic kidney diseases.

O400

Two populations of long-lived interstitial macrophages and NR4A1-dependent local precursors populate the lung CD64$^+$compartment

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Immune homeostasis is crucial in the lung mucosa that faces constant exposure to a wide range of external non-pathogenic stimuli. Our previous reports demonstrated that lung interstitial macrophages (IM) were important guardians of lung immune homeostasis by protecting the host against the development of aberrant type 2 immune responses against allergens such as seen during allergic asthma. Yet, an unbiased characterization of the lung IM compartment has been lacking until recently. In recent study, we analyzed 3,596 tissue CD64$^+$mononuclear cells by single-cell RNA-sequencing (scRNA-seq) and identified two phenotypically distinct subpopulations of IM, i.e. MHC-II^{lo}CD206$^+$IM and MHC-II^{hi}CD206$^-$ IM, as well as an as-yet unknown discrete population of NR4A1-dependent extravasating CD64$^+$CD16.2$^+$monocytes. Fate-mapping experiments supported that both IM subsets had an extended lifespan and were slowly replaced by monocytes in adults, with MHC-II^{lo}CD206$^+$IM exhibiting an increased self-maintenance potential as compared to MHC-II^{hi}CD206$^-$IM. MHC-

DOI: 10.1002/eji.201970400

II^{lo}CD206^{+}IM were larger, mainly found in the bronchial interstitium and produced high levels of immunoregulatory cytokines, including IL-10, and chemokines. Conversely, MHC-II^{hi}CD206^{-}IM preferentially populated the alveolar interstitium and exhibited a typical antigen-presenting cell profile. Interestingly, additional scRNA-seq and RNA velocity analyses of lung monocyte populations provided evidence that CD64^{+}CD16.2^{+}monocytes arose from intravascular Ly-6C^{lo}patrolling monocytes that underwent an extensive specification program upon entering the lung tissue to become local precursors of MHC-II^{hi}CD206^{-}IM.
This study identifies CD64^{+}CD16.2^{+}monocytes as tissue-programmed IM progenitors and reveals new knowledge about the diversity of lung IM, an important step towards understanding of the mechanisms underlying lung immune homeostasis and preventing immune-mediated diseases in the airways.

LB010

New insights into macrophage dynamics in the mouse diaphragm

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Macrophages are crucial for skeletal muscle health. Tissue-resident macrophages (TRM) are macrophages residing in the tissue at steady state. The origin of muscle TRM and the dynamics of TRM from different origins at steady state and upon muscle injury are poorly understood. Injury of diaphragm (DIA), the main respiratory muscle, occurs in several disease conditions and contributes to respiratory insufficiency. Here we sought to determine: 1) the contribution of bone marrow/monocyte-derived macrophages (BMDM) to TRM maintenance in the DIA at steady state; and 2) the responses of BMDM versus TRM at different phases after acute DIA muscle injury. Bone marrow chimeric mice (CD45.1/2) shielded with lead on DIA to protect TRM from radiation effects were used to distinguish TRM from BMDM.
We found that at steady state, BMDM are the predominant contributor (≈55%) to TRM maintenance in the muscle; however, there also appears to be a substantial BMDM-independent population. At 4 days after muscle injury the intramuscular macrophages strikingly increased. Differences in local proliferation of TRM versus BMDM, as detected by Ki67 and BrdU staining, do not appear to account this increase in macrophages, which is primarily composed of BMDM (>90%). After long-term (60 days) recovery from diaphragm damage, the large predominance of BMDM observed in the acute phase of injury is reversed such that the pre-injury steady state levels and ratio of BMDM to TRM are re-established. These data provide new information regarding the relative roles of BMDM and TRM in the DIA under steady state and pathological conditions.

LB011

High-dimensional analysis of the murine liver identifies two distinct subsets of Kupffer cells

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Tissue-resident macrophages have been extensively studied recently and are now recognized as populations with specific origin and functions strongly dictated by their tissue of residence. For example, it is now established that microglial cells that are surrounded by neurons and astrocytes are different from Kupffer cells surrounded by hepatocytes and hepatic stellate cells, different from lung alveolar macrophages and so forth.
However, heterogeneity of tissue-resident macrophages within a same organ has been overlooked, assuming that whole organs constitute a homogeneous “niche”. But the most recent advances are

DOI: 10.1002/eji.201970400

challenging this paradigm with notably the rise of powerful new technologies such as single-cell RNA sequencing and mass cytometry that are now revealing a lot of heterogeneity in cells previously assigned to a so-called uniform population.
Herein, we report the presence of two distinct populations of Kupffer cells in the mouse liver. Although these cells share a common embryonic origin clearly identifying them as bona fide Kupffer cells, we have identified numerous genes and proteins such as esam and mrc1 (CD206) that are differentially expressed in these two populations. Such heterogeneity is not explained at the spatial level as both subpopulations are uniformly distributed within the liver. Furthermore, Esam^{+}CD206^{+}KCs express endothelial markers, indicating a functional convergence between liver endothelial cells and this subset of liver macrophages.
Our study reveals that Kupffer cells are more heterogeneous than anticipated and that several "micro-niches" can exist in a given organ. This knowledge should shed light on the complex interactions between cells and their environment.

LB047

Notch signaling modulates peroxisome proliferator-activated receptor gamma level in interleukin 4-stimulated human macrophages through AKT and NEDD4L

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Macrophages in various diseases exhibit distinct phenotypes, depending on the stimuli and microenvironment. Macrophage phenotypes are classified at least into 2 types that are pro-inflammatory type induced by pathogen associated molecular patterns and wound healing type induced by interleukin (IL)-4. These phenotypic polarizations strongly associate with severity of some chronic diseases such as atherosclerosis and cancer. Notch signaling and a nuclear receptor, peroxisome proliferator-activated receptor (PPAR) g, are known to regulate macrophage polarization and their crosstalk was reported in some cell types such as adipocytes. Whether Notch signaling and PPARg interact during macrophage polarization have not been documented. In this study, human monocytic cell line THP-1 and primary human monocytes derived macrophages activated Notch signaling upon IL-4 stimulation. Inhibition of Notch signaling reduced phosphorylation of Akt downstream of IL-4/IL-4R and also one of its target *PPARG* mRNA. Hyperactive Notch signaling by overexpression of Notch1 intracellular domain (NIC1) increased PPARg protein stability by decreasing proteasome degradation, but did not change the level or stability of mRNA. Transcriptomic analysis uncovered the links between Notch and PPARg through NEDD4L, an E3 ubiquitin ligase. Deletion of *NEDD4L* in the presence of NIC1 in THP-1 significantly reduced Akt phosphorylation and *PPARG* mRNA transcription upon IL-4 stimulation. Activation of Notch signaling affected lipid metabolism in IL-4-stimulated macrophages by increasing lipid accumulation via CD36, one of the targets of PPARg. Collectively, we present evidences linking Notch signaling and PPARg via Akt and NEDD4L in IL-4-stimulated macrophages that play a critical role in macrophage lipid uptake.

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DOI: 10.1002/eji.201970400

LB048

IGF-2 preprograms maturing macrophages to acquire oxidative phosphorylation-dependent anti-inflammatory properties

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Recent investigations revealed that macrophages could be trained with an altered responsiveness, raising the possibility of combating autoimmune diseases by imparting anti-inflammatory capabilities to these cells. While investigating the effect of mesenchymal stem cells on experimental autoimmune encephalomyelitis (EAE), we found a critical role of insulin-like growth factor 2 (IGF-2) in training macrophages to become anti-inflammatory during their maturation. IGF-2 exerts its effects by preprogramming maturing macrophages to commit oxidative phosphorylation (OXPHOS). IGF-2-preprogrammed macrophages maintained the mitochondrial complex V activities even upon pro-inflammation stimulation, thus enabling an elevated programmed death-ligand 1 (PD-L1) expression. PD-L1 neutralization abolished the beneficial effect of IGF-2 on EAE. Furthermore, adoptive transfer of IGF-2-preprogrammed macrophages to EAE mice increased Tregs and alleviated the diseases. Our results demonstrate that shaping macrophage responsiveness by IGF-2 is effective in managing inflammatory diseases, and the OXPHOS commitment can be preset to determine the anti-inflammatory fate of macrophages.

LB049

Role of tumor-associated macrophages (TAMs) in the absence of the interleukin 4 receptor during the development of colitis-associated colon cancer in a murine model.

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In inflammation associated colon cancer (CAC), both immune cells and cancer cells produce interleukin 4 (IL4) in high amounts. The IL4 through the interleukin 4 receptor (IL4R) can active macrophages to tumor associated macrophages (TAMs). TAMs are one of the main cells infiltrates in colon cancer. These cells allow the establishment of the tumor by suppressing the immune response, proliferation and survive of cancer cells. In CAC models, mice lacking IL4R (*IL4R KO*$^{-/-}$) develop fewer tumors than wild mice (WT). However, it is unknown if the lower number of tumors is due to the absence of IL4R on macrophages or on other cells. The aim of our work is to determine the role of the macrophages during the CAC in *IL4R KO*$^{-/-}$ mice. Results. *IL4R KO*$^{-/-}$ mice with CAC had lower histopathology damage and mucus production was maintained. In circulation the percentage of Ly6C^{hi} cells was preserved, while the percentage of Ly6G^{+} cells increased. In spleen and mesenteric nodules the percentage of CD4^{+} and CD8^{+} lymphocytes was maintained while in WT CAC mice the percentage of CD4^{+} and CD8^{+} lymphocytes decreased. On the other hand, we observe that the macrophages participate in tumor development and their absence its associated with an increase in mortality as well as a greater histopathological damage and a lower mucus production in both *IL4R KO*$^{-/-}$ mice and in WT mice with CAC.

DOI: 10.1002/eji.201970400

LB084

Effect of P27 and PE_PGRS33 proteins on the mitochondrial function of alveolar macrophages an their response to *Mycobacterium tuberculosis*

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This study aims to determine the functional and structural abnormalities of mitochondria induced by mycobacterial proteins P27 and PE_PGRS33 on MH-S alveolar macrophages.
Mitochondria of *Mycobacterium tuberculosis* (Mtb)-infected macrophages display numerous changes respecting ATP production, membrane potential, cytochrome C release, mitochondrial cristae morphology, and relocalization around phagosomes. These changes are often associated with mycobacterial virulence. Evidence shows that the specific mycobacterial proteins, P27 and PE_PGRS33, target mitochondria on human monocyte-derived macrophages, and induce fission and fusion, respectively. Although, their precise role in mitochondrial function remains unknown.
PE_PGRS33 belongs to the Pro-Glu/Pro-Pro-Glu protein family and it is expressed during pulmonary TB infection. This protein can induce cell death and innate immune response modulation via TLR-2.
P27 is a lipoprotein that binds and transports mycobacterial triacylglycerides, critical in lipid metabolism regulation, cell wall integrity, and virulence.
By electron microscopy, we evaluated mitochondrial morphology changes of alveolar macrophages after incubation with PE_PGRS33 and P27 proteins. To assess mitochondrial oxygen consumption, we used a Clark-type microelectrode and MH-S cells were loaded after 4 hours of stimuli with both proteins.
Our results show that these proteins induce fusion (PE_PGRS33) and fission (P27) on MH-S cells with different oxygen consumption profiles.
Our data suggest that PE_PGRS33 may increase mitochondrial mass and ATP production, therefore improving macrophage bactericidal activity by modulation of macrophage function. In contrast, the opposite effect could be expected due to the induction of fission by P27.
Cytokine and protein quantification will be determined to understand the immune response profile of stimulated macrophages.

LB085

Plasmacytoid dendritic cell differentiation is distinct from the myeloid lineage and occurs from Ly6D$^+$ early lymphoid progenitors

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The dendritic cell (DC) family currently comprises conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). However, pDCs and cDCs exhibit distinct origins, phenotypes and functions, questioning whether or not pDCs belong to the DC lineage. Here, we combined single cell transcriptomic analysis with mass cytometry, *in vitro* and *in vivo* assays, to show that the priming of mouse hematopoietic progenitor cells towards the pDC lineage occurs at the common lymphoid progenitor (CLP) stage, indicative of an early divergence of the pDC and cDC lineages. We found

DOI: 10.1002/eji.201970400

transcriptional signatures of pre-pDCs within the CLP and CD115$^-$ CDP (common DC progenitors) populations, and identified Ly6D, IL-7Rα, CD81, and CD2 as key markers of pDC differentiation, distinguishing pre-pDCs from pre-cDCs. Thus, pDCs developed in the bone marrow from a Ly6D^{hi}CD2^{hi} lymphoid progenitor cell and differentiated independently of the myeloid cDC lineage. We propose that pDCs represent a distinct family of innate type I IFN-producing lymphoid cells, distinct from the cDC lineage.

LB086

Selective expression of DNAM-1 (CD226) is required for small peritoneal macrophages to prime CD4$^+$ T cell

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There are two distinct subsets of mouse macrophages in peritoneal cavity which are (CD11b^{hi} F4/80^{hi} large peritoneal macrophages (LPMs) and CD11b$^+$ F4/80^{lo} small peritoneal macrophages (SPMs)). However, we found that SPMs, but not LPM, have the ability to prime CD4$^+$ T cells both *in vitro* and *in vivo*. Interestingly, we revealed that SPMs, but not LPMs, strongly expressed an immune activating receptor DNAM-1, suggesting us to examine the contribution of DNAM-1 in the distinctive function of SPMs. Unlike wild type SPMs, DNAM-deficient SPMs did not prime antigen specific CD4$^+$ T cells. Although antigen phagocytosis and antigen processing were comparable between wild type and DNAM-1-deficient SPMs, we found that blocking the interaction between DNAM-1 on SPMs and its ligand CD155 on CD4$^+$ T cells with a neutralizing anti-CD155 mAb inhibited an antigen-specific CD4$^+$ T cell proliferation. Moreover, mice transferred with DNAM-1-deficient SPMs showed decreased CD4$^+$ T cell proliferation and germinal center formation compared with mice transferred with wild type SPMs after immunization. Taken together, we propose that SPMs are functionally distinct from LPMs, and DNAM-1 plays an important role in CD4$^+$ T cell costimulation by SPMs via its direct interaction with the ligand CD155 on CD4$^+$ T cells.

P0140

Tie2 expression on macrophages is required for blood vessel reconstruction and tumor relapse after chemotherapy

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Tumor relapse after chemotherapy is a major hurdle for successful cancer therapy. Chemotherapeutic drugs select for resistant tumor cells and reshape tumor microenvironment, including the blood supply system. Using animal models, we observed on macrophages in tumor tissue a close correlation between upregulated Tie2 expression and tumor relapse upon chemotherapy. Conditional deletion of Tie2 expression in macrophages significantly prohibited blood supply and regrowth of tumors. Tie2$^+$ macrophages were derived from tumor-infiltrating Tie2$^-$CD11b$^+$cells and hypoxia-induced Tie2 expression on these cells. Mechanistically, expression of Tie2 prevented macrophages from apoptosis in stress conditions via the AKT-dependent signaling pathway. Together, these results demonstrate that Tie2 expression by macrophages is necessary and sufficient to promote the reconstruction of blood vessels after chemotherapy, shedding new light on developing novel strategies to inhibit tumor relapse.

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DOI: 10.1002/eji.201970400

P0141

Skeletal stem cell-mediated suppression on inflammatory osteoclastogenesis occurs via concerted action of cell adhesion molecules and osteoprotegerin

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Objective: Here we investigated how skeletal stem cells (SSCs) modulated inflammatory osteoclast (OC) formation/bone resorption. We determined a synergistic role of intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) as well as ostetoprotegerin (OPG) in SSCs mediated suppression of inflammatory osteoclastogenesis.
Methods: The effects of SSCs on inflammatory osteoclastogenesis were investigated by using lipopolysaccharide-induced mouse osteolysis model in vivo and human osteoarthritis synovial fluid in vitro. The OC formation was determined by tartrateresistant acid phosphatase staining. Bone resorption was assayed by micro-computerized tomography, serum C-terminal telopeptides assay, and pit formation on dentine slices. The expression of ICAM-1, VCAM-1 and OPG in SSCs and their contribution to suppression of osteoclastogenesis were explored by flow cytometry. Gene modifications and neutralization antibodies were used for further mechanism exploration.
Result: SSCs remarkably inhibit inflammatory osteoclastic activity in vivo and in vitro. Additionally, inflammatory cytokines stimulated ICAM-1 and VCAM-1 expression as well as OPG secretion by SSCs. Furthermore, ICAM-1 and VCAM-1 recruited $CD11b^+$ OC progenitors into proximity with SSCs, which strengthened the inhibitory effects of SSCs-derived OPG on osteoclastogenesis.
Conclusion: In the inflammatory microenvironments, SSCs expressed higher level of ICAM-1 and VCAM-1 as well as produced more OPG that are sufficient to inhibit osteoclastogenesis in a “capture and educate” manner. This may represent a synergistic mechanism to limit bone erosion during joint inflammation by SSCs.

P0142

Effect of gap junctions on Raw264.7 macrophages infected with H37RV

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Background: Apoptosis and inflammation have been shown to play an important role in the mechanisms involved in the pathogenesis of Mycobacterium tuberculosis(MTB) infection. When macrophages undergo apoptosis and polarization, gap junctions(GJs) may be needed to provide conditions for their functions. Connexin 43 (Cx43) and connexin 37(Cx37) are the main connexins in macrophages that participate in the formation of GJ channels.
Methods: An H37Rv infection RAW264.7 macrophage model was established to investigate the associate between connexins and host macrophage immune defense response after MTB infection. First, Real-time Polymerase Chian Reaction (RT-PCR) was used to detect the mRNA expression of Cx43 and Cx37. Cx43 protein expression and location was detected by western blotting and immunofluorescence. Confocal microscope was used to assay the gap junctional intercellular communication (GJIC). Then, electron microscope used to observe the morphology of macrophages. Finally, RAW264.7 macrophage apoptosis and mitochondrial membrane potential was detected byflow cytometry, and the expression of inflammation factors such as CD86, CD206, and IL-6, IL-10, TNF-α, and TGF-β were detected by Real-time PCR and enzyme-linked-immunosorbent serologic assay (ELISA).
Results: H37Rv infection significantly promoted host macrophage Cx43 mRNA and protein

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DOI: 10.1002/eji.201970400

expression (increased 1.6-fold and 0.3-fold respectively), and enhanced host macrophage GJIC. When host macrophage cell-to-cell communication induced by H37Rv infection, the apoptosis rate and inflammatory factors expression also increased.
Conclusions: The results confirm that H37Rv infection can obviously induce host macrophage Cx43 expression and enhance GJIC, which may implicated in host macrophage inflammatory reaction, to regulate the release of inflammatory factors and/or initiate apoptosis to activate host immune defense response.

P0143

Macrophages reprogrammed by lung cancer microparticles promote tumor development via releasing IL-1β

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Despite macrophage is known to contributed for inhibitory immune microenvironment of tumor (tumor associated macrophage, TAM), the origin and function of TAM are remaining poorly understood. Here we show that lung cancer cells derived microparticles (L-MPs) polarized macrophage to release IL-1β which not only leading to facilitate tumor growth but also promoting cancer stem cells development. Furthermore, we demonstrated the underlying mechanism is involved in TLR3 signal and inflammasome NLRP3 activating by tumor microparticles. Finally, in addition to mouse tumor MPs, we show that therapy induced human L-MPs also presented consistent effect on human TAMs polarization in a humanized mouse model. Importantly, these findings uncovered the mechanism of tumor cells microparticles mediated mature IL-1β release on macrophage and form the basis for exploring the reasons of tumor therapy resistant and tumor recurrence.

P0144

Cypermethrin promotes lung cancer metastasis via modulation of macrophage polarization by targeting MicroRNA-155/Bcl6

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Cypermethrin (CYM), a type II pyrethroid, is widely used as an insecticide for agriculture and household. Cumulative evidence indicates that acute and chronic exposure to CYM might cause a number of health problems, such as cancer and neuronal system diseases. However, the molecular mechanism underlying this pathology is unknown. The main objective of our study was to define the effects of CYM on macrophages and the implication of such effects in cancer metastasis and the potential mechanism involved. The effects of CYM on the macrophages were evaluated by detecting the expression of M1 and M2 macrophage polarization markers through ELISA, quantitative RT-PCR and Western blot assay. Transwell and wound healing assay were used to test the migration of lung cancer cells after exposure to CYM *in vitro* and a metastasis animal model *in vivo*. CYM significantly suppressed lipopolysaccharide (LPS)-induced M1 macrophage polarization and promoted a shift toward M2 macrophage status. Mechanistically, CYM downregulated miR-155 significantly, leading to enhanced expression of its target gene *Bcl6*, thereby reducing the expression of Mitogen-activated protein kinase kinase 4 (MKK4), an upstream kinase of c-Jun N-terminal kinases (JNK), and inhibiting

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DOI: 10.1002/eji.201970400

JNK activation. Impaired JNK activation thus promoted a shift in macrophage polarization from the M1 to the M2 phenotype. Finally, CYM-treated macrophages promoted metastasis of Lewis lung cancer cells *in vitro* and *in vivo* models. Taken together, our findings show that CYM is able to inhibit the M1 polarization and promote the macrophages to the M2 phenotype, which plays an important role in tumor metastasis.

P0145

CCL2 promotes macrophage- associated chemoresistance via MCPIP1 dual catalytic activities in multiple myeloma

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We previously showed that the chemokine CCL2 can recruit macrophages (Mφs) to the bone marrow (BM) in myeloma and that myeloma-associated Mφs are important in drug resistance. Here, we explored the role of increased CCL2 expression in the BM microenvironment of myeloma and uncovered the underlying mechanism. Our results showed that CCL2 expression is associated with the treatment status of myeloma patients, when newly diagnosed patients received 4 courses of PCD combined therapy, CCL2 expression in their BM was much lower. Interestingly, CCL2 did not affect the proliferation and drug responses of MM cells neither in vitro nor in vivo. However, CCL2 promotes Mφs to protect myeloma cells from chemotherapy drug-induced apoptosis and polarizes Mφs to the M2-like phenotype. Mechanistic studies showed that MCPIP1 mediated the protective effect of CCL2-Mφs via its dual catalytic activities and that MCPIP1 also affected Mφs' polarization in vivo. In addition, we found that CCL2 induces MCPIP1 expression via the JAK2- STAT3 signaling pathway. Thus, this study provides novel insight into the mechanism of Mφs-mediated drug resistance and the underlying molecular mechanisms.

P0146

Biodegradable Mg-based alloy affected vascular smooth muscle cells (VSMCs) by immunoregulation of macrophage

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Mg-based alloys had emerged in biomedical application such as bone fixation or vascular stent. Little is known whether the degradable products of Mg-based alloy could impact the communication of cell-to-cell. In this study, we explored possible effects of Mg-based alloy on interaction of macrophage and VSMCs. The 5% and 15% extracts of Mg-Nd-Zn-Zr alloy (denoted as JDBM) as similar Mg^{2+} concentration as 2mM and 8mM $MgCl_2$ were added in macrophage after 2 h LPS stimulation for another 24h or 72h, respectively. the supernatants (CM) were harvested at above time to add into VSMCs for culture indicated times. No obvious cytoxicity were observed in macrophage and VSMCs cultured with extract or $MgCl_2$ or CM. Macrophage toward to a M2 polarization as well as the decrease tendency of pro-inflammatory cytokines were in both time and concentration dependence.

DOI: 10.1002/eji.201970400

Interestingly, the growth factors (e.g. VEGF, TGFβ, BDGF-bb) and osteogenesis factors (e.g. BMP-2, OPG, Runix-2) of macrophage expressed in 2mM $MgCl_2$ and 5% extract were more than 24h or 72h control group respectively, whereas lower or no difference expression of these factors in 8mM $MgCl_2$ and 15%extract were observed compared with control group. Additionally, the VSMCs were inhibited proliferation and migration, calcification in the 8mM $MgCl_2$ and 15%extract rather than 2mM $MgCl_2$ and 5%extract compared with 24h or 72h control group respectively. Thus, our results demonstrated high concentration extract could inhibit VSMCs activation by regulation of macrophage, whereas low concentration extract seemed to promote their activity, which need to take into before clinical application.

P0147

Colon cancer cell-derived colony stimulating factor 1 involves in the interaction between cancer cells and tumor-infiltrated macrophages

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Objective: Macrophages are highly represented in the colorectal cancer microenvironment. Colony stimulating factor 1 (CSF1) is a primary chemoattractant and functional regulator for macrophages, and therefore would be a feasible intervention for the macrophage-targeting therapeutics. However, the expression of CSF1 in colon cancer microenvironment and its roles are largely unknown.
Methods: The expression patterns of CSF1 and macrophages were explored in colon cancer patients. The effects of macrophages on CSF1 expression of colon cancer cells were analyzed, and the relevant mechanisms were also explored. A CSF1 over-expression tumor xenografts model was established to futher determine the roles of cancer cell-derived CSF1 in cancer development.
Results: CSF1 was over-expressed exclusively in colon cancer cells. The high CSF1 expression and macrophages infiltration were related to the TNM stage and survival of colon cancer patients. Co-culture with macrophage promoted CSF1 production in colon cancer cells depending on PKC pathway, which was activated by IL-8, mainly produced by macrophages. Colon cancer cell-derived CSF1 drove the recruitment of macrophages and re-directed their secretion profile, including the augment of IL-8 production. The mice tumor xenografts also found that over-expression of CSF1 in colon cancer cells promoted intratumoral infiltration of macrophages, and partially suppressed tumor growth.
Conclusion: Our results reported a positive feedback loop between colon cancer cells and tumor infiltrated macrophages, which led to the formation of a high CSF1 microenvironment.

P0148

High-mobility group nucleosome-binding protein 1 as endogenous ligand induces innate immune tolerance in a TLR4-sirtuin-1 dependent manner in human blood peripheral mononuclear cells

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High-mobility group nucleosome-binding protein 1 (HMGN1) functions as a non-histone chromatin-binding protein in the cell nucleus. However, extracellular HMGN1 acts as an endogenous danger-associated inflammatory mediator (also called alarmin). We demonstrated that HMGN1 not only

DOI: 10.1002/eji.201970400

directly stimulated cytokine production but also had the capacity to induce immune tolerance by a TLR4-dependent pathway, similar to lipopolysaccharide (LPS)-induced tolerance. HMGN1-induced tolerance was accompanied by a metabolic shift associated with the inhibition of the induction of Warburg effect (aerobic glycolysis) and histone deacetylation viaSirtuin-1. In addition, HMGN1 pre-challenge of mice also downregulated TNF production similar to LPS-induced tolerance in vivo. In conclusion, HMGN1 is an endogenous TLR4 ligand that can induce both acute stimulation of cytokine production and long-term tolerance, and thus it might play a modulatory role in sterile inflammatory processes such as those induced by infection, trauma, or ischemia.

P0149

Histone deacetylase 4 is not required for epidermal Langerhans cell development

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Langerhans cells (LCs) are special tissue-resident dendritic cells (DCs) in the epidermis. Despite many factors were involved in the homeostasis and function of LCs, the underlying molecular mechanisms remain elusive. Histone deacetylases (HDACs), known to mediate the acetylation of lysine residues on histone proteins, play a vital role in the development and activation of immune cells. Our previous study has been shown that HDACs were required for LCs homeostasis, maturation and phagocytosis by using trichostatin A in vitro, which is a well-known HDAC inhibitor. However, the specific function of individual HDACs in LCs remains largely unexplored. To investigate the role of HDAC4 in LCs maintenance and function, HDAC4 knockout (HDAC4 KO) mice were generated. We found that epidermal LCs (MHC Ⅱ$^+$ CD45$^+$) in HDAC4 KO mice were comparable to that of WT mice, suggesting that HDAC4 was dispensable for LCs homeostasis. Phagocytic capacity of LCs in HDAC4 KO mice had no difference compared to that of WT mice based on the ratio and MFI of FITC$^+$ LCs, indicating that HDAC4 deficiency did not affect LCs phagocytosis. After being cultured for 65 h in vitro, the ratio of MHC Ⅱ in LCs from HDAC4 KO was slightly increased, but expression levels of CD80 and CD86 had no significant differences. Thus, HDAC4 was not required for LCs maturation. Overall, our data highlight that HDAC4 might not be the key molecule of histone deacetylase activity for LCs homeostasis, phagocytosis and maturation, or some members of HDAC family were redundantly essential for LCs development and function.

P0150

Late onset of nlrc3-like deficiency triggers microglia cell death through activating inflammatory pathway in zebrafish

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Microglia are tissue-resident macrophages resided in the central nervous system (CNS) and they play critical roles in removing cellular debris and infectious agents as well as regulating neurogenesis and neuronal activities. Yet, the molecular basis underlying the establishment of microglia pool and the maintenance of their homeostasis in the CNS remain largely undefined. Here we report the

DOI: 10.1002/eji.201970400

identification and characterization of a mutant zebrafish, which harbors a point mutation in the nucleotide-binding oligomerization domain (NOD) receptor gene *nlrc3-like*, resulting in the loss of microglia in a temperature sensitive manner. Temperature shift assay reveals that the late onset of *nlrc3-like* deficiency leads to excessive microglia cell death. Further analysis shows that the excessive microglia death in *nlrc3-like* deficient mutants is attributed, at least in part, to aberrant activation of canonical inflammasome pathway. Our study indicates that proper regulation of inflammasome cascade is critical for the maintenance of microglia homeostasis.

P0151

Down-regulation of MCT2-mediated lactate transport by Notch signaling promotes MDSCs differentiation into anti-tumor TAMs

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Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) are pathologically activated myeloid cells in the tumor microenvironment and play critical roles in regulating tumor growth. However, the relationships among these myeloid subsets and the underlying mechanisms remain unclear. In this study, we found Notch activation in myeloid cells repressed tumor growth by re-modeling the development of myeloid cells, especially by decreasing the number of G-MDSCs and increasing mature M1-like TAMs, which were also validated in *ex vivo* experiments. To further explore the mechanism, we performed a transcriptome microarray using bone marrow-derived macrophages (BMDMs) isolated from Notch-activated and control mice, and found that monocarboxylate transporters 2 (MCT2), a lactate transporter responsible for lactate import, was significantly down-regulated by Notch activation. Furthermore, reporter assay and ChIP results indicated that Notch directly regulated MCT2 transcription. Using both pull-down and HPLC-MS assays, we further identified c-Jun as a novel intracellular sensor of lactate in myeloid cells. The interaction with lactate protected c-Jun from degradation mediated by FBW7 and subsequently promoted COX-2 expression. *In vivo* experiments finally suggested that the combination of Notch activation with MCT2 inhibitors could significantly suppress tumor growth through reducing lactate intake by MDSCs. Most importantly, the relationship between Notch-MCT2-c-Jun axis in myeloid cells and tumor progression was also observed in clinical lung cancer patients. Taken together, our study revealed that down-regulation of MCT2-mediated lactate transport by Notch signal re-modeled myeloid cells development and repressed tumor progression. Targeting Notch signal and lactate metabolism represents a potential therapeutic option for cancer.

P0152

Identification of a macrophage subset involved in hepatic fibrosis

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Recent studies suggest that hepatic macrophages are a remarkably heterogeneous population of immune cells that fulfill diverse functions in homeostasis, disease progression, and regression from injury, indicating that macrophage subtypes should be redefined depending on their characteristics and corresponding disorders. CD24, a small glycosylphosphoinositol-anchored protein, is widely used as a marker for differentiation of multiple lineages of cells. We found that CD24-/- mice had a more severe liver damage and fibrosis than that of WT mice in liver fibrosis model induced by bile duct-ligated (BDL model). Interestingly, using FACS analysis, we observed a significantly increase of CD24

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DOI: 10.1002/eji.201970400

expression and secretion of TGF-β1, a crucial factor for fibrosis, in $CD45^{+}CD11b^{hi}F4/80^{int}Ly6C^{lo}$ hepatic macrophage subset. To define this population, we sort the $CD11b^{hi}F4/80^{int}Ly6C^{lo}$ macrophages in BDL model and co-cultured with HSCs. We found that this macrophage population may be crucial for the induction of activated HSCs followed by the beginning of fibrosis, at least in part, through production of TGF-β1. Our results show this macrophage population may be critical for clarifying molecular mechanisms of liver fibrosis negatively regulated by CD24 via hepatic macrophages subsets.

P0153

The immunoregulatory roles of melatonin on diets-induced nonalcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) has been recognized as one of the leading causes of cirrhosis in adults worldwide. However, there is no specific treatment for NASH currently. Inflammation in the liver is crucial for the progression from hepatic steatosis to NASH, and infiltration of different subsets of inflammatory cells is the hallmark of steatohepatitis. Melatonin has been reported to have immunoregulatory effects for both innate and adaptive immune cells. In this study, in a methionine and choline-deficient diet (MCD) induced mouse NASH model, we demonstrated that oral administration of melatonin ameliorated NASH development. Compared with control mice, MCD-fed mice with melatonin intervention exhibited significantly lowered plasma ALT and AST, decreased liver steatosis, lobular inflammation and focal liver necrosis. Melatonin treatment mainly inhibited $CD11b^{int}F4/80^{low}$ monocytes liver infiltration, TNF-a secretion and MHC II expression during NASH development. No significant difference in frequency of Kupffer cells and liver infiltrated lymphocytes in MCD-fed mice with or without melatonin treatment. These changes of monocytes and Kupffer cells were further verified in $Rag1^{-/-}$ mice. Mechanistically, monocytes expressed markedly higher melatonin receptors than lymphocytes. Melatonin inhibited M1 monocytes polarization and TNF-a secretion, induced M2 monocytes proportion, which resulted in the shifting of the M1/M2 balance towards regulation. In conclusion**,** we elucidates that melatonin regulates intrahepatic innate immune cell, mainly M1 monocytes, suppresses the hepatic inflammation and improves diets induced NASH. Melatonin might serve as a new treatment for NASH.

DOI: 10.1002/eji.201970400

P0154

Histone deacetylase 1 and 2 play essential roles in alveolar macrophage homeostasis

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Alveolar macrophages (AM) are the major lung-resident macrophages that play a critical role in pathogen clearance and surfactant homeostasis. The molecular mechanisms that maintain AM homeostasis are not yet fully understood. Histone deacetylases (HDAC) are pleiotropic enzymes removing acetyl moieties from histone proteins, mediating transcriptional quiescence via chromatin condensation. In this study we found that HDAC1 and HDAC2 function as essential regulators for AM homeostasis. By using *Cd11c*-cre mouse strain to mediate deletion of HDAC1 and HDAC2 in AMs, we found that loss of either HDAC1 or HDAC2 evoked no obvious defect in AMs under homeostatic conditions. However, simultaneous loss of both HDAC1 and HDAC2 caused profound defect of AMs, accompanied by significant accumulation of pulmonary surfactants and increased susceptibility to influenza virus infection. We subsequently used *Lyz2*-cre mouse strain to mediate myeloid cell-restricted deletion of HDAC1 and HDAC2. We found specific requirement of HDAC1 and HDAC2 for AM homeostasis but not for other tissue macrophages. Based on kinetic analysis, dual inactivation of HDAC1 and HDAC2 disrupts commitment from fetal monocytes to perinatal AM. These observations shed light on the roles of HDAC1 and HDAC2 acting as epigenetic regulators in maintaining integrity of the AM, which will facilitate the comprehension of epigenetic regulation on tissue specific macrophage.

P0155

Tumor produced adenosine promotes self-renewal of less differentiated macrophages in human hepatocellular carcinoma

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Macrophages are a major component of the tumor microenvironment, and their density is generally correlated with an unfavorable outcome among patients in a variety of cancers. It has long been considered that macrophages are terminally differentiated and tightly linked to cell cycle exit. Until recently, several studies disclosed that under certain pathological conditions, such as inflammation and cancer, tissue macrophages can massively expand and substantially influence the disease progression. However, most of these findings were based on murine models, and the mechanisms of macrophage replication are disparate depend heavily on local environments. Little is known about the presence, phenotype, clinical relevance and the regulating mechanism of proliferating macrophages in human cancer. To address these questions, we used human hepatocellular carcinoma (HCC) tumor samples and established a macrophage maintenance model *in vitro*. We observed that HCC tumor-infiltrating macrophages exhibit considerable *in situ* proliferative capacity, as determined by proliferation marker Ki67 staining. The presence of self-replicating macrophages is inversely associated with patient prognosis. Furthermore, proliferating macrophages from both HCC tumor tissue and *in vitro* model appear to be a less differentiated phenotype. Mechanistically, we found that adenosine, which is abundant in the tumor microenvironment, acting exclusively through its receptor A2A to elicit macrophage expansion via PI3K/Akt/mTOR signaling pathway. Our findings not only reveal a distinct mechanism that regulating macrophage replenishment in human cancer but also make proliferating macrophage a promising target for restoring the anti-tumor immune microenvironments by selectively modulating the accumulation of macrophages.

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DOI: 10.1002/eji.201970400

P0156

Acceleration of transcription factor CBP degradation reduces lung fibrosis by disturbing the GSK-3b/OTUA interaction in macrophages

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Pulmonary fibrosis (PF) is a devastating disease with high death rate and unclear etiology. A rare of anti-fibrotic therapeutics are available for treating this devastating disease as yet. Understanding the underlying mechanisms is critical for developing effective therapeutics. Chronic or repeated lung injury results in chronic inflammation, which work together to drive the development of PF. Alveolar macrophages (AMs) are close proximity to the activated myofibroblast and can trigger chronic inflammation and PF. Here, we report that chronic lung injury inactivated the ubiquitin-modifying enzyme OTUA, causing a progressive accumulation of the transcription factor CBP in alveolar macrophages (AMs) from PF patients and mice. The CBP accumulation shifted AMs from a classical phenotype to a profibrotic phenotype, which produced a large quantity of profibrotic factors to activate lung fibroblasts into PF effector cells, myofibroblasts. Mechanistically, protein kinase GSK-3b, whose activity was enhanced in response to chronic lung injury, interacted with and phosphorylated OTUA to suppress its enzymatic activity, which interfered with the CBP degradation. The overexpression of OTUA or pharmacological-restored OTUA activity accelerated CBP degradation and exhibited a potent therapeutic efficacy against experimental PF. Our study provides a concept insight into the regulation of AMs phenotypes and the pathogenesis of PF that may be of potential therapeutic significance for the treatment of PF and other fibroproliferative lung diseases.
This work was supported by the National Natural Science Foundation of China (81530093 to ZWH; 81803604 to SSL).

P0157

Batf3-dependent dendritic cells are required for natural killer cell trafficking and killing in melanoma

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Batf3-dependent dendritic cells (DCs) play a critical role in cross-priming CD8+ T cell to initiate an anti-tumor adaptive immunity. However, whether Batf3-dependent DCs promote the capability of natural killer (NK) cells to infiltrate into tumor mass and kill tumor cells is poorly described. Here, we assessed the potential effects of Batf3-dependent DC on regulating the anti-tumor innate immunity of NK cells by using an implant melanoma model in Batf3-/- mice. Tumor growth kinetics combined with flow cytometric assays indicated the decreased trafficking of NK cells into tumors contributed to tumor overgrowth. Mechanistically, this was due to the absence of XCL1 and CXCL10, which is important for Batf3-dependent DC and NK cells accumulating in inflamed tissues, respectively. Furthermore, the absent of Batf3-denpendent DC directly conducted the decreased NK cell activation, which was reflected by the downregulated expression of some NK activation and killing function-related receptors, such as NKG2D, 2B4 and FasL. Our data indicate that in addition to cross-prime CTL, Batf3-dependent DCs is also critical for NK recruitment and activation to fully initiated an anti-tumor immunity.

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DOI: 10.1002/eji.201970400

Acknowledgements: This work was supported by grants from the National Basic Research Program of China (2015CB943203)

P0158

Effects of opium (Taryak) detoxification on blood CD11c⁺ myeloid and CD123⁺ plasmacytoid dendritic cells

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Background and aim: Evidence from in vivo and in vitro experiments on human and animal subjects suggests that opioid abuse may exert adverse immunomodulatory effects on innate and adaptive immune responses. The aim of this research was to understand the effects of detoxification on percentage of dendritic cells (DCs) in opium addicts.
Material and methods: In this study, 38 chronic opium addicts were divided into two groups. One of the groups was treated with methadone, while the other group received buprenorphine. Percentage of myeloid DCs ($CD11c^+$) and plasmacytoid DCs ($CD123^+$) were measured before and after detoxification program.
Results: There was a significant difference in the percentages of peripheral blood myeloid DCs between the addict subjects and the control group. However, there wasn't a significant change in the percentage of plasmacytoid DCs between the two groups. Detoxification with methadone, intensified the reduction of myeloid DCs in peripheral blood of addicts, while the buprenorphine detoxification regimen corrects this decrease.
Conclusion: Dendritic cells play an important role in innate and adaptive immune responses, therefore the impairment caused by opium in these cells could increase the occurrence of infectious diseases. The results indicate that buprenorphine might be beneficial in modifying the immunological disorders resulted from opium abuse.

P0159

The relevance of CD83 in HSV-induced inflammation in a mouse model

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Behçet's disease (BD) is an autoimmune/autoinflammatory disease leading to systemic vascular complications. Dendritic cells (DCs) are the most potent antigen presenting cells that regulate multiple inflammatory pathways. This study investigated the association of DCs co-stimulatory molecules in BD. The frequencies of co-stimulatory molecules expressed in DCs were measured in peripheral blood leukocytes (PBL) by flow cytometry (FACS) and the severity of symptoms was assessed in Herpes Simplex Virus type 1 induced BD symptomatic mice. The frequencies of CD83 positive cells were significantly increased in BD mice compared to asymptomatic mice. Abatacept, as known for CD80/86 blocker, significantly decreased the frequencies of CD83 positive cells in PBL of normal mice in a time-

DOI: 10.1002/eji.201970400

and dose-dependent manner. BD symptomatic mice treated with Abatacept gradually reduced the severity score of symptoms. Inhibition of CD83 by CD83 siRNA significantly reduced the frequencies of CD83 positive peritoneal macrophages and decreased serum IL-17 level in BD symptomatic mice. BD symptoms were improved and disease severity score was decreased after CD83 siRNA injection. Discontinuation of CD83 siRNA treatment deteriorated symptoms, and re-administration of CD83 siRNA improved again the BD symptoms of mice. By the transmission electron microscopy, bone marrow derived and cultured DCs treated with CD83 siRNA expressed down-regulation of dendrites on the cell surface membrane. These results clearly indicate the involvement of CD83 expressing cells to inflammatory symptoms of BD.

P0160

The superior capacity of fetal monocytes in the development and function of tissue-resident macrophage provide a novel system for the manipulation of alveolar macrophages

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Tissue-resident macrophages ($M\Phi_{TR}$) develop during embryogenesis and are self-maintained throughout adulthood independent from bone marrow hematopoietic stem cells. However, the relative potential of different embryonic precursors in determining development and functional capacity of $M\Phi_{TR}$ remains unclear and $M\Phi_{TR}$ remain difficult to study because of their limited availability. Here, we studied alveolar macrophage (AM) development after adoptive transfer of embryonic precursors into neonatal *Csf2ra*$^{-/-}$ mice, which lack AM and develop pulmonary alveolar proteinosis (PAP). Comparing fetal monocytes and primitive macrophages, we found that the latter have a poor glycolytic capacity and are therefore strikingly impaired in development to AM. Furthermore, mice containing primitive macrophage-derived AM failed to efficiently prevent PAP and protect from mortality following influenza infection, in contrast to mice containing fetal-monocyte derived AM. Notably, we were able to establish culture conditions that allowed long-term (up to one year) *in vitro* maintenance of fetal liver monocytes, that remained the potential to develop into bona fide fully functional AM upon transfer into *Csf2ra*$^{-/-}$ mice. Harnessing the potential of this approach for genome editing and gene therapy, we restored a disrupted Csf2ra gene in fetal liver monocytes and their capacity to develop to AM *in vivo*, by retroviral expression of a wild-type gene.

P0161

Repeated administration of diuretics combined with captopril to healthy mice differently modulates macrophage-induced innate and humoral immune responses

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Introduction: The over-activated inflammatory responses of immune cells, such as macrophages infiltrating perivascular tissues, is proposed to correlate with the risk of hypertension development. Whereas, still little is known about the possible immunomodulatory effects of clinically relevant diuretics administered alone or in combination with antihypertensive drugs. Thus, we undertook current research aiming at investigation of the effect of these drugs on macrophage-mediated immunity in healthy mice.
Methodology: CBA mice were treated intraperitoneally with captopril (5 mg/kg) with or without hydrochlorothiazide (10 mg/kg) or furosemide (5 mg/kg) by 8 days. On the third day, mice were intraperitoneally injected with mineral oil. Five days later macrophages were harvested to assess their

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DOI: 10.1002/eji.201970400

capability to generate reactive oxygen intermediates (ROIs) and nitric oxide (NO), as well as to activate humoral immunity, after pulsing with sheep red blood cells (SRBC).
Results: Both diuretics, when administered alone or with captopril, enhanced ROIs, but decreased NO production by macrophages. Furthermore, SRBC-pulsed macrophages from mice treated with captopril combined with diuretics augmented the secretion of antigen-specific antibodies by recipient B cells. Finally, SRBC-pulsed macrophages of mice treated with hydrochlorotiazide alone or furosemide with captopril increased the number of antigen-specific B cells in recipient spleens.
Conclusions: Our research results showed that diuretics with or without captopril enhance the Th2-dependent humoral immune response by affecting the function of macrophages. Further studies should investigate the clinical effect of these observations.

The study was supported by budget funds for science in 2017-2021 under the “Diamond Grant” program (0168/DIA/2017/46).

P0162

Transcription factor BATF2-mediated prevention of intestinal inflammation

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Inappropriate activation of the IL-23 signaling pathway causes chronic inflammation through the induction of immunopathological Th17 cells in the intestine. IL-23 is primarily produced by innate myeloid cells including dendritic cells (DCs) and macrophages (MΦs) in the intestinal lamina propria. However, the molecular mechanisms underlying the regulation of IL-23 production by these cells remains poorly understood. In this study, we demonstrated that transcription factor BATF2 regulates intestinal homeostasis by suppressing IL-23-driven T cell responses. *Batf2* was highly expressed in intestinal innate myeloid subsets, such as monocytes, CD11b$^+$ CD64$^+$ MΦs, and CD103$^+$ DCs. *Batf2*$^{-/-}$ mice spontaneously developed colitis and ileitis with altered microbiota composition. In this context, IL-23 was produced in high quantities by intestinal CD11b$^+$ CD64$^+$ MΦs from *Batf2*$^{-/-}$ mice compared with wild-type mice, while production of TNF-α and IL-10 was normal. Moreover, increased numbers of IFN-γ$^+$, IL-17$^+$, and IFN-γ$^+$ IL-17$^+$ CD4$^+$ T cells, but not IL-10$^+$ CD4$^+$ T cells, accumulated in the lamina prorpia of the colons and small intestines from *Batf2*$^{-/-}$ mice. In addition, RORγt-expressing innate lymphoid cells were also increased in the intestine of *Batf2*$^{-/-}$ mice. *Batf2*$^{-/-}$ *Rag2*$^{-/-}$ mice showed a reduction in intestinal inflammation present in *Batf2*$^{-/-}$ mice. Furthermore, the high numbers of intestinal IL-17$^+$ and IFN-γ$^+$ IL-17$^+$ CD4$^+$ T cells were markedly reduced in *Batf2*$^{-/-}$ mice when introducing *Il23a* deficiency, which was associated with the abrogation of intestinal inflammation. These results indicated that BATF2 in innate myeloid cells is a key molecule for the prevention of IL-23/IL-17 pathway-mediated adaptive intestinal pathology.

P0163

Effects of acetamiprid on Toll-like receptor 7-mediated inflammation

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Neonicotinoid insecticides have been used in agriculture all over the world. Neonicotinoid insecticides selectively interact with insect nicotinic acetylcholine receptors (nAChRs) to interfere with the nervous system, causing insect paralysis and death. It has been reported that the toxicity of neonicotinoid

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DOI: 10.1002/eji.201970400

insecticides to mammals is lower since relatively low affinity to mammalian nAChRs. Acetamiprid is one of the major neonicotinoid insecticides. Recently, several studies indicated that toxicity of neonicotinoid insecticides, including acetamiprid, to human and animals. However, the underlying mechanisms how neonicotinoid insecticides affect human health remain unclear. Since inhalation is one of the exposure routes of acetamiprid, we investigated the effects of acetamiprid on the function of alveolar macrophages (AMs) under Toll-like receptor (TLR)7-mediated inflammatory conditions. Acetamiprid inhibited the levels of TNF-α and IL-6 mRNA expression induced by imiquimod, a ligand of Toll-like receptor TLR7 in MH-S cells, a AM cell line. Imiquimod-induced phagocytosis was also suppressed by the exposure to acetamiprid in MH-S cells. However, the expression levels of IL-1β mRNA and cell-surface molecules (CD40, CD80, CD86, MHC class II) induced by imiquimod exhibited little effect by acetamiprid in MH-S cells. These results suggested that acetamiprid could modulate the activation of AMs. We are now investigating the effects of other neonicotinoid insecticides on AM functions.

P0724

HLA-DR regulate tumor-associated macrophage phenotype to promote invasion and metastasis of esophageal cancer

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Tumor-associated macrophages (TAM) are important components of tumor microenvironment. HLA-DR as a marker for TAMs phenotype detection, may participate in the clinical progression of esophageal cancer through regulation of TAMs. This study showed that the low expression of HLA-DR in esophageal squamous cell carcinoma (ESCC) was correlated with the increased of CD163+ M2 TAMs density, indicating high invasiveness and poor prognosis of patients with ESCC, suggesting that HLA-DR down-regulates promote the progression of ESCCs may be achieved through regulation of TAMs. Down-regulation of HLA-DR in TAMs by shRNA in vitro revealed that the TAMs phenotype was transformed into M2 phenotype, and the expression of VEGF and MMP9 in TAMs was up-regulated, and the ability of ESCC cells invasion and migration was significantly increased in TAMs co-culture system. The tumor tissue in vivo and nude mice experiments showed that the down-regulation of HLA-DR in stromal TAMs was positively correlated with the expression of VEGF and MMP9 in ESCC tissues, predicting the high invasiveness and poor prognosis of tumors in patients with ESCCs. Down-regulation of HLA-DR promotes phenotypic transformation of TAMs, promotes the secretion of VEGF and MMP9, and participates in the invasion and migration of ESCCs, which is closely related to the poor prognosis of patients.

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DOI: 10.1002/eji.201970400

P0725

Tumor fibroblasts recruit and polarize macrophages through the interaction of TEM1 and CD68 in HCC

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Fibroblasts and macrophages play a key role in the development of hepatocellular carcinoma (HCC). In this study, we performed IHC staining in 125 cases of HCC and found that **TEM1 was highly expressed in the membrane of fibroblasts** and perivascular cells in HCC. **A large number of macrophages were found in TEM1 positive fibrous septum**, but few macrophages existed in TEM1 negative fibrous septum. We also found that **TEM1 can be secreted by fibroblasts** isolated from tumor specimens and **TEM1 has chemotaxis ability on macrophages**. In vitro assay showed that both **TEM1 positive fibroblasts and rhTEM1 can adhere macrophages**. We explored TEM1-binding proteins by mass spectrometry, pull-down assay and co-IP assay and found **the interaction between TEM1 and CD68.**
Furthermore, we confirmed that **TEM1 positive fibroblasts can promote the polarization of macrophages to M2 phenotype**. We performed transcriptome sequencing and found that, among the factors secreted by fibroblasts, GAS6 is an optimal polarization candidate. We demonstrated that **fibroblasts were the main source of GAS6 in HCC and the GAS6 expression was tightly regulated by TEM1**. when treated with rhGAS6, the TNFα and the mature form of IL-1β of macrophages decreased significantly, indicating that **GAS6 can effectively promote the polarization of macrophages to M2 phenotype**.
The **fully human TEM1 antibody IgG223 could induce TEM1 to internalize into fibroblasts**, weakening the recruitment and polarization of macrophages, thus exhibiting anti-tumor activity in vivo. Therefore, TEM1 is an ideal target for HCC treatment, and **IgG223 provided a promising candidate therapeutics for HCC**.

P0727

The three members of the Vav family proteins form complexes that concur to foam cell formation and atherosclerosis

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Objective: During foam cell formation and atherosclerosis development, the scavenger receptor CD36 plays critical roles in lipid uptake and triggering of inflammatory response via activation of the guanine nucleotide exchange factor Vav1. The Vav protein family is made of 3 highly conserved members known as Vav1, Vav2 and Vav3. Vav3 was recently found to exert function in atherosclerosis development comparable to Vav1. It remains thus to decipher whether Vav2 also plays a role in the development of atherosclerosis.
Approach and Results: In this study, we found that the three Vav family proteins showed distinct intracellular distribution. Vav2 deficiency in RAW264.7 macrophages diminished significantly Oxidized low-density lipoprotein (Ox-LDL) uptake and CD36 signaling, demonstrating that each Vav protein family member was required for foam cell formation. Genetic disruption of Vav2 in ApoE-deficient C57BL/6 mice significantly inhibited the severity of atherosclerosis. Strikingly, we further found that genetic deletion of each member of the Vav protein family by CRISPR/Cas9 resulted in an almost similar alteration of transcriptomic profiles of a macrophage cell line. The three members of the Vav

DOI: 10.1002/eji.201970400

proteins were found to form ternary complex, and genetic ablation of each single Vav molecule was sufficient to prevent endocytosis of CD36, explaining the coincident requirement for the three members of the Vav proteins in foam cell formation and atherosclerosis.
Conclusions: The functional interdependence of the three Vav family members in foam cell formation was due to their indispensable roles in transcriptomic programing, lipid uptake, and activation of the JNK kinase in macrophages.

P0728

The immunosuppressive effects of umbilical cord mesenchymal stem cells on dendritic cells through regulating of DC´s differentiation and cytokines

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Background: Mesenchymal stem cells derived from umbilical cord (UC-MSCs) exert significant immunosuppressive effects on multiple immune cells, including dendritic cells, and therefore have great potential in the treatment of immune-related diseases. In this study, we evaluated whether UC-MSCs regulate the differentiation and maturation of human monocytes into dendritic cells (DCs). The mechanisms responsible for UC-MSCs´ effects on DCs were also investigated.
Methods: Human monocyte-derived DCs were induced from $CD14^+$ cells by adding diverse cytokines and co-cultured with or without UC-MSCs. The morphology of DCs was detected under a light microscope . Flow cytometry was used to analyze the phenotypes and function of DCs. ELISA was used to study cytokine production.
Results: In this study, we successfully induced DCs from $CD14^+$ monocytes. Monocytes were co-cultured with UC-MSCs in the transwell from the first day. During monocyte induced into immature DCs (iDCs), the proportion of immature DCs which exhibited expression of CD1a, was lower than it of control after UC-MSCs were co-cultured. However, UC-MSCs showed no effects on matural process of DCs when they were co-cultured with iDCs from the fifth day of induction. Furthermore, endocytosis and morphology of DCs were also interfered by UC-MSCs. ELISA found that UC-MSCs inhibited the differentiation of human monocyte-derived DCs by the production of interleukin (IL)-10 and IL-6.
Conclusions: Our results showed that UC-MSCs played an important role on DC differentiation and function instead of maturation, suggesting that they can be involved in the immune responses at multiple levels.

P0729

Inactivated EV71 induces nonspecific protection from reinfection via memory of monocytes and macrophages

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DOI: 10.1002/eji.201970400

“Trained immunity” refers to the memory characteristics of the innate immune system. Monocytes/macrophages have the ability of innate immune memory. But few studies examine the defense effect of intestinal memory macrophages. Trained immunity can be induced by β-glucan, BCG, LPS, modified LDL (oxLDL, oxidized low-density lipoprotein or acetylated LDL) or IL-1β. This study investigated intestinal-associated viruses: heat-killed EV71 virus induced “trained immunity” in monocytes/macrophages. In vitro cell experiments, heat-killed EV71 virus pretreatment significantly induced "training effect" in mouse macrophages, which was shown that pre-incubation of mouse peritoneal macrophages or bone marrow-derived macrophages with heat-killed EV71 virus on day 0 augmented production of IL-1β and TNFα upon re-stimulation 6 days later with poly(I:C). In the human monocyte THP-1 cell line, heat-killed EV71 virus also induced a similar “trained immunity" effect. Pre-incubation of THP-1 cells with heat-killed EV71 virus on day 0 augmented production of IL-1β and TNFα upon re-stimulation 6 days later with poly(I:C)(mimicking RNA virus infection), heat-killed EV71(mimicking EV71 virus infection) or human rotavirus live attenuated vaccine(mimicking human rotavirus infection). Further studies have shown that heat killed EV71 virus enhances the sensitivity of mice macrophages to poly(I:C) stimulation by inducing TLR3 expression in macrophages, and TLR3 partially mediates the "trained immunity" effect. This study observed and validated the phenomenon of intestinal virus-induced innate immune memory in macrophages, suggesting that intestinal macrophages may also have innate immune memory characteristics. This study may open the door for novel vaccine approaches, new therapeutic strategies for the treatment of intestinal pathogenic microbial infections.

P0730

Repurposing proton pump inhibitors as a novel class of Toll-like receptor inhibitors for acute lung injury

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common acute respiratory diseases with high mortality. To date, there are still no effective pharmacologic treatments for ALI/ARDS. Toll-like receptor (TLR) activation in macrophages plays an essential role in ALI/ARDS pathogenesis and may serve as a promising target to control the overwhelming inflammatory reactions in the lung. Previously, we found that inhibition of the endosomal acidification process in macrophages by nanoparticles effectively reduced the TLR activation and inflammatory cytokine production, indicating the significance of endosomal pH modulation in TLR activation. As the most widely used common drugs to control gastric acid secretion, proton pump inhibitors (PPIs) are known to inhibit proton pumps and modulate endosomal pH. Since the proton pumps (V-ATPase) are expressed in alveolar macrophages and airway epithelial cells, we hypothesized that PPIs may be able to reduce lung inflammation by regulating TLR signaling in macrophages through endosomal pH modulation. In this work, we first tested the effect of several clinical used PPIs on TLR4 activation *in vitro* and found that they could inhibit endosomal TLR signaling and the downstream proinflammatory cytokine production. Using a lipopolysaccharide (LPS)-induced ALI mouse model, we then demonstrated that PPIs could effectively reducing lung inflammation and injury by decreasing inflammatory cell infiltration and cytokine production 24h after intratracheal instillation. Interestingly, PPIs also promoted long-term tolerance to LPS. This study provides a new strategy for repurposing clinically available pharmacological agents as new treatments for ALI/ARDS.

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DOI: 10.1002/eji.201970400

P0731

CKIP-1 limits macrophage foam cell formation and vascular inflammation in atherosclerosis

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Atherosclerosis is the underlying pathological process of coronary artery disease (CAD) and cerebrovascular disease, which are severe vascular diseases. Atherosclerosis is recognized as a chronic inflammatory disease of large and medium arteries including lipid metabolism disorder and recruitment of immune cells to the artery wall. Macrophages uptake modified lipoproteins and transform into the foam cells, triggering inflammatory response and thereby promoting plaque formation. Here we show that casein kinase 2-interacting protein-1 (CKIP-1) is a suppressor of macrophage foam cell formation and atherosclerosis. Aortic plaque burden is significantly higher in *Apoe*$^{-/-}$ *Ckip-1*$^{-/-}$ mice on Western diet than in *Apoe*$^{-/-}$ controls with increased lipoprotein uptake, macrophage foam cell formation and vascular inflammation, indicating a protective role of CKIP-1 in this process. Ablation of *Ckip-1* specifically upregulates the transcription of scavenger receptor LOX-1, but not that of CD36 and SR-A. Mechanistically, CKIP-1 interacts with the proteasome activator REGγ and targets the transcriptional factor Oct-1 for degradation, thereby suppressing the transcription of LOX-1 by Oct-1. Moreover, *Ckip-1*-deficient mice undergo accelerated atherosclerosis, and bone marrow transplantation experiments showed that *Apoe*$^{-/-}$ *Ckip-1*$^{-/-}$ donors derived hematopoietic cells are sufficient to increase atherosclerotic plaque formation when transplanted to the recipient mice. Therefore, CKIP-1 plays an essential anti-atherosclerotic role through regulation of foam cell formation and inflammatory response. These data extended our understandings of CKIP-1 as a regulator of inflammatory response as well as atherogenesis progression, suggesting a potential strategy for atherosclerosis treatment based on targeting Oct-1-LOX-1 axis.

P0732

Sp-1 negatively regulates miR-20b expression in macrophages

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miR-20b is a member of the miR-106a-363 gene cluster, located on the X chromosome. miR-20b regulates the expression of multiple genes in vivo and it is closely related to the occurrence and development of many diseases, including inflammatory diseases and tumor development amongst others. However few studies have focused on the regulation of the miR-20b gene itself. In this study, **we used the miRBase database to obtain the upstream 2000 bp sequence of the miR-20b precursor**. **BioinformaticssoftwarePMATCH 1.0 and AliBaba2 were used to predict transcription factor binding in the upstream region. Sp-1 was identified as one of the most probable transcription factors regulating miR-20b gene expression. After treatment with a Sp-1 siRNA, the expression of miR-20b was significantly increased in RAW264.7 cells as well as in peritoneal and alveolar macrophages. In addition, the interference with Sp-1 gene expression also reversed the decrease in miR-20b expression in RAW264.7 cells induced by TNF-α.** These results indicate that Sp-1 negatively regulates the expression of miR-20b in macrophages. This finding suggests the potential of Sp-1 as a target for therapies in diseases associated with miR-20b overexpression.
Keywords: miR-20b, sp-1, macrophage, siRNA.

DOI: 10.1002/eji.201970400

P0733

TNFAIP8 regulates macrophage migration and infiltration via activation of NF-κB pathway

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The migration and infiltration of macrophages are key processes for a variety of physiological function and pathological reaction, such as pathogen clearance, inflammation or tissue homeostasis. TNFα induced protein 8 (TNFAIP8) is a member of TNFAIP family proteins that regulate the proliferation, invasion, metastasis and progression of malignant tumors. It expresses in most of immune cells including the macrophage. However, its roles in macrophages have not been completely understood. In present study, using the immunoblot, shRNA techniques, qRT-PCR, transwell and infiltration assays, we studied the roles of TNFAIP8 in macrophages. The results showed that knocking down TNFAIP8 by shRNA dampened the pseudopod formation and adhesion of mouse macrophages, reduced the expression of chemokine receptor CCR5 and elevated MIP-1α secretion. Moreover, silencing of TNFAIP8 inhibited the gelatin degradation ability and the secretion of MMP-9 of macrophages and suppressed macrophages migration and gel filtration. Furthermore, we found that knocking down TNFAIP8 suppressed the LPS-induced NF-κB activation by down-regulation of P65 phosphorylation and nuclear translocation. Taken together, TNFAIP8 plays important role in regulating the migration, chemotaxis and infiltration of macrophages via activation of NF-κB, which suggests that TNFAIP8 is a novel potential therapeutic target of autoimmune diseases and infectious diseases.

P0734

Regulation of autophagy by the Rab GTPase network

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Autophagy (macroautophagy) is a highly conserved intracellular and lysosome-dependent degradation process in which autophagic substrates are enclosed and degraded by a double-membrane vesicular structure in a continuous and dynamic esicle transport process. The Rab protein is a small GTPase that belongs to the Ras-like GTPase superfamily and regulates the vesicle traffic process. Numerous Rab proteins have been shown to be involved in various stages of autophagy. Rab1, Rab5, ab7, Rab9A, Rab11, Rab23, Rab32, and Rab33B participate in autophagosome formation, whereas Rab9 is required in on-canonical autophagy. Rab7, Rab8B, and Rab24 have a key role in autophagosome maturation. Rab8A and Rab25 are also involved in autophagy, but their role is unknown. Recent studies have found that LC3-associated phagocytosis (LAP) defects, rather than classical autophagy, may contribute to the pathogenesis of SLE. Our studies have found that low expression of Rab33a inhibits the production of LAP in macrophages, which has preliminarily confirmed that, compared with patients with low dsDNA, the expression of Rab33a in mononuclear macrophages of SLE patients with high dsDNA is down-regulated, suggesting that Rab33a may regulate the ability of macrophages to phagocyte apoptosis cell and anti-inflammatory function through the LAP pathway.

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DOI: 10.1002/eji.201970400

P0735

Exosomal transfer of tumor-derived miR-183-5p promotes tumor growth and metastasis by regulating the proinflammatory phenotype of macrophages

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Tumor-associated macrophages (TAMs) play a critical role in the tumor inflammatory microenvironment and facilitate tumor growth and metastasis. Most types of tumors aberrantly express microRNAs (miRNAs), which can be transferred between cells by exosomes and can regulate gene expression in recipient cells, but it remains unclear whether tumor-derived miRNAs are transferred by exosomes and regulate the TAM phenotype. We report that 4T1 breast-cancer-cell-derived exosomes enhanced TAM expression of the proinflammatory cytokines IL-1β, IL-6, and TNF-α, and that inhibition of 4T1-cell exosome secretion through shRNA-mediated Rab27a/b depletion repressed tumor growth and metastasis and markedly downregulated IL-1β, IL-6, and TNF-α in a 4T1 breast-tumor model. Furthermore, miRNA-expression profiling revealed that 3 miRNAs (miR-100-5p, miR-183-5p, and miR-125b-1-3p) were considerably more abundant in 4T1-cell exosomes than in bone marrow-derived macrophages, indicating potential exosome-mediated transfer of the miRNAs, and, notably, miR-183-5p was found to be transferred from 4T1 cells to macrophages through exosomes. Moreover, *PPP2CA* was verified as a miR-183-5p target gene, and PPP2CA downregulation enhanced NF-κB signaling and promoted macrophage expression of IL-1β, IL-6, and TNF-α. Lastly, when miR-183-5p was downregulated in exosomes through miR-183-5p-sponge expression in 4T1 cells, these 4T1-cell-derived exosomes triggered diminished p65 phosphorylation and IL-1β, IL-6, and TNF-α secretion, and the miRNA downregulation also led to repression of tumor growth and metastasis in the 4T1 breast-tumor model *in vivo*. Thus, miR-183-5p expressed in tumor cells was transferred to macrophages by exosomes and promoted macrophage inflammatory phenotype by inhibiting PPP2CA expression, which contributed to tumor progression in a breast-cancer model.

P0736

High salt diet aggravates ischemic stroke outcome by alleviating efferocytosis of macrophage

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Background and Purpose: High salt diet (HSD) is one of the risk factors of ischemic stroke. Macrophage removes dead neuron after ischemic stroke and then alleviates neuroinflammation. High salt primes macrophage towards a proinflammatory phenotype. The efferocytosis of macrophage is weakened on proinflammatory phenotype. The study explores whether high salt diet alleviates efferocytosis of macrophage after ischemic stroke.
Method: Wild-type male C57bl/6 mice were fed with fodder containing 8% sodium chloride and subjected to transient middle cerebral occlusion (tMCAO). Brain tissue were collected 3 days after experimental ischemia and flowcytometry and immunostaining were performed to detect the neuron decrease, macrophage polarization and efferocytosis. Primary bone marrow derived macrophages were used for additional mechanism study. Macrophages were treated with extra 40mM sodium chloride compared with complete medium.
Result: High salt diet increased infarct volume after ischemia and aggravated neural function deficiency. In brain lesion, HSD upregulated several inflammatory mediators, especially those secreted by macrophage, and caused more immune cells infiltration including macrophages. Macrophage/microglia in brain lesion presented weakened efferocytosis. The expression of CD206 on

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DOI: 10.1002/eji.201970400

macrophage, the marker of an anti-inflammatory phenotype, is downregulated in HSD mice compared with normal diet mice. Triggering receptor expressed on myeloid cells 2 (Trem2), a receptor relevant with phagocytosis, were downregulated in HSD stroke mice.
Conclusion: HSD aggravated ischemic stroke outcome and activated macrophages. Efferocytosis of macrophage was decreased in HSD mice. Trem2 is an important receptor on high macrophage for it is consistent with phagocytosis.

P0737

IRF3 activation is associated with its O-GlcNAcylation state in anti-viral innate immunity

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O-GlcNAcylation plays an important role in many fundamental biological activities. However, little attention was paid to the function of O-GlcNAcylation in immune system. Whether O-GlcNAc transferase (OGT) can regulate virus-triggered immune response remains largely unknown. Our results showed that there is an interaction between OGT and IRF3. Moreover, IRF3 is O-GlcNAcylated by OGT. Furthermore, OSMI-1, a highly potent and selective inhibitor of OGT, inhibited O-GlcNAcylation. OSMI-1 treated show defective antiviral immune responses upon VSV challenge and IRF3 phosphorylation and dimerization. These results indicated that OGT is involved in the positive regulation of innate anti-viral immune response via IRF3 phosphorylation and dimerization. However, the mechanism of IRF3 o-GlcNAcylation regulating its activation needs to be further determined. Our study shed a light on the molecular mechanism by which OGT-mediated O-GlcNAcylation regulating IRF3 function and highlighted the important role of glucose metabolism in antiviral innate immunity.

P0738

Role of histone deacetylase 3 in the development and function of alveolar macrophages

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Alveolar macrophages (AMs) are the major constituents of lung resident macrophages. They originate from fetal monocytes and possess specialized gene expression profiles. Proper function of AMs assures the homeostasis of pulmonary immune system and the defense against pathogens. However, the epigenetic regulatory mechanisms controlling the development and function of AMs remain unclear. Here we report that histone deacetylase HDAC3 is indispensable for the development and function of AMs in lung. Conditional deletion of HDAC3 in macrophages impaired the AM populations and their replenishment after irradiation. It is also required for the proliferation of AMs induced by GM-CSF. Mice with HDAC3 conditional knockout in macrophages were more susceptible to influenza virus infection. Transcriptome analysis showed that numerous AM specific genes and genes that increased during the development of AMs were downregulated in HDAC3 knockout AMs. The protein interaction network showed that HDAC3 interact with multiple proteins that are involved in metabolic regulation, indicating that deletion of HDAC3 in AMs may results in metabolic disorders in AMs. Although further investigation is required on the mechanism of how HDAC3 regulates the metabolism in AMs, these results all suggested that HDAC3 has an essential role in orchestrating the development and function of AMs.

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DOI: 10.1002/eji.201970400

P0739

Roquin-1 regulates macrophage immune response and participates in hepatic ischemia-reperfusion injury

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With the development of liver surgery, ischemic reperfution (IR) injury has received increasing attention. Roquin-1 has been shown to play an important role in innate immune and immune balance. We demonstrate that Roquin-1 expression increased at 1 hour after IR and then decreased in C57B/L mice. The immunofluorescence double-label showed that Roquin-1 was mainly expressed in mø. Furthermore, we used Clodronate Liposomes to remove mø,and injected the bone marrow derived macrophage (BMDM) through the tail vein in 1 hour before IR. We found that liver IR injury was aggravated by Roquin-1 interference. The results of PCR and ELISA suggested that after interference with Rqouin-1, mø increased toward M1 and decreased toward M2. Then, interference with Roquin-1 promoted the polarization of mø to M1 and inhibited the polarization of M2. By WB technology and AMPKα and mTOR inhibitors, we found that Roquin-1 promotes the phosphorylation of mTOR and STAT3 by inhibiting the phosphorylation of AMPKα. We used AICAR to activate AMPKα in mø and found that the level of ubiquitination of AMPKα was decreased after activation of AMPKα. Furthermore, by bioinformatics methods, we identified potential ubiquitination sites on AMPKα. By the point mutation experiments in vitro, we confirmed that the ubiquitination of these sites is regulated by Roquin-1. Meanwhile, Roquin-1 interference inhibited the activation and function of AMPKα. This topic describes the protection of liver IR injury by Roquin-1 and discusses its main mechanism for regulating AMPKα activity through ubiquitination and affecting the polarization of mø.

P0740

Candida albicans cell wall mannoprotein synergizes with lipopolysaccharide to affect RAW264.7 proliferation, phagocytosis and apoptosis

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Background: In recent years, the infection of candida albicans has increased by more than 20 times due to the widespread use of immunosuppressant, broad-spectrum antibiotics and anti-tumor drugs. Candida albicans infection is not only difficult to diagnose, but also has a high mortality rate. In this report, we examined the effects of the C. albicans cell wall mannoprotein (MP) on macrophage immunity.
Methods: The MTS assay was used to detect cell proliferation activity and neutral red staining to observe cell phagocytosis. The Griess method was used to detect NO secretion in culture supernatants and apoptosis of macrophages were determined with use of FITC-Annexin V and PI staining. mRNA and protein expressions of JAK2, STAT3, IL-1β, IL-6, TNF-α and iNOS in RAW264.7

DOI: 10.1002/eji.201970400

cells were determined with use of RT-PCR and western blot.
Results: MP significantly promoted the proliferation of RAW264.7 cells, inhibited their phagocytic capacity, but exerted no significant effects on apoptosis of macrophages. In addition, MP not only up-regulated the expression of cytokines, but also the expressions of p-stat3 and p-jak2. Interestingly, when MP was combined with lipopolysaccharide (LPS) a markedly accentuated release of inflammatory cytokines was observed.
Conclusion: MP participates in the inflammatory response by promoting LPS-induced macrophage inflammatory response. The JAK2/STAT3 signaling pathway is one of the important signaling pathways for MP to participate in LPS-induced macrophage inflammatory response. Our study also found that MP participates in LPS-induced inflammation by promoting the transformation of RAW264.7 from M0 to M1.

P0741

The structural feature and biological activity of Igμ in mouse macrophages

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According to the classic immunology theory, immunoglobulin (Ig) could only be produced by B lymphocytes and plasma cells. However, many evidence have found Ig could be expressed by non-B cells. In this study, we found that IgM heavy chain (Igμ) was expressed in macrophages of mice. By the Next Generation Sequencing, we demonstrated the Igμ were functional. Furthermore, like the B-Ig, most sequences of macrophage-derived Igμ showed germline-encoded or with minimal somatic hypermutations. However, the analysis results of macrophage-derived Igμ repertoires revealed that the Igμ did not showed diversity, but displayed restricted VDJ pattern, which suggests that Igμ may have a unique biological function. Functionally, we found that when knockdown of Igμ in macrophage, the migration of macrophage was promoted, moreover, the results of proteome analysis displayed that increased motility may be related to activation of Src/FAK signaling pathway.
Considering the role of macrophages in inflammatory, we stimulated the macrophages with LPS and found Igμ expression was significantly reduced. When Igμ was silenced, the proinflammatory cytokines, such as IL-6 and MCP-1 induced by LPS were increased, which may be mediated by activation of Akt and STAT3 (Ser727). In addition, after knockdown of Igμ, the phosphorylation of IRE1α and the splice of XBP-1 in Unfolded Protein Reaction (UPR) pathway was increased, especially, the BiP expression , which is a target of spliced XBP-1, was markedly up-regulated.

P0742

Lung inflammation is attenuated by intratracheal administration of quercetin

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Acute respiratory distress syndrome (ARDS) is characterised by widespread injury of the alveolar-capillary membrane, resulting in a life-threatening form of respiratory failure. Since little or no effective treatments were available, ARDS is associated with high rates of mortality. Quercetin is one of the flavonoids found in fruits and vegetables. We have previously reported that quercetin had potent cytoprotective effects through heme oxygenase (HO)-1 in various cells, including mast cells,

DOI: 10.1002/eji.201970400

fibroblasts, and epithelial cells. In this study, we investigated the effects of intratracheal administration of quercetin on lipopolysaccharide (LPS)-induced ALI in mice. We also investigated the involvement of HO-1 on in quercetin's suppressive effects.
Intratracheal administration of quercetin reduced the wet lung-to-body ratio and interstitial infiltration in LPS-challenged mice. The expression of HO-1 was significantly increased in lungs, especially alveolar macrophages (AM) and bronchial epithelial cells, after administration of quercetin. Quercetin showed marked suppression in the production of TNF-α, IL-1β, and IL-6 induced by LPS was observed, and SnPP, an inhibitor of HO, canceled these effects in AMJ2-C11 cells, a mouse AM cell line. Furthermore, quercetin decreased the activity of matrix metalloproteinase (MMP)-9 and the production of pro-inflammatory cytokines in bronchoalveolar lavage fluid cells activated by LPS in advance. These results suggested that quercetin could suppress LPS-induced lung inflammation via HO-1-dependent pathway.

P0743

Artificially cultured Polyporus alveolaris mycelium extract (PAME) exhibits anti-tumor effects via macrophages

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We have previously shown that the components extracted from Hachinosutake (*Polyporus alveolaris*), PAE, is the good activator for macrophages to anti-tumor. In fact, it has been confirmed in an *in vitro*system that PAE activates RAW264.7 and THP-1 to exhibit an antitumor effect. In order to ensure the stability of the sample, an artificial culture method was established. Here, we report the results of the Mφ activation effect of *Polyporus alveolaris* Mycelia Extract (PAME), which is extracted from the artificial cultured mycelium of Anthracis bovis comparison with PAE on. As expected, the anti-tumor activity index (Cytostasis index to P815 tumor target) of RAW264.7 stimulated with PAME showed a high anti-tumor activity of 75.7%. Next, we compared PAE and PAME by examined the effects of PAE and PAME to the production of TNF-α and Nitric oxide production, from RAW264.7. As a result, it was confirmed to induce TNF-α production and Nitric Oxide production in a concentration dependent manner with PAE at 0.5-2mg/mL. On the other hand, only TNF-α production was induced with PAME on the same condition. Furthermore, the fraction that showed anti-tumor activity had PAE of 100 kDa or more, while PAME was 30 kDa or less. Interestingly, PAE was significantly decreased its activity by β1-3 glucanase treatment, whereas PAME was hardly affected by this enzyme. These results indicate that PAE and PAME contain different components and exert an anti-tumor effect via macrophage activation through different pathways despite being extracted in the same way from mushrooms of the same origin.

P0744

The origin of osteoclasts in pannus in arthritis

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Background: In rheumatoid arthritis, significant synovial inflammation eventually leads to destruction of the bone caused by osteoclasts in pannus. It is not entirely clear whether the osteoclast precursors seen in pannus arises from division of local precursors or differentiation from newly recruited blood monocytes.
Objective: To identify the origin of osteoclasts in pannus in arthritis.

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DOI: 10.1002/eji.201970400

Methods: Osteoclast precursors were identified by RANKL-induced osteoclastogenesis in vitro using joint tissues of collagen-induced arthritic mice. Irradiated wild-type (WT) DBA1/J mice were transplanted with bone marrow cells from TRAP-Tdtomato transgenic mice. WT and TRAP-Tdtomato transgenic mice were conjoined for parabiosis. The transplanted mice and parabionts were subjected to the development of collagen-induced arthritis. Flow cytometry analysis, immunohistochemistry, and two-photon microscopy imaging in vivo were applied to discern whether osteoclast precursors and osteoclasts were originated from bone marrow through circulation or not.
Results: Immunohistochemistry analysis of arthritic knees of bone marrow chimeric mice showed that the osteoclasts in pannus were Tdtomato+ and exclusively derived from bone marrow. In vivo imaging of the bone marrow using two-photon microscopy showed the increased motility of osteoclast precursors, some of them entering the blood stream.
Conclusion: Osteoclasts in pannus in collagen-induced arthritis are exclusively derived from monocytes in bone marrow through circulation.

P0745

Type II interferon synergize with either CD40 or TLRs signaling to efficiently render macrophages tumoricidal

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Introduction: Re-polarization of tumor-infiltrating macrophages into an efficient anti-tumor M1-like phenotype has raised great interest lately in cancer immunotherapy. IFNγ and/or LPS have been described as the typical inducers of the classical M1 activation, however less is known about other molecules and their potential functional and phenotypic-induced differences on macrophages.
Materials and methods: INFγ, IFNβ, LPS, Pam3 and soluble CD40L were used alone or in combinations to activate murine macrophages. Growth inhibition of co-cultured Lewis-lung carcinoma (LLC) cells was investigated using thymidine incorporation. Cancer cytotoxicity *in vivo* was investigated using co-injections of activated macrophages with LLC cells in BL6 mice. Endocytosis, production of NO and cytokines, expression of cell markers, as well as, the metabolic and intrinsic properties of polarized BMDM were also analyzed.
Results and discussion: We found that in addition to interferons plus TLR agonists activation, also sCD40L+IFNγ was sufficient, in inducing the NO-depended macrophage-mediated growth-inhibition of LLC. Importantly, macrophages treated with IFNγ+Pam3 hampered tumor establishment when co-injected with LLC in vivo. In addition, activated macrophages upregulated NO, as well as TNFα, IL-6, IL-12, IL-15 and IL-18. Interestingly, in vitro endocytosis was downregulated in tumoricidal macrophages. Expression of CD38, CD40, CD80/86, MHCI/II and PD-L1 were induced in different patterns depending on the applied stimuli. Finally, oxidative phosphorylation and proliferation was suppressed in tumoricidal macrophages. Herein, we have shown that TLR-agonists, CD40 activation and/or interferons promote distinct functional and phenotypic properties in so-called M1 macrophages. Our results point to the potential importance of multiple signal activation in the development of macrophage-mediated cancer immunotherapeutics.

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DOI: 10.1002/eji.201970400

P0746

Pathogenesis of fatty liver disease by Ssu72-mediated functionality of Kupffer cell

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Ssu72 is a dual protein phosphatase which have activity about tyrosine or serine/threonine residues. Ssu72 is known to dephosphorylate Ser5-P and Ser7-P of an RNA polymerase II (RNAP II) carboxy-terminal domain (CTD). The amino acid of Ssu72 have a protein tyrosine phosphatase motif, CX5RS, but possess a variant 'aspartate loop' suggest of a new class of phosphatase. We found that Ssu72 function as a cohesin-binding phosphatase and interacts with Aurora B kinase for regulate duplicated sister chromatid cohesin and separation. Recently we reported that hepatocyte specific Ssu72 deletion led to onset of fatty liver disease with high incidence. In addition, we observed not only dramatic decrease of macrophage population but also defective M2 macrophage generation in BAT of adipose tissue specific Ssu72 knockout mice. In this aspect, we generated myeloid cell specific Ssu72 deletion mouse model, and we will introduce the physiological relevance of Ssu72 in regulating the function of Kupffer cells during the development of fatty liver disease.

P0747

How obesity changes the dynamics, diversity and function of macrophages in the adipose tissue: search for culprits

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Immune cell infiltration in visceral adipose tissue (VAT) during obesity is associated with local chronic inflammation and the development of metabolic syndrome, a plague of aging societies. Adipose tissue macrophages (ATMs), one of the major leukocyte fractions in the VAT, are not only the key cells for maintenance of VAT homeostasis, but they could also play a major role in a metabolic dysregulation during aging and obesity. Similarly what we have observed in a tumour microenvironment, at least three phenotypically distinct subpopulations of ATMs are present in the VAT showing different frequencies during the progression of obesity suggesting that the tissue environment drives ATM heterogeneity and vice versa that ATMs contribute to VAT homeostasis and inflammation. We will present data on the turn-over kinetics and potential roles of ATMs during development of obesity probing beyond the superficial M1/M2 dichotomy.
This work is supported by a Ministry of Education (MOE) MOE2016-T2-1-012 and MOE2018-T2-2-016 to C.R.

P1327

Effects of tofacitinib on DC subsets in rheumatoid arthritis

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DOI: 10.1002/eji.201970400

Objective: Tofacinitib is an oral Janus kinase inhibitor, recently approved for the treatment of rheumatoid arthritis (RA) . Altered DC subsets subsets have been reported in RA patients pathology . This study was undertaken to evaluate the effects of tofacitinib on DC subsets in RA patients.
Methods: Peripheral pDC cells (Lin-HLADR+CD123+CD11C-)and mDC cells (Lin-HLADR+CD123-CD11C+)were analyzed by using flow cytometry in 8 RA patients at baseline and at the 3 month treatment and 15 healthy subjects.
Results: Compared with the healthy subjects, active RA patients had significantly lower proportion of PB pDC cells and mDC cells ($P < 0.05$,respectively).Tofacitinib treatment significantly reduced the patient´s disease activity($P < 0.05$), and resulted in an increase in pDC cells(Lin-HLADR+CD123+CD11C-)and mDC cells (Lin-HLADR+CD123-CD11C+)($P < 0.05$,respectively) .
Conclusion: Our findings indicate that RA patients showed lower proportion of PB pDC cells (Lin-HLADR+CD123+CD11C-)and mDC cells (Lin-HLADR+CD123-CD11C+),and.monitoring of pDC cells and mDCcells appears to be adequate to assess RA disease activity in tofacitinib-treated patients .

P1328

ATP6V0d2 mediates leucine-induced mTORC1 activation and polarization of macrophages

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mTORC1 senses the cellular nutrition, energy status and extracellular stimuli and regulates cell growth, differentiation and functions. The activation of mTORC1 requires amino acids and localization of mTOR to the lysosome membrane. Although numerous amino acid sensors have been identified, the regulation of mTORC1 activation by amino acids remains largely elusive. ATP6V0d2 is a macrophage-specific vacuolar ATPase subunit and dispensable for lysosome acidification. In this study, we found that only leucine, neither arginine nor glutamine, induced mTORC1 activation in bone marrow derived macrophages (BMDMs), though all three amino acids induced mTORC1 activation in HEK293T. Deletion or overexpression of ATP6V0d2 resulted in impaired or enhanced leucine-induced mTORC1 activation; and deletion of ATP6V0d2 inhibited the recruitment of mTOR to the lysosome membrane in BMDMs. Compared to WT counterpart, M1 polarization was impaired, but M2 polarization was enhanced in *Atp6v0d2*$^{-/-}$ BMDMs *in vitro*. Furthermore, the phosphorylation of S6 in F4/80^{+} macrophages from fasted *Atp6v0d2*$^{-/-}$ mice was significantly reduced upon leucine feeding, compared with macrophages from control animals; and deletion of ATP6V0d2 significantly reduced splenic F4/80^{+}CD11c^{+} M1 polarization but enhanced F4/80^{+}CD206^{+} M2 polarization. In summary, we identified that ATP6V0d2 mediates leucine-induced mTORC1 activation in a macrophage specific fashion, which subsequently regulates macrophage differentiation.
Key： mTORC1, leucine, macrophage, polarization

P1329

A case of blastic plasmacytoid dendritic cell neoplasm and immunophenotype analysis

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The patient was a 20-year-old male whose retrooccipital lymph node enlargement was found with no pain for half a year.Multiple lymph nodes were enlarged half a month ago,and some rash appeared on

DOI: 10.1002/eji.201970400

the front chest wall and abdominal skin,repeated fever. Later,he came to the hospital with increased lymph nodes and left upper abdomen dragging pain.Bone marrow smear showed 85% of the original cells.Bone marrow flow cytometry showed abnormal juvenile cells accounted for 94.7% and CD38,HLA-DR,CD4,CD123,CD56,CD7 were positive.Tumor cells were found in bone marrow,supraclavicular lymph nodes and upper abdominal skin biopsy,and immunohistochemistry showed that CD4,CD123,CD56,Ki-67 were positive.
Diagnosis: Blastic plasmacytoid dendritic cell neoplasm.CHOP regimen was given for chemotherapy and was included in clinical follow-up.

P1330

Effects of interleukin-10 on the protein expression profile of human mature dendritic cells

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Dendritic cells (DCs) are the most important antigen-presenting cells due to their professional and extremely efficient antigen-presenting function. Evidences show that tumor-derived factors could impair DCs' immune functions, which lead to inefficacy of DCs-based immune therapies. Our previous studies found that IL-10, as one of the suppressive cytokines from tumor microenvironment (TME), could deteriorate DCs' motility and immune functions while the underlying mechanism is unknown. In this study, we have compared the proteomic changes of mDCs treated by IL-10 and control group via two-dimensional electrophoresis combine with MALDI-TOF/TOF MS. Then we analyzed the function of differentially expressed proteins through bioinformatics methods to explore the molecular mechanism of IL-10 which has inhibitory effect on mDCs. The results indicated that IL-10 interfered with the oxidative metabolic process, glycolytic metabolism, and expression of cytoskeleton -related proteins in mDCs, and disturbances in these physiological processes resulted in reduced immune function and motility of mDCs. Our research lays the foundation for further revealing of the role of mDC in the tumor microenvironment.
Keyword: dendritic cells, IL-10, proteomic, immune function, metabolism

P1331

Systematic biological study on the expression of cytoskeleton-binding proteins in dendritic cells at different differentiation stages

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Dendritic cells (DCs) are specialized antigen presenting cells, which play an important role in innate and adaptive immune responses. The functionality and morphology of monocytes (MOs) in the process of differentiation into mature dendritic cells (mDCs) via immature dendritic cells (imDCs) display great differences, but the underlying mechanism is still elusive. The synergistic effect of cytoskeletal binding proteins is the structural basis for cells to exert their biological functions. In the present studies, the expression levels and localization of cytoskeletal binding proteins in DCs at different differentiation stages were investigated by gene chip, protein chip, western blotting and immunofluorescence, the data were analyzed by system biology algorithms in order to find the key cytoskeleton-binding proteins which affect the DCs' differentiation. The results showed that MOs,

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DOI: 10.1002/eji.201970400

imDCs and mDCs had significantly different expression profiles and colocalization of cytoskeleton-binding proteins, p130Cas, merlin, fascin and Src could play crucial roles in the differentiation of DCs. It's most significant for further understanding of the immunological behavior of DCs and immune response initiation process.
Keywords: dendritic cells, differentiation, cytoskeleton-binding proteins, systematic biology

P1332

The regulations of osmotic pressure to the biomechanical properties and immune functions of immature dendritic cells

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Dendritic cells (DCs) are powerful antigen presenting cells in the body and play an important role in activating immune response or immune tolerance. The main function of immature dendritic cells (imDCs) is to parasitize peripheral tissues and take up antigens, which undergo the complex physical and chemical microenvironment. Under some pathological conditions, extracellular osmotic pressure changes significantly. The effect of osmotic pressure on imDCs is still unknown. The biomechanical properties of cells can reflect the relationship between their structure and function. In this study, we explored the effects of high and low osmotic extracellular environments on the phagocytic and biomechanical properties of imDCs. The results showed that the biomechanical properties of cells were changed, such as electrophoretic mobility and cell membrane fluidity, and the F-actin cytoskeleton was remodeled under different osmotic pressures, the capability of antigen phagocytosis was enhanced, suggesting that the physical factors could also be a non-negligible aspect in regulating immune cell function. It is significant for further understanding of the immunoregulatory functions of DCs.
Keywords: immature dendritic cells; osmotic pressure; biomechanical properties; immune function

P1333

Biomechanical characteristics closely related with immune functions of dendritic cells

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As potent antigen presenting cells, dendritic cells (DCs) are utilized to deliver the signals essential for the initiation of immune responses. The motility of DCs is crucial for migration of immature DCs (imDCs) in peripheral tissue and the interaction between mature DCs (mDCs) and naïve T cells in the secondary lymph node. From biomechanical viewpoint, the deformability of cells is necessary for their motility. Deformation of cells can be divided into active deformation (e.g. chemotaxis) and passive deformation (e.g. migration under shear stress of blood flow). However, there is no detailed study on the deformability of DCs including imDCs and mDCs. In this study, the active and passive deformations of DCs under various culture conditions were respectively measured by atomic force microscopy and micropipette. The results showed that DCs at different differentiation stages displayed various deformabilities, their passive and active deformation depended on matrix stiffness, mDCs had higher active deformation and Young's modulus as well as lower passive deformation. It's significant for further understanding the immunoregulatory functions of DCs.

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DOI: 10.1002/eji.201970400

P1334

Acidic microenvironment affects the migration and biophysical properties of dendritic cells

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Dendritic cells (DCs) are the most powerful antigen-presenting cells known to date and play an important role in anti-tumor immune responses. The tumor microenvironment plays an important role in the occurrence and development of tumors, and acidity is an important feature. The effect of the acidic tumor microenvironment on the immunoregulatory function of dendritic cells is still too clear. The biophysical properties of cells can reflect the relationship between their structure and function, including membrane fluidities, viscoelastic properties, osmotic fragilities, membrane negative charges, cytoskeleton (F-actin) organization and motility. This study investigated the effects of acidic tumor microenvironment on the biophysical and immunological functions of DCs from the perspective of biophysics and tumor immunology. We found that both acidity (pH6.5 and 5.8) and lactic acid (5mM, 10 mM and 20mM) could inhibit the migration, membrane negative charges and F-actin expression of DCs. Importantly, only in 1 hour of the treatment, acidity could significantly inhibit the migration ability and F-actin expression levels, and lactic acid could significantly reduce the amount of membrane negative charges and F-actin expression levels of DCs. It's significant for understanding the immune regulatory function of DCs and the immune escape mechanism of tumors.
Keywords: tumor microenvironment, acidity, dendritic cells, biophysical property, immune function

P1335

Effects of Fumonisin on the cytoskeletal structure of dendritic cells

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Dendritic cells (DCs) are the most potent professional antigen-presenting cells of the immune system. In nature, Fumonisins are important mycotoxins and detrimental to human and animal health as its serious acute toxicity, cytotoxicity, immunotoxicity and carcinogenicity, etc. The understanding of its toxicological mechanism is essential for preventing and controlling the occurrence of mycotoxicosis. Therefore, it is of great significance for elucidating the toxicity mechanism through studying the influence of fumonisin on DCs. As biophysical properties of cells can reflect their relationship between structure and function, the effects of fumonisin on the biophysical properties and F-actin cytoskeleton of murine DCs were studied from the perspective of biophysics and immunology. Our results showed that the biophysical characteristics of murine mDCs were affected, such as membrane fluidity and electrophoretic mobility were weakened. Moreover, fumonisin could reorganize the cytoskeletal structure of mDCs accompanied with the expression level changes of actin-binding proteins. It is significant for further understanding of the fumonisin toxicological mechanism.

DOI: 10.1002/eji.201970400

P1336

Inflammatory compound lipopolysaccharide promotes the survival of GM-CSF cultured dendritic cell via PI3 kinase dependent up-regulation of Bcl-x

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As professional antigen-presenting cells, dendritic cells (DCs) initiate and regulate immune responses against inflammation. The invasion of pathogens into the body, however, can in turn cause the change of DCs in both activity and viability, which ultimately affect immune homeostasis. The exact mechanisms that the bacteria utilize to alter the lifespan of DCs, however, are far from clear. In this study, we found that the bacterial wall compound lipopolysaccharide (LPS) can promote the survival of GM-CSF-differentiated DCs (GM-DCs). At molecular levels, we demonstrated that GM-DCs had distinct pattern of mRNA expression for anti-apoptotic BCL-2 family members, of which, Bcl-x increased significantly following LPS stimulation. Interestingly, specific inhibition of BCL-XL protein alone was sufficient to remove the anti-apoptotic effects of LPS on BM-DCs. Further study of the signaling mechanisms revealed that although LPS can activate both Erk MAP kinase and PI3 kinase pathways, only blocking of PI3K abolished both Bcl-x up-regulation and the enhanced survival phenotype, suggesting the PI3K signaling mediated the up-regulation of Bcl-x for the LPS-induced pro-survival in GM-DCs. Collectively, this study unveils a molecular mechanism that DCs adapt to maintain innate immunity against the invasion of pathogens.

P1337

Cannabinoid receptor 2 deletion promotes proliferation and activation of hepatic macrophages in mice with acute liver injury induced by concanavalin A

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Cannabinoid receptor 2 (CB2) play a crucial role in the regulation of immune inflammatory inflammation in the body. Studies have shown that CB2 agonists can protect liver ischemia-reperfusion injury by mitigating oxidative stress, and can control the release of inflammatory factors by regulating immune balance, thereby reducing acute hepatitis damage and altering liver cholesterol metabolism and lipids. Dependent on intestinal transport. In immunological liver disease, activation and proliferation of hepatic macrophages are closely related to the degree of liver damage. At present, the basic pathogenesis of autoimmune diseases is the immune imbalance disorder and the release of inflammatory factors. Hepatic macrophages are rich in CB2, and the protective effect of CB2 gene expression on acute immune liver injury and its mechanism are not clear. The results showed that after the CB2 gene was knocked out, the ALT in the serum of the mice was higher under the stimulation of ConA, and the F4/80, CD68 and TNF-α in the liver tissues were higher than those in the wild type mice. Moreover, we also observed that liver knockout in mice with knockout was more serious by HE staining. This is important for the preliminary study of the effect of CB2 gene deletion on hepatic macrophages in acute immune liver injury in mice.
Keyword: cannabinoid receptor-2(CB2); acute liver injury; liver macrophages

DOI: 10.1002/eji.201970400

P1338

IL-4-polarized BV2 microglia cells promote tube generation of HUVEC by secreting exosomes

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The microglia cells may play nerve-protective role via the promotion of angiogenesis, and their transfer has been shown to play a protective role in ischemic stroke. However, the underlying mechanisms are largely unknown and need further investigation. We studied the proangiogenic effects of IL-4 and LPS-polarized BV2 microglia by in vitro experiments and the underlying mechanisms of these pro-angiogenic effects. We used phosphate buffered saline (PBS), LPS or IL-4 to polarize BV2 cells, respectively. The gene expression pattern was analyzed by reverse transcription polymerase chain reaction (RTPCR). We examined the angiogenesis effect of polarized BV2 cells and their exosomes through 3-dimensional co-cultures in vitro. Finally, the microRNA (miRNA) profiles of exosomes released by BV2 cells under different polarization conditions were examined using miRNA microarray. Interleukin-4-polarized microglia increased the tube formation of endothelial cells by secreting exosomes. The miRNA profiles of exosomes released by BV2 cells under different polarization conditions were different. Exosomes from IL-4-polarized BV2 cells contained higher amounts of miRNA-26a compared to those from the LPS-polarized and unpolarized BV2 cells. Interleukin-4-polarized microglia cells might promote angiogenesis through the secretion of exosomes containing miRNA-26a.

P1339

Macrophage p38α promotes nutritional steatohepatitis through M1 polarization

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Background and aims: p38 mitogen-activated protein kinases are important inflammatory factors. We aimed to characterize the cell type-specific role of p38α in non-alcoholic steatohepatitis (NASH).
Methods: NASH was established and compared between hepatocyte-specific p38α knockout ($p38\alpha^{\Delta Hep}$), macrophage-specific p38α knockout ($p38\alpha^{\Delta M\Phi}$) and wild-type ($p38\alpha^{fl/fl}$) mice fed with high-fat diet (HFD), high-fat/high-cholesterol diet (HFHC), or methionine-and choline-deficient diet (MCD). p38 inhibitors were administered to HFHC-fed wild-type mice for disease treatment.
Results: p38α was significantly upregulated in the liver tissues of patients with NAFLD. Compared to $p38\alpha^{fl/fl}$ littermates, $p38\alpha^{\Delta Hep}$ mice developed significant nutritional steatohepatitis induced by HFD, HFHC or MCD. Meanwhile, $p38\alpha^{\Delta M\Phi}$ mice exhibited less severe steatohepatitis and insulin resistance than $p38\alpha^{fl/fl}$ mice in response to a HFHC or MCD. The effect of macrophage p38α in promoting steatohepatitis was mediated by the induction of pro-inflammatory factors (CXCL2, IL-1β, CXCL10 and IL-6) secreted by M1 macrophages and associated signaling pathways. $p38\alpha^{\Delta M\Phi}$ mice exhibited M2 anti-inflammatory polarization as demonstrated by increased M2 macrophages and enhanced arginase activity in liver tissues. Primary hepatocytes from $p38\alpha^{\Delta M\Phi}$ mice showed decreased steatosis and inflammatory damage. In a co-culture system, p38α deleted macrophages attenuated steatohepatitic changes in hepatocytes through decreased secretion of pro-inflammatory cytokines (TNF-α, CXCL10 and IL-6), which mediate M1 macrophage polarization in $p38\alpha^{\Delta M\Phi}$ mice. Restoration of TNF-α, CXCL10 or IL-6 induced lipid accumulation and inflammatory responses in $p38\alpha^{fl/fl}$ hepatocytes co-cultured with $p38\alpha^{\Delta M\Phi}$ macrophages. Moreover, pharmacological p38 inhibitors suppressed HFHC-induced steatohepatitis.
Conclusions: Macrophage p38α promotes the progression of steatohepatitis by inducing pro-inflammatory cytokine secretion and M1 polarization. p38 inhibition protects against steatohepatitis.

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DOI: 10.1002/eji.201970400

P1340

Severe fever with thrombocytopenia syndrome virus-induced macrophage differentiation is regulated by miR-146

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging hemorrhagic fever with a high mortality rate in humans, which is caused by SFTS virus (SFTSV), a novel phlebovirus in the Bunyaviridae family, is tick borne and endemic in Eastern Asia. Previous study found that SFTSV can infect and replicate in macrophages in vivo and in vitro. However, the role of macrophages in virus replication and the potential pathogenic mechanisms of SFTSV in macrophage remain unclear. In this study, we provided
evidence that the SFTSV infection drove macrophage differentiation skewed to M2 phenotype, facilitated virus shedding, and resulted in viral spread. We showed evidence that miR-146a and b were significantly upregulated in macrophages during the SFTSV infection, driving the differentiation of macrophages into M2 cells by targeting STAT1. Further analysis revealed that the elevated miR-146b but not miR-146a was responsible for IL-10 stimulation. We also found that SFTSV increased endogenous miR-146b induced differentiation of macrophages into M2 cells mediated by viral non-structural protein (NSs). The M2 skewed differentiation of macrophages may have important implication to the pathogenesis of SFTS.

P1341

Macrophages promote DNA repair of double strand break in multiple myeloma cells by non-homologous end joining(NHEJ), nevertheless sacrifice the accuracy of NHEJ

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Multiple myeloma (MM) is a hematological malignancy, characterized by clonal proliferation of malignant plasma cells. DNA damage and genomic instability play an important role in the pathogenesis of MM. Based on the characteristics of high heterogeneity and genomic instability of MM, and the protective effect of MΦs on MM cells (MMCs), our study intended to further clarify whether MΦs affect MMCs DNA damage response and DNA repair, and the relationship between MΦs and genomic instability of MMCs. We found that the content of MΦs in bone marrow biopsy of MM patients is related to the results of cytogenetics. The higher the content of MΦs, the more complicated the cytogenetic abnormalities of patients. In our study, MΦs were harvested from peripheral blood monocytes, which were incubated for 7 days with M-CSF. The results showed that MΦs reduces the baseline γH2AX of MMCs, contributing to MMCs survive in the case of genomic instability. In the case of severe injury of MMCs' DNA, MΦs promote the DDR and DNA damage

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DOI: 10.1002/eji.201970400

repair. We examined the effects of macrophages on HR and NHEJ using U2OS cells with HR/NHEJ reporter, and found that macrophages increased NHEJ but have no sense on total HR. Furthermore, we adopt paired gRNA-CRISPR/Cas9 system to detect NHEJ level in endogenous genes. The results showed that MΦs significantly promoted the NHEJ in MMCs both in vitro and in vivo, however reduced accuracy of NHEJ repair, and increased the length of base loss in NHEJ meanwhile promoted genomic instability of MMCs.

P1342

The role of Notch signaling in regulating different hepatic macrophages subsets in progression and regression of hepatic fibrosis

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Hepatic fibrosis is a morbid consequence of chronic liver injury, featured as excess extracellular matrix (ECM) deposition. Identifying key molecules involved in this process is of great significance in combatting fibrosis. Hepatic macrophages are a remarkably heterogeneous population of immune cells with different origins, which fulfill diverse functions in the initiation, progression and regression of hepatic fibrosis. However, how different subsets of macrophages function in different stages of fibrosis still remains unclear.
By using mouse hepatic fibrosis models, we found that myeloid-specific Notch signaling activation aggravated the progression of hepatic fibrosis, featured as excessive ECM deposition, severe disruption of hepatic parenchymal cells, elevated activation and diminished apoptosis of hepatic stellate cells, as well as increased inflammation in the liver. During progression, Notch activation could repress the restoration of Kupffer-like cells, increase Ly6C^{int} bone marrow-derived inflammatory macrophages and decrease Ly6C^{lo} restorative macrophages that are better capable of matrix degradation. Furthermore, Notch signaling was engaged in the transition from Ly6C^{hi} inflammatory macrophages to Ly6C^{lo} restorative macrophages through regulating C/EBPα. The underlying mechanism is still under investigation.
During regression of hepatic fibrosis, we found that Kupffer-like cells-specific Notch signaling blockade could accelerate the regression, with increased proliferation of Kupffer-like cells. Elimination of Kupffer-like cells reversed the regression, suggesting the importance of tissue-resident Kupffer-like cells in this process.
Collectively, our findings suggested that Notch signaling could respectively regulate bone marrow-derived macrophages and tissue-resident macrophages in the progression and regression of fibrosis, which may provide new antifibrotic strategies by targeting defined macrophages subsets.

P1343

Fbxw7 exacerbates colitis by increasing CCL2/7 in CX3CR1^{hi} macrophages to promote intestine inflammation

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Resident and inflammatory mononuclear phagocytes (MPh) with functional plasticity in the intestine are critically involved in the pathology of Inflammatory Bowel Diseases (IBD), in which the mechanism remains incompletely understood. In the present study, we found that increased expression of E3

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DOI: 10.1002/eji.201970400

ligase FBXW7 in the inflamed intestine was significantly correlated to IBD severity in both human diseases and mice model. Myeloid-*Fbxw7* deficiency protected mice from dextran sodium sulfate (DSS) and 2,6,4-trinitrobenzene sulfonic acid (TNBS) induced colitis. *Fbxw7* deficiency resulted in decreased production of chemokines CCL2 and CCL7 by colonic resident macrophages and reduced accumulation of CX3CR1^{int} pro-inflammatory MPh in colitis colon tissue. Mice received AAV-shFbxw7 administration showed significantly improved survival rate and alleviated colitis. Mechanisms screening demonstrated that FBXW7 suppresses H3k27me3 modification and promotes CCL2 and CCL7 expression via degradation of histone-lysine N-methyltransferase EZH2 in macrophages. Taken together, our results indicate that FBXW7 degrades EZH2 and increases CCL2/CCL7 in CX3CR1^{hi} macrophages, which promotes the recruiting CX3CR1^{int} pro-inflammatory MPh into local colon tissues with colitis. Targeting FBXW7 might represent a potential therapeutic approach for intestine inflammation intervention.

P1344

mTORC2 deficiency alters the metabolic profile of conventional dendritic cells

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Background: Mechanistic target of rapamycin complex 2 (TORC2) deficiency in dendritic cells (DC) results in their enhanced pro-inflammatory function; however, underlying mechanisms remains unclear.
Methods: A Seahorse XFe96 Bioanalyzer was utilized to measure metabolic flux for bone marrow-derived DC generated from wild-type control (Ctrl) C57BL/6 (B6) or CD11c-CreRictor$^{f/f}$ (herein referred to as TORC2$^{-/-}$ DC) B6 mice, in the absence or presence of rapamycin. ATP concentrations and mitochondrial mass were determined using an ATP determination kit and flow cytometry. The Golgi apparatus was visualized by confocal microscopy. A mouse nCounter immunology panel was used to analyze their transcriptional profiles.
Results: Compared to Ctrl DC, TORC2$^{-/-}$ DC showed an enhanced baseline glycolytic function, increased dependence on glycolytic ATP production and higher viability following LPS stimulation. TORC2$^{-/-}$ DC exhibited an increased spare respiratory capacity (SRC) compared to Ctrl DC. This metabolic phenotype corresponded with increased mitochondrial mass and failure of TORC2$^{-/-}$ DC mitochondria to depolarize following stimulation. TORC2$^{-/-}$ DC displayed more compact Golgi stacks with less perinuclear localization compared with Ctrl DC. Rapamycin-mediated inhibition of mTORC1 activity in TORC2$^{-/-}$ DC led to loss of their enhanced SRC and glycolytic activity. The altered metabolic activity of TORC2$^{-/-}$ DC could be ascribed to enhanced TORC1 activity, namely increased expression of multiple genes upstream of Akt/TORC1 activity, culminating in increased expression of the transcription factors peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding transcription factor 1.
Conclusions: These findings suggest a novel role for TORC2 in restricting TORC1-driven metabolic functions and mitochondrial regulation in DC.

DOI: 10.1002/eji.201970400

P1345

Unraveling pathogen proliferation-related signaling pathways in Leishmania major-infected monocytes in vivo

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Inflammatory phagocytes recruited to the skin tissue upon infection with *Leishmania major* (*L. major*) represent a main host cell for the parasite. Our previous study suggests that CD11c-expressing Ly6C+ monocytes constitute a reservoir for efficient *Leishmania* proliferation and cell-to-cell transmission. However, there is a large heterogeneity in pathogen proliferation rates even among cells of individual subtypes. Here, combining single-cell RNA-sequencing (scRNA-seq) and a biosensor for measuring pathogen proliferation in the living tissue, we investigated the transcriptomic differences between CD11c-expressing monocytes harboring rapidly versus slowly proliferating *L. major* in the ongoing infection. The preliminary analysis suggests that only Ly6C-negative CD11c-expressing monocytes express genes differentially depending on pathogen proliferation rates. Strikingly, low pathogen proliferation relates to differentially expressed genes (DEGs) involved in inhibition of phagocyte migration and attraction of T-effector cells. On the other hand, the high proliferation-specific DEGs seems to be involved in the activation of inflammatory responses, with pattern recognition signaling are predicted to be involved in the upstream regulation of the observed transcriptomic changes. This ongoing study will shed light on the causality underlying the correlations between pathogen proliferation and host cells.

P1346

Expression and function of Podoplanin in *Helicobacter pylori*-infected macrophages

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Podoplanin is a mucin type transmembrane protein that has been implicated in cancer metastasis through regulation of cellular motility. Expression of Podoplanin has been reported in certain macrophages and T cell subsets, but its role in these immune cells remains unappreciated. In this study, we investigated the role of Podoplanin in the immunity against *Helicobacter pylori*, a *Gram*-negative microaerophilic bacterium that is associated with the onset of gastric cancer. Interestingly, we found that the expression of Podopanin was significantly elevated for >10-fold in RAW264.7 macrophages following *H. pylori* infection. Podoplanin depleted RAW264.7 cells demonstrated reduced ability to perform cell migration as compared to wildtype. This finding suggests that Podoplanin may play an important role in controlling immune cell migration during infection but further investigation is required to elucidate the mechanism underlying its association with cell migration.

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DOI: 10.1002/eji.201970400

P1347

Macrophage subpopulation phenotypic shift in response to *Rhizopus delemar* spores

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The frequency of lethal opportunistic fungal infections particularly due to Zygomycosis including *Rhizopus* has increased recently in particular among immunocompromised individuals. While recent findings highlighted the significance of macrophages in clearing fungal infections, the exact mechanism of clearance and the innate immune responses are still poorly investigated. The present study demonstrated the phenotypic changes in macrophages enriched from peripheral blood mononuclear cells obtained from healthy donors in response to *R. delemar* and its extracted pathogenic determinant. Classical-inflammatory, intermediate and non-classical macrophage populations were identified based on the expression of CD14 and CD16. Macrophage subpopulations M1 and M2 were also identified based on the expression of HLADR, CD86, CD80, CD163 and CD206. Both *R. delemar* spores and its pathogenic determinant caused significant reduction in the absolute cell numbers. Classical macrophages were significantly reduced by ~75% associated with significant reduction in HLADR expression. Activation markers including CD86 and CD80 showed no significant change. Non-classical macrophages were significantly increased when compared to untreated and lipopolysaccharides (LPS)-treated macrophages. Additionally, HLADR was downregulated and CD163, a known receptor for scavenging Hemoglobin-haptoglobin complex, was significantly upregulated in non-classical macrophage subpopulation. Our findings suggest a potential macrophage phenotypic modulation in response to *R. delemar* infection. Further studies are required to delineate the exact fungal substances responsible for such phenotypic shift.

P1348

Transgelin-2 is required for migration and T-cell stimulation in dendritic cells

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Actin cytoskeletal rearrangement and stabilization are the critical mechanisms of regulation of migration and immune synapse formation between immune cells. TAGLN2 is a small 22-kDa actin-binding protein which stabilizes actin structures and participates in actin-associated signaling pathways. Although the contribution of TAGLN2 in various types of immune cells has been well defined, the scope of TAGLN2 function in dendritic cells has not been addressed. Herein, we examined the role of TAGLN2 in DCs. Although TAGLN2 expression was upregulated in response to lipopolysaccharide (LPS), TAGLN2 KO DCs were differentiated normally and displayed normal LPS-induced upregulation of surface marker and cytokine production. However, activation of CD4+T lymphocytes by TAGLN2 KO DCs with preloaded antigen was significantly decreased dose dependently. We further confirmed impaired priming activity of TAGLN2 KO DCs in vivo after adoptive transfer into OTII recipient mice. However, activation of transferred DCs was not affected by TAGLN2 deficiency in vivo as shown as LPS-induced maturation. Interestingly, homing to draining lymph nodes in vivo were dramatically reduced in TAGLN2 KO DCs and exhibited severely decreased membrane dynamics in response to chemokine. Importantly, TAGLN2 KO DCs showed lower chemotactic activity toward chemokine including CCL-3, -5, -19, -21, and CXCL12. Furthermore, two photon microscopy

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DOI: 10.1002/eji.201970400

study showed decreased sustained T-cell interaction and clonal expansion in TAGLN2 KO DCs injected mice. In summary, our findings indicate that TAGLN2 positively regulates migration of DCs into lymph nodes and is essential for several aspects of DC-mediated adaptive immune responses.

P1349

Lactoferrin induces tolerogenic property during the development of bone marrow-derived dendritic cells (BMDCs) in allogenic CD4+T cell activation

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Tolerogenic DCs (tDCs) are generally known as regulatory DCs that suppress immune response through the induction of T cell apoptosis/anergy and regulatory T cells (Tregs). Since we recently found that LF strongly stimulated activated $CD4^+$T cells to differentiate into $Foxp3^+$ Tregs, we explored whether LF affects the generation of tDCs. In LF-treated BMDCs, expression of CD86 (a major costimulatory molecule) was diminished while levels of Arg-1 & IDO-1 (known as key molecules of tDC) was elevated, as compared to those in untreated BMDCs. Furthermore, allogenic $CD4^+$T cells were less proliferative under the influence of LF-treated BMDCs. Collectively, these results suggest that LF causes developing BMDCs to be tolerogenic presumably through enhancement of Arg-1 and IDO-1 expression.

P1350

Transcriptome profiling of human alveolar macrophages to probe phenotypic heterogeneity and control of Mycobacterium tuberculosis infection

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Introduction: *Mycobacterium tuberculosis* (Mtb) infection remains a global health challenge. The problem is exacerbated by our incomplete understanding of the nature of protective immunity against Mtb in the lung in humans. Due to this limited understanding of what constitutes protective immunity at the primary site of Mtb infection and disease, we lack effective correlates of vaccine efficacy and treatment response beyond long-term outcome.
Alveolar macrophages (AMs) are the predominant innate immune cells in the lung which are critically important in the early control and pathogenesis of Mtb, Their response to intracellular Mtb likely determines the course and outcome of the infection. Recent studies have shown that AMs are a heterogeneous population, however, none have probed into their biology, hence the inter-relationship between AMs and how this impacts control of Mtb infection in the human lung is unknown.
Methods: Surface immunophenotyping against cell surface proteins and RNA-seq will be used to generate a phenotypic and functional definition of the AM subsets in the human lung of 15 healthy adults.
Expected results: We will present cell surface immunophenotyping and RNA-Seq data that will allow an in-depth understanding of human AM heterogeneity and function, as well as contribute novel discoveries to be placed into context with current understanding of pulmonary immune responses to Mtb infection.
Conclusion: This study will form the foundation for understanding what constitutes protective immunity to Mtb in the lung in humans through characterization of the mechanisms responsible for resistance or permissiveness for Mtb growth and survival by human AMs.

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DOI: 10.1002/eji.201970400

P1351

D-series resolvins regulate Phospholipase D during inflammation and resolution by modulating phagocyte functions

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A successful acute inflammatory response results in the elimination of infectious agents by neutrophils and monocytes, followed by resolution and repair by tissue-resident and recruited macrophages. D-series resolvins are pro-resolving mediators involved in resolution and tissue repair, whose signaling mechanism remains of interest. In this study, we found that resolvins activate phospholipase D (PLD), a ubiquitously expressed membrane lipase that hydrolyzes phosphatidylcholine (PC) into free choline and phosphatidic acid (PA), and has a pivotal role in various phagocyte functions. During inflammation in hind limb ischemia/reperfusion injury (HLIR), RvD5 reduced lung neutrophil myeloperoxidase (MPO) levels in WT and PLD1$^{-/-}$ mice, but not in PLD2$^{-/-}$ mice, pointing to a novel role of PLD2 as the relevant isoform in RvD5-mediated neutrophil functions. Resolvin-D4 (RvD4) and, to a larger extent, Resolvin-D5 (RvD5) regulated macrophage PLD gene expression (*Pld1-Pld6*), in naïve (M0), pro-inflammatory (M1) and anti-inflammatory (M2) macrophages, as well as increased PLD protein expression, enzyme activity, phagocytosis and efferocytosis. PLD2 itself induces macrophage polarization from CD80^{+}-M1 to CD206^{+}-M2 phenotypes, resulting in increased efferocytosis by M2 macrophages, enhanced by RvD5. The mechanism for PLD-mediated actions of RvD5 in polarizing macrophages (M1-M2) is two-pronged: *(a)* RvD5 inhibits post-transcriptional mechanisms such as miRs and 3'exonulceases that process PLD2 mRNA transcripts in the cytoplasm, thus increasing PLD2 expression and activity and *(b)* RvD5 enhances PLD2-S6K signaling and Actin expression required for membrane expansion and efferocytosis. This study provides compelling evidence that RvD5-PLD2 may be an attractive target for therapeutic intervention in inflammatory conditions like I/R injury, cardiovascular diseases.

P1920

Macrophages are important source of PD-L1 and PD-L1 expressing in macrophages affect the prognosis of NSCLC patients with tumor PD-L1 negative

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PD-1 antibody has achieved significant clinical efficacy for advanced non-small cell lung cancer(NSCLC) , but the ORR is not optimistic for the non-selected patients. PD-L1, as the therapeutic target, is still controversial to be a predictor of efficacy. It was noticed that some patients with tumor cell PD-L1 negative (TC PD-L1-) could still benefit from anti PD-1/PD-L1 therapy. Besides the tumor cells, PD-L1 was also known to express in immune cells. Therefore, in this study, we aimed to clarify the factors that affect the PD-L1 expression in tumor immune microenvironment (TIME), and their functions on the NSCLC prognosis. With the multiple quantitative immunofluorescence staining on 137 tissue specimens of NSCLC patients,we screen out 91 patients with TC PD-L1- and found that

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DOI: 10.1002/eji.201970400

macrophages, especially M2 macrophages, were the main source of PD-L1 for patients with TC PD-L1$^-$. Furthermore, PD-L1 expressed in centralM2 (M2 macrophages infiltrating into tumor islets) and PD-L1 expressed in marginM1 (M1 macrophages in tumor margin) were independent prognostic factors for NSCLC patients with TC PD-L1$^-$, suggesting that the expression of PD-L1 in macrophages may also be associated with the benefit of anti-PD-1 therapy, which is an important factor to be considered in evaluating the efficacy of immunotherapy in the future.

P1921

Renal tubular epithelial cell regulates macrophage phenotype differentiation after acute kidney injury

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Background and objectives: Excessive macrophage infiltration after acute kidney injury is an important prognostic mala factor. Our study aims to observe the regulation of injured tubular epithelial cells(MPTCs) on macrophage phenotype differentiation.
Methods: Lentivirus was used to overexpress or knockout of KIM-1 in MPTCs(KI-KIM-1/KO-KIM-1). Supernatant of injured MPTCs was made into conditioned medium to stimulate macrophages. The expression of macrophage subtype markers was detected by qPCR and flow cytometry. Macrophages were injected into the mouse renal cortex to observe the physiological changes in vivo.
Results: After hypoxia injury, compared with KO-KIM-1 group, KI-KIM-1 group stimulated the expression of Arg-1, CCL1, CCR2 and CD150 increased in macrophages. After hypoxia-reoxygenation injury, KI-KIM-1 group could stimulate the expression of CD206, IL-10, CD150, CXCL13 and CCR2 increased. Number of M2 phenotype cells in KI-KIM-1 group was significantly higher than that in KO-KIM-1 group, and KI-KIM-1 group could differentiate macrophage to M2c phenotype. Compared with KO-KIM-1 group, KI-KIM-1 group had no change in cell viability and phagocytosis after normal culture and hypoxia injury. However, after hypoxia-reoxygenation injury, the cell viability of KI-KIM-1 group stimulated macrophage was significantly reduced but the phagocytic ability was enhanced. Level of renal fibrosis induced by KI-KIM-1 group after hypoxia-reoxygenation injury was significantly lower than that of KO- KIM-1 group on the seventh day after surgery.
Conclusion: Injured MPTCs induce macrophage differentiation into M2c phenotype by high expression of KIM-1, and this macrophage do not aggravate renal fibrosis.

P1922

Monoacylglycerol lipase in macrophages suppresses melanoma by potentiating TLR4-Sirpα axis-mediated tumor phagocytosis

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Lipid metabolic reprogramming greatly contributes to macrophage activation and cancer progression. We have previously reported that monoacylglycerol lipase (MGLL) could activate macrophage towards a M1 phenotype and thus stimulate the anti-tumor activity of CD8+ T cells in colorectal cancer. However, whether MGLL would regulate phagocytosis of tumor associate macrophages (TAMs) remains unclear. Here by injecting B-16 cells subcutaneously, we set up a melanoma models in C57BL/6 mice, in which we investigate the roles of MGLL in TAM phagocytosis and melanoma

DOI: 10.1002/eji.201970400

development. We demonstrate that expression of MGLL in TAMs was decreased. Forced expression of MGLL largely enhances tumor-phagocytosis by peritoneal macrophages. Mechanistically, MGLL suppresses signal regulatory proteins α (Sirpα), the receptor of "don´t eat me" checkpoint, by stimulating TLR4 signal transduction. Functionally, we confirm that conditional transgene of MGLL in macrophages notably inhibits melanoma progression in subcutaneous xenograft models. Taken together, we identify MGLL as a stimulator of phagocytosis via potentiating TLR4-Sirpα axis in TAMs.

P1923

Expanding roles for immune adaptors in regulation of pro-inflammatory signaling in macrophages

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Introduction: Immune adaptors are widely expressed in immune cells and play multifunction in various signaling pathways. The roles of immune adaptors in T cells involved in TCR-mediated activation, proliferation and adhesion have been well studied. However, the function of immune adaptors in macrophages largely remains unclear. The present study expanded roles of immune adaptors in regulation of TLR4-mediated inflammatory responses in macrophages.
Methods: We isolated primary macrophage cells from immune adaptor knockout mice and established immortalized bone marrow-derived macrophages as well as stable knockdown THP-1 cell line for the following study. The roles of immune adaptor were examined based on both the underlying molecular mechanism with routine approaches and in vivo study with macrophage-adoptive transferred and *Listeria monocytogene* infected mice models.
Results: In our study, we were the first to identify the phosphorylation state of the immune adaptor downstream of TLR4 signaling. We found the immune adaptor physically interacts with transcription factor and is involved to modulate a variety of transcription factors activation such as STAT3, IRF3 and MAPKs with different patterns, collectively repressing the pro-inflammatory phenotype triggered by LPS in macrophages. Furthermore, we demonstrated the immune adaptor suppresses M1 macrophage plasticity, antimicrobial capacity and antigen presentation capacity therefore mediating downstream Th1 effector T cell function.
Discussion: Our study suggests a pivotal role of immune adaptors in immunoregulation of macrophages that prevents macrophages from excessive inflammation and autoimmunity, identifying immune adaptors as potential molecular targets for macrophage-specific therapeutic interventions.

P1924

p55PIK promotes LPS-induced inflammation by inhibiting autophagy

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Autophagy, which can be induced by the recognition of pathogen-associated molecular patterns (PAMPs), e.g. lipopolysaccharide with pattern recognition receptor, such as TLR4, controls inflammation through limiting the activation of inflammasome or regulating the secretion of immune mediators. Class IA PI3K inhibits autophagy through p110-Akt-mTORC1 pathway to suppress the

DOI: 10.1002/eji.201970400

formation of ULK1 complex, while class III phosphatidylinositol-3-phosphate kinase (PtdIns3K) VPS34 promotes autophagy initiation. p55PIK is a regulatory subunit of Class IA PI3K encoded by PIK3R3, the functions of p55PIK in inflammation and autophagy are still unclear. In this study, we demonstrated that p55PIK, an isoform of phosphoinositide 3-kinase (PI3K), promoted LPS-induced pro-inflammatory cytokine expression through activating NF-κB signaling pathway in LPS-stimulated bone marrow derived macrophage (BMDM) and THP-1 cell line. p55PIK also inhibit the formation of autophagosome in both LPS-stimulated BMDM and THP-1 cell line. Furthermore, knockout of p55PIK promoted autophagy and using autophagy inhibitor 3-MA can partially rescue the suppression of pro-inflammatory cytokine expression in p55PIK knockout cell line. Conversely, Overexpression of p55PIK suppressed autophagy and using autophagy promoter Rapamycine partially rescue the promotion of pro-inflammatory cytokine expression in over-expressed p55PIK cell line. These data suggests that p55PIK promotes LPS-induced inflammation response by inhibiting autophagy. The applying of a p55PIK specific inhibitor, TAT-N15 may be a potential strategy for the treatment of septic shock mediated by LPS through inhibiting inflammatory response by promoting autophagy.

(This research was funded by the National Natural Science Foundation of China, No.31670891 and No. 81874148)

P1925

Tailoring nanomaterials for targeting tumor-associated macrophages

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Advances in the field of nanotechnology together with an increase understanding of tumor immunology have paved the way for the development of more personalized cancer immuno-nanomedicines. Nanovehicles, due to their specific physicochemical properties, are emerging as key translational moieties in tackling tumor-promoting,
M2-like tumor-associated macrophages (TAMs). Cancer immunonanomedicines
target TAMs primarily by blocking M2-like TAM survival or affecting their signaling cascades, restricting macrophage recruitment to tumors and re-educating tumor-promoting M2-like TAMs to the tumoricidal, M1-like phenotype. Here, the TAM effector mechanisms and strategies for targeting TAMs are summarized, followed by a focus on the mechanistic considerations in the development of novel immuno-nanomedicines.
Furthermore, imaging TAMs with nanoparticles so as to forecast a patient's clinical outcome, describing treatment options, and observing therapy responses is also discussed. At present, strategies that target TAMs are being investigated not only at the basic research level but also in early clinical trials. The significance of TAM-targeting biomaterials is highlighted, with the goal of facilitating future clinical translation.

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DOI: 10.1002/eji.201970400

P1927

Macrophage-mediated vascular permeability regulates ascites formation in ovarian cancer via VCAM-1 pathway

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The volume of High-grade serous ovarian cancer (HGSOC) ascites has prognostic significance and contributes to cachexia with eventual compromise of the patient's mobility. However, targeting macrophages abrogated the formation of ascites, but resulted in an increase of permeability at the same time. Differentially polarized macrophages have disparate impact on vascular permeability. In this study, we investigated the molecular mechanism of macrophage-mediated vascular permeability in ovarian cancer. We compared different subtypes of macrophages and their impact on regulating different types of endothelial cells via the expression of VCAM-1, VLA-4 and ROS in co-culture system. Our results showed that M2 macrophages down-regulated VCAM-1 pathway, which reduced ROS level and the phosphorylation of VE-cadherin, to protect the vascular barrier. Downregulating VLA-4 expression level in macrophages resulted in hyperpermeability. Endothelial cells co-cultured with M2 macrophages produced less ROS showed that ROS was involved in the vascular barrier regulated by macrophages. Macrophages depletion using either clodronate liposomes or antibodies unexpectedly resulted in hyperpermeability in vivo. This effect could be rescued when VLA-4 overexpression macrophages were injected into macrophages-deficient mice. As seen in the ovarian cancer specimens, the expression levels of VCAM-1 and VLA-4 were significantly increased and positively correlated with the ascites volume. Taken together, M2 macrophages protect vascular barrier through VCAM-1 that regulates ROS level and the phosphorylation of VE-cadherin. For the first time we have unraveled the molecular mechanism of macrophage-mediated vascular permeability, which could provide new strategies to treat ascites in OC patients.

P1928

Single-cell analysis of mouse plasmacytoid dendritic cells during an in vivo viral infection unravels their activation trajectory and their molecular regulation

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Plasmacytoid dendritic cells (pDC) are a major source of type I (IFN-I) in response to viral-type stimuli. What other functions pDC exert during viral infections is controversial. Bulk analysis of human pDC responses to in vitro stimulations was recently reported to lead to a bifurcation between IFN-I production versus activation of CD4 T lymphocytes. Other studies reported the contamination of both human and mouse pDC by novel types of conventional dendritic cells proposed to account for all the T cell activation properties formerly reported for pDC. Hence, more studies are needed to address the functional specialization of pDC and their molecular regulation. To address this issue, we combined an *Ifnb1* reporter mouse strain with flow cytometry and single cell RNA sequencing to characterize pDC activation states. This strategy enabled us to dissect the activation trajectory of mouse pDC in vivo during the course of a viral infection, including by following the fate of the cells after they had terminated their IFN-I production. We showed that IFN-I production and T cell activation were

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DOI: 10.1002/eji.201970400

performed by the same individual cells, but sequentially in time and in different micro-anatomical locations. In addition, we showed that TNF signaling promoted pDC IFN-I production. Hence, we propose a novel model of how individual pDC are endowed to exert different functions in vivo during a viral infection in a manner tightly orchestrated in time and space.

P1929

ADRB3 stimulation induced M2-like phenotype in in vitro primary human macrophages

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Background: Inflammatory bowel diseases (IBD) are a worldwide health-care problem with a continuously increasing incidence, caused by an impaired or inappropriate inflammatory response toward intestinal bacteria that is mainly headed by the recruitment of inflammatory macrophages. Among environmental factors, stress, *via* the secretion of cathecholamines, has been shown to activate catecholaminergic receptors. a-adrenoreceptors boost inflammation/oxidative stress while b2-adrenergic (b2-AR) signaling suppresses immunity. We demonstrated that b3-AR, is also expressed and functional in human macrophages. Thus, the investigation of b3-AR pathway could be of interest for the treatment of IBD.
Methods: In this study we characterized macrophages maturation status after *in vitro* stimulation with β3-agonists and identify the signalling pathways induced.
Results: Using flow cytometry, we showed that b3-AR stimulation leads to anti-inflammatory and anti-oxidant responses, polarizing macrophages toward a M2-like phenotype. Cell surface marker CD206 is upregulated for 2 ± 0,24 fold ($p< 0.05$) while HLA-DR is down-regulated for 1.4 ± 0.3 fold ($p< 0.05$) after b3-AR stimulation compared to control group. We also demonstrated that these effects depend upon an ERK1/2- activation. Finally, we showed that b3-AR is resistant to desensitization thanks to a Gs/Gi switch and can induce its own expression after long term stimulation since we showed an overexpression of ADRB3 (2 to 3 ± 0.6 fold $p< 0.05$) and a 6 to 8 ± 1.7 log2-fold increase ($p< 0.05$) of *mRNAADRB3* expression after 24 hours of treatment.
Conclusion: These preliminary results suggest that the use of b3-agonists may be of interest for the pharmacological managementof IBD.

P1930

mTORC1 promotes the differentiation of bone marrow-derived dendritic cells via Stat3/Smad3/Id2 axis

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Dendritic cells (DCs) are important antigen presenting cells (APCs) and play a crucial role in the regulation of immune responses. Previous reports have found that mTOR is involved in DCs differentiation, and inhibitor of DNA-binding protein 2 (Id2) has a close relationship with the differentiation of DCs. However, the exact mechanism between them has not been fully elucidated. Here we demonstrated that rapamycin (RAPA), an inhibitor of mTOR, represses the maturation of bone marrow-derived DCs (BMDCs) via inhibiting the expression of Id2. By direct interacting with Stat3, mTORC1 up-regulates the activity of Stat3, thereby inhibiting the nuclear translocation and phosphorylation of Smad3. The phosphorylated Smad3 promotes the degradation of Id2 via activating

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DOI: 10.1002/eji.201970400

the autophagy in a p62-dependent manner. Thus, mTORC1 inhibits the autophagy, up-regulates Id2 via Stat3/Smad3/p62 axis and promotes the maturation of BMDCs.

P1931

CD1d highly expressing on DCs reduces lung tumor burden by enhancing anti-tumor immunity

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Dendritic cells (DCs), as professional antigen presenting cells are essential for the initial activation of adaptive anti-tumor immunity. CD1d is considered to present phospholipid and glycosphingolipid antigen. However, it's as yet unknown whether CD1d on DCs is possible to enhance anti-tumor immunity, especially T cells related immunity.
We observed *that CD1d is predominantly expressed on DCs* in 3LL tumor-bearing mice, whilst a *deficiency of CD1d promoted tumor growth*. Interestingly, CD1d expressing on DCs was not only required for presenting antigen to NKT cells, *but also dramatically promoted $CD4^+$T and $CD8^+$ T cells activation, especially cytotoxic T cells*. All the T cells (NKT, $CD4^+$ T and $CD8^+$ T cells) upregulated CD69, CD107a, IFN-γ after the adoptive transfer of $CD1d^+$DCs, and tumor growth were repressed. In mechanism, we identified that *$CD1d^+$DCs were concomitant with a higher expression of costimulatory molecules (CD40, CD80 and CD86) and MHCI/II*, which are essential for DCs to present antigens to T cells. Consistently, *$CD1d^+$DCs displayed stronger activation-associated-ERK1/2 and NF-κB signals; whereas JAK2-STAT3/6 signaling was required for maintaining high level of CD1d on DCs.* In lung cancer patients, the anti-tumor activities of all the T cells were enhanced with the increase of $CD1d^+$DCs. Analysis of TCGA data revealed that high levels of *CD1d indicate better outcomes for patients. Collectively, CD1d enhances DCs-based on anti-tumor immunity*, not only by targeting NKT, but also by activating $CD4^+$T and $CD8^+$ T cells. $CD1d^+$DCs may be superior to the bulk DCs in cancer immunotherapy.

P1932

Brain microglia is maintained and regenerated from a specialized cellular subset

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Microglia, macrophages of the brain parenchyma, are essential for normal brain development and function as well as local immune responses. However, they can also induce and perpetuate severe brain pathology in certain conditions . Microglia are derived from early, fetal yolk sac (YS) progenitors and they are then maintained locally behind the blood brain barrier (BBB) without any external cellular input. It is thought that all microglia cells, like many other tissue-resident macrophages , have self-renewal capacity to maintain their cellularity. Here we, however, report an identification of a minor microglial stem cell-like cells (MgSCs) that are responsible for microglia expansion and maintenance during development and during steady state. MgSCs resemble somatic, unipotent stem cells: they are self-renewing and able to differentiate to the bulk of sessile microglia which, on the need, can dedifferentiate back to MgSCs. Due to their central role in microglia turnover MgSCs can become valuable, therapeutic targets in neuropathological conditions.

This work is supported by MOE Academic Research Fund grant MOE-T2-013 to K.K.

DOI: 10.1002/eji.201970400

P1933

Stimulation of FasL induces production of proinflammatory mediators relevant to the activation of mitogen-activated protein kinases in dendritic cells

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FasL is a type II transmembrane protein and belongs to the tumor necrosis factor family, which is well investigated for its effect on cell apoptosis. Previous researches have uncovered that FasL severs as a T cell co-stimulatory signal through its reverse signaling. Though DCs also express FasL, the role of FasL-mediated reverse signaling in dendritic cell function has been mystic. To find out the function of FasL on DC, we applied Fas-Fc fusion protein to stimulate BMDC, and the results showed that stimulation of FasL induced the secretion of proinflammatory mediators such as TNF-a, IL-6, increased expression of the costimulatory molecules, enhanced antigen presentation capability and decreased phagocytosis capability in WT BMDC. Meanwhile the results that BMDC from FasLgld or FasL△Intra mice (mutation in the intracellular segment of FasL and the extracellular segment still works) secreted less proinflammatory mediators than WT BMDC verified the effect of FasL in DC. Further investigation showed that FasL-mediated reverse signaling is relevant to the mitogen-activated protein kinases. Furthermore, we found that FasL activation may aggravates inflammation in mice with systemic fungal or bacterial infection. FasL- mutant mice had a higher survival rate than wild-type control mice in response to infection to some extent. Therefore, deep investigation on the reverse signals of FasL will shed new light on the functional regulation of DC and a therapeutic target for treating systemic infection.

P1934

Liver-resident macrophages contribute to tumor microcirculation construction for rapid growth of liver metastases in a mouse model

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Background: Tumor-associated macrophages (TAMs) are highly heterogeneous cell populations in the tumor microenvironment. Macrophages may originate from bone marrow-derived monocytes or yolk sac progenitor-derived tissue-resident macrophages, yet the exact origin, manifestation within tumor microenvironment and function of TAMs remains to be elucidated.
Methods: The liver metastasis model in C57BL/6 mice was used to imitate the rapid growth of colorectal liver metastases (CRCLM) via splenic injection of Mc38 metastatic colorectal cancer cells. Immunohistochemistry, HE staining pathological analysis were intensively applied to examine specific cells and tumor microcirculation on serial sections of liver biopsies, on which Flow Cytometry was used additionally.
Results: Locally proliferated F4/80$^+$ liver-resident macrophages, acting as TAMs, greatly contributed to microcirculation construction and integration within the liver metastases during the malignant conversion period, which ultimately boosted rapid CRCLM growth. Bone marrow-derived CD11b$^+$ TAMs may facilitate the expansion of F4/80$^+$ TAMs, as TMP195 treatment-directed M1 macrophage polarization resulted in breakdown of the tumor vasculature and significant decrease of F4/80$^+$ TAMs.
Conclusions: We delineated an essential role of F4/80$^+$ liver-resident macrophages as perivascular TAMs in promoting rapid growth of CRCLM in the mouse liver metastasis model.

DOI: 10.1002/eji.201970400

CRCLM progressed in the liver in a replacement growth pattern incorporating the innate liver vessels and tumor vasculogenic mimicry to construct the tumor microcirculation.
Keywords: Liver-resident macrophages; Colorectal liver metastases; Tumor microcirculation; Replacement growth pattern; Tumor-associated macrophages

P1935

The interaction between Wnt2b and TLR signals in HCC-associated macrophage polarization

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Backgound: Tumor-associated macrophages (TAMs) are one of the most prominent components in the hepatocellular carcinoma (HCC) microenvironment. The crosstalk between HCC cells and TAMs are considered to be involved in HCC progression. TLR singnals can regulate macrophage polarization. Wnt signaling plays important roles in liver development and tumorigenesis. Our previous studies have found that Wnt2b promotes HCC. However, its role in macrophage polarization is not well understood.
Aims: To investigate the interaction between Wnt2b and TLR signals in polarization of macrophages and find the new promising strategy for HCC therapy.
Methods: We used condition media of HCC cells to induce TAMs. Intervention of Wnt2b expression in macrophages. Flow cytometry and quantitative RT-PCR were utilized to detect the expression of the markers.
Results: The condition media of HCC cells could up-regulate the expression of M2 markers accompanied with Wnt2b in TAMs. Overexpression of Wnt2b in macrophages lead to M2 polarization and induced the EMT in HCC. Silencing the expression of Wnt2b could inhibit the M2 polarization. TLR9 agonist, CPG ODN could down-regulate the expression of Wnt2b in TAMs and inhibited the M2 polarization.
Conclusions: It suggests that the interaction between Wnt2b and TLRs signals affects the polarization of HCC-related macrophages. Downregulating Wnt2b expression in TAMs by using CPG may be potential strategy for HCC therapy.
Keywords: HCC; TAMs; Wnt2b; TLRs

P1936

Intestinal tumor infiltrating macrophages regulate the occurrence of chronic schistosomiasis colorectal cancer

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In this study, we try to investigate the characteristics between **colorectal cancer**(CRC) patients with *Schistosomiasis* and without *Schistosomiasis* from clinical information and Clinical pathology experiment, and research on the connection between *Schistosomiasis* and CRC. In addition, we try to understand whether macrophage polarization plays a crucial role in the development of CRC with *Schistosomiasis.*From 2010 to 2016, the prevalence of schistosomiasis decreased from 5367.95/10^5 to 1227.47/10^5 in Wuhu, PR. China. The incidence of CRC significantly increased from 23.55/10^5 to 32.38/10^5 in Wuhu from 2011 to 2015. The morbidity of CRC in transmission control districts was the highest from ages 61 to 70 and this peak occurred approximately 10 years earlier than that in the transmission interruption district. The mean numbers of macrophages in patients with schistosomiasis were significantly higher than those in patients without schistosomiasis. In contrast, the proportions of

DOI: 10.1002/eji.201970400

M2 macrophages were significantly higher in CRC patients than in colitis patients. Chronic schistosomiasis significantly affects the occurrence of CRC, and mainly promotes the early progression of CRC. Macrophage polarization plays a pivotal role in the development of CRC. Chronic schistosomiasis preferentially induces the differentiation of macrophages into the M2 phenotype. Our results show chronic schistosomiasis patients need regular colonoscopy examination to find out CRC and take treatment early; these patients should take examination at least 10 years early than individuals without schistosomiasis.

P1937

The dendritic cells' immunological behaviors modulated by the spatial confinements of deposited fibrin matrix

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The responses of dendritic cells (DCs) to the mechanical microenvironment caused by implanted materials are highly correlated to the host immune responses and largely determines the outcome of tissue regeneration. In the early stage of the inflammations following injury or implantation, a large amount of fibrin would deposit around the implanted materials and form a microporous fibrous-liked network structure, which can provide mechanical microenvironment with different spatial confinement in dimensions for following recruited DCs. Herein, we have established a useful model by salmon fibrin to mimic the deposited fibrin matrix and found that DCs cultured on or in fibrin hydrogels exhibit various morphological features (*e.g.* shapes and dendritic branch). The spatial confinements cause the reorganizations of DCs' cytoskeleton and generate mechanical stimuli to DCs, leading to significant impacts on the biomechanical characteristics and the immune functions of DCs. Thus, the mechanical stimuli deriving from the spatial confinements of deposited fibrin matrix is an important mechanical factor to modulate the biological behaviors of DCs. It is significant for further understanding the effects of the remolded mechanical microenvironment caused by inflammations on DCs' immunologic behaviors.

P1938

Semaphorin 3E regulates macrophages response to lipopolysaccharide-induced systemic inflammation

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Semaphorin 3E (Sema3E) is a secreted protein that was originally discovered as neuronal guidance cue. Recent evidences showed that Sema3E plays an important role in regulating the activities of various immune cells. However, the exact role of Sema3E in macrophage function, particularly during inflammation is not fully understood. Here, we studied the impact of *Sema3E* gene deletion on macrophage function during lipopolysaccharide-induced acute inflammatory response. We found that Sema3E-deficient (*Sema3e*$^{-/-}$) mice were better protected from LPS-induced acute inflammation as exemplified by their superior clinical score and effective temperature control compared to their wild type littermates. This superior control of inflammatory response in *Sema3e*$^{-/-}$ mice was associated with significantly lower phosphorylation of STAT1, STAT3, and ERK1/2 and a concomitant reduction in iNOS expression and production of TNF and IL-6 compared to their *Sema3e*$^{+/+}$ littermates. *Sema3e*$^{-/-}$ mice also contained significantly higher numbers of alternatively activated macrophages compared to

DOI: 10.1002/eji.201970400

their *Sema3e*$^{+/+}$ littermates at both baseline and after LPS challenge. *In vivo* specific deletion of the Sema3E high affinity receptor, plexinD1, on macrophages led to improvement in clinical disease following exposure to lethal dose of LPS. Collectively, our data show that Sema3E plays an important role in dampening early inflammatory response to LPS by regulating macrophage function, suggesting an important role of this pathway in macrophage inflammatory response.

P1939

Cross-talk between mesenchymal stem cells from periapical lesion and macrophages gained from peripheral blood monocytes by different differentiation protocols

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Periapical lesions (PL) are chronic inflammation around the dental root, which is result of spreading the infections from the inflamed dental pulp. Macrophages (MØ) have an important role in the inflammatory processes within PL, which is primarily reflected in the production of cytokine which promote bone resorption, but also a cytokine which has a role in reducing inflammation and healing. Recently, the presence of mesenchymal stem cells (MSC) has been shown in the PL but their role in PL still remains unclear. The aim of this work was to examine cross-talk between PL-MSC and MØ in direct and indirect contact. With a method of dual immuhistochemistry and confocal microscopy we have shown that CD146+ PL-MSC (pericytes) stay in close contacts with CD68+ MØ in PL. In the co-culture of PL-MSC and MØ obtained by different procedures of differentiation (spontaneous, sMØ), M-CSF induced (mMØ) and GM-CSF induced differentiation (gmMØ) we have shown that MØ, independently of the type, increase the production of IL-6 by PL-MSC while differently modulating their phenotypic properties. On the other hand, the PL-MSC modulates the expression of surface markers and production of cytokines by MØ, which results in sMØ polarization toward M1, while mMØ and gmMØ leads toward their M2 polarization. The conclusion is that in PL there are different two-way communication between PL-MSC and MØ. The result of these interactions is certainly an increase in IL-6 production, but also different polarization of MØ, where they partially boost proinflammatory and partially immunoregulatory response inside PL.

P1940

First description of human innate response immunomodulation by Uropathogenic Escherichia coli (UPEC)

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Uropathogenic *Escherichia coli* (UPEC) is the most common agent isolated in 75-95% of acute urinary tract infections of bacterial origin. Once there is a lack of information regarding human DCs (dendritic cells) and UPEC interactions, we decided to perform experiments to reveal such data. DCs were obtained from healthy donors, cultured with the V27 (urosepsis) and J96 (pyelonephritis) strains of UPECs for 24 hours. Next, we observed that both strains showed the same ability to infect DCs, with no significant difference concerning colony forming unit (CFU). Furthermore, both strains induced changes in DC morphology, causing cell death, especially for V27 strain. In J96 recovered cells, we observed a significant presence of cells PI+ (1,87 ±0,38% in control DCs to 6,07 ±1,28%). Cells were also analyzed by flow cytometry to evaluate the expression of important surface molecules. Among these molecules, J96 caused a significant decrease in CD11c expression (from 96,67 ±1,24% to 66,77 ±10,44 %) and CD1a expression (from 42,28 ±10,23% to 24,32 ±5,66%). DC-SIGN (CD209) was

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DOI: 10.1002/eji.201970400

significantly inhibited by UPEC (from 62,20 ±6,45% to 22,57 ±4,96%), CD86 (from 60,67 ±8,19% to 36,59 ±10,80%) and HLA-DR (from 86,56 ±5,58% to 36,59 ±10,80%). In conclusion, we observed that both UPECs strains were able to infect DCs, inducing morphologic alterations and cell death for some of these. UPECs seem to negatively regulate the expression of relevant DC markers and costimulatory molecules, including HLA-DR. Therefore, these phenomena may reflect in a poor antigen presentation and T lymphocyte activation, favoring pathogen establishment and disease development.

P1941

Using of immunomagnetic MicroBeads conjugated with mouse anti-human CD14 antibodies for isolation of bovine blood monocytes

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The experiment was focused on possibility of using of immunomagnetic MicroBeads conjugated with mouse anti-human CD14 antibodies for isolation of bovine blood monocytes. Then, these monocytes were used for developing of dendritic cells. Peripheral blood mononuclear cells were isolated from whole blood of healthy cattle using Histopaque (density 1.077). Monocytes were isolated from the population of mononuclear cells by immunomagnetic MicroBeads conjugated with mouse anti-human CD14 antibodies. The purity of monocytes was higher than 95 %. That population of CD14 positive cells were used for developing of dendritic cells. For that purpose, there were used granulocyte-macrophage colony stimulating factor and interleukin-4. In three days, the CD14 positive cells changing into dendritic cells which were observed by morphological changes including developing of typical dendrites.

P1942

Identification of components of the unfolded protein response (UPR) in human DC hematopoietic progenitors and DC subsets

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Background: The mechanisms of DC differentiation, function and homeostasis are of great interest due to the therapeutic potential of DCs in different inflammatory settings. The unfolded protein response (UPR), in charged of maintaining protein homeostasis and ER biogenesis, appears to be essential for DC survival and function. However, knowledge regarding the interplay between DCs and ER stress in human DCs is very scarce. Our aim is to pinpoint UPR components that are activated in human DCs subsets as well as human DC progenitors, and to identify the role and relevance of these factors in DC biology.
Methods: We established an expansion protocol for DC hematopoietic progenitors and differentiation of DC populations, from CD34+ cells isolated from umbilical cord blood. DC precursors as well as DC subsets were identified and sorted using multi-parametric flow cytometry, followed by RNA isolation for further analysis of gene expression of components from all three UPR branches using real time PCR.
Results: We were able to identify each step of human DC hematopoietic differentiation, as well as all three main DC subsets, and found that distinct components of the UPR are differentially expressed during DC development as well as between DC subsets. Furthermore, the IRE1a/XBP1s branch of the UPR appears to be more activated in the cDC1 subset compared to other cell types.

DOI: 10.1002/eji.201970400

Conclusion: We have successfully established an analytical approach to study UPR activation on human DCs, thus contributing to the understanding of the role of ER stress responses to human DC biology and differentiation.

P1943

Functionalization-dependent effects of cellulose nanofibrils on tolerogenic mechanisms of human dendritic cells

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Cellulose nanofibrils (CNF) are very attractive for use in biomedicine due to their good biocompatibility and biomimetic properties. We have shown previously that native (n)CNF induces tolerogenic properties the immunological effects of functionalised CNF have not been investigated so far, which is an imperative for their safe biomedical application. Considering the role of dendritic cells (DC) as key regulators of adaptive immunity, we hypothesised that phosphonation of oxidised CNF (cCNF) can alter DC tolerogenic properties and their capacity to polarise T-cell mediated immune response. We found that CNF, functionalised with 3-AminoPropylphosphoric Acid (ApA; ApAcCNF), inhibited down-regulation of CD14 and up-regulation of CD1a expression significantly during their differentiation, whereas nCNF and cCNF only inhibited the up-regulation of CD1a. Additionally, ApAcCNF was found to be more potent in inhibiting maturation and allostimulation potential of DCs, which was accompanied by higher production of regulatory cytokines and increased ability to induce suppressive $CD8^+CD25^+IL\text{-}10^+$ regulatory T cells in immunoglobulin-like transcript (ILT)-3 and ILT-4-dependent manner. In contrast, DCs treated with nCNF increased the expression of indolamine-dioxygenase 1 (IDO-1), which in culture with allogeneic T cells induced predominantly an increase in the percentage of suppressive $CD4^+CD25^{hi}FoxP3^{hi}$ T cells and TGF-β production. Diverse tolerogenic effects of CNF were in correlation with their different sizes and functionalization, as well as the redistribution of the expression of CD209 and actin filaments in DCs at the contact site with the CNF. These results widen their potential application for controlled modulation of chronic inflammatory conditions, as well as in tissue engineering.

P1944

Microbiota-associated myeloid TNF production dictates the balance between tolerogenic and protective neonatal pre-cDC1

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Introduction: Induction of immune protection against pathogens is particularly crucial during the neonatal period dominated by anti-inflammatory and tolerance immunity. We have previously shown that precursors of type 1 conventional dendritic cells (pre-cDC1) are key players in this dynamic equilibrium thanks to their unique ability to secrete both pro- (IL12p40) and anti-inflammatory (IL10) cytokines.
Materials and Methods: C57BL/6, $TNF^{M\text{-}KO}$, $IL10^{reporter/GFP}$ and antibiotics-treated neonates were analyzed by flow cytometry and RNA sequencing to evaluate the differentiation and function of pre-cDC1 during the first week of life at steady state and upon *Listeria monocytogenes* infection.

DOI: 10.1002/eji.201970400

Results: We demonstrated that microbiota colonization promotes TNF secretion by splenic monocytes and macrophages during the first hours of life. This cytokine production is involved in the differentiation and function of neonatal pre-cDC1. Indeed, in the absence of myeloid TNF, splenic neonatal pre-cDC1 adopted a newly characterized regulatory phenotype, based on the expression of C1q and β-catenin that are correlated with their IL-10 production. We further demonstrated that during neonatal *Listeria monocytogenes* infection, microbiota-associated myeloid TNF production increased the ability of pre-cDC1 to induce protective $CD8^+$ T cells responses, by favoring their IL12p40 secretion and inhibiting their IL10 production.
Conclusions: Early life environmental factors such as microbiota-associated myeloid TNF promote the adaptive T-cell response later in life by dictating the balance between neonatal tolerogenic and protective pre-cDC1.

P2607

Seselin ameliorates inflammation via targeting Jak2 to suppress the proinflammatory phenotype of macrophage

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Background and Purpose: Sepsis is a serious clinical condition with high mortality rate. Emerging evidence proved that inhibition of inflammation was beneficial for the treatment of sepsis. Here, we evaluated the anti-inflammation activity of seselin in septic mice and further detected underlying molecular mechanism.
Experimental Approach: The in vivo therapeutic effect of seselin was evaluated in septic C57BL/6 mice. Sepsis was induced by cecal ligation and puncture or injection of LPS. The in virto anti-inflammation activity of seselin was determined in LPS and IFN-gamma stimulated macrophages. The mechanism was investigated by co-immunoprecipitation, cellular thermal shift assay and molecular docking.
Key Results: In vivo study showed that seselin obviously ameliorated cecal ligation and puncture induced sepsis in mice. In septic mice lung tissue and cultured macrophages, seselin downregulated level of pro-inflammatory factors and activity of STAT1 and p65, the master signal pathway molecules for proinflammatory macrophages polarization. Importantly, adoptive transferred with seselin pretreated BMDM significantly descended system proinflammatory factors in LPS challenged mice. The further mechanism was that seselin targeted Jak2 to block interaction with IFNR and downstream STAT1.
Conclusion and Implications: Our study suggested the anti-inflammatory activity of seselin and identified its molecular target, Jak2. These results indicated the potential possibility of seselin in treatment of inflammatory disease via blocking the proinflammatory phenotype of macrophages.

P2608

Ontogeny of synovial macrophages and the roles of synovial macrophages from different origins in arthritis

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The ontogeny of macrophages in most organ/tissues in human body has been proven. Due to the limited number and inaccessibility of synovial macrophages (SM), the origin of SM has not been fully

DOI: 10.1002/eji.201970400

illuminated. The objective of this study was designed to investigate the ontogeny of SM and to evaluate the role of SM from different origins in arthritis. Two origins of SM, embryonic SM (ESM) and bone marrow SM (BMSM) were identified in Cx3cr1-EGFP mice, CCR2$^{-/-}$ mice and bone marrow (BM) chimera model by using a stringent sorting strategy. The cellular features, including dynamic total cell number, *in situ* proliferation, phagocytosis and expressions of pro-inflammatory and anti-inflammatory genes, of ESM and BMSM were compared. In addition, ESM and BMSM showed different expression patterns in Rheumatoid Arthritis (RA) patients' synovium and during the developmental process of collagen-induced arthritis (CIA) mice. Taken together, these results demonstrated that the SM at least has two origins, ESM and BMSM. The different cellular property and dynamic expression patterns in RA patients/CIA mice highlight the notion that ESM and BMSM might play different role in arthritis.

P2609

Carbon quantum dots-mediated reactive oxygen species clearance and dexamethasone targeted delivery for liver fibrosis treatment

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Liver fibrosis is a process of inflammatory reaction and fibrogenesis caused by chronic liver injury, and is a common pathological process of cirrhosis and hepatocellular carcinoma. Liver parenchymal cell damage caused by reactive oxygen species (ROS) can activate downstream inflammatory cells (such as Kupffer cells), produce a large number of inflammatory mediators and cytokines, and further activate hepatic stellate cells, causing collagen deposition, which leads to liver fibrosis. Therefore, avoiding hepatocyte damage and its downstream inflammatory response should be the main strategy for the treatment of chronic liver injury. Herein, we have rationally designed nano-sized carbon quantum dots (CDs) as a carrier of anti-inflammatory drug dexamethasone. We found that dexamethasone-loaded CDs can rapidly target to the liver parenchyma and achieve effectively ROS clearance. In addition, we proved that dexamethasone-loaded CDs can release drugs in response to enzymatic reactions in the microenvironment of fibrosis. Overall, our results showed the clinical potential of CDs as nano-drug carrier for the highly efficient and rapidly targeted treatment of liver fibrosis.

P2610

Effect of IL-33 defect on the polarization and migration of mouse peritoneal macrophages induced by *Toxoplasma Gondii* infection

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IL-33 was a newly member of IL-1 family and its function on macrophages in mice infected with *Toxoplasma* is poor understood. In this study, the effect of IL-33 defect on the polarization of peritoneal macrophages from mice with acute *Toxoplasma* infection was investigated. The expression of M1 makers (NO, MHC II,CD86 and TLR4) were increased in IL-33$^{-/-}$ mice compared with WT group (NO, MHC II and CD86, $P<0.01$; TLR4, $P<0.05$); the infection rate of peritoneal macrophages was deceased in IL-33$^{-/-}$ mice compared with wild type mice ($P<0.05$); the expression of M1 makers (NO, MHC II, CD86 and TLR4) were increased ($P<0.01$) while the M2 maker (CD206) was down-regulated in IL-33$^{-/-}$ mice infected with *Toxoplasma* than that in the wild type infected group; the secretion of NO, IL-10, TNF-α and the expression of MHC II and CD86 were higher in infected group of both IL-33$^{-/-}$ and wild type mice compared with uninfected control group respectively ($P<0.01$). These finding

DOI: 10.1002/eji.201970400

suggested that the peritoneal macrophages were polarized to M1 direction in IL-33$^{-/-}$ mice ; IL-33 deficiency promoted the secretion of NO and the expression of MHC II, CD86 and TLR4 via driving polarization of M1 macrophages, thereby enhancing the immune protection in acute *Toxoplasma* infection.
Keywords: interleukin-33; macrophage; acute *toxoplasma* infection; polarization

P2611

Phagocytic activity in human immunodeficiency virus type 1 infection

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Phagocyte is one of the main leukocyte that include monocytes/macrophages and ploymorphonucleated(PMNS.Activated macrophayes produce some cytokines and other humeral mediators that intervene in defensive inflammatory processes and reactive oxygen and nitrogen Species to skill microorganisms.HIV-1 gp120, p24, Nef reduce the activation of macrophages and neutrophils and induce apoptosis. In the late stage of AIDS with low level of CD4T cell easily lead to coinfection or superinfections (such as respiratory, gastroenteric and HIV-1 demenia).

P2612

Intrinsic, cancer cell-selective toxicity of organic photothermal nano-agent: a simple formulation for combined photothermal-chemotherapy of cancer

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Nano-agent-mediated photothermal therapy (PTT) combined with chemotherapy has been proposed as an effective strategy against cancer. However, chemotherapeutic agents often cause serious side effects. Herein, a novel PTT nanoagent (Cy5.5-MSA-G250) with unanticipated intrinsic tumor-selective cytotoxicity is developed. The Cy5.5-MSA-G250 nanoparticles (NPs) are created by mixing mouse serum albumin (MSA) and coomassie brilliant blue (G250), and then conjugated with cyanine 5.5 (Cy5.5). As expected, Cy5.5-MSA-G250 NPs can efficiently kill cancer cells *in vitro* and *in vivo* by PTT. Meanwhile, we accidentally discover that Cy5.5-MSA-G250 have intrinsic specific cytotoxicity against tumor cells but not against normal cells. Moreover, the tumor-specific cytotoxicity of Cy5.5-MSA-G250 is much stronger than that of cytarabine, an FDA-approved anticancer drug. *In vivo* experiments also prove that Cy5.5-MSA-G250 NPs can effectively eliminate residual tumor cells and prevent metastasis. Further study indicates that selective induction of G1 cell cycle arrest and inhibition of DNA duplication in tumor cells may be the possible mechanism of the tumor cell-selective cytotoxicity of Cy5.5-MSA-G250 NPs. In addition, direct visualization, low systematic toxicity, good biodegradation, and efficient body excretion further make Cy5.5-MSA-G250 NPs attractive for *in vivo* applications. Taken together, Cy5.5-MSA-G250 NPs are proven to be a promising platform for combined photothermal-chemotherapy.
This work was financially supported by the National Science Foundation of China (No. 81660592)

DOI: 10.1002/eji.201970400

P2613

CSF1 mediated decrease of CD206 positive macrophage in endometrium contributes to Asherman´s syndrome

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Problem: Asherman´s syndrome (AS) is a worldwide disease that charactered by endometrial fibrosis and regeneration disorder. Macrophages are key regulators of inflammation, tissue repair, regeneration and fibrosis. However, the role of macrophage in AS is still unclear.
Method of Study: Endometrial biopsies of AS patients and controls were collected during the late proliferating phase of the menstrual cycle. Fibrosis of endometrial biopsies was detected by Masson's trichrome staining and a-SMA immunostaining. Cell proliferation was examined by Ki67 immunostaining. Macrophages were detected by immunostaining of CD68 and CD206. The expression level of CSF1, CSF1R, CCL2 and Ki67 were also detected by q-PCR and immunohistochemistry. For in vitro study, Ishikawa (IK) cells and THP1-derived macrophage were used. M2 culture supernatant (M2-S) was applied to H2O2 or bleomycin treated IK cells.
Results: In the endometrium of AS patients, the stroma was replaced by fibrous tissue and cell proliferation was reduced. Macrophages in endometrial tissue were mainly CD206 positive M2 macrophages and their number was significantly decreased in endometrium of AS patients. In vitro experiments showed that M2-S promoted the growth and migration of Ishikawa (IK) cells, and inhibited their apoptosis. M2-S also protected IK cells from bleomycin-induced fibrosis. The expression level of CSF1 was reduced in endometrium of patients with AS.
Conclusions: Macrophages are critical cells involved in the process of endometrial repair and fibrosis. The decrease of macrophage may be due to the reduced expression level of CSF1. Manipulation of macrophage activation/function may provide a novel therapeutic target for AS.

P2614

Macrophage specifically expressed IRAK-M affected macrophage polarity to facilitate the survival of Mycobacterium tuberculosis

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Background: IRAK-M, a member of IRAK family, is a negative regulator in TLR signaling and specifically expresses in monocytes and macrophages. *Mycobacterium tuberculosis* (*M. tb*), infects specifically macrophages as host cells. The role of IRAK-M in intracellular growth of *M. tb* and macrophage polarization was explored, for deeply understanding the pathogenesis of *M. tb*, the significance of IRAK-M to innate immunity and pathogen-host interaction.
Methods: U937 was induced with PMA into macrophages. IRAK-M expression was detected in *M. tb* infected U937 cells and in human lung tissue of pulmonary tuberculosis. Intracellular survival of *M. tb* was determined in IRAK-M knock-down U937 cells and over-expressing Jurkat cells. Molecular markers of M1- and M2-type macrophages were detected in IRAK-M knockdown U937 cells infected with *M. tb* H37Rv. U937 cells were stimulated with immunostimulant CpG7909 into M1 and infected with H37Rv. Expression of IRAK-M, IRAK-4 and iNOS was detected to evaluate the effect of IRAK-M

DOI: 10.1002/eji.201970400

to M1 polarization of macrophages during *M. tb* infection.
Results: IRAK-M increased in *M. tb* infected macrophages and human lung tissue of pulmonary tuberculosis. IRAK-M over-expression resulted in higher bacterial load, while IRAK-M interference resulted in lower bacterial load in *M. tb* infected cells. During *M. tb* infection, IRAK-M knockdown induced M1-type, while inhibited M2-type polarization of macrophages. M1-type polarization of U937 cells induced by CpG7909 was inhibited by *M. tb* infection, which was reversed by IRAK-M knockdown.
Conclusions: IRAK-M might alter the macrophage polarity, facilitating intracellular survival of *M. tb* and affecting Th1-type immunity of the host.

P2615

Counter regulation of ATP6V0d2 expression by IL-4 and LPS

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Macrophages sense tissue environments and polarize into different phenotypes that execute tissue specific functions. ATP6V0d2, a V0 subunit of V-ATPase, is expressed in macrophages and plays critical roles in regulation of inflammation, infection and autoimmunity in a lysosome acidification independent manner. However, the regulatory mechanism of *Atp6v0d2* expression in macrophages remains largely elusive. We found that *Atp6v0d2* mRNA and protein expression were highly induced by IL-4 and STAT6 binding to multiple sites within *Atp6v0d2* promoter was enhanced after IL-4 stimulation. In contrast, TLR agonists including LPS, poly (I:C), Pam3CSK4, Gardiquimod strongly suppressed ATP6V0d2 expression, which can be reversed by the addition of a NF-kB inhibitor. The suppression of IL-4-induced ATP6V0d2 by LPS was dose dependent and the addition of LPS resulted in significantly reduced STAT6 binding to the *Atp6v0d2* promoter. Together, these data demonstrate that ATP6V0d2 expression is counter regulated by IL-4 and LPS, suggesting it may serve as a focal point for macrophage polarization and function.

P2616

Chemokines CCL2, 3, 14 stimulate macrophage bone marrow homing, proliferation, and polarization in multiple myeloma

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Previous work showed that macrophages (MΦs) infiltrate the bone marrow (BM) of patients with myeloma and may play a role in drug resistance. Our study analyzed chemokines expressed by myeloma BM that are responsible for recruiting monocytes to the tumor bed. We found that chemokines CCL3, CCL14, and CCL2 were highly expressed by myeloma and BM cells, and the levels of CCL14 and CCL3 in myeloma BM positively correlated with the percentage of BM-infiltrating MΦs. In vitro, these chemokines were responsible for chemoattracting human monocytes to tumor sites and in vivo for MΦ infiltration into myeloma-bearing BM in the 5TGM1 mouse model. Surprisingly, we also found that these chemokines stimulated MΦ in vitro proliferation induced by

DOI: 10.1002/eji.201970400

myeloma cells and in vivo in a human myeloma xenograft SCID mouse model. The chemokines also activated normal MΦ polarization and differentiation into myeloma-associated MΦs. Western blot analysis revealed that these chemokines promoted growth and survival signaling in MΦs via activating the PI3K/Akt and ERK MAPK pathways and c-myc expression. To summarize, our results showed that chemokines CCL3, CCL2, and CCL14 as functional chemokines that were responsible for increased MΦ infiltration in the MM BM microenvironment. We also found that these chemokines promoted mMΦ proliferation within the tumor bed and polarization of normal MΦs to mMΦs via activation of the PI3K-Akt and MAPK/ERK pathways. Because mMΦs confer MM chemoresistance, targeting those chemokines may prevent MΦ infiltration in the tumor bed, and therefore may improve the efficacy of MM chemotherapy.

P2617

Pattern recognition receptors on macrophages for recognition and phagocytosis of nanomaterials

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Nanomaterials (NMs), new materials in nanometer scale with unique properties, have been reported to induce a variety of toxic effects in the body. One of the toxicological mechanisms is involved in monocyte/macrophage phagocytosis and subsequent inflammatory reactions and oxidative stresss. Once upon entering the blood stream, NMs are coated with sveral layers of serum proteins (protein corona), and Ig, copmplements and other opsonins in the corona will mediate monocyte/macrophage uptake via opsonin receptors. How macrophages recognize and phagocytose NMs with different chemical compositions and 3 dimensional structures in the absence of serum proteins is still elusive. In this study, we focused on pattern recognition receptors (PRRs) on macrophages in the recognition and phagocytosis of NMs. Four kinds of representative NMs, i.e. titanium dioxide, multi-walled carbon nanotubes, fullerene C60 and sillica, each having unique prperties (size, chemical composition, 3-D structure and surface property), were chosen for analysis of phagocytosis and cytokine production by mouse Raw264.7 macophages. High-throughput sequencing was engaged to analyze and screen candidate PRRs and cytokines, and results suggested scarvenger receptors a3 and f1 (Scar-a3 and Scar-f1) and formylpeptide receptor Fpr1 were involved in the recognition of different NMs. Next, we established corresponding reseptors knock-down stable cell lines by ShRNA. Observation by polarized microscopy and qPCR analysis of candidate cytokines (IL-1b, CXCL9, CCL20, TNFSF-13) indicated that these PRRs were related to recognition and phagocytosis of different NMs.

P2618

Crosstalk between adipocyte differentiation and immunoregulation compensates for osteopenic phenotype in mice with loss-of-function mutation in Lrp5

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A loss-of-function mutation in the Lrp5 gene in mice leads to low bone mass disorder because of the inhibition of the canonical Wnt signaling, and these mice are used as a homologous model of human osteoporosis-pseudoglioma syndrome. However, the complex effects of the systemic microenvironment in the bone marrow (BM) on mice with a loss-of-function mutation in Lrp5 remain unclear. In the present study, we evaluated the proliferation and osteogenic activity of mice osteoblasts by MTT assay and Alizarin red staining. The concentrations of osteoblastic ALP,

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DOI: 10.1002/eji.201970400

osteoclastic TRAP, and adiponectin in the supernatant were measured by enzyme-linked immunosorbent assay. The number and area of bone resorption pits were determined by toluidine staining to evaluate the osteoclastic activity. Reverse transcription-PCR was conducted to measure the expression of genes related to inflammation, osteolysis, osteogenesis, and adipogenesis. A flow cytometry assay was conducted to analyze the functional phenotype of immune cells. We observed increased osteogenesis in osteoblasts co-cultured with BM-derived myeloid cells compared to that in osteoblasts cultured alone. An increased frequency of M2 macrophages and reduced expression of inflammatory cytokines was found in myeloid cells derived from the BM of whole loss-of-function mice. The increased adipogenic tendency and higher levels of adiponectin in the whole loss-of-function mice may contribute to the promotion of M2 macrophage differentiation. We identified a novel systemic regulatory mechanism in bone formation and degradation, which partly ameliorated osteopenia because of the loss of Wnt signaling, which depends on crosstalk between adipocytes and M2 macrophages in the BM.

P2619

A novel Fas-binding outer membrane protein and lipopolysaccharide of Leptospira interrogans induce macrophage apoptosis through the Fas/FasL-caspase-8/-3 pathway

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Leptospira interrogans is the major causative agent of leptospirosis, an emerging, globally spreading zoonotic infectious disease. The pathogen induces macrophage apoptosis, but the molecular basis and mechanism remain unknown. In the present study, we found that *L. interrogans* caused apoptosis of phagocytosis-inhibited macrophages, and the product of the *L. interrogans* LB047 gene (Lep-OMP047) was the unique protein captured by mouse and human Fas proteins. The recombinant expressed Lep-OMP047 (rLep-OMP047) strongly bound mouse and human Fas proteins with equilibrium association constant (K_D) values of 5.20×10^{-6} to 2.84×10^{-9} M according to surface plasmon resonance measurement and isothermal titration calorimetry. Flow-cytometric examination showed that 5 µg rLep-OMP047 or 1 µg lipopolysaccharide of *L. interrogans* (Lep-LPS) caused 43.70% or 21.90% early apoptosis in mouse J774A.1 macrophages and 28.41% or 15.80% for PMA-differentiated human THP-1 macrophages, respectively, but the apoptosis was blocked by Fas-antagonizing IgGs, Fas siRNAs and caspase-8/-3 inhibitors. Moreover, Lep-OMP047 was significantly up-regulated during infection of macrophages. Lep-LPS promoted the expression and cytomembrane translocation of Fas and FasL in macrophages. The JNK and p38 MAPK but not ERK signaling pathways, as well as the transcription factors c-Jun and ATF2 but not CHOP, mediated Lep-LPS-induced Fas/FasL expression and translocation. TLR2 but not TLR4 mediated Lep-LPS-induced JNK/p38 MAPK activation. Therefore, we demonstrated that a novel Fas-binding OMP and LPS of *L. interrogans* induce macrophage apoptosis through the Fas/FasL-caspase-8/-3 pathway.

P2620

Increased glycolysis of tumor associated macrophages accelerates breast cancer progression

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DOI: 10.1002/eji.201970400

Tumor associated macrophages (TAMs) play a key role in accelerating tumor progression. The function of TAMs can be reshaped by their metabolic reprogramming. The glucose metabolism status in TAMs have aroused intensive attention. Its effects on the function of TAMs and the underlying mechanisms are still mysterious. In this study, TAMs were isolated from 4T1 tumor-bearing mice. The RNA sequencing and metabolomics results demonstrated that glycolysis and glycolysis related genes were upregulated in TAMs. The expression of lactate dehydrogenase (LDHA), one glycolysis associated enzyme, was confirmed to be increased in mouse bone marrow derived macrophages (BMDMs) cultured with 4T1 conditional medium, which were used to mimic TAMs in vitro. In this co-culture system, glucose consumption, production of lactate and ATP were also increased. So was the lactate producing rate. Moreover, reduced LDHA activity or expression decreased the levels of TAMs markers. After screening, GM-MCF in 4T1 supernatants was proven to be one major cause for the upregulated glycolysis of TAMs. Further analysis found that PI3K/AKT pathway was activated in TAMs. Inhibition of PI3K/AKT pathway by LY294002 notably reduced the expression of glycolysis related genes and TAMs markers. Additionally, the supernatant from TAMs with reduced LDHA decreased sphere-formation and migration of 4T1 cells. This was partially ascribed to the less production of lactic acid and urea. Co-injection of RAW264.7 cells with reduced LDHA and 4T1 cells into the mouse mammary fat pad could obviously decrease the tumor volume and lung metastasis. The specific mechanisms will be further elucidated.

P2621

ID4 regulates polarization of tumor-associated macrophage to promote colorectal cancer stemness

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Although immunotherapy has been used to treat some patients with microsatellite instability-high, most of the colorectal cancer patients show poor response to immunotherapy. Tumor cells interact closely with the surrounding immune cells and stromal cells, which together form the complicated tumor microenvironment and participate in the regulation of immune response. Tumor-associated macrophages (TAM), the highest proportion of immune cells in the tumor microenvironment, impede cytotoxicity against tumor cells by different mechanisms. In colon cancer, the inhibitor of DNA binding protein 4 (ID4) is highly expressed in tumor tissues and associated with lymph node metastasis and poor prognosis. In this study, we found that the expression of ID4 in TAM of colon cancer tissues was significantly higher than that in normal tissues, and TAM with high expression of ID4 played an important role in promoting tumorigenesis. On one hand, high expression of ID4 in TAM reduced $CD8^+$ T cell infiltration and activity via inhibiting the transcription of several chemokines such as CXCL-9; on the other hand, it works through enhancing the stemness traits of tumor cells via regulating the Wnt/b-catenin signaling pathway. Besides, immune effector molecule TNF-α, a key cytokine in colitis, increased ID4 expression in TAM through affecting ID4 protein stability but not transcriptional level. This study provides insights of crosstalk between tumor microenvironment and tumor cell intrinsic properties in the promotion of colorectal carcinogenesis and progression.

DOI: 10.1002/eji.201970400

P2622

TRIM21 ubiquitylates IRF5 and mediated macrophaged-induced Bortezomib resistance in Myeloma

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Bortezomib (bort) has improved overall survival in multiple myeloma (MM) patients, but the majority of them develop drug resistance. We previously demonstrated that PBMC-induced macrophages (MΦs) are insensitive to bort and protect MM cells against bort-induced apoptosis. In this study, we aimed to characterize the role of TRIM21, an E3 ligase, in MΦs-mediated resistance of MM cells to bort and to futher understand the molecular mechanism involved in the process. We found that the expression of TRIM21 was obviously increased during MΦs differentiation using RT-PCR and Western blot. The small-interfering RNA knockdown of TRIM21 in MΦs resulted in reduced ability of protecting MM cells against bort-induced apoptosis. And flow cytometry analyses showed TRIM21-knocked down MΦs preferentially to be M1 type, with upregulated levels of CD80, CD86 and downregulated levels of CD206, CD163. Furthermore, mass spectrometry identified a transcription factor IRF5 as a substrate protein of TRIM21, and we delinated a IRF5-dependent mechanism of MΦs polarization. These data both define a critical role for TRIM21 in M1 MΦs polarization and thus mediated MM bort resistance, and provide the basis for a novel targeted therapeutic approach.

P2623

Switch from WT1 to EGR-1 binding on TGF-β1 promoter contributes to the committed differentiation of leukeia cells into macrophage lineage

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TGF-β/SMAD signaling pathway was considered as the breakthrough point of research. To observe the levels of TGF-β1 and its' downstream genes, such as Early growth response gene 1(EGR-1) and the Wilms' tumor 1 (WT1) in macrophage differentiation which was induced by phorbol ester (PMA). As well as the regulation function of EGR-1 and WT1 to TGF-β1 and the transduction of TGF-β/SMAD signaling pathways .Then further discussion the influence of them to TGF-β/SMAD signaling pathway. Our previous study revealed that PMA could inhibit the proliferation of leukemia cell lines and promote cell differentiation into macrophages in vitro. It was noted that cytoplasm staining decreased and the number of mature cells was increased. The expression of CD11b and CD14 in K562, HL-60 and THP-1 cells were increased, either the mRNA and protein expression of TGF-β1, SMAD3, SMAD4 were gradually increased in response to treatment with PMA as compared with that of control while the level of WT1 was decreased. The expression of EGR-1 was quickly increased, then appeared a downward trend and maintained a period of time. PMA treatment suppressed the binding of WT1 on TGF-β1 promoter while promoting the binding of EGR-1 to TGF-β1 promoter which was indicated by ChIP assay. In conclusion, PMA plays an important role in inhibition of proliferation and induction of committed macrophage differentiation of leukemia cells. EGR-1 can competitively inhibit WT1 bind on TGF-β1 promoter so as to promote TGF-β/SMAD signaling pathway transduction during the process of the differentiation.

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DOI: 10.1002/eji.201970400

P2624

Effect of macrophage TGF-β1 deletion on testicular spermatogenesis in mice

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The testis produces sperm throughout the male reproductive lifespan by differentiation of spermatogonial stem cells. Macrophages are abundant in the adult mammalian testis, and play an important role during the testicular spermatogenesis. Depletion of macrophages leads to a disruption in spermatogonial differentiation. However, it is not clear which cytokines secreted by macrophages play a key role in sperm differentiation. TGF-β superfamily signaling play an important role in regulating testis development and spermatogenesis. TGF-β regulates the development and homeostasis of several tissue-resident macrophage populations. Nevertheless, the role of TGF-β secreted by macrophages in spermatogenesis remains undefined. Using CX3CR1-Cre-TGFβ1 mouse model, we found that the TGF-β1 level was downregulated in the testis. There was no significant change in the number of macrophages. Interestingly, the depletion of TGF-β1 in testicular macrophages lead to significant upregulation of *Sox9*、*Fgf9* and *Gata4* expressions. This indicates the abnormal function of Sertoli cells in CX3CR1-Cre-TGFβ1 mouse. Histopathological examination revealed a disruption in spermatogonial differentiation. These findings reveal an unexpected role for TGF-β1 secreted by macrophages in the spermatogenesis in the testis and raise the possibility that TGF-β1 of macrophages play a role in development and function maintain in other tissues.
Acknowledgements: This work was supported by grants from the National Basic Research Program of China (2015CB943203) and the Natural Science Foundation of Shandong Province (ZR2017BC030).

P2625

Function and mechanism study of Kisspeptin/GPR54 in macrophage mediated tumor microenvironment

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Therapies targeting immune checkpoints have shown great clinical potential in a subset of patients with cancer but may be hampered by failure to reverse the immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs) are the most abundant immune cells in TME, and play important roles in restricting antitumor immunity. However, the key regulatory genes of TAMs and their specific mechanisms still need to be further studied. G protein-coupled receptors, as the largest family of cell membrane receptors, are important for sensing extracellular signals and regulating intracellular signal transduction. Among them, GPR54, as a specific receptor for the neuropeptide hormone Kisspeptin, has been found to play an important role in adolescent development and tumor metastasis. Our previous study demonstrated that Kisspeptin/GPR54 also involved in regulation of antiviral innate immune responses. However, whether Kisspeptin/GPR54 inhibit tumor progression by regulating the function of immune cells in TME remains largely unknown. Here, we found that expression of the neuropeptide hormone receptor GPR54 was significantly up-regulated in M1 macrophages through high-throughput screening, while the M1 polarization of macrophages was reduced obviously by knocking out GPR54 and led to enhanced growth of tumors. Furthermore, we found that GPR54 promotes the IFN-γ induced M1 polarization of macrophages by enhancing the JAK/STAT signaling pathway, and then changes the tumor microenvironment in the

DOI: 10.1002/eji.201970400

mouse B16F10 melanoma model and eventually inhibits the development of tumor. Thus, our data indicated vital roles of Kisspeptin/GPR54 in host antitumor immunity and a potential therapeutic target in cancer immunotherapy.

P2626

LncRNA NR_109 regulating TAM polarization to rebuild the microenvironment of gastric cancer

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Tumor associated macrophages (TAM) is a critical kind of immune cells in tumor microenvironment and play vital roles in promoting tumor progression and invasion. However, the molecular mechanisms underlying TAM regulation remain to be further investigated and may make significant contributions to cancer treatment. Long non-coding RNAs (lncRNAs) as important regulators of gene expression, play an important role in human diseases, including cancers and have an undefined role in the polarization of macrophage. In this study, we found that lncRNA-NR_109 were shown to be highly expressed in M2 macrophages and gastric cancer (GC) - related macrophages, and were associated with unfavorable outcome of patients with GC. NR_109 affected the expression of surface markers on TAMs and the level of cytokines in the cultural supernatant of M2. In vitro, the M2 with NR_109 knocking down could decreased the proliferation and migration of GC cells. NR_109 also influenced the growth of GC cells through controlling M2 in vivo. Mechanistically, NR_109 bound to FUBP1, which is the transcript factor of c-Myc and inhibited the ubiquitination of FUBP1. At the meantime, NR_109 promoted the transcription of c-Myc and resulted in the polarization of M2. Overall, our observations suggest that NR_109 may exert an important role in controlling the polarization and function of TAMs through binding with FUBP1 and promoting the expression of c-Myc.

P2627

mTORC1 is required for alveolar macrophage terminal differentiation, self-renewal and function

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Alveolar macrophages are the primary phagocytes of the innate immune system, it is one of the first lines of defense against microbial invasion and clean dead cell debris, and release numerous secretory products (pro-inflammation and anti-inflammation) to balance lung homeostasis. When the function of AMs impaired develop pulmonary alveolar proteinosis, an intra-alveolar accumulation of surfactant protein lead to progressive dyspnea and increased the pulmonary infection. AM differentiation is essential for tissue homeostasis, but molecular mechanisms controlling AM generation have not been fully defined. Here, we show that the mTORC1 is required for the perinatal development of alveolar macrophages. Mice expressing a conditional allele of mTORC1 (Lysm-Raptor$^{f/f}$ and CD11c-Raptor$^{f/f}$) exhibited significant reductions in alveolar macrophages and failed to effectively clear dead cell debris, showing that immunodeficiency results from reduced alveolar macrophage numbers. Moreover, Conditional deletion of mTORC1 in mice at different time points halted the development and differentiation of AMs. In adult mice, mTORC1 deficiency in AMs increased infiltration of inflammatory cells and apoptotic cell in the alveolar space, and developed pulmonary alveolar proteinosis, which indicate mTORC1 contribute to the maintenance of tissue integrity and hemostasis by the phagocytosis and processing of opsonized apoptotic cells. These findings reveal an additional layer of complexity regarding the guidance cues, which govern the

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DOI: 10.1002/eji.201970400

genesis, maturation, and survival of AMs, and will lay a solid foundation for the application of AMs in lung immune dysfunction and pulmonary alveolar proteinosis.

P2628

A novel strategy to strengthen lung cancer immunotherapy by silencing IDO2 in dendritic cells

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While the DC-based immunotherapy has achieved satisfactory results in animal models, it was not as good as expected in clinical application despite being shown to be safe to apply. Improving the efficiency of the DC-based vaccine is essential for cancer immunotherapy. In the present study we explored to amplify and strengthen the antitumor immune response of a DC-based tumor vaccine by silencing expression of indoleamine 2,3-dioxygenase2 (IDO2), a tryptophan rate-limiting metabolic enzyme in DCs. In vitro studies showed that silencing IDO2 in DCs did not affect the differentiation but increased the expression of costimulatory molecules when stimulated by TNF-a plus tumor cells (LLC) lysate. In a mixed cocultrue system, compared to the control DCs, IDO2 silenced DCs can promote the proliferation of allogeneic T cells and reduce the induction of Tregs. Further in vivo experiments showed that silencing IDO2 in DCs have stronger power in inhibiting the growth of tumors than the control DCs group. Moreover, treatment with IDO2 silenced DC-based cancer vaccine enhanced the CTL activity while decreasing the T cell apoptosis and percentage of $CD4^+CD25^+Foxp3^+$Treg. Our research provides evidence that silencing of tryptophan rate-limiting metabolic enzyme (IDO2) has the potential to enhance the efficacy of DC-based cancer immunotherapy.

P2629

The influence of miR-26a on Rab32-associated vesicles trafficking during *Listeria monocytogenes* infection in macrophage

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To study the roles of miRNA in innate immunity, we profiled miRNA of RAW264.7 cell lines infected of *Listeria monocytogenes (Lm)* through high throughput qPCR. From miRNA profile, we select miR-26a, miR-27a and miR-196b for next investigation, which were steadily decrease during *Lm* infection. We established a series of miRNA overexpression transgenic (TG) mice by lentiviral virus, including miR-26a TG mice, miR-27a TG mice and miR-196b TG mice, to explore the roles of these miRNA in innate immunity. Experimental data from BMDM and *in vivo* of miRNA TG mice suggested miR-26a and miR-196b may involve *Lm* infection. Our previous reported that Rab32 plays a critical role in innate immunity, Rab32 can formed a complex with other proteins to encompass bacteria in dendritic cell lines DC2.4. So, we speculated that miR-26a and miR-196b whether influence Rab32-associated vesicles trafficking during *Lm* infection. Then, we found the number of Rab32-associated vesicles increase in miR-26a overexpressed RAW264.7 during *Lm* infection by fluorescence microscopy. To study the roles of Rab32 in macrophage against *Lm*, Rab32 knockout in RAW264.7 cell lines by CRISPIR/Cas9 and conditional knockout Rab32 in myeloid cells mice were used to evaluate for the roles of Rab32 in macrophage *in vitro* and *in vivo*. Further to explore the regulatory mechanism of miR-26a on Rab32-associated vesicles trafficking during *Lm* infection. We performed transcriptome of RAW264.7 control and RAW264.7 overexpressed miR-26a infected with *Lm* for 5h, RNA-seq

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DOI: 10.1002/eji.201970400

suggested that miR-26a may influence on Rab32-associated vesicles through targeting EPHA2, MAP3K9, NHSL1 and OTUD1 during *Lm* infection.

P2630

Edaravone protects against progression of liver fibrosis via decreasing the IL-1β secretion of macrophage

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Background and aims: Edaravone, a strong novel free radical scavenger, and clinical and experimental studies have demonstrated neurovascular protective effects. Since neurosurgical patients are usually followed by a state of comatose and deep sedationand need to be treatmented for a long time, which prone to result in the liver injury. It makes sense to illuminate the impact of EDA in the development of liver fibrosis.
Methods: EDA inhibition of the TAA-induced liver fibrosis was evaluated by western blot, quantitative RT-PCR, flow cytometry and Pathological section test.
Results: EDA had a hepatic protective role to TAA-induced chronic liver fibrosis via inhibiting monocyte-derived macrophages recruitment and decreasing hepatic inflammation in vivo; In vitro assay, EDA suppressed LPS-induced pro-IL-1b and IL-1b maturation of macrophages. This inhibitory activity is involved in the inhibition of NF-κB signal pathway and reactive oxygen species (ROS) production. Moreover, EDA decreased the activation of HSCs via decreasing the IL-1β secretion of macrophage
Conclusions: Edaravone protects against progression of liver fibrosis via decreasing the IL-1β secretion of macrophage. Therefore, this study has an important guiding significance for drug-induced hepatic fibrosis, reducing liver damage and saving medical resources.

P2631

PPA1 promotes breast cancer metastasis by stimulating EMT and regulating macrophage polarization

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Inorganic pyrophosphatase (PPA1) catalyzes the hydrolysis of pyrophosphate to inorganic phosphate, thereby providing extra energy for metabolism, and its expression is significantly higher in many tumors, especially in lung and ovarian tumor tissues, which suggests that PPA1 may plays an important role in carcinogenesis and in the development of some tumors. Our study aimed to explore the expression and predictive role of PPA1 in breast cancer progression. We firstly examined PPA1 expression in human breast cancer samples by IHC, and found that PPA1 was overexpressed, as well as correlated with advanced grades, stages, and poor survival in tumors from breast cancer patients. Moreover, suppressing PPA1 inhibited tumor invasion and migration in vitro and in vivo. And overexpression of PPA1 promoted metastasis of breast cancer cells by stimulating epithelial-to-mesenchymal transition (EMT) through WNT/β-catenin, but not TGF-β or Snail signaling. In addition, western blot and immunofluorescence revealed that PPA1 in breast cancer cells may regulate polarization of macrophages which are one of the most important immune cells. Taken together, these

DOI: 10.1002/eji.201970400

results demonstrated that β-catenin as one of vital downstream molecules that mediate the EMT induced by PPA1. Therefore, strategies for downregulating PPA1 may have therapeutic potential for the treatment of breast cancer.

P2632

M2-like tumor-associated macrophages drive vasculogenic mimicry through amplification of IL-6 expression in glioma cells

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Vasculogenic mimicry (VM) has offered a new horizon for understanding tumor angiogenesis, but the mechanisms of VM in glioma progression have not been studied explicitly until now. As a significant component of immune infiltration in tumor microenvironment, macrophages have been demonstrated to play an important role in tumor growth and angiogenesis. However, whether macrophages could play a potential key role in glioma VM is still poorly understood. Herein we reported that both VM and CD163+ cells were associated with WHO grade and reduced patient survival, and VM channel counting was correlated to the number of infiltrated CD163+ cells in glioma specimens. In vitro studies of glioma cell lines implicated that M2-like macrophages (M2) promoted glioma VM. We found that conditional medium derived from M2 amplified IL-6 expression in glioma cells. Furthermore, our data indicated that IL-6 could promote glioma VM, as blocking IL-6 with neutralizing antibodies abrogated M2-mediated VM enhancement. In addition, the potent PKC inhibitor bisindolylmaleimide I could prevent M2-induced IL-6 upregulation and further inhibited glioma VM facilitation. Taken together, our results suggested that M2-like macrophages drove glioma VM through amplifying IL-6 secretion in glioma cells via PKC pathway.
This work was supported by grants from the National Natural Science Foundation of China (No. 81772879) and National Key Research and Development Program of China (2018YFC1002803).

P2633

Abberent expression of nor1 protein in tumor associated macrophages contributes to the development of DEN-induced hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary tumor of the liver and the sixth most common lethal cancer worldwide. Recent evidences demonstrated that oxidored nitro domain containing protein 1(NOR1), a putative tumor suppressor gene, is overexpressed in human HCC tissues. However, the role of NOR1 in HCC development remains unclear. Here, we described that NOR1 protein level is elevated in HCC and is associated with poorer clinical outcome. However, ecotopic overexpression of NOR1 protein in human HCC cell line HepG2 cells had no effect on cells proliferation, migration and clonality. Immunofluoresence assay indicates NOR1 protein is mainly expressed at CD163 positive M2 tumor associated macrophages(TAMs). To explore the role of NOR1 in the development of HCC, we interrogated the susceptibility of mice lacking the NOR1 gene to DEN-induced hepatocarcinogenesis. NOR1 deficient mice displayed resistance to DEN-induced HCC. We also demonstrate that mNOR1 protein is enriched in F4/80 positive Kupffer cells (KCs) infiltrated in DEN induced murine HCC tissues. Loss of NOR1 led to increase of iNOS whereas decrease of Arg1,Ym1 expression in KCs. Overexpression of NOR1 in THP-1 macrophages led to decrease of iNOS but increase of Arg1. Mechanistic investigations showed that inflammatory cytokines IL-6, TNF-α

production and NF-κB activation were also decreased in NOR1 knockout mice exposed to DEN treatment. Our data suggested that NOR1 is overexpressed in HCC associated TAMs and promotes M2 alternative polarization. Genetic deletion of NOR1 in mice leads to impairment of IL-6 production and NF-κB activation, which in turn attenuates DEN-induced HCC development.

P2634

Galectin-9 positive tumor-associated macrophages associate with immunoevasive contexture but favorable adjuvant chemotherapeutic response in muscle-invasive bladder cancer

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Purpose: Tumor-associated macrophages (TAMs) exhibit heterogeneous subsets and dichotomous roles in cancer-immune evasion. This study aimed to assess clinical effects of galectin-9$^+$TAMs in muscle-invasive bladder cancer (MIBC).
Materials and methods: We identified gal-9$^+$TAMs by immunohistochemistry (IHC) of a tumor microarray (n=141) from Zhongshan Hospital and by flow cytometric analysis (FACS) of tumor specimens (n=20) from the Shanghai Cancer Center. The survival benefit of platinum-based chemotherapy in this subpopulation was evaluated. The effect of tumor-immune microenvironments with different levels of gal-9$^+$ TAMs was explored.
Results: Frequency of gal-9$^+$TAMs increased with tumor stage and grade. Gal-9$^+$TAMs predicted poor OS and RFS and showed better abilities to discriminate prognostic groups. Patients with high gal-9$^+$TAMs showed a prominent survival benefit with adjuvant chemotherapy (ACT). High gal-9$^+$ macrophage infiltration was correlated with increasing Treg and mast cells and decreasing CD8$^+$T and DC cells. Dense infiltration of Gal-9$^+$TAMs was related to reduced cytotoxic molecules, enhanced immune checkpoints or immunosuppressive cytokines expressed by immune cells and active proliferation of tumor cells. Additionally, the accumulated subpopulation was strongly associated with PD-1$^+$TIM3$^+$CD8$^+$T cells.
Conclusions: Gal-9$^+$TAMs predict OS and RFS and response to ACT in MIBC patients. High gal-9$^+$TAMs were associated with a pro-tumor immune contexture concomitant with T cell exhaustion.
Keywords: galectin-9$^+$TAMs; muscle-invasive bladder cancer; adjuvant chemotherapy; immune contexture

P2635

C5aR1$^+$ tumor associated macrophages impede CD8$^+$T cell function and predict poor clinical outcomes in gastric cancer

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Objective: Recent studies have reported the role of C5a-C5aR1 signaling in limiting T cell response during carcinogenesis. This study aims to investigate the effects of C5aR1$^+$ tumor associated macrophages on immune contexture and clinical outcomes in gastric cancer.

DOI: 10.1002/eji.201970400

Experimental Design: A total of 440 tumor tissue microarray specimens and 55 fresh tumor tissue samples of gastric cancer patients from Zhongshan Hospital, Fudan University were analyzed. Clinical outcomes were compared between subgroups of gastric cancer patients by Kaplan-Meier curves and Cox regression model. C5AR1$^+$ TAMs and CD8$^+$T cell landscape were determined by immunohistochemistry, flow cytometry, and CIBERSORT. In vitro culture of fresh tumor tissue was performed to evaluate the anti-tumor effect of blocking C5aR1.
Results: C5aR1$^+$ cell infiltration correlates with tumor progression and poor prognosis in patients with gastric cancer. Intratumoral C5aR1$^+$ cells are predominantly tumor associated macrophages (TAMs) with an immunosuppressive phenotype in gastric cancer. Decreased CD8$^+$T cells infiltration, decreased GZMB, IFN-γ, PRF1 and increased PD-1 expression suggest CD8$^+$T cells dysfunction in high proportion of C5aR1$^+$ TAMs. Moreover, blockade of C5aR1 with PMX205 in vitro increases the anti-tumor immunity of CD8$^+$T cells. Accordingly, high level of C5aR1$^+$ TAMs infiltration indicates poor overall survival (OS, $P < 0.001$), progression-free survival (PFS, $P < 0.001$) and inferior chemotherapeutic benefit in gastric cancer patients after surgery.
Conclusions: C5AR1$^+$ TAMs dominate in C5aR1$^+$ cells and contributes to the immunosuppressive microenvironment by disrupting CD8$^+$T cell function in gastric cancer. C5AR1$^+$ TAMs predict poor clinical outcomes, providing potential immunotherapeutic approach for gastric cancer patients.

P2636

Drug-loaded microparticles modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype

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Resetting tumor-associated macrophages (TAMs) is a promising strategy to ameliorate the immunosuppressive tumor microenvironment and improve innate and adaptive antitumor immunity. Here we show that drug-loaded microparticles (drug-MP), can function as an antitumor immune modulator that switches TAMs from M2 to tumor-killing M1 phenotype. Mechanistically, drug-MP increases macrophage lysosomal pH, causing Ca2+ release via the lysosomal Ca2+ channel mucolipin-1 (Mcoln1), which induces the activation of p38 and NF-κB, thus polarizing TAMs to M1 phenotype. In parallel, the released Ca2+ activates transcription factor EB (TFEB), which reprograms the metabolism of TAMs from oxidative phosphorylation to glycolysis. As a result, drug-MP-reset macrophages ameliorate tumor immune microenvironment by decreasing immunosuppressive infiltration of myeloid-derived suppressor cells and Treg cells, thus enhancing antitumor T-cell immunity. These data illuminate a previously unrecognized antitumor mechanism of drug-MP, suggesting a potential new macrophage-based tumor immunotherapeutic modality

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DOI: 10.1002/eji.201970400

P2637

CD68- and CD163-positive tumor infiltrating macrophages in non-metastatic breast cancer: a retrospective study and systematic review

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Studies have indicated the significance of tumor associated macrophages (TAMs) in breast cancer; however, inconsistent results still exist. We retrospectively reviewed the macrophage distribution in 1579 breast cancer specimens with anti-CD68 or anti-CD163 immunohistochemical staining, and further analyzed the overall survival data. Furthermore, we performed a retrospective study and systematic review of the published studies on CD68- and CD163-positive macrophages in non-metastatic breast cancer. 13 studies with 5116 patients were included in this meta-analysis. Our own data revealed a high density of both CD68- and CD163-positive TAMs that was significantly related to lymph node metastasis (CD68, $P = 0.003$; CD163, $P < 0.001$); high Ki67 (CD68, $P = 0.026$; CD163, $P < 0.001$), poor histological grade (CD68, $P < 0.001$; CD163, $P < 0.001$) and hormonal receptor negativity (CD68, $P < 0.001$; CD163, $P < 0.001$); only CD163-positive TAMs were associated with poor overall survival ($P = 0.003$). Nonetheless, the meta-analysis only found that CD68- and CD163-positive TAMs were associated with high Ki67 [CD68, Relative risk (RR): 1.18, 95% confidence interval (CI): 1.09-1.28; CD163, RR: 1.75, 95% CI: 1.39-2.20], advanced histological grade (CD68, RR: 1.72, 95% CI: 1.46-2.03; CD163, RR: 1.99, 95% CI: 1.35-2.94) and low hormonal receptor levels (CD68, RR: 0.75, 95% CI: 0.69-0.82; CD163, RR: 0.82, 95% CI: 0.74-0.90), but not lymph node metastasis and HER2 expression. This meta-analysis further supports the clinical significance of TAMs in breast cancer, and both CD68- and CD163-positive TAMs could be prognostic markers in non-metastatic breast cancer.

P2638

The transcription factor Foxp1 regulates the differentiation and function of dendritic cells

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Dendritic cells (DCs) are the sentinels of the immune system and play a critical role in initiating adaptive immune responses against pathogens. As the most powerful antigen presenting cells, DCs are also important in maintaining immune homeostasis and participating in the development of autoimmune diseases. How the maturation and function of DCs is regulated in these conditions and what is the function of various transcription factors is still unclear. In this study, **we found that the expression of the transcription factor Foxp1 gradually increased during the maturation of DCs.** Then, we constructed a recombinant adenovirus carrying Foxp1-interfering RNA (Ad-simFoxp1) and transfected murine bone marrow-derived DCs *in vitro*. **DCs transfected with Ad-simFoxp1 exhibited markedly lower costimulatory molecules, and decreased cytokines. And Ad-simFoxp1 greatly inhibited mature DC-induced T cell responses. Moreover, *in vivo* infusion with Ad-simFoxp1-modified DCs significantly delayed the onset of experimental autoimmune encephalomyelitis (EAE).** Therefore, **adoptive transfection of Ad-simFoxp1 in DCs may be a potential treatment strategy against autoimmune diseases.**

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DOI: 10.1002/eji.201970400

P2639

Vitamin B2 metabolism regulates macrophage polarization

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Macrophages can polarize to two distinct phenotypes: classical M1 and alternative M2 states. How vitamin metabolism impacts macrophage polarization is largely unknown. Vitamin B2 (Riboflavin,RF) is converted to FMN by riboflavin kinase (RFK) and FAD which are essential cofactors for dehydrogenases, reductases and oxidases. Here we show that T-helper cytokines IFNg decreases while IL-4 upregulates macrophage RFK expression levels. Knockdown of RFK can inhibit IFNg-induced M1 and promote IL-4-mediated polarization of macrophages, while supplement of FAD or NO donor rescue this phenotype. Overexpression of RFK could enhance M1 state and iNOS inhibitor treatment reduces proinflammatory cytokine expression. Our study reveals vitamin B2 metabolism can regulate macrophage polarization.

P2640

The role of C1QBP in CSF-1 dependent macrophage adhesion

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Background: C1QBP is originally identified as a pre-mRNA splicing factor SF2-associated protein and mediates many biological responses in the immune system. CSF-1 is a principle macrophage developmental molecule that stimulates proliferation and activation of macrophage. In addition, the physiological role of C1QBP in immune response remains to be established. **In this study, we carried out experiments to investigate the role of C1QBP in macrophage adhesion.**
Objective: To investigate the C1QBP expression and its influence on CSF-1 dependent cell adhesion in murine macrophage RAW-264.7 cell.
Methods: Lentivirus mediated C1QBP knockdown stable cell line was constructed. Cell adhesion assay was used to detect the role of C1QBP knockdown in colony stimulating factor-1(CSF-1) induced cell adhesion ability. The level of p-integrinβ1 after C1QBP knockdown was determined by immunofluorescence. The change of adhesion related protein integrinβ1 and p-integrinβ1 after C1QBP knockdown was tested using Western blotting.
Results: Lentivirus mediated C1QBP knockdown stable cell line was constructed, and confirmed that the expression of C1QBP was significantly decreased. **After C1QBP knockdown, CSF-1 induced cell adhesion ability significantly decreased (*P*< 0.05). P-integrinβ1 significantly decreased after C1QBP knockdown according to immunofluorescence.The total expression level of integrin-β1 were not affected, but the level of integrin-β1 phosphorylation decreased.**
Conclusions: C1QBP knockdown impared adhesion ability of macrophage RAW-264.7 cell, and may play a biological role by inhibiting phosphorylation of integrinβ1.
Keywords: C1QBP; macrophage; cell adhesion; integrinβ1

DOI: 10.1002/eji.201970400

P2641

Exosomes derived from rAAV/AFP-transfected dendritic cells Elicit specific T cell-mediated immune responses against hepatocellular carcinoma

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Dendritic cell (DC)-derived exosomes (Dex) have been proved to induce and enhance antigen-specific T cell responses in vivo, and previous clinical trials have shown the feasibility and safety of Dex in multiple human cancers. However, there is little knowledge on the efficacy of Dex against hepatocellular carcinoma (HCC) until now. In this study, human peripheral blood-derived DCs were loaded with recombinant adeno-associated viral vector (rAAV) carrying alpha-fetoprotein (AFP) gene (rAAV/AFP), and high-purity Dex were generated. Then, naïve T cells were stimulated with Dex, to investigate the specific T cell-mediated immune responses against HCC. Our findings showed that Dex were effective to stimulate naïve T cell proliferation, and induce T cell activation to become antigen-specific cytotoxic T lymphocytes (CTLs), thereby exhibiting anti-tumor immune responses against HCC. In addition, Dex-sensitized DC precursors seemed more effective to trigger major histocompatibility complex class I (MHC I)-restricted CTL response and allow DCs to make full use of the minor antigen peptides, thereby maximally activating specific immune responses against HCC. It is concluded that Dex, which combine the advantages of DCs and cell-free vectors, are promising to completely, or at least in part, replace mature DCs to function as cancer vaccines or natural antitumor adjuvant.

P2642

Constitutively expressed MHC class Ib molecules regulate macrophage M2b polarization and sepsis severity in irradiated mice

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Macrophages can change their physiology in response to microenvironmental signals. This differentiation into classically activated M1 or alternatively activated M2 macrophages is known as polarization. In this study, we isolated bone marrow-derived macrophages from β2m-deficient (deficient in both MHC class Ia and Ib) and K^bD^b-deficient (deficient only in MHC class Ia) mice and found that β2m-deficient macrophages showed a significantly lower M2b polarization efficiency. In addition, the absence of constitutive MHC class Ib expression decreased the stability of the Notch-1 transmembrane domain (NTM). Finally, we found that β2m-deficient mice exposed to irradiation showed reduced bacterial translocation and sepsis severity. Overall, our study demonstrates that MHC class Ib molecules are essential for M2b macrophage polarization and suggests that MHC class Ib molecules play an important role during infection-induced innate immunity.

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DOI: 10.1002/eji.201970400

P2643

Proinflammatory response induced by Cy5.5-MSA-G250 nanoparticles in RAW264.7 mouse macrophages

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Cy5.5-MSA-G250 nanoparticles (NPs) had been proved to have unique advantages for cancer treatment, including excellent photothermal performance, tumor cell-selective cytotoxicity, direct visualization, low systematic toxicity, good biodegradation, and efficient body excretion. However, to cellular systems, Cy5.5-MSA-G250 NPs are considered as foreign particles, and the effect on immunity system of the NPs is still unknown. Therefore, more efforts are needed to understand the role of Cy5.5-MSA-G250 NPs on the immunity system. In this study, we aim to evaluate the toxicity of immune cells under NPs exposure, and screen the pro-inflammatory responses in RAW264.7 mouse macrophage-like cell line, established from the ascites of a tumor induced in a male BALB/c mouse, after treatment of Cy5.5-MSA-G250 NPs. *In vitro* experiments clearly showed that Cy5.5-MSA-G250 NPs can promote M1 polarization, associated with changes in cell morphology and enhanced production of inflammatory cytokines. This inducing M1 polarization capacity will help Cy5.5-MSA-G250 NPs to better anticancer effects in clinical trials. And the observed M1 macrophages' polarization triggered by NPs can be abolished after adding TLR4 inhibitor, CLI095, suggesting that TLR4 is involved in Cy5.5-MSA-G250-induced inflammation.

This work was financially supported by the National Science Foundations of China (No. 81660592).

P2644

Alternatively activated (M2) macrophage express SP to promote tumour growth and invasiveness can be inhibited by aprepitant in Esophageal carcinoma

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Background and aims: The substance P (SP)/neurokinin-1 receptor (NK1R) complex has been discovered to be pivotal in the development of a variety of human cancers, and NK1R antagonists, such as the clinical drug aprepitant, are promising future anticancer agents. Yet, the role of the SP/NK1R complex as a potential anticancer target in esophageal carcinoma (ESCA) is still unknown.
Methods: The pro-tumour functions of M2 macrophages in ESCA were investigated in vitro co-culture system. Human esophageal carcinoma cell lines TE1, KYSE150, and KYSE170, human tumor samples from 25 adult with esophageal carcinoma were analyzed regarding the SP/NK1R complex as a potential new anti-tumor target in esophagus cancer.
Results: M2 macrophages can express SP to promote tumor growth and enhance tumor migration. Therapeutic targeting with the NK1R antagonists aprepitant, led to growth inhibition , migration weakened and apoptosis in esophageal carcinoma cells in a dose-dependent manner. Intriguingly, esophageal carcinoma cells predominantly expressed the truncated splice variant of NK1R. Expression analysis of 25 human esophageal carcinoma samples confirmed the clinical relevance of NK1R, we found the expression were significantly higher for the tr-NK-1R. Most importantly the NK1R antagonists induce apoptosis in Esophagus cancer cells
Conclusions: For the first time, we describe the NK1R in its truncated splice variant as a potent target in human esophagus cancer cells and an inhibitory effect in vitro by NK1R antagonists.
Key words: Esophageal carcinoma; truncated neurokinin-1 receptor; aprepitant

DOI: 10.1002/eji.201970400

P2645

New anti-tumor mechanisms of lithium carbonate: Reeducate tumor associate macrophage through regulating of glycogen metabolism

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Cancer Immunotherapy that lies on innate immune responses has not been well established yet. Macrophages play critical roles in innate immune system. Although macrophages are capable of killing tumor cells, typically for so-called M1 macrophages, they are commonly educated into M2 macrophages, which promote tumor angiogenesis, tumor escaping and metastasis. Targeting these M2 macrophages holds great potential in cancer immunotherapy. Previous studies have revealed that there are obvious differences between M1 and M2 macrophages in metabolic mode. Our investigations also indicated that M1 phenotype is regulated by glycolysis pathway. Increased glycolysis may contribute to the reprogramming of M2 to M1 phenotype. Based on the above findings, we utilize lithium carbonate (LC) which is a classical inhibitor of GSK-3β to reeducate M2 tumor-promoting macrophages into M1 tumor-inhibiting macrophages by restoring Gys1 activity and increasing glycogen synthesis. Our results showed that LC resets tumor-associated M2 macrophages to M1 phenotype in vitro. LC also markedly inhibit the growth of B16 melanoma, MC38 colon cancer, 4T1 breast cancer and H22 hepatocarcinoma in vivo. Next, we validated that the tumor-inhibitory effect of LC is macrophage dependent and relies on the mobilizing of CD8 T cell antitumor immunity. Taking together, these findings reveal the anti-tumor immune responses and mechanisms of LC, and pave the way for its tumor immunotherapy based on macrophages.

P2646

Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype

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Resetting tumor-associated macrophages (TAMs) is a promising strategy to ameliorate the immunosuppressive tumor microenvironment and improve innate and adaptive antitumor immunity. Here we show that chloroquine (CQ), a proven anti-malarial drug, can function as an antitumor immune modulator that switches TAMs from M2 to tumor-killing M1 phenotype. Mechanistically, CQ increases macrophage lysosomal pH, causing Ca2+ release via the lysosomal Ca2+ channel mucolipin-1 (Mcoln1), which induces the activation of p38 and NF-κB, thus polarizing TAMs to M1 phenotype. In parallel, the released Ca2+ activates transcription factor EB (TFEB), which reprograms the metabolism of TAMs from oxidative phosphorylation to glycolysis. As a result, CQ-reset macrophages ameliorate tumor immune microenvironment by decreasing immunosuppressive infiltration of myeloid-derived suppressor cells and Treg cells, thus enhancing antitumor T-cell immunity. These data illuminate a previously unrecognized antitumor mechanism of CQ, suggesting a potential new macrophage-based tumor immunotherapeutic modality.

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DOI: 10.1002/eji.201970400

P2647

Lipid accumulation in tumor-associated macrophages endow pro-tumor potential in gastric cancer

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Tumor-associated macrophages (TAMs) achieve the key homeostatic function that countenances tumor maintenance and growth. Here we report that a substantial proportion of TAMs in tumor-bearing mice and patients with gastric cancer have high amounts of lipid as compared with macrophages from tumor-free mice and healthy individuals. TAMs with high lipid levels are characterized by an M2-like polarized profiling. The high lipid level in TAMs correlates negatively with phagocytic potency against tumor cells. Moreover, TAMs with high lipid content are not able to effectively stimulate allogeneic T cells, and promote the migration of tumor cells. In our studies, lipid accumulation in TAMs might be caused by increased uptake of extracellular lipids from tumor cells or tumor microenvironment, which induces the increased expression of PI3Kγ, highly expressed in myeloid cells, polarizing to an M2-like profiling. Furthermore, we demonstrate that this effect of lipid accumulation on TAM to M2-like polarization could be reversed by the inhibitor of PI3Kγ. Pharmacological targeting PI3Kγ in high-lipid TAMs with a selective inhibitor, like IPI549, restore the functional activity of macrophages and substantially enhance the effects of phagocytosis and anti-tumor immunity activity. This cross-talk most probably evolves to be engaged in gastric cancer growth by manipulating the lipid levels in TAMs.

P2648

MyD88 exacerbates immunological pathology in experimental viral fulminant hepatitis

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Viral fulminant hepatitis (FH) is a serious liver disease with high mortality due to excessive inflammation in liver tissues. The lack of knowledge on inflammatory signal dysregulation attributing to FH pathogenesis has been an obstacle for seeking effective interventions. We report that mice deficiency in MyD88 (MyD88-/-) are resistant to murine hepatitis virus strain-3 (MHV-3)-mediated FH. The expression of multiple pro-inflammatory cytokines causing the recruitment of inflammatory NKp46-Lin-Thy2.1+Roγt+ group-3 innate lymphoid cells (ILC3) to the livers was severely impaired in MyD88-/- mice as compared to wild-type (WT) littermates, resulting in reduced liver pathology, viral replication and prolonged mortality post-infection. Additionally, MHV-3 markedly augments expression of high-mobility group box 1 (HMGB1) in infected hepatocytes/macrophages and induces HMGB1 protein migration from the nucleus to the extracellular milieu, where it activates MyD88-dependent inflammation. Conversely, WT animals treated with the HMGB1 blocking antibodies are resistant to MHV-3-caused FH. These results demonstrate that the MyD88 is essential in controlling the pathogenesis of viral FH, and interruption of this signaling pathway sheds light on a novel therapeutic strategy for the disease.

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DOI: 10.1002/eji.201970400

P2649

LKB1 deficiency in dendritic cells promote the development of gastrointestinal polyposis

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Peutz-Jeghers syndrome (PJS), a cancer predisposition syndrome characterized by benign gastrointestinal (GI) polyps, is caused by a germline mutation in LKB1, the gene that encodes the tumor suppressor LKB1. Patients with PJS show an increased risk of developing cancer at relatively young ages. Multiple cell types and signaling pathways underlying tumor formation has been found to be involved, however, whether LKB1-deficient Dendritic cells (DC) also promote PJS remains elusive. Here, we report that homozygous deletion of LKB1 in DC is sufficient to promote GI polyposis. we generated mice with a conditional knockout of the LKB1 gene in DC, LKB1fl/fl; CD11c-Cre+ mice (KO mice). Unexpectedly, we observed full penetrance of polyp development in the KO mice already at 4 months of age. The histopathology of Polyps observed in KO mice displayed an arborizing smooth-muscle core. However, in the KO mice, we did not detect any alterations in the expression of hepatocyte growth factor (HGF), IL-6, IL-11 or TGF-β which were deregulation in previous reports. By contrast, LKB1-deficient DC led to excessive Treg expansion in multiple organs and dampened effector T cell immunity. Furthermore, we demonstrated that deletion of AMPKα1 in DC did not result in polyp formation. In conclusion, our results show that LKB1 deficiency in DC promotes the development of gastrointestinal polyposis which was independent of inflammatory and AMPK signalling.

This work was supported by grants from National Basic Reseach Program of China (2015CB943203)

P2650

LKB1 regulates AMP kinase independent epidermal LC homeostasis via β-catenin signaling pathway

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Emerging data show that liver kinase B1 (LKB1) is critical for some immune cells development or function. Langerhans cells (LCs) are a unique type of dendritic cell subset residing in stratified squamous epithelia, where they play a key role in regulating the balance between immunity and tolerance. **Whether LKB1 is critical for LC development is unclear.** Here we show that the epidermal LC homeostasis is profoundly regulated by the LKB1. As CD11c is highly expressed by LC, we crossed mice carrying loxP-flanked LKB1 alleles with CD11c-Cre mice to specifically delete LKB1 in LC (KO mice). **The number of epidermal LC was significantly decreased in KO mice.** Then, we observed LKB1-deficient LCs undergo spontaneous maturation and enhanced migratory potential

DOI: 10.1002/eji.201970400

based on the increased surface expression of MHC class II and costimulatory molecules, and the downregulated expression of E-cadherin and langerin. Ki-67 and annexin V staining revealed a faster turnover rate and increased apoptosis in LKB1-deficient LCs, which might additionally affect the preservation of the LC network. **Furthermore, we demonstrated that the function of LKB1 in LC was β-catenin signaling pathway dependent, not the conventional AMP-activated protein kinase (AMPK) signaling dependent.** In conclusion, our results show that **LKB1-β-catenin axis is a critical signaling pathway for controlling LC homeostasis under the steady state.**
This work was supported by grants from the National Basic Research Program of China (2015CB943203) and Shanghai Public Health Clinical Center (KY-GW-2018-09).

P2651

B cell adapter for Phosphoinositide 3-kinase regulates dendritic cell homeostasis through the dual-activation of NF-κB and PI3K/AKT signaling pathways

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The maturation of dendritic cells (DCs) is a crucial process in antigen presentation and the shaping of effective adaptive immune responses. Complicated signaling pathways and delicate regulatory networks are involved in modulating the maturation of DCs. B cell adapter for Phosphoinositide 3-kinase (BCAP) plays a distinct role in regulating the activation of different immune cells. While BCAP is also expressed by DCs, its function in this important subset of immune cells is still unclear. Here, we report that BCAP negatively regulates Toll-like receptor-induced DC maturation and their ability to present antigen. Aged BCAP-deficient mice consistently exhibited excessive DC maturation and a shortened life-span. Interestingly, BCAP simultaneously modulated the activation of PI3K/AKT and NF-κB signaling pathways by dynamically interacting with MyD88 and the p85α subunit of PI3K. Our results reveal the non-redundant role of BCAP in regulating DC homeostasis and uncover a novel bilateral mechanism for BCAP-regulated MyD88-dependent TLR signal transduction.

Granulocytes and Mast Cells

O181

Wip1 dephosphorylates Coronin1A to affect neutrophil activation through calcium signaling

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It is well known that neutrophils play a critical role in innate immune system. Wip1 (*PPM1D*) is a serine/threonine specific protein phosphatases that belongs to protein phosphatase 2C (PP2C) family and regulates important signaling pathways through dephosphorylating numerous signaling molecules. In our previous study, it was found Wip1 was crucial for the development, inflammatory activation and migration of neutrophils. Functional characterization of Wip1 in neutrophils, however, has been hampered by the paucity of known substrates. Here, the expression level and phosphorylation sites of proteins in LPS-induced neutrophil activation of Wip1$^{+/+}$ and Wip1$^{-/-}$ mice was profiled by array and quantitative phospho-proteomics experiments. Characterization of one Wip1 substrate, the Coronin1A (Coro1a), showed that Wip1-mediated phosphorylation promoted Ca^{2+} mobilization from intracellular stores due to Coronin-1-dependent calcium release via PLC-γ1

DOI: 10.1002/eji.201970400

signaling, which affects neutrophil activation. In addition, gene set enrichment analysis (GSEA) of expression microarray data provides evidence that the increased calcium signaling pathway and Wip1-deficiency-promoted neutrophil activation are coupled. These data point to the prospect of a previously unrecognized Wip1-dependent function during neutrophil activation through limiting the Coronin1A-mediated calcium signaling pathway. That will advance our understanding of neutrophil biology and will hopefully render promising new therapeutic for sepsis, asthma and graft rejection.

O182

Energy metabolic reprogramming of neutrophil accelerates brain damage in traumatic brain injury

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Over-inflammation plays an important role in the secondary injury of traumatic brain injury (TBI),such as brain edema, cerebral infarction, and neurogenic acute lung injury. Neutrophils, as the most important component of the innate immune system, are regarded as the earliest but short-lived player role in acute inflammation. However, in the present study, we found that the concentration of glutamate (Glu) was significantly increased in the peripheral blood after the mouse model of TBI. This microenviroment induced specific expression of glutamate transportor 1 (GLT1) on neutrophils which is not expressed on normal neutrophils. Moreover, GLT1 mediated the intake of Glu into neutrophils and switched the energy metabolic mode of neutrophils from glycolysis to aerobic oxidation. Post TBI, this energy metabolic reprogramming delayed neutrophil apoptosis significantly and accelerate brain damages including increase neurological deficit scores, brain inflammatory responses and so on. These results were confirmed in TBI patients. Accordingly, it demonstrates that high concentration of glutamate may delay apoptosis of neutrophils by changing the energy reprogramming, thus triggering excessive inflammatory response after TBI. These results provide a novel metabolism regulatory mechanism of neutrophils in central nervous system (CNS) injury and indicate a new linkage between peripheral immune system and CNS.

(This work was supported by National Natural Science Foundation of China No. 81771693.)

O183

Tissue persistence of mast cell subsets in mice is regulated by Fcgamma receptor-mediated apoptosis

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With their centennial-long discovery, mast cells are still being regarded as an enigmatic cell type. Rodent mast cells can be divided into two major subtypes: mucosal mast cells (MMC) and connective tissue mast cells (CTMC). A decade-old observation, which has never been mechanistically accounted for, revealed a longer lifespan for CTMC compared with MMC. We have discovered that IgG immune complexes may sensitize mouse mast cells for apoptosis if they only express one of the two Fcg receptors, i.e., FcgRIIB and FcgRIIIA. It is well documented that MMC only express FcgRIIB, whereas CTMC express both FcgRIIB and FcgRIIIA. We propose that FcgR-mediated cell apoptosis may account for differential tissue persistence of mast cell subsets. As a proof of concept, we recorded progressively lower frequencies of peritoneal mast cells (a type of CTMC) and nasal tissue CTMC in

DOI: 10.1002/eji.201970400

FcgRIIIA-/- and FcgRIIB-/- mice compared with wild-type mice during aging. Importantly, we reproduced these results using the mast cell engraftment model, which ruled out possible confounding effects of mast cell recruitment or FcgR expression by other cells on mast cell number regulation. We also confirmed that cell proliferation was not a confounding element in our data interpretation. In conclusion, our work has uncovered an FcgR-dependent mast cell number regulation paradigm that can provide a mechanistic explanation for the long-observed differential mast cell subset persistence in tissues.

O184

Immunomodulatory roles of erythroid cells in physiological and pathological conditions

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The main role of red blood cells is gas-transportation ($O_2$and CO_2) however our recent studies have unveiled immunomodulatory functions for these highly abundant cells in particular their immature counterparts ($CD71^+$erythroid cells) in different physiological and pathological conditions.Although these cells are very low in healthy adults, stresses such as anemia, pregnancy, chronic infection, late stage cancer result in a process called extramedullary erythropoiesis, which results in the abundance of immature red blood cells in the periphery.
We have shown that $CD71^+$erythroid cells play an essential role in feto-maternal tolerance. However, lower frequency of these cells is associated with pregnancy complications in pregnant women with inflammatory bowel disease (IBD). More importantly $CD71^+$erythroid cells from either placenta or cord blood of IBD mothers exhibit impaired immunosuppressive activities which is associated with lower expressions of regulatory molecules such as PD-1H (VISTA), TGF-band ROS. In addition, we have observed a subpopulation of $CD71^+VISTA^+$erythroid cells promote the development and function of regulatory T cells.Furthermore, we have found expansion of these immunosuppressive cells in cancer patients, which may impact their disease outcome. Finally, we have found that $CD71^+$erythroid cells via mitochondrial ROS exacerbate HIV infection/replication and trans-infect HIV into $CD4^+$T cells. Thus, our studies highlight the important role of $CD71^+$erythroid cells in different physiological and pathological conditions.

LB012

Neutrophils deficient in innate suppressors enhance anti-tumor immune responses

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Emerging evidence reveals immune-suppressive roles of tumor-associated neutrophils in promoting tumor growth, invasion and metastasis in various cancer including colorectal cancer. However, mechanisms responsible for the generation of immune-suppressive neutrophils are not well defined. Our current study tested hypothesis that the removal of innate suppressor “check point” in neutrophils may facilitate tumor-immune surveillance *in vitro*and *in vivo*. To this regard, we examined two key innate signaling suppressors, Tollip and IRAK-M, and their roles in modulating neutrophil anti-tumor immune functions. We observed that selective deletion of Tollip or IRAK-M exhibited reduced tumor counts in the AOM-DSS chemically induced colon cancer model. The suppressor deficiency neutrophils demonstrated enhanced capability to promote, instead of suppress, the proliferation and activation of effective T cells. Functionally, we observed that the transfusion of Tollip deficient neutrophils can potently enhance anti-tumor immune response and reduce tumor burden. Together,

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DOI: 10.1002/eji.201970400

our data reveal a novel anti-tumor immune-enhancement strategy through utilizing reprogrammed neutrophils with targeted removal of innate signaling suppressors.

LB050

Autonomous regulation of mast cell degranulation through an inhibitory receptor CD300a

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Although phosphatidylserine (PS) confined to the inner leaflets of plasma membrane is exposed on the cell surface when cells undergo apoptosis, viable cells also externalize PS in certain cellular states. However, the pathophysiological significance of PS exposure on viable cells remains elusive. Through imaging analyses, we found that PS was exposed on the plasma membrane of live mast cells (MCs) during degranulation and colocalized with CD300a, an inhibitory immunoreceptor that recognizes PS. Fluorescence resonance energy transfer (FRET) experiment reveals the direct cis-binding between CD300a and PS during degranulation. Functionally, we observed that degranulation was greater after stimulation with an IgE-antigen complex in MCs deficient in CD300a than in wild-type MCs. Pretreatment of MCs with a neutralizing anti-CD300a antibody efficiently upregulated the degranulation of wild-type MCs as a result of interference of *cis*-interaction between CD300a and PS as evidenced by decreased FRET efficiency. Consistently, CD300a-deficient mice or *in vivo* treatment with a neutralizing CD300a antibody showed slower recovery of body temperature compared with wild-type mice in MCs-dependent passive systemic anaphylaxis. Our study suggests self-regulation of MC degranulation through cis-interaction of PS with CD300a to down regulate allergic response, adding another layer of regulation in allergic responses.

P0164

HI *E.coli*-elicited acute infection promotes hematopoietic recovery by enhancing granulopoiesis without affecting the long-term reconstitution activity of HSCs

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Radiotherapy and chemotherapy are regular clinical options to treat malignant neoplasms, but leading to neutropenia, anemia or thrombopenia, and high mortality. Especially neutropenia is an independent risk factor for recovery neutropenic patients. Shorten the time of neutropenia is very important for the recovery of patients treated with radiotherapy and chemotherapy. In our study, we used acute peritonitis model induced by HI *E.coli* (heat-inactivated *E.coli*) to study the effect of acute infection and inflammation to hematopoietic recovery. We found HI *E.coli*-elicited acute infection could dramatically induce emergency granulopoiesis and rescue the survival of lethally irradiated mice. Also, we demonstrated that HI *E.coli*-elicited acute infection could trigger “expansion” of LSK cells during emergency granulopoiesis. Although the frequency of functional HSCs in expanded LSK cells was reduced, the number of functional HSCs in BM did not alter, and first and second transplantation assay indicated that HI *E.coli*-elicited acute infection did not affect the long-term reconstitution activity

DOI: 10.1002/eji.201970400

of HSCs. Our research indicated HI *E.coli* vaccination could be a potential therapeutic approach for accelerating BM recovery in neutropenic patients.

P0165

The origin and development route of mast cells

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Mast cells are tissue-resident immune cells with many physiological and pathophysiological functions. Although mast cell progenitors are found in the yolk sac, their origin and contribution to adult mast cells are unclear. Our reconstitution assays provided strong evidence that unlike mucosal mast cells, connective tissue mast cells originate from fetal mast cells that seed the peripheral tissues before birth and are maintained independently of born marrow-derived definitive hematopoietic stem cells (HSCs) throughout life. We further utilized fate-mapping systems to study the ontogeny of mast cells. We showed that early erythro-myeloid progenitors (EMPs), late EMPs and HSCs all give rise to fetal mast cells in succession. They all pass through an intermediate stage of integrin b7^{+} mast cell progenitors that appear at E11 and colonize peripheral tissues, where they complete their maturation with the production of metachromatic granules at E16.5. Although early EMP-derived mast cells evolve first and populate most tissues during early embryogenesis, they are subsequently replaced by late EMP-derived mast cells in most connective tissues during late embryogenesis and early adulthood, with exceptions in the pleural cavity and adipose tissue. Thus, mast cells with distinct origin display tissue-location preferences in adult mice: early EMP-derived mast cells are limited in adipose tissue and pleural cavity, late EMP-derived mast cells dominate most connective tissues while HSC-derived mast cells are a leading population in the mucosa. Furthermore mast cells with distinct origin display distinct transcriptional profiling. Therefore, embryonic origin shapes the heterogeneity of adult mast cells.

P0166

Metabolic switch of neutrophils promotes metastasis of human hepatocellular carcinoma via neutrophil extracellular traps

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Neutrophils (PMN) constitute a major component in human hepatocellular carcinoma (HCC) and play important roles in regulating disease progression. However, the underlying mechanisms regulating phenotypes and functions of HCC-associated PMN have not been fully understood by now. In this study, we found that PMN significantly up-regulated the release of neutrophil extracellular traps (NETs) in HCC tumor tissues. PMN-related NETs induced the migration and extravasation of cancer cells and down-regulated the expression of adhesion molecules on vascular endothelial cells, thus increasing vascular permeability and facilitating tumor metastasis of HCC. Mechanically, glycolytic activation of PMN by tumor microenvironments enhanced their levels of pentose phosphate pathway (PPP) flux, which subsequently up-regulated the release of NETs through the NADPH-ROS pathway. Accordingly, levels of PMN-related NETs in tumor tissues were positively associated with tumor metastasis and negatively correlated with disease progression of patients with HCC. These results unveiled a novel mechanism by which cellular metabolic switch regulates the pro-tumor functions of PMN in a specific human tumor microenvironment, and NETs represent a potential target for future immune-based anti-cancer therapies of human HCC.

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DOI: 10.1002/eji.201970400

P0167

Neutrophils remold breast cancer cell glycometabolism via direct contact

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Breast cancer is one of the most common malignant disease in women. Abnormal tumor microenvironment is the main causes of tumor cell malignant transformation, while its composition is complex. Our previous studies have shown that tumor infiltrating neutrophils (TINs) are abundant and surrounding tumor cells. The effect of TINs is controversial that play both tumor-promoting and tumor-suppressing role. We found that mice high-invasive 4T1 breast cancer cells can recruit neutrophils by producing considerable GM-CSF , and then interact with neutrophils in a direct contact way, but low-invasive EMT6 breast cancer cells could not. Further, the metabolic pattern of the tumor cells that are in direct contact with neutrophils has changed. Among them, the most prominent change is hexokinase, which is not present on tumor cells that indirect contacting with neutrophils. Hexokinase is a key enzyme in the first step of glycolysis that phosphorylates glucose to 6-phosphate-glucose. Tumor cells with high production of hexokinase are able to accelerate the rate of glycolysis, and inhibit tumor autologous apoptosis, thereby causing tumor progression. In summary, Tumor cells activate hexokinase of themselves by recruiting neutrophils via directly contacting with TINs. Tumor cells elevate glycolysis owing to highly productive hexokinase that would explain Warburg effect in malignant tumor progression.

P0168

A novel *in vitro* approach to generate N2-like neutrophils permissive for *Leishmania donovani* infection

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The intracellular protozoan parasite *Leishmania donovani* is the causative agent of visceral leishmaniasis. After infection with *Leishmania* neutrophils are rapidly recruited to the site of infection and encounter the pathogen. Although neutrophils possess potent antimicrobial effector mechanisms, *Leishmania* parasites can survive in neutrophils. How the parasites evade of killing by neutrophils, is poorly understood.
In tumor models two functionally distinct neutrophil subsets has been described, the pro-inflammatory N1 cells with anti-tumor activity and the tumor-promoting anti-inflammatory/immunosuppressive N2 phenotype. We hypothesize that *Leishmania* parasites induce the polarization of neutrophils towards an anti-inflammatory/immunosuppressive phenotype to evade neutrophil defense mechanisms. In this study we attempted to polarize primary human neutrophils towards a N1 or N2 phenotype *in vitro*. A cocktail of pro-inflammatory stimuli was used to polarize N1 cells whereas mimicking a tumor microenvironment in vitro was applied to polarize neutrophils to an N2 phenotype. Expression of typical N1 and N2 cell surface markers and cytokine profiles were used to assess the phenotype of the *in vitro*-generated neutrophil subsets. N1-polarized neutrophils expressed CD95 and CD54 (ICAM-1) and secreted IP-10 at a higher level whereas N2-polarized cells showed enhanced CD10 expression and IL-8 production. These observations, among others, suggest that our approach was successful to polarize neutrophils towards N1- and N2-like phenotypes in vitro. The finding that *in vitro* polarized N2-like neutrophils had a markedly lower capacity to kill intracellular *Leishmania* supports the hypothesis that the parasites induce the polarization of N2 neutrophils in order to survive inside neutrophils.

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DOI: 10.1002/eji.201970400

P0169

Altered metabolism of phospholipases, diacylglycerols, endocannabinoids and N-acylethanolamines in patients with mastocytosis

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Background: Mastocytosis is characterized by mast cells (MCs) expansion and accumulation in various organs. The symptoms are related to the increased release of MC-derived mediators that exert local and distant effects. MCs are source and target of phospholipase enzymes (PLs), that catalyzed the cleavage of membrane phospholipids releasing lipid mediators [e.g. diacylglycerols (DAGs) and the endocannabinoid (EC) 2-arachidonoylglycerol (2-AG)]. To date, there are no data on the role of these lipid mediators in mastocytosis. Here we analyzed plasma levels of PLA_2, PLC, DAG, ECs and EC-related N-acylethanolamines in patients with mastocytosis.
Methods: In 23 patients with mastocytosis and 23 healthy individuals we measured plasma PLA_2 and PLC activities, DAG, 2-AG, anandamide (AEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA).
Results: Plasma PLA_2 and PLC activities were increased in mastocytosis patients compared to controls. Concentrations of DAG 18:1/20:4 and 18:0/20:4, two second messengers produced by PLC, were higher in mastocytosis compared to controls, whereas the concentrations of their metabolite, 2-AG, were not altered. The anandamide (AEA) was decreased in mastocytosis patients compared to controls; by contrast, AEA congener, palmitoylethanolamide (PEA), was increased. PLA_2 and PLC activities were increased only in patients with mediator-related symptoms. Moreover, PLC activity was positively correlated with disease severity and tryptase concentrations. By contrast AEA was negatively correlated with tryptase concentrations.
Conclusions: PLs and some lipid mediators are altered in patients with mastocytosis. Our results may pave the way for investigating the functions of these mediators in the pathophysiology of mastocytosis and provide new potential biomarkers and therapeutic targets.

P0170

Activation of cannabinoid receptor type-1 (CB_1) and type-2 (CB_2) inhibits the release of vascular endothelial growth factor (VEGF-A) from human neutrophils

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Background: Neutrophils (PMNs) play a role in the acute phase of inflammation and resistance against invading pathogens. Activation of cannabinoid receptor type-1 (CB_1) and -2 (CB_2) has been suggested as a new strategy to modulate angiogenesis *in vitro* and *in vivo*. Angiogenesis, the formation of new blood vessels is complex processes that require the coordinated action of several factors such as vascular endothelial growth factors (VEGFs). These factors are produced by several cells, included PMNs. We sought to investigate whether endocannabinoids and their receptors

DOI: 10.1002/eji.201970400

modulate PMNs production of VEGF-A and CXCL8 and reactive oxygen species (ROS) production.
Methods: PMNs were isolated from healthy donors. Cells were stimulated with CB_1 and CB_2 agonists/antagonists alone and in association with LPS. Mediators concentrations were measured using ELISA, whereas ROS production was evaluated by fluorescence assay. Finally, the evaluation of the formation of neutrophil extracellular traps (NETS) was detected employing Zeiss Microscopy.
Results: CB1 and CB2 agonists did not induce VEGF-A and CXCL8 release from PMNs but reduced spontaneous release of VEGF-A. LPS activated PMNs causing VEGF-A and CXCL8 release. Preincubation of PMNs with agonists inhibited in concentration dependent manner VEGF-A release induced by LPS but did not affected CXCL8 production. The effect of CB agonists of LPS induced VEGF-A was reverted by the treatment with CB antagonists. Finally, CB agonists and antagonists promoted ROS production conversely did not induced NETS formation by PMNs.
Conclusions: Modulation of CB_1 and CB_2 receptors expressed by PMNs might be a novel strategy to modulate immune cell-assisted angiogenesis.

P0749

The tumor-associated neutrophils secrete LOX and MMP to promote tumor angiogenesis in gastric cancer bearing mice model

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Objective : To explore the role of tumor-associated neutrophils (TANs) on angiogenesis in gastric cancer bearing mice and the relationship with lysyl oxidase (LOX) and matrix metalloproteinases (MMPs).
Methods: Gastric cancer bearing mice model were established by injecting human gastric cancer cell AGS into BALB/c-nude mice (model group). For intervention group, anti-LY6G MAb 1A8 were intratumorally injected to delete the neutrophils. Immunohistochemistry was used to detecte the number of TANs, microvessel density (MVD) and the levels of LOX, MMP-2 and MMP-9 in tumors tissues. Flow cytometric method (FCM) was used to analyze the amounts of neutrophils. Neutrophils were separated from tumor tissues. The mRNA and protein expression of LOX, MMP-2, MMP-9 in neutrophils were quantitated used real-time PCR assays and western blotting.
Results: Compared with model group, the number of TANs was lower ($P< 0.01$) in intervention group; and the number of MVD was decreased ($P< 0.05$); the protein levels of LOX, MMP-2 and MMP-9 were down-regulated ($P< 0.05$). Compared with model group, mRNA expressions of LOX, MMP-2, MMP-9 in TANs were lower in intervention group ($P< 0.01$); and protein levels of LOX, MMP-9 in TANs were also lower in intervention group ($P< 0.05$). The number of TANs were positively correlated with the levels of LOX, MMP-2, MMP-9 and MVD.
Conclusions: In gastric cancer bearing mice model, the majority TANs exist in tumor tissues are N2 type and promote tumor angiogenesis by secreting LOX, MMP-2 and MMP-9.
This research was supported by National Natural Science Foundation of China (31860255 and 81260314)

DOI: 10.1002/eji.201970400

P0750

Tumor domesticated neutrophils metabolic remodeling accelerates tumor pre-metastatic niche formation

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The concept of pre-metastatic niche is originated from the seed-soil theory that is considered to be one of the essential aspect for metastasis, but the mechanism is still unclear. Our study shows that the primary tumor secrete damage associated molecular patterns (DAMPs) acting on peripheral neutrophils that contribute to the pre-metastatic niche formation. The domesticated neutrophils are abnormally activated with increasing production of reactive oxygen species (ROS), which could damage mitochondrial function easily. These factors lead to a decrease in the ability of neutrophils to oxidative phosphorylation. Further research showed damaged mitochondria bring about the mitochondrial membrane potential altered, inducing mitochondria migrate from the peri-nucleus to the edge in cytoplasm. By scanning electron microscopy and transmission electron microscopy, we confirmed that tumor-educated neutrophils have a stronger ability to form neutrophil extracellular traps (NETs), which is mtDNA-dependent owing to the mitochondria in cytoplasmic margin. By producing NETs, neutrophils capture circulating tumor cells in the bloodstream, thereby forming distant metastasis. In summary, our finding reveal that primary tumors domesticate peripheral neutrophils forming mtDNA-dependent NETs which assists tumor cells keeping in distant organs without cell kiling, and finally promote tumor metastasis formation.

P0751

AGM-S3 stromal cells augment survival of neutrophils and preserve its immune functions

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AGM-S3, a cell line derived from the aorta-gonad-mesonephros (AGM) region, is shown to be able to support the development of hematopoietic stem cells (HSCs). In vitro, AGM-S3 cells can promote the differentiation of HSCs into erythroid and megakaryocytic cells. However, the role of AGM-S3 cells in maintaining the survival and function of terminally differentiated blood cells remains unclear. Therefore, we established a co-culture system in vitro to study the effect of AGM-S3 cells on the neutrophils. We found that co-culture with AGM-S3 cells could significantly delayed neutrophils death (the half-life was extended from 20 hours to 48 hours), and maintained the functions of chemotaxis, phagocytosis and the release of reactive oxygen species as well. Further studies indicated that AGM-S3 cells mainly activate pro-survival signaling pathways (such as Akt) in neutrophils by secreting cytokines (such as IL-6, MCP-1, and IL-1α, etc.), which promote the continuous expression of Mcl-1 and Bcl-xl, resulting in inhibition of Caspase-3. In the mouse model of peritonitis, neutrophils co-cultured for two days were reinfused from the tail vein into mice, and they could be recruited to the abdominal inflammation. In conclusion, our results indicated that AGM-S3 cells are durable in maintaining the survival and function of neutrophils in vitro and those neutrophils bear normal immunological activity after being refused to mice.
This study will provide a new strategy and experimental basis for the future research of neutrophil function modification and infusion for the treatment of granule deficiency and refractory infection.

DOI: 10.1002/eji.201970400

P0752

Effect of zebrafish Notch1a/Notch1b on neutrophils challenged by Vibrio parahaemolyticus

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Neutrophils play a major role in fighting extracellular pathogens and acute inflammation. To further investigate the regulation of Notch1a and Notch1b in zebrafish on neutrophil production and function, local infection model was established by the hindbrain ventricle (HBV) of 3 days post fertilization (dpf) zebrafish larvae infected with *Vibrio parahaemolyticus* Vp57^{RFP}. We have found that more neutrophils in the brain of *notch1b*$^{-/-}$ than that in WT and *notch1a*$^{-/-}$. The median lethal dose (LD_{50}) and survival percent of *notch1a*$^{-/-}$ and *notch1b*$^{-/-}$ zebrafish larvae were significantly lower than WT after infected with Vp57^{RFP}. Compare to WT and *notch1a*$^{-/-}$ group, the *notch1b*$^{-/-}$ group recruited quickly and more neutrophils to the infection site. Moreover, the expression level of *cxcl8a* was up-regulated in *notch1b*$^{-/-}$ than that in WT and *notch1a*$^{-/-}$ group, before infection and 2 hours post infection (hpi). To detect the role of *notch1a* and *notch1b* in demand-driven granulocytogenesis, we use Vp57^{RFP} to infect the HBV of 3 dpf zebrafish larvae to 48 hpi, and observed the dynamic process of producing neutrophils in the aorta-gonad-mesonephros (AGM) and caudal hematopoietic tissue (CHT). Compared with WT, the number of AGM and CHT neutrophils of *notch1a*$^{-/-}$ were decreased, and the same in *notch1a*$^{-/-}$ and *notch1b*$^{-/-}$ infection group. The qRT-PCR showed that the expression of *gcsf* was significantly down-regulated in *notch1a*$^{-/-}$ and *notch1b*$^{-/-}$ zebrafish larvae than that of WT at 4, 6 and 12 hpi, respectively. These findings will provide us a better clue for understanding the function of Notch1a/Notch1b on neutrophils during infection *in vivo*.

P0753

Basophils promote the differentiation of monocytes into M2-like macrophages which dampen IL-1α-mediated aggravation of allergic inflammation

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Basophils are the rarest granulocytes and play significant roles in allergy and protective immunity against parasitic infections through interaction with other immune cells. We recently discovered that basophil-derived IL-4 licenses inflammatory monocytes to acquire anti-inflammatory M2-like phenotypes in the IgE-dependent delayed-onset skin allergic inflammation model (IgE-CAI model). However, it remains ill-defined how these anti-inflammatory M2-like macrophages dampen allergic inflammation.
CCR2-deficient mice fail to generate M2-like macrophages in the IgE-CAI skin lesion owing to the defect in monocyte recruitment to the skin, and hence display aggravated skin inflammation with massive cellular infiltration. We detected the formation of neutrophil-rich clusters of inflammatory cells in the skin lesion, and TUNEL-positive dead cells accumulated in these clusters. Previous report showed that IL-1α plays a crucial role in cluster formation of dendritic cells in the inflamed skin, implying that IL-1α may also contribute to the neutrophil-rich cluster formation in CCR2-deficient mice. Indeed, antibody-mediated blockade of IL-1α significantly attenuated ear swelling and infiltration of inflammatory cells including neutrophils in the IgE-CAI skin lesion of CCR2-deficient mice. Thus, IL-1α appears to play an important role in aggravated inflammation observed in these mice. Given that IL-1α is commonly released from necrotic cells, we hypothesized that monocyte-derived M2-like macrophages dampen the IL-1α-mediated aggravated inflammation through cleaning up necrotic cells.

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DOI: 10.1002/eji.201970400

In accordance with this, macrophages stimulated ex vivo with culture supernatants of activated basophils showed enhanced phagocytosis of dead cells. These results strongly suggest that the clearance of dead cells by M2-like macrophages dampens IL-1α-mediated aggravation of allergic inflammation.

P0754

Correlation between release of neutrophil microparticles (MPs) and neutrophil extracellular traps (NETs) during systemic inflammation in mice

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Microparticles (MPs) are microstructures secreted by various cells, including neutrophils. They transport bioactive molecules thus impact intracellular communication, both in homeostasis and inflammatory state. Neutrophils are also potent producers of neutrophil extracellular traps (NETs) that can be induced by MPs and/or can contain them. The aim of the study was to investigate secretion of neutrophil MPs and correlate their release with NETs during sepsis. As MPs are present in numerous body fluids they were studied not only in blood but also in peritoneal fluid. *In vivo*, MP and NET formation was evaluated with intravital microscopy (IVM) in vasculature of cremaster muscle and liver sinusoids. MPs were additionally analyzed with Nanoparticle Tracking Analysis (NTA), and healthy and septic (lipopolysaccharide) mice were compared. The NTA revealed that low amounts of microparticles were present in fluids of healthy mice and their numbers increased upon sepsis. During systemic inflammation, MPs peaked at first in peritoneal fluid and then in plasma. Kinetics of MPs were confirmed with IVM and the studies also showed correlation between their release and formation of NETs. Namely, whereas NETs started to be formed earlier than MPs, the highest levels of both NETs and MPs were detected within the first 8 hours of sepsis. Further studies are required to verify if this concomitant release is beneficial or pathological for sepsis outcome, but strongly indicate an interplay between the two types of structures. This study was funded by the National Science Centre of Poland (grants No. 2018/29/N/NZ6/01073 and 2018/29/B/NZ6/00713).

P0755

Pulmonary tuberculosis is accompanied by the accumulation of low-density neutrophils

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Pulmonary tuberculosis is accompanied by the accumulation of low-density neutrophils
Tuberculosis (TB) is one of the most common infections in the world. TB pathogenesis is not fully understood. Particularly, neutrophils were shown to contribute to both, TB protection and pathology raising a question on their exact role during TB. We have hypothesized that the dual role for neutrophils during TB is due to the heterogeneity of this innate immune cell population. In this study, we performed pilot analysis of neutrophil heterogeneity during TB.
Patients with pulmonary TB (n=27) and healthy *Mtb*-exposed individuals (n=20) were included in the study. Blood samples were fractionated into high- and low-density populations and analyzed by flow cytometry. High-density $CD66^+CD14^-$ cells were considered as “typical” normal-density granulocytes (NDG). $CD66^+CD14^-$ cells present in low-density population were considered as low-density granulocytes (LDG). LDG frequencies, phenotype and functional characteristics were evaluated.
TB patients differed from healthy participants by a higher frequency of LDG (p=0.01). LDG expressed

DOI: 10.1002/eji.201970400

$CD15^+CD11b^+CD33^+HLA\text{-}DR^-$ phenotype, and differed from NDG by: (i) higher percentages of $CD62L^-$ and $MPO^{low/int}$ cells ($p < 0.01$), which likely represented activated/degranulated granulocytes; (ii) higher percentages of $CD10^{low}CD16^{low}$ cells reminiscent of recent bone marrow emigrants; (iii) higher heterogeneity of cell nuclear morphology. The study documents the heterogeneity of neutrophil population and suggests the accumulation of activated and low-differentiated subpopulations during pulmonary TB.

The study is part of the Central Tuberculosis Research Institute´s stream 0515-2019-0018

P1352

Increased neutrophil extracellular traps promote metastasis potential of hepatocellular carcinoma via provoking tumorous inflammatory response

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Inflammation bridges metastasis and host. Emerging evidences highlight role of neutrophils, as the most abundant host inflammatory cells, in cancer metastasis. But their role in metastasis confuses. Neutrophil extracellular traps (NETs), as the decondensed chromatin in web-like structure coated with protease released by activated neutrophils, is the most unique function of neutrophils first found to trap and kill invading pathogens. Beyond innate immune defensive function, NETs have proven their importance in immune regulation and various inflammation-related conditions including cancer. Hepatocellular carcinoma (HCC) is one of the leading cause of cancer-related death with limited treatment options. Through clinical samples and functional studies, we found NETs formation is enhanced in neutrophils from patients with HCC, especially metastatic HCC. The increased NETs fuel HCC metastasis. In detail, NETs trap disseminated HCC cells in pre-metastasis site, and then offer the trapped HCC cells a higher metastasis potential with boosted invasiveness and resistance to cytotoxicity of NETs themselves. In mechanism, these enhanced metastasis potential gained from NETs is due to an triggered vigorous inflammatory response in the trapped HCC cells featured as Cox2 up-regulation following activation of Toll-like receptor (TLR)-4/9. Abolishing TLR4/9 or Cox2 well abrogates the enhanced metastasis potential gained from NETs. Further, we suggest that targeting NETs-aroused metastasis potential with well available anti-inflammation drugs aspirin and hydroxychloroquine combined with DNase by digesting NETs can effectively inhibit HCC metastasis in mice. Our study add to a novel perspective of how inflammation act with HCC metastasis and a way of old drugs in new use.

P1353

Interleukin-3-induced GITR promotes human basophil activation

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Human basophils play important roles in regulating allergic immune responses by releasing histamine and effector cytokines such as interleukin 4 (IL-4) and IL-13. This process is controlled through a variety of surface receptors e.g. FcεRI, and IL-3R. Glucocorticoid-induced TNFR family-related protein

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DOI: 10.1002/eji.201970400

(GITR), a TNF receptor family member, has been implicated in regulating allergic reactions by modulating the functions of T cells and ILCs. In the present study, we aim to elucidate whether GITR is expressed by human basophils and contributes to basophil activation. Flow cytometric analysis revealed that GITR was not constitutively expressed on human primary basophils. However, activation by IL-3 rather other established modulators e.g. anti-IgE, fMLP and C5a induced high expression of GITR at both mRNA and protein level. Moreover, increased GITR expression was due to de novo protein synthesis evidenced by treatment with protein synthesis inhibitor Cycloheximide. In agreement with this, we identified that GITR increased significantly in allergen-challenged PBMCs from patients with seasonal allergic rhinitis by microarray analysis. Further analysis showed that induction of GITR by IL-3 was partially inhibited by JAK2 inhibitor (AZD1480) and fully suppressed by PI3K inhibitor (LY294002), MEK1/2 inhibitor(U0126). Activation of GITR by its ligand, GITRL, promoted the release of IL-4 and IL-13 by IL-3-treated basophils. Moreover, IgE-mediated degranulation was enhanced by GITRL in the presence of IL-3. In conclusion, increased expression of GITR on human basophil is regulated by IL-3 via multiple signaling pathways and contributes to enhanced activation and degranulation of human basophils induced by IL-3.

P1354

Neutrophil elastase: a doble edged sword in topheous gout

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Objective: Neutrophil extracellular traps (NETs) has been thought to be play an important role in the stability of tophus. Urate lowering therapy (ULT) often induces recurrent episodes of acute gout. In this study, we try to explore the underlying mechanism of recurrent gout flares after ULT from the perspective of NETs.
Methods: Neutrophil Elastase (NE), dsDNA and α1-antitrypsin (AAT) levels were compared among the patients with or without tophus. A murine gouty model with MSU crystal injected intraperitoneally was established and AAT was injected *i.p.* after spontaneous resolution. IL-1β and IL-6 levels were detected in the peritoneal lavage fluids. The chalk like substances in the peritoneal cavity were identified by immunofluorence and treated with AAT, followed by IL-1β and IL-6 detection, *in vitro*.
Results: In patients with tophi, serum dsDNA in those who suffered 4 flares/a were higher than those with 0-2 flares/a. In acute gout, dsDNA, NE and AAT levels in patients with and without tophi were elevated after urate lowering therapy. During stable phase, ATT level did not change even after urate lowering therapy. The levels of IL-1β and IL-6 in the peritoneal fluid was significantly higher in the AAT group. Immunofluorescence identified large amounts of IL-1β and IL-6 associated with the NETs in the chalk like substances. The IL-1β and IL-6 level increased significantly with time when the chalk like substances treated with AAT.
Conclusion: Urate lowering therapy induced shrinkage of the tophus. AAT blocked the activity of NE, leading to a massive release of inflammatory cytokines.

DOI: 10.1002/eji.201970400

P1355

Mechanism of S100A10 inhibiting the migration of basophils from bone marrow to peripheral blood

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Basophils are derived from bone marrow hematopoietic stem cells and released into peripheral blood after maturation. Basophils have an important role in the pathogenesis of allergic reaction, parasitic infection, autoimmune diseases and myeloid leukemia. Therefore, it is important to regulate basophil migration from bone marrow for the therapy of basophils-related diseases. Our previous studies showed that S100A10 inhibited the migration of basophils from bone marrow. However, the mechanism remains unclear. It has been reported that transmembrane protein beta 1 Integrins can inhibit cell migration from bone marrow, and its localization on cell membrane is regulated by intracellular transporters. S100A10 is an important intracellular transporter for ion channel and receptors. **Therefore, we speculate that S100A10 inhibits the migration of basophils from bone marrow via regulating the location of beta 1 Integrins on basophil membrane**. In this study, the mechanism of S100A10 inhibiting basophil migration will be revealed by bone marrow transplantation, protein imprinting, flow cytometry, confocal microscopy and bimolecular fluorescence complementarity assay and so on. This study will provide a new therapeutic idea for basophil-related diseases.

P1356

Short chain fatty acids as stimuli of neutrophil extracellular traps

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Introduction: SCFAs are metabolites produced from gut bacterias through fermentation of dietary fiber. Have been described as an important player in the immune response due that are ligands of receptors expressed in host cells.
Objective: Determine whether physiological levels of short chain fatty acids are able to induce the formation of neutrophil extracellular traps and whether this mechanism is dependent on the Free Fatty Acid Receptor.
Methodology: Neutrophils were obtained from healthy individuals and were stimulated with acetic, propionic, and butyric acid at levels found in the colon (60 mM, 20 mM, and 20 mM, respectively), as well as in peripheral blood in a fasting state (100µM, 4µM and 2µM). To test the role of the receptor in NETs induction pathway in response to SCFAs, we used FFA2R pathway inhibitors: CATPB; a receptor level inhibitor, and genistein; against the Gα/q11 proteins.
Results: The release of NETs was observed in response to stimulation of neutrophils with each of the SCFAs, at concentrations found in the intestine, acetic acid preserved this effect at blood concentration in a fasting state, 100 µM. The use of a competitive inhibitor against the receptor or blocking the signaling of G proteins decreased the release of NETs by up to 50%. Additional results showed that conditions of pH 6.0, as well as autologous serum, could inhibit SCFA-induced NETs.
Conclusion: SCFAs act through FFA2R to induce NETs.

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DOI: 10.1002/eji.201970400

P1357

Discovery of RhoH as a negative regulator in neutrophil extracellular trap formation

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Neutrophil extracellular trap (NET) formation is a novel function of neutrophils that facilitates the immobilization and killing of invading microorganisms. In spite of its protective role in microbial infection, if dysregulated, NETs contribute to the pathogenesis of inflammatory diseases. Thus, exploring the regulation mechanisms of NET formation could not only add knowledge to neutrophil biology but may also provide new effective therapeutic targets for the related diseases.
RhoH, an atypical small GTPase, is exclusively expressed in the hematopoietic cells. Our group found that freshly isolated neutrophils contained only little or no detectable RhoH protein, but its expression was induced by GM-CSF under in vitro and in vivo inflammatory conditions, such as cystic fibrosis (CF). Interestingly, the aberrant expression of RhoH in CF neutrophils led to less NET formation upon activation compared with control neutrophils. In addition, we also isolated primary neutrophils from bone marrow of wild type and *Rhoh*-knockout mice. Results showed that GM-CSF induced RhoH expression inhibited NET formation in primary mouse neutrophils, while lack of RhoH restored the ability to form NETs, indicating that RhoH is a negative regulator in NET formation. This notion is further supported by overexpression of RhoH blocked NET formation in differentiated Hoxb8 neutrophils.
Research in signaling pathways for NET formation demonstrated that RhoH had no effects on ROS production but prevented actin rearrangement instead of tubulin polymerization possibly through inhibiting cofilin activity. Although the underlying mechanism is still under investigation, it's sufficient to conclude that RhoH plays an inhibitory role in NET formation.

P1358

Video-rate bioluminescence imaging of mast cell degranulation

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Degranulation refers to the secretion of inflammatory mediators, such as histamine, serotonin, and proteases, that are stored within the granules of mast cells and that trigger allergic reactions. The amount of these released mediators has been measured biochemically using cell mass. To investigate degranulation in living single cells, fluorescence microscopy has traditionally been used to observe the disappearance of granules and the appearance of these discharged granules within the plasma membrane by membrane fusion and the movement of granules inside the cells. Here, we developed a method of video-rate bioluminescence imaging to directly detect degranulation from a single mast cell by measuring luminescence activity derived from the enzymatic reaction between *Gaussia* luciferase (GLase) and its substrate coelenterazine. The neuropeptide Y (NPY), which was reported to colocalize with serotonin in the secretory granules, fused to GLase (NPY-GLase) was efficiently expressed in rat basophilic leukemia (RBL-2H3) cells, a mast-cell line, using a preferred human codon-optimized gene. Bioluminescence imaging analysis of RBL-2H3 cells expressing NPY-GLase and adhered on a glass-bottomed dish showed that the luminescence signals from the resting cells were negligible, while the luminescence signals of the secreted NPY-GLase were repeatedly detected after the addition of an antigen. These results indicated that video-rate bioluminescence imaging using GLase will be a useful tool for detecting degranulation in single mast cells.

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DOI: 10.1002/eji.201970400

P1359

Neutrophil interferon-induced, double-stranded RNA-activated protein kinase (EIF2AK2) mediates vascular adhesion and transmigration in non-viral inflammatory disease

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Neutrophils accumulate in inflamed tissues and organs for primary host defense. Regulating properly the cell activity could direct therapeutic strategies to limit the scope of t the inflammation and subsequent damage in autoimmune diseases such as rheumatoid arthritis, glomerulonephritis, and systemic lupus erythmatosus. Here we focused on neutrophil interferon-induced, double-stranded RNA-activated protein kinase (EIF2AK2), known to be activated by double-stranded RNA in viral infection. We found that neutrophil EIF2AK2 is activated (auto-phosphorylated) even by sterile inflammatory stimuli. In an in vitro human system, chemical inhibition of the enzyme reduced neutrophil adhesion on TNF-α-activated HUVECs under flow conditions. Also, genetic silencing or pharmacological inhibition of the enzyme attenuated neutrophil trans-well migration. Mice treated with the chemical inhibitor showed reduced recruitment of CD45+Ly6G+ neutrophils in a dermal air pouch model of TNF-α-induced inflammation. In summary, neutrophil EIF2AK2 mediates vascular cell adhesion and transmigration, and thus a disruption of this enzyme activity may decrease neutrophil influx in autoimmune diseases.

P1945

PRAK is required for the formation of neutrophil extracellular traps

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Neutrophil extracellular traps (NETs) are one of the most powerful and specific tools for neutrophils to clean up extracellular microbes, but the mechanisms of NETosis under infection are scarcely studied. In this study, by examining the neutrophils from human peripheral blood and mouse abdomen, we demonstrated that PRAK dysfunction resulted in a significantly reduced NET formation and elevated apoptotic cells. Furthermore, PRAK dysfunction could lead to impaired NET-mediated antibacterial activity and shorten the survival of mice with CLP-induced sepsis. Mechanism studies revealed that attenuated NET formation in PRAK dysfunctional neutrophils correlated with overproduction reactive oxygen species (ROS), which triggered apoptosis through excessive autophagy. The imbalance of NET formation and apoptosis was further regulated by treatment with lower ROS in hypoxia. Here, we propose a novel candidate, PRAK, which can sense the oxidative stress and regulate the releasing of ROS, may be the master molecular switch to regulate the NETosis-apoptosis axis of neutrophils.

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DOI: 10.1002/eji.201970400

P1946

Improving granulocyte transfusion in neutropenic pneumonia by inhibiting neutrophil spontaneous death in vitro

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Granulocyte transfusion is hampered by the short shelf life of collected cells in vitro and rapid death of transfused neutrophils in vivo. We previously reported that neutrophil death is a heterogeneous process and mediated by multiple pathways. By targeting multiple death mechanisms, we recently identified a treatment (we call it “CLON-G treatment“ which means “Caspase +Lysosomal membrane permeabilization + Oxidant + Necroptosis” inhibiting plus G-CSF treating) that significantly prolonged neutrophils´ survival in vitro, to increase cells' half-life from ~24 hr. to more than 5 days. This is the longest storage time of neutrophils in vitro as far as our knowledge. Additionally, the morphology and functions (phagocytosis, bacterial killing, chemotaxis, and reactive oxygen species production) of CLON-G treated neutrophils were shown to be comparable to freshly isolated neutrophils even after 5 days. Extending neutrophils' life span by CLON-G treatment also allowed efficient transfection of plasmids. In vivo, CLON-G treatment improved the survival of neutrophils, transfusion of treated neutrophils in neutropenic pneumonia mouse model increased bacterial clearance of Escherichia coli (E. coli) upon infection and prolonged survival time of model mice. Together, CLON-G treatment might be a novel clinical procedure for the long-term storage and application of neutrophils in transfusion medicine.

P1947

Hemolysin-α positive *E.coli* induces neutrophils secreting amounts of IL-1β

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Interleukin-1β (IL-1β) is one of the most important mediators of inflammation and host responses to infections. Augmented IL-1β production has been found in patients with viral, bacterial, fungal and parasitic infections. IL-1β primarily is produced by cells of the monocytic lineage, including monocytes, macrophages and dendritic cells. However, increasing papers contested that IL-1β secreted by neutrophils might also play a major role in inflammation response. Here we found that CFT073, one of the hemolysin-α positive *Escherichia coli* (*E.coli*), could induce more IL-1β secretion from neutrophils than macrophages. Firstly, we found that CFT073 could induce neutrophil death by co-incubation purified neutrophils with opsonized CFT073. Moreover, we detected amounts of IL-1β secretion from the supernatant by western blot and Elisa. We supposed that the cell death and IL-1β secretion was hemolysin-α dependent through comparing the gene sequence of 15 different *E.coli* strains. This result was also confirmed by hemolysin-α knockout CFT073 strain. Further, we found that CFT073 induced neutrophil death and IL-1β secretion was NLRP3/caspase-1/gasdermin D signaling pathway dependent. The in vivo assay also showed hemolysin-α knockout CFT073 could improve survival in abdominal inflammation mouse model. Thus, our results firstly found the key factor, hemolysin-α,

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DOI: 10.1002/eji.201970400

could induce more IL-1β secretion from neutrophils, which suggested a dominated role of neutrophils in *E.coli* induced inflammatory response.

P1948

Impact of the oral allergic syndrome on basophil activation in birch-pollen sensitized patients

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Background: Our study aimed to evaluate the predictive value of the basophil activation test (BAT) in pollen-associated food allergy.
Materials and methods: 30 patients from 12 to 59 years [Me = 30; ±σ=11,12], 18 males and 12 females**,** were involved in the study and formed 3 groups. 1st group: 12 patients sensitized to birch pollen with OAS to apple. 2nd group: 14 patients sensitized to birch pollen without OAS. 3rd group: 4 non-allergic subjects.
BAT was performed with BeckmanCoulter Allergenicity Kit with 2 allergens (Betv1 and Mald1) in 3 concentrations each (1 ng/ml, 10 ng/ml, 100 ng/ml). Flow cytometry analysis was performed with BD FACSCanto II.
Results: in the 1st group highest basophil activation was registered for Betv1 in dose of 100 ng/ml (64,55 [47,3; 84,6]%) and for Mald1 in dose of 100 ng/ml (11,65 [8,94; 34,5]%). In the 2nd group highest basophil activation was registered for Betv1 in dose of 100 ng/ml (31,2 [4,84; 44,68]%) and for Mald1 in dose of 10 ng/ml (1,95 [1,37; 2,97]%).
Conclusion: Betv1 significantly activates basophils in group of patients with allergy to birch pollen and OAS to apple compared to patients with allergy to birch pollen only. Mald1 significantly activates basophils in group of patients with allergy to birch pollen and OAS. Betv1 in dose of 100 ng/mL elicits higher basophil activation than the same dose of Betv1 in the group of patients with allergy to birch pollen and OAS (64.5% vs 31,2%), P=0.0014 (U -test).

P1949

Pivotal role of STIM2, but not STIM1, in IL-4 production by IL-3-stimulated basophils

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Recent studies have identified non-redundant roles for basophils, that are distinct from those played by mast cells, in a variety of immune responses. Although the importance of Ca^{2+} influx has been demonstrated in the activation of basophils, the molecular mechanism regulating Ca^{2+} influx remains

DOI: 10.1002/eji.201970400

ill-defined. Here we examined the role of two Ca^{2+} sensors, stromal interaction molecule (STIM) 1 and STIM2, in basophil activation *in vitro* and *in vivo* by comparing basophils deficient for either of them with wild-type basophils. STIM1- but not STIM2-deficient basophils showed impaired IL-4 production and degranulation *in vitro* when stimulated with IgE plus allergens. In contrast, when stimulated with IL-3, STIM2- but not STIM1-deficient basophils displayed diminished IL-4 production, indicating differential roles for STIM1 and STIM2 in IL-4 production by basophils, depending on the stimuli, through FceRI and IL-3 receptor (IL-3R), respectively. This difference appeared to associate with distinct time courses of Ca^{2+} influx and transcriptional upregulation of the *Il4* gene elicited by two different stimuli. The differential function of STIM1 and STIM2 was also observed *in vivo* between FceRI- and IL-3R-mediated immune responses, both of which basophils are crucially involved in. Thus, the present study illustrates the vital role of STIM2 that has often been considered as a lesser relative of STIM1.

P1950

Involvement of mast cell in the development of inflammation

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While researching inflammation great attention is paid to mononuclear cells and neutrophils, but mast cells (MS) are also involved in the inflammation development. Interest is in the assessment of the composition of the cells and degranulation of mast cells of the peritoneal cavity during inflammation. Experiments were performed on 16 white male rats. The 1 group consisted of animals were injected intraperitoneally with 3% thioglycolic broth , the 2nd group - broth diluted 1:10. Control animals were injected with physiological solution. Peritoneal exudate was obtained within 5 days and samples were fixed and stained by the Romanovsky-Giemsa method and toluidine blue. Statistical analysis of the data was performed using Student´s *t*-test. It was shown that the number of leukocytes increases according to the concentration of the stimulator (r=0.86). In the 1st group the number of MC was 35.5±4.6, in control 9.0±2.2% ($p< 0.05$), amount of macrophages and neutrophils increased. The composition of exudate cells in the 2nd group was not statistically different from that in the control. There was an equal amount (40.0±5.4 and 49.5±4.8%) of partially and completely degranulated MCs in the 1st group. With less pronounced inflammation, MC with partial degranulation prevailed (49.6±3.9 vs. 28.0±6.5%; $p< 0.05$). In general, with a small amount of stimulator, there was no intensive and complete degranulation of MC, and, consequently, release of inflammatory mediators, which may be associated with a less clear clinical picture.
Thus, we made hypothesis about the essential role of MC in the manifestation of the inflammatory clinical signs.

P1951

Allergen induced tolerance and modulation of mast cell responses involves specific signaling

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The rise in allergic diseases of all kinds is a matter of concern worldwide including developing countries. Existing therapies for such disorders remain unaffordable for patients belonging to low-income developing countries. There is a dire need for cost effective and affordable therapies for allergies. Mast cells (MCs) are the major mediator of IgE-mediated allergic disorders. During this study, the effect of primary challenge and response on mast cell mediators' release during secondary challenge was studied. RBL-2H3 or BMMCs were challenged by allergen+IgE combinations during

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DOI: 10.1002/eji.201970400

primary and secondary challenge. First, the recovery of MCs after a primary allergen challenge in terms of surface expression of the high-affinity IgE receptor, FcεRI, granule content and proliferation capacity was confirmed. Pre-stored mediator release was measured as β-hexosaminidase and histamine release whereas production and secretion of cytokines was analyzed as late-phase mediators at mRNA and protein level. Role of signaling pathways and epigenetic modifications in modulation of mast cell secondary responses was assessed by use of various inhibitors like wortmannin, tranylcypromine and pargyline. Secondary challenge resulted in reduced mediator release and pro-inflammatory cytokine expression at mRNA and protein level. Use of inhibitors negated the inhibition caused by primary challenge resulting in enhanced responses. Our study revealed the MC responses can be modulated *in vitro* after a primary challenge, to become tolerant to allergen by specific signaling and chromatin modifications. Modulation of MC responses may provide insight into new therapeutic avenues for treating allergic disorders targeting MCs.

P2652

Neutrophil-to-lymphocyte ratio correlates with prognosis and response to chemotherapy in patients with de novo acute myeloid leukemia excluding acute promyelocytic leukemia

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Neutrophil-to-lymphocyte ratio (NLR) is recognized as a potential prognostic marker to predict survival outcome in several tumors. However, the predictive value of pretreatment NLR in acute myeloid leukemia (AML) remains unknown. A retrospective review of 181 patients with *de novo* AML excluding acute promyelocytic leukemia (M3) was conducted in our institute. We categorized the patients into two groups by defining NLR = 2.0 as the cut-off point. At a median follow-up for 28.0 months, AML patients with low NLR (< 2.0) had longer OS and DFS than those with high NLR (≥ 2.0) using Kaplan-Meier survival curves with log-rank tests. Similar results were also observed in AML cases of WHO subtypes AML with maturation and acute myelomonocytic leukemia. The univariate Cox regression analysis showed that NLR, absolute neutrophil count (ANC), and absolute lymphocyte count (ALC), Log(WBC) were significantly associated with OS and DFS in all AML patients or its WHO subtypes AML with maturation and acute myelomonocytic leukemia, whereas only ALC was associated with OS and DFS in the multivariate Cox regression analysis in these patients. Furthermore, the median NLR was dramatically increased in low NLR group but no statistical change in high NLR group when patients achieved complete remission (CR). In conclusion, our results suggest that pretreatment NLR as a marker can predict the prognosis of patients with non-M3 AML.

P2653

Chemotaxis-driven delivery of nano-pathogenoids for complete eradication of tumors post-phototherapy

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The efficacy of nano-mediated drug delivery for a complete eradication of solid tumors has been impeded by multiple biological barriers such as the mononuclear phagocyte system (MPS), as well as vascular and interstitial barriers. To overcome the above mentioned obstacles, we report a nano-pathogenoid (NPN) system that can *in situ* hitchhike circulating neutrophils and supplement conventional photothermal therapy. Cloaked with bacteria-secreted outer membrane vesicles (OMVs)

DOI: 10.1002/eji.201970400

inheriting pathogen-associated molecular patterns (PAMPs) of native bacteria, NPNs can be effectively recognized by pattern recognition receptors (PPRs) on circulating neutrophils. Once internalized, neutrophils laden with NPNs will then migrate towards tumor sites, extravasate across the blood vessels, and penetrate the interstitial tissue of the inflamed tumors. In the tumor tissue, NPNs can be rapidly released from neutrophils upon the formation of neutrophil extracellular traps (NETs) and be subsequently taken up by tumor cells to exert anticancer effects. Due to the excellent targeting efficacy, cisplatin-loaded NPNs combined with photothermal therapy completely eradicated solid tumors in all treated mice. Overall, our combinatorial approach represents an efficient and generalizable strategy towards enhanced tumor targeting and therapy.

P2654

Relationship between abnormal expression of immunopheno-types in acute myeloid leukemia and karyotypic abnormalities

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Objective: To investigate the relationship between the expression of immunophenotypes in acute myeloid leukemia (AML) and karyotypic abnormalities.
Methods: We selected a total of 131 patients with acute myeloid leukemia in our department of Hematology from January 2017 to June 2018. There were 75 males and 66 females with an average age of 58 years (age distribution 22-88 years), including 6 cases of M1 , 68 cases of M2, 15 cases of M3, 30 cases of M4, 10 cases of M5, 1 case of M6, and 1 case of acute mixed cell leukemia.
Results: Among the 131 patients, 62 cases were associated with mistranslation expressed, Abnormal expression were accompanied with 31 cases of CD56+ ,15 cases of CD15+, 15 cases of CD7+,10 cases of CD19+,1 case of CD2+, 1 case of CD5+, and all of 19 cases of two or more concomitant lines. In this group 31 patients had no abnormal mitotic phase changes, which were not included in the statistics. Fifteen patients with M3 were not counted because of immunophenotyping and chromosome specificity. Finally, 85 patients were enrolled. Statistical analysis of chi-square test was statistically significant (=3.994, p=0.0457).
Conclusion: The abnormal expression of immunophenotypes in acute myeloid leukemia is mainly CD56, CD15, CD7, CD19, etc. Patients with abnormal expression are more likely to have chromosomal abnormalities.
Keywords: acute myeloid leukemia; lineage expression; chromosomal abnormalities

P2655

Grx1 controls tumor growth through regulating neutrophils function

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In addition to the first responder to tissue damage and infection, neutrophils are also involved in cancer initiation and progression. However, the role of neutrophils in the tumor microenvironment remains controversial, with evidence for both pro- and anti-tumor roles. Recent study showed that deletion of Glutaredoxin 1 (Grx1), an enzyme that catalyzes actin deglutathionylation thioltransferase,

DOI: 10.1002/eji.201970400

increased actin glutathionylation, attenuated actin polymerization, and consequently impaired neutrophils function. However, whether *Grx1*gene ablation has effect on tumor growth and the role of neutrophils has not been explored. In this study, we generated a mice line with *Grx1* gene deficiency and conditional knockout of *Grx1* in the neutrophils. Mice were subcutaneously inoculated with several tumor cells line including B16F0, E.G7 and LLC1. Surprisingly, we found that above mentioned tumor almost completely absent in the Grx1-deficient mice. Our findings demonstrated that Grx1deletion have a antitumorigenic role in the tumor growth, but the mechanism of how Grx1 regulates the neutrophils in the tumor microenvironment remains investigation.
Keywords: Grx1, neutrophils, tumor

P2656

Research on the antibacterial function and the influence on neutrophils function of the combination of Xiyanping and Ceftriaxone

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Some of Traditional Chinese Medicine (TCM) is used to treat infections with antibiotics in China. Did the combination of the treatment have some synergistic effect? We investigated antibacterial function and mechanism of combination of Xiyanping and Ceftriaxone. 30 healthy rabbits was divided into Xiyanping, Ceftriaxone, and Xiyanping + Ceftriaxone group (combination group) randomly. They were injected Xiyanping, Ceftriaxone and Xiyanping + Ceftriaxone respectively trough the marginal ear vein for 7 days. Then drug serums were separated, and their antibacterial function were tested by Microbiological assay of antibiotics. Xiyanping had no antibacterial function alone. However, the antibacterial function increased in combination group than Ceftriaxone group($P < 0.05$). The influence on neutrophils function of them was discussed too. 10 healthy mice were into blank group, and 40 staphylococcus aureus-infected mice were divided into model, Xiyanping, Ceftriaxone and Xiyanping + Ceftriaxone group (combination group). Mice in blank and model group were injected normal saline, those in other groups were injected Xiyanping, Ceftriaxone and Xiyanping + Ceftriaxone respectively. Chemotaxis was tested by transwell assay, and respiratory burst was measured by Flow Cytometry (FCM). The chemotaxis of neutrophils weren't difference amongst all groups ($P > 0.05$). The reactive oxygen species(ROS) of neutrophils in blank, Xiyanping, and combination group were higher than in model and Ceftriaxone group($P < 0.01$). There was no difference in blank, Xiyanping and combination group($P > 0.05$) and between model and Ceftriaxone group($P > 0.05$). In conclusion, the combination of Xiyanping and Ceftriaxone can enhance the antibacterial function by through raising the respiratory burst of nertrophils.

P2657

Neutrophils mobilization by MTX-packaging tumor microparticles confers treatment efficacy for obstructive cholangiocarcinoma

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Neutrophils elicited by Coley toxin and Bacillus Calmette-Guérin have been recognized as a powerful weapon to treat cancers. Nevertheless, those triggered neutrophils are often coerced by tumor-derived factors into tumor-promoting ones. Thus, approaches that can mobilize neutrophils without tumor

DOI: 10.1002/eji.201970400

coercion are highly desirable. Here, we show that methotrexate (MTX)-packaging, tumor cell-derived microparticles (MP) acts as an effective immunotherapeutic agent to treat patients with obstructive extrahepatic cholangiocarcinoma (ECCA) via mobilizing and activating neutrophils. We find that MTX-MP perfusion via percutaneous transhepatic biliary drainage (PTBD) elicits marked recruitment of neutrophils through a glucose-UDPG signaling, leading to the rapid degradation of stromal barrier of ECCA. Thus, MTX-MPs gain access to and are taken up by ECCA cells, rendering the cells undergoing pyroptosis through a gasdermin E-dependent pathway. This pyroptotic death combined with MTX-MP-educated macrophages can further trigger a secondary wave of neutrophil recruitment and confers neutrophils an antitumor trait. These findings reveal self-derived materials packaging drugs which can transform into an antitumor compound weapon, leading to treatment efficacy against obstructive ECCA with low adverse effect.

P2658

Circulating neutrophil dysfunction in patients with HBV-related acute-on-chronic liver failure (ACLF)

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Background and aims: Acute-on-chronic liver failure (ACLF) is one of the major causes of death in patients with chronic hepatitis B virus (HBV) infection. Derangement of innate immune plays a vital role in the pathogenesis of ACLF. The aim of the study is to characterize the quantitative, transcriptomic and functional alterations of circulating neutrophils in HBV-related ACLF patients.
Methods: The association between circulating neutrophil count/neutrophil-to-lymphocyte ratio (NLR) and ACLF severity and prognoses were analyzed in an ACLF cohort. The phenotype, transcriptional landscape, free components, phagocytosis and oxidative burst capacity of neutrophils were determined in health controls, compensated cirrhosis or HBV-related ACLF patients.
Results: Circulating neutrophil counts and NLR were significantly higher in the HB-ACLF patients than in the No-ACLF patients ($P< 0.001$) and independently associated with ACLF prognosis. Neutrophil TLR1, TLR2, TLR4 and CEACAM1 expression was higher in HBV-ACLF patients, as well as plasma MPO compared to No-ACLF controls. Transcriptome analyses revealed that neutrophil in HB-ACLF patients were enriched with genes related to activation, degranulation and defense to bacterium, but depleted with regulation of lymphocyte response. And neutrophil in HB-ACLF patients displayed enhanced oxidative burst to E.Coli but impaired phagocytosis compared to No-ACLF controls.
Conclusions: Our study demonstrates a primed phenotype of neutrophils in HBV-ACLF, which was associated with disease progression and mortality.

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DOI: 10.1002/eji.201970400

Cell Death, Immunity and Inflammation

O137

Extracellular sphingomyelinase Rv0888 of *Mycobacterium tuberculosis* contributes to pathological lung injury of *Mycobacterium smegmatis* in mice via inducing formation of neutrophil extracellular traps

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Mycobacterium tuberculosis is the causative agent of tuberculosis, which mainly causes pulmonary injury and tubercles. Although macrophages are generally considered to harbor the main cells of *M. tuberculosis*, new evidence suggests that neutrophils are rapidly recruited to the infected lung. *M. tuberculosis* itself, or its early secreted antigenic target protein 6 (ESAT-6), can induce formation of neutrophil extracellular traps (NETs). However, NETs trap mycobacteria but are unable to kill them. The role of NETs formation in the pathogenesis of mycobacteria remains unclear. Here, we report a new *M. tuberculosis* extracellular factor, **bifunctional enzyme Rv0888**, with both nuclease and sphingomyelinase activities. **Rv0888 sphingomyelinase activity can induce NETs formation in *vitro* and in the lung of the mice,** and enhance the colonization ability of *M. smegmatis* in the lungs of mice. **Mice infected by *M. smegmatis* harboring Rv0888 sphingomyelinase induced pathological injury and inflammation of the lung, which was mainly mediated by NETs, induced by Rv0888 sphingomyelinase, associated protein (MPO) triggered caspase-3**. In summary, the study sheds new light on the pathogenesis of mycobacteria and reveals a novel target for tuberculosis treatment.

O138

DLL4 conveys Notch-dependent signals achieving selective macrophage polarization or death

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Molecular mechanisms underlying vascular and inflammatory cell network at endothelial and macrophage levels are still unclear. Here we found that microvascular inflammation associates with changes in Notch signaling at endothelium/monocyte interface including loss of endothelial Notch4 and the acquisition of the Notch ligand Dll4 in both cell types. We showed that endothelial DLL4 induces circulating monocytes to polarize into a M1-type pro-inflammatory macrophages ($CD40^{high}CD64^{high}CD200R^{low}$ $HLA\text{-}DR^{low}CD11b^{low}$) eliciting the production of IL-6. Dll4 and IL-6 are both Notch-dependent and are required for macrophage polarization through selective down and upregulation of M2- and M1-type markers, respectively. Subsequently, we investigated the ability of DLL4 to interfere with M2 polarization. We found that DLL4 triggers a specific alteration of the IL-4 induced M2 phenotype through a significant inhibition of M2 markers (CD11b, CD206, CD200R). DLL4 also induces caspase3/7-dependent apoptosis specifically in M2 differentiating macrophages while DLL1 had no effect. DLL4 signals via Notch1 and DLL4-mediated apoptosis is Notch-dependent. Fully differentiated M2 macrophages became resistant to DLL4 action**.** DLL4 upregulates gene expression, upon M2 upon differentiation, affecting the Notch pattern (Notch1, 3, Jag1) and activity (Hes1), transcription (IRF5, STAT1) that associates with decrease in Akt but not STAT6 phosphorylation. In conclusion, our findings reveal an interplay between DLL4/Notch and IL-6/IL-6R or IL-4/IL-4R signaling pathways supporting M1 differentiation and impairing M2 differentiation via apoptosis.

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DOI: 10.1002/eji.201970400

O139

K63-linked ubiquitination regulates RIPK1 kinase activity to prevent cell death during embryogenesis and inflammation

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Receptor-interacting protein kinase 1 (RIPK1) is a critical regulator of cell death through its kinase activity. However, how its kinase activity is regulated remains poorly understood. Here, we generated *Ripk1*$^{K376R/K376R}$ knock-in mice in which the Lys(K)63-linked ubiquitination of RIPK1 is impaired. Surprisingly, the *Ripk1*$^{K376R/K376R}$ knock-in mice displayed an early embryonic lethality at E13.5 due to liver degeneration mediated by massive cell death, which is resulted from reduced TAK1-IKK-mediated suppression on RIPK1 kinase activity and enhanced formation of more apoptosome and necroptosome in *Ripk1*$^{K376R/K376R}$ cells in response to TNFα. Although TNFR1 deficiency delayed the lethality, concomitant deletion of RIPK3 and Caspase8 fully prevented embryonic lethality of *Ripk1*$^{K376R/K376R}$ mice. Notably, *Ripk1*$^{K376R/-}$ mice were viable but developed severe systemic inflammation that was mainly driven by RIPK3-dependent signaling pathway, indicating that K63-linked ubiquitination on Lys376 residue of RIPK1 also contributes to inflammation process. Together, our study provides new insights into the in vivo function and mechanism by which K63-linked ubiquitination on K376 regulates RIPK1 kinase activity to control embryonic development, inflammation and host immune homeostasis.

O140

CD38 regulates central and effector memory T lymphocyte generation in chronic inflammation model

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The chronic inflammation participates in the development of several diseases like AIDS, where the activation and reduction of T lymphocytes determinate HIV progress. The presence of circulating Lipopolysaccharide (LPS) has been correlated with CD38 overexpression in T cells. A correlation between the circulating LPS and CD38 activation protein, expressed by central and effector memory T lymphocytes has been described, nonetheless, the function of CD38 has not been elucidated. In order to analyze the relationship between CD38 expression on T lymphocytes in the presence of LPS, we induced chronic inflammation in a murine model via LPS administration in wild type and CD38 deficient mice twice a week for 23 wks. Then, naïve, central and effector memory T lymphocytes were analyzed from total splenocytes by flow cytometry. In addition, we evaluated the ex vivo and in vitro IFN-γ production. Our data suggest that CD38 regulate negatively the development of effector T lymphocytes and promotes the generation and activation of central memory T lymphocytes in a chronic inflammation state. Under inflammation conditions, CD38 deficient mice have lower numbers of interferon-producing cells, but, stimulation through its TCR, increases the amount of IFN-γ

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DOI: 10.1002/eji.201970400

producing cells. Altogether, the results establish a role of CD38 in the immune response of T lymphocytes in chronic inflammation, both regulating T cells responses and cytokines production. It is necessary to keep on this research, to consider CD38 as a new alternative in the regulation of chronic inflammation in HIV disease.

O141

SPHK1 deficiency protects acetaminophen-induced acute liver injury mice from ER stress and mitochondrial permeability transition

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Acetaminophen (APAP) is the leading cause of drug-induced acute liver failure worldwide. S1P, whose formation is catalyzed by SPHK1 or SPHK2, is a bioactive lipid implicated in human health and disease. Here, we show that APAP-treated sphK1-deficient mice exhibited markedly less liver damage with reduced inflammation compared to wild-type mice. This is associated with reduced JNK activation and hepatocyte death in the absence of SPHK1. Mechanistically, SPHK1 deficiency alleviates APAP-induced ER stress through affecting the phosphorylation of inositol requiring enzyme 1α (IRE1α), Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK)- (PERK)-eukaryotic translation initiation factor 2α (EIF2α)-activating transcription factor (ATF-4), and the activition and level of activating transcription factor 6 (ATF6). In addition, SPHK1 deficiency inhibits mitochondrial permeability transition as evidenced by the impaired phosphorylation of JNK, apoptosis signal-regulated kinase 1 (ASK1) and glycogen synthase kinase 3β (GSK3β). SPHK1 deficiency also reduced level of histone deacetylase, acetylation of p65 and STAT1, thereby affecting the transcription of inflammatory genes. The exogenous supplementation of S1P significantly reversed the activation of EIF2α and ATF6 during ER stress as well as the activation of GSK3β, ASK1, and JNK during the mitochondrial permeability transition (MPT). FTY720, a functional antagonist for S1P receptor, significantly ameliorated APAP-induced liver injury and improved animal survival. Our study reveals a critical role for SPHK1 in mediating APAP-induced hepatotoxicity by promoting ER stress and MPT.

O142

A novel prevention of erythrocyte fragility and anaemia induced by extracellular histones

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Neutrophil extracellular traps (NETs) are released from neutrophils following activation by pathogens and/or tissue injury and exhibit anti-microbial activity. Uncontrolled formation of NETs can, however, become pathogenic, particularly via their associated histones, that can be cytotoxic in the vasculature causing endothelial cell death, systemic vascular obstruction, multiple organ failure and initiate

DOI: 10.1002/eji.201970400

coagulation by both activating platelets and damaging erythrocytes such that they become pro-thrombotic.
Recently we discovered an additional effect of histones on erythrocyte, which is to enhance their fragility when they are subjected to sheer stress associated with blood flow through the vasculature and spleen in particular. We have synthesised a family of small polyanions (SPAs) which have been screened for their ability to neutralise the pathogenic effects of histones and NETs.
We have used a novel mechanically-induced sheer stress method as well as an *in vitro* spleen filtration model to investigate the effects of mammalian histones ± SPAs, on RBC fragility. The *in vitro* findings were mirrored *in vivo* with the injection of histones and SPAs in mice.
Our results revealed that free histones bind to erythrocytes with high affinity and render erythrocytes susceptible to lysis by shear stress in a dose-dependent manner and some SPAs could totally inhibit or reverse this phenomenon.
Based on these data we propose that erythrocytes can neutralise the damaging effects of histones in the circulation and also transport them to the spleen for disposal. Overload of this disposal system could cause the unexplained anaemia associated with sepsis, cancer and other pathological conditions.

O143

Plasma exosomal miR-375-3p regulates ferroptosis in keratinocytes by targeting GPX4 in SJS/TEN

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening, cutaneous adverse drug reactions that are accompanied by keratinocyte cell death. Ferroptosis is a recently recognized form of regulated cell death driven by lipid-based reactive oxygen species (ROS) accumulation. However, the molecular mechanisms of ferroptosis regulation are still largely unknown in SJS/TEN. Exosomes are nanometre-sized vesicles with a lipid bilayer membrane and present in body fluids, containing functional proteins, mRNAs and miRNAs. Nevertheless, the potential roles of plasma exosomes and the underlying mechanisms in SJS/TEN have yet to be explored. In our study, we uncovers that exosomes isolated from plasma of SJS/TEN patients showed the expected size between 30 and 150nm. Then, exosomes were shown to be positive for exosomal markers CD9, CD63 and TSG101. Deep sequencing analysis and qRT-PCR of plasma exosomal derived miRNAs demonstrated that miR-375-3p level was the markedly upregulated in SJS/TEN patients and was positively correlated with clinical features. Then, plasma derived exosomes from patients were internalized by human primary keratinocytes and promoted malondialdehyde (MDA) and lipid ROS accumulation and ferroptotic cell death. Additionally, ectopic expression of miR-375-3p suppressed glutathione peroxidase 4 (GPX4), an enzyme converts lipid hydroperoxides to lipid alcohols, resulting in increased ferroptotic cell death. Importantly, knockdown of miR-375-3p inhibited ferroptosis, which completely prevented SJS/TEN-like responses in a mouse model of SJS/TEN.
Our results indicate that GPX4 downregulated by the overexpressed miR-375-3p mediates keratinocyte ferroptosis in SJS/TEN patients. Circulating exosomal miR-375-3p might be used as a potential disease marker for the diagnosis of SJS/TEN.

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DOI: 10.1002/eji.201970400

O144

The scavenger circulating protein CD5L controls acute and systemic inflammation in sepsis

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Inflammation is a protective response of the immune system to harmful challenges like wounds and pathogens. However, the system sometimes overreacts and triggers an incontrollable inflammatory response known as sepsis that often leads to death. CD5L is a member of the Scavenger receptor cysteine-rich superfamily of proteins with varying functions such as apoptosis inhibitor, microbial sensor and is an important mediator in different infectious or inflammatory diseases. We explored the role of CD5L in sepsis using our newly generated CD5L knockout mice by CrispR/Cas9 engineering. When challenged with sub-lethal doses of LPS, CD5L KO mice had a much higher mortality rate compared with wild-type animals (WT). Similarly, the CD5L deficient animals succumb in higher number when subjected to polymicrobial sepsis induced by cecal ligation and puncture (CLP). Further dissection of the molecular mechanisms underlying the susceptibility of CD5L KO mice in CLP indicated that these mice have impaired immune responses translated by lower levels of several cytokines and chemokines. This is accompanied by increased CFU counts in the peritoneal cavity and also in blood, associated with decreased numbers of infiltrating leukocytes, namely neutrophils, to the peritoneal cavity comparing with WT mice. Interestingly, we have found that, contradicting the previously described literature, CD5L can be expressed and secreted by different additional leukocyte populations other than monocytes/macrophages, such as neutrophils infiltrating the peritoneal cavity upon CLP induction. Our results ascribe an important role for CD5L in orchestrating the inflammatory processes during sepsis.

P0171

Improving granulocyte transfusion in neutropenia-related pneumonia

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Patients receiving chemotherapy and radiotherapy are always accompanied by neutropenia. Neutropenia related infections greatly lead to high mortality and morbidity. Broad spectrum anti-bacterial and anti-fungal drugs and/or G-CSF are used to treat neutropenia related infections. However, part of the patients don't respond to these treatment. Neutrophil transfusion has been applied since 1930s as an alternative way, but it has never been widely used in clinical. Short lifespan of neutrophils is the key rate limiting factor. **Here, we cultured neutrophils with NAC, Hsp70, G-CSF, Q-VD-oph, DFO and RIP1-inhibitor to inhibit all the known death related pathway and then assessed death of cultured neutrophils. Both mouse and human neutrophil lifespan were expanded from 18-24 hours to 5-7 days *in vitro*.** To investigate whether our NHGQ-treated neutrophils can work as freshly isolated neutrophils, we conducted neutrophil *in vitro* function assays including ROS production, bacterial killing, phagocytosis and chemotaxis. Our results indicated that NHGQ-treated neutrophils had similar functions as the freshly isolated neutrophils. To further determine whether transfusion with NHGQ-treated neutrophils could enhance the host defense as transfusion with freshly isolated neutrophils, we conducted neutrophil transfusion on our established

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DOI: 10.1002/eji.201970400

neutropenia related pneumonia mouse model. Consistent survival rates were observed between groups tansfused with NHGQ-treated neutrophils and freshly isolated neutrophils. Together, our data demonstrate NHGQ-treated neutrophils with longer lifespan can function normally as freshly isolated neutrophils and this provide promising possibility for improving neutrophil transfusion efficiency in neutropenia related infections.

P0172

PDCD4 deficiency aggravated colitis and colitis-associated colorectal cancer via promoting IL-6/STAT3 pathway in mice

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Background: Although programmed cell death (PDCD) 4 is generally considered to be a new tumor suppressor, the consequence of Pdcd4 deficiency in tumorigenesis is not well established. The role of PDCD4 in colitis-associated colorectal carcinoma (CRC) remains unknown.
Methods: Experimental colitis and CRC were induced by dextran sodium sulfate and dextran sodium sulfate with azoxymethane, respectively, in wild type and Pdcd4 knockout (Pdcd4(-/-)) mice and were evaluated by clinical examination and histopathology. Levels of cytokines were detected by enzyme-linked immunosorbent assay. Changes in signaling pathways were examined by Western blot and immunofluorescent staining. Cell proliferation was determined by BrdU incorporation and Cell Counting Kit-8 staining.
Results: Pdcd4 deficiency not only aggravated the dextran sodium sulfate-induced acute colitis but also promoted the development of colitis-induced CRC. Mechanically, Pdcd4 deficiency accelerated epithelial cell proliferation during tumorigenesis, markedly up-regulated the expression of proinflammatory cytokines, such as interleukin (IL)-6, and enhanced the activation of signal transducer and activator of transcription (STAT3), a IL-6 downstream effector. Using purified cells, we found that Pdcd4 deficiency increased IL-6 expression in vitro and the susceptibility to IL-6/STAT3 pathway-mediated cell proliferation significantly. Furthermore, blockade of IL-6/STAT3 pathway through sgp130Fc reversed the promoting effect of Pdcd4 deficiency on colonic epithelial cell proliferation in vivo.
Conclusion: The Pdcd4 deficiency accelerates colitis and colitis-associated CRC presumably through up-regulating IL-6/STAT3 pathway, suggesting that PDCD4 plays a protective role in inflammation-associated carcinoma and might be a potential target for the treatment of CRC.

P0173

HPV E7 inhibited cell pyroptosis by promoting TRIM21 mediated degradation and K33 linked-ubiquination of IFI16 inflammasome

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Bachground: Human papillomavirus (HPV) is a DNA virus that causes sexually transmitted infections. HPV oncoprotein E7 is critical in regulating host immunity to promote self-escape, occurrence of cervical cancer and genital warts. Pyroptosis, which is a highly inflammatory form of programmed cell death, can be induced by the inflammasomes and acts as a defense against infection when the host is infected with pathogens.

DOI: 10.1002/eji.201970400

Objective: This study focused on the role and mechanisms of HPV E7 in cell pyroptosis for promoting HPV immune escape and tumor development.
Materials and methods: This study explored the affection of HPV E7 in regulating cell pyroptosis through flow cytometry analyses, microarray, mass spectrum, immunoprecipitation and western blot.
Results: We found HPV E7 could inhibit cell pyroptosis induced by transfection with dsDNA. Also, HPV E7 could inhibit the activation of inflammasomes and the production of IL-18 and IL-1b. Mass spectrum and immunoprecipitation showed that HPV E7 could interact with IFI16 and TRIM21. We also found HPV E7 promoted K33-linked ubiquitination and degradation mediated by E3 ligase TRIM21.
Conclusions: HPV E7 could recruit the E3 ligase TRIM21 to ubiquitinate and degradate IFI16 inflamasome, leading to inhibit the cell pyroptosis and promote HPV immune escape and tumor development.

P0174

The Hippo effectors YAP and TAZ integrate microenvironmental cues to modulate inflammatory responses

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The Hippo pathway transduces microenvironmental cues to regulate cell behaviors and mediates cell density-dependent proliferation. Based on the study in H358, HeLa, and HEK293T cells, we discovered cell density modulates inflammatory responses through the Hippo signaling pathway. Specifically, we found cyclooxygenase 2 (COX-2), the crucial inflammatory mediator is induced much less at low cell densities than at high cell densities. However, NF-kB activation through the proinflammatory cytokines interleukin-1 (IL-1β) and tumor necrosis factor (TNFα) is not affected by cell density. Mechanistically, using techniques of co-immunoprecipitation and chromatin immunoprecipitation, we observed that the Hippo effectors Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are localized to the nucleus, bind to TEA domain transcription factors (TEADs), recruit histone deacetylase 7 (HDAC7) to the promoter region of COX-2, and repress its transcription at low cell density and that high cell density abrogates this YAP/TAZ-mediated transcriptional repression. Of note, IL-1β stimulation promotes cell migration and invasion mainly through COX-2 induction, but cell proliferation is not affected. YAP inhibited COX-2 induction and thus cell migration and invasion in vitro. Moreover, the KEGG analysis of gene transcription of H358 cells stably expressing YAP(5SA), the constitutively active form of YAP or GFP, as a control group, established YAP as a bona fide negative regulator of inflammatory responses. Our findings highlight that the cellular microenvironment significantly influences inflammatory responses via the Hippo pathway.

P0175

Hepatomas are exquisitely sensitive to pharmacologic ascorbate via multiple mechanisms

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DOI: 10.1002/eji.201970400

Ascorbate is an essential micronutrient known for redox functions at normal physiologic concentrations. In recent decades, pharmacological ascorbate has been found to selectively kill tumour cells. However, a difficulty of using pharmacologic ascorbate is dosing frequency in humans. Herein, we determined that among four liver cell lines, Huh-7 cells were the cells most sensitive to ascorbate. Ascorbate induced a significant increase in the percentage of cells in the G0/G1 phase, the apoptosis rate and intracellular ROS in Huh-7 cells. We further found that high doses of ascorbate served as a pro-drug that kills hepatoma cells by altering mitochondrial respiration, but a low dose of ascorbic acid did not. Furthermore, in a Huh-7 cell xenograft tumour mouse model, a high dose of ascorbate (4.0 g / kg / 3 days) by intraperitoneal injection significantly inhibited tumour growth and changed transcript levels of 192 genes and ncRNAs, whereas the lower dose of IP ascorbate (2.0 g/kg/3 days) had different effects. Moreover, qRT-PCR and immunohistochemical staining analysis verified that RAGE and DGKK were up-regulated in HCC tumour tissue from mice treated with a high dose of ascorbate, which implied that RAGE and DGKK activation might be relative to oxidative stress, leading to hepatoma cell death. Our studies of ascorbate in a xenograft tumour mouse model involved in high dose ascorbate treated HCC patients illuminated a plausible mechanism of the anti-tumour activity of high-dose ascorbate as a novel potential therapeutic agent for liver cancer.

P0176

The farnesoid X receptor regulates the development and function of monocyte/macrophage in inflammation and infection

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As a nuclear receptor for bile acid, Farnesoid X receptor (FXR) is one of the key transcription factors known to regulate immune cell development and function. By regulating genes involved in many signaling pathways, FXR is emerging as an attractive drug target for many inflammatory and infectious diseases. However, the molecular mechanism underlying FXR mediated regulation remains elusive. In this study, utilizing $FXR^{-/-}$mice and multiple in vitro assays, we investigated the role of FXR in the development and function of monocyte/macrophage lineage. We found the ablation of FXR in mice leads to a defective monocyte/macrophage proliferation in bone marrow, which associated with decreased expression of c-Fos-CREB via dephosphorylation of MAPK proteins Erk/p38/JNK. Intriguingly, we further noted that in cells treated with lipopolysaccharide in vitro, BMDM from $FXR^{-/-}$ mice expressed lower anti-inflammatory cytokine IL-10, but a comparable level of pro-inflammation cytokines, such as IL-1β, IL-6, and TNF-αcomparing to BMDM from WT littermates. Moreover, BMDM from $FXR^{-/-}$ mice displayed elevated NO production and accumulated more LPS-induced inducible Nitric oxide synthase (iNOS), which potentially promotes inflammation in vivo. Monocytes are essential in host defense to *Listeria* infection. We further found decreased liver and spleen CFU in $FXR^{-/-}$mice comparing to the WT littermates in *L.monocytogene* infection, which may through the contribution of enhanced antibacterial function from monocytes as well as reduced monocytes as a reservoir for *Listeria*. Together, we for the first time found that FXR regulates the development and function of monocyte/macrophage in inflammation and infection.

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DOI: 10.1002/eji.201970400

P0177

Inhibition of HtrA2 alleviated dextran sulfate sodium (DSS)-induced colitis by preventing necroptosis of intestinal epithelial cells

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Necroptosis of intestinal epithelial cells has been indicated to play an important role in the pathogenesis of inflammatory bowel disease (IBD). The identification of dysregulated proteins that can regulate necroptosis in dextran sulfate sodium (DSS)-induced colitis is the key to the rational design of therapeutic strategies for colitis. Through Tandem Mass Tag (TMT)-based quantitative proteomics, HtrA2 was found to be downregulated in the colon of DSS-treated mice. UCF-101, a specific serine protease inhibitor of HtrA2, significantly alleviated DSS-induced colitis as indicated by prevention of body weight loss and decreased mortality. UCF-101 decreased DSS-induced colonic inflammation, prevented intestinal barrier function loss and inhibited necroptosis of intestinal epithelial cells. *In vitro*, UCF-101 or silencing of HtrA2 decreased necroptosis of HT-29 and L929 cells. UCF-101 decreased phosphorylation of RIPK1 and subsequent phosphorylation of RIPK3 and MLKL during necroptosis. Upon necroptotic stimulation, HtrA2 translocated from mitochondria to cytosol. HtrA2 directly interacted with RIPK1 and promoted its degradation during a specific time phase of necroptosis. Our findings highlight the importance of HtrA2 in regulating colitis by modulation of necroptosis and suggest HtrA2 as an attractive target for anti-colitis treatment.

P0178

The preventive and therapeutic effects of Piper longum extract in liver cirrhosis induced by CCL4 in rats

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To observe and study liver injury mechanism though tumor necrosis factor a (TNF-α), Interleukin-6 (IL-6) in rats with liver cirrhosis induced by carbon tetrachloride (CCL_4) complex factors and to investigate the preventive effect and mechanism of piper longi fructus. 60 SD rats purchased from Inner Mongolia Medical University were randomly divided into blank control group, cirrhosis model group, high, medium and low dose groups of Piper extract. Except blank control group, rats in other groups were induced with 0.35g/l phenobarbital sodium solution. 40% and 50% of the carbon tetrachloride rapeseed oil solution were injected subcutaneously at multiple points. At last 60% of the concentration was injected subcutaneously at multiple points. In cirrhosis model group, a large number of hepatocytes appeared degeneration and vacuolar necrosis. Fatty degeneration was more serious, accompanied by inflammatory cell infiltration, disordered arrangement of hepatocyte cord, a large number of fibrous tissue proliferation, and the destruction of hepatic lobule structure was replaced by pseudolobule formation. Compared with the model group, the hepatic injury of the rats treated with Piper chinensis extract was significantly alleviated and the hepatic steatosis was gradually aggravated with the decrease of drug concentration. Compared with the model group, the degree of hepatic fibrosis in the Piper extract treatment group was decreased, hepatocyte steatosis and fibrosis were gradually alleviated with the increase of drug dosage. Proper dosage of piper longi fructus extract can

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DOI: 10.1002/eji.201970400

prevent and improve liver injury in rats. It can prevent cirrhosis by reducing the expression of TNF-α and IL-6.

P0179

TIPE2 regulates RAC1-MTORC1 axis and impairs autophagy flux

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Tipe2, tumor necrosis factor-alpha-induced protein 8 (TNFAIP8)-like 2 (TNFAIP8L2), is an identified immune negative regulator of innate and adaptive immunity and plays an important role in inflammatory homeostasis. It has been proposed that murine Tipe2 binds directly and blocks Rac1 GTPase activity to determine the strengths of phagocytosis and oxidative burst in innate immune. Besides, Rac1 is reported as a crucial activator of mammalian target of rapamycin (mTOR) by mediating mTOR localization at specific membranes via binding to mTOR under nutrient stimulation. Here, we find that Tipe2 can compete with mTOR for binding to GTP bound state of Rac1 directly and disassociate active Rac1 from lysosomal Rac1-mTORC1 complex, thereby diminishing mTORC1 activity. Conversely, loss or down-regulation of Tipe2 exhibit enhancement of mTOR activation. Interestingly, Tipe2 fails to increase active autophagy flux by suppression of mTOR activity with glutamine and serum starvation. Instead it appears to especially impair autophagic lysosome reformation (ALR), rather than autophagy initiation, in starvation induced autophagy. Finally, we demonstrate that Tipe2 leads to inability of mTOR reactivation during late stage of autophagy so as to disrupt autophagy flux, thereby leading to cell death. Our study reveals a novel role of Tipe2 in autophagy by regulating the Rac1-mTORC1 axis and supports it as a target for potential therapeutic treatment.

P0180

Thrombin contributes to anti-myeloperoxidase antibody positive IgG-mediated glomerular endothelial cells activation through SphK1-S1P-S1PR3 signaling

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Background: Activation of coagulation system plays an important role in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) pathogenesis. Thrombin, generated during coagulation could disrupt endothelial barrier integrity through protease-activated receptor 1 (PAR1). Our previous study found that sphingosine-1-phosphate (S1P) contributed to myeloperoxidase (MPO)-ANCA-positive IgG-induced glomerular endothelial cell (GEnC) activation through a S1P receptor (S1PR)-dependent route. In recent years, S1P signaling was reported to be involved in thrombin effects on endothelial cells. This current study investigated whether the interaction between thrombin-PAR and S1P-S1PR signaling contributed to MPO-ANCA-positive IgG-induced GEnC dysfunction.
Methods: The effect of thrombin on GEnC activation was analyzed from three aspects. First, morphological alteration of GEnCs was observed. Second, permeability assay was performed to determine GEnC monolayer activation quantitatively. Third, endothelin-1 (ET-1) levels were measured. Expression levels of sphingosine kinases (SphKs) and S1PRs were detected. In addition, antagonists of PAR1 and S1PR3 were employed to determine their roles. Eventually, PAR1 and tissue factor (TF) expression levels as well as TF procoagulant activity were analyzed.
Results: Thrombin induced further damage of tight junction, increase in endothelial monolayer permeability as well as upregulation of ET-1 levels in GEnCs stimulated with MPO-ANCA-positive IgG.

DOI: 10.1002/eji.201970400

Blocking PAR1 downregulated ET-1 levels in the supernatants of GEnCs treated by thrombin plus MPO-ANCA-positive IgG. Expression levels of SphK1, S1PR3 increased significantly in GEnCs treated with thrombin plus MPO-ANCA-positive IgG. S1P upregulated PAR1 and TF expression, and enhanced procoagulant activity of TF in MPO-ANCA-positive IgG-stimulated GEnCs.
Conclusion: Thrombin synergized with SphK1-S1P-S1PR3 signaling pathway to enhance MPO-ANCA-positive IgG-mediated GEnC activation.

P0181

The neutrophil chemoattractant peptide proline-glycine-proline is associated with acute respiratory distress syndrome

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Acute respiratory distress syndrome (ARDS) is characterized by unrelenting polymorphonuclear neutrophil (PMN) inflammation and vascular permeability. The matrikine proline-glycine-proline (PGP) and acetylated PGP (Ac-PGP) have been shown to induce PMN inflammation and endothelial permeability in vitro and in vivo. In this study, we investigated the presence and role of airway PGP peptides in acute lung injury (ALI)/ARDS. Pseudomonas aeruginosa-derived lipopolysaccharide (LPS) was instilled intratracheally in mice to induce ALI, and increased Ac-PGP with neutrophil inflammation was noted. The PGP inhibitory peptide, arginine-threonine-arginine (RTR), was administered (it) 30 min before or 6 h after LPS injection. Lung injury was evaluated by detecting neutrophil infiltration and permeability changes in the lung. Pre- and posttreatment with RTR significantly inhibited LPS-induced ALI by attenuating lung neutrophil infiltration, pulmonary permeability, and parenchymal inflammation. To evaluate the role of PGP levels in ARDS, minibronchoalveolar lavage was collected from nine ARDS, four cardiogenic edema, and five non-lung disease ventilated patients. PGP levels were measured and correlated with Acute Physiology and Chronic Health Evaluation (APACHE) score, Pao_2 to Fio_2 (P/F), and ventilator days. PGP levels in subjects with ARDS were significantly higher than cardiogenic edema and non-lung disease ventilated patients. Preliminary examination in both ARDS and non-ARDS populations demonstrated PGP levels significantly correlated with P/F ratio, APACHE score, and duration on ventilator. These results demonstrate an increased burden of PGP peptides in ARDS and suggest the need for future studies in ARDS cohorts to examine correlation with key clinical parameters.

DOI: 10.1002/eji.201970400

P0182

Activation of NLRP3 inflammasome as a key indicator of carbon nanotubes proinflammatory potential

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The aim of our study was to evaluate pro-inflammatory potential of multi-walled carbon nanotubes (MWCNTs). Despite the fact, that some of these nanotubes are not acutely cytotoxic in vitro, mechanical disruption of some organelles like lysosomes can trigger NLRP3 assembly leading to production of proinflammatory cytokines IL-1β and IL-18. We regard NLRP3 activation as an essential step in induction of chronic inflammation in vivo.
Pristine PMWCNTs and functionalized NDMWCNTs and COOHMWCNTs were tested in our study. Detailed observation of shape and length of MWCNTs was assessed using electron microscopy. Human monocyte-derived cell lines THP-1, THP1-null and THP1-defNLRP3 were used as in vitro model. Cell viability was evaluated via LDH assay. Localization of MWCNTs was detected by electron microscopy. Activation of NLRP3 and release of IL-1β was measured by cell-based assay using HEK-Blue™ IL-1β cells. ELISA kits were used for detection of other cytokines in supernatants.
After 24h, MWCNTs were located as free particles in the whole cytoplasm. Rarely, some individual tubes were observed to reach the nucleus membrane. Interestingly, while all types of MWCNTs activated NLRP3, only COOHMWCNTs caused release of proinflammatory cytokines IL-6 and TNF-α. Increased LDH levels showed higher cytotoxicity in the case of functionalized MWCNTs only.

Supported by: PROGRES Q-09/Q-10, ERDF/ESF projects NANOBIO CZ.02.1.01/0.0/0.0/17_048/0007421 and FIT CZ.02.1.01/0.0/0.0/15_003/0000495 (JT), the project RO0519 of the Czech Ministry of Agriculture (JT). NanoPharm (join project of Veterinary Research Institute and International Clinical Research Center (FNUSA-ICRC)(JT) is acknowledged, as well as support by Education and Research Centre VRI-Malvern (JT).

Keywords: Inflammasome, Cytotoxicity

P0183

CCL5 binding to its receptor CCR1 alleviates apoptosis of lung epithelial cells by inhibiting MAPK kinase pathway during respiratory syncytial virus infection

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Lung epithelial cells play an essential role on maintaining the stability of the pulmonary environment by secreting a variety of mediators and expressing a variety of membrane molecules. When pathogens invade, how do lung epithelial cells react following the challenge？Respiratory syncytial virus (RSV) is the cause of ~70% of all cases of viral bronchiolitis. In this study, A549 cells, Hep2 cells, and beas-2b

DOI: 10.1002/eji.201970400

cells were infected with RSV respectively, and the expressions of some inflammatory factors and membrane molecules were detected by RT-qPCR, ELISA and Western blot. The results showed that high expression of CCL5 significantly in the above infected-cells. After different concentrations of CCL5 was added to the infected-cells, the activation level of MAPK, NF-κB and PI3K/AKT pathways were detected separately, and the result showed that p-P38 expression was gradually decreasing with the increasing concentration of CCL5. Subsequently, we overexpressed and knocked down CCL5 respectively in A549 cell, and found that the level of apoptosis was reduced or increased following overexpression or knockdown of CCL5. Meanwhile, CCL5 and CCR1 were overexpressed by A549 cell, and viral load was significantly reduced after 24 hours of RSV infection.Our results suggested that CCL5 combination with its receptor CCR1 protects lung epithelial cells from RSV infection partly by inhibiting the activation of MAPK signaling.

P0756

Rho GTPases are involved in S1P-enhanced glomerular endothelial cells activation with anti-myeloperoxidase antibody positive IgG

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Background: Sphingosine-1-phosphate (S1P) is a key regulator in vascular inflammation. Our previous study found that under pathophysiological concentration in active AAV patients, S1P participated in MPO-ANCA-positive IgG induced GEnC activation through a S1PR-dependent way. However, the downstream signaling pathways are still not fully clear.
Methods: The activation of Rac1 and RhoA in GEnCs stimulated by S1P in the presence of MPO-ANCA-positive IgG were first analyzed. Then GEnCs were pre-incubated with selective agonists and/or antagonists of S1PRs before stimulation by S1P plus MPO-ANCA-positive IgG so as to determine which S1PR mediated Rac1 or RhoA activation. Further, the effect of Rac1 or RhoA on S1P-induced ICAM-1/VCAM-1 expression of GEnCs in the presence of MPO-ANCA-positive IgG was determined by employing inhibitors of Rac1 and RhoA.
Results: Under pathophysiological concentration in active AAV patients, S1P increased the activity of both RhoA and Rac1 in GEnCs compared with controls. Pre-incubation of GEnCs with the S1PR1 selective agonist SEW significantly increased the activity of Rac1 in GEnCs treated by S1P plus MPO-ANCA-positive IgG, while the inhibition of S1PR1 with the antagonist W146 attenuated the Rac1 activity. The S1PR2-4 selective antagonists significantly downregulated the relative activity of RhoA in GEnCs treated by S1P plus MPO-ANCA-positive IgG, while the S1PR5 agonist A97 upregulated the RhoA activity. RhoA inhibition significantly downregulated ICAM-1/VCAM-1 levels in GEnCs treated by S1P and MPO-ANCA-positive IgG, whereas Rac1 inhibition exerted opposite effect.
Conclusion: RhoA and Rac1 signaling pathways were implicated in MPO-ANCA-positive IgG-mediated GEnCs activation enhanced by S1P of pathophysiological concentration in AAV.

P0757

Subtyped cell-in-cell structures associate with patient survival of human pancreatic ductal adenocarcinoma

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DOI: 10.1002/eji.201970400

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Cell-in-cell structures (CICs) were documented in various human tumors for decades, however, the prognostic contributions of subtyped CICs are poorly understood. In this study, we explored this question in human pancreatic ductal adenocarcinoma (PDAC) by the multispectral "EML method". Overall CICs (oCICs), at cutoff of 5 CICs/core, was identified as an independent adverse prognostic factor (HR: 1.917, *p*= 0.002) superior to other clinicopathological factors examined, including TNM stage (HR: 1.798, *p*=0.044) and lymph node metastasis (HR: 1.510, *p*=0.056). Homotypic CICs (hoCICs, also as TiT) between tumor cells, the major subtype (75.6%) out of the fours identified, also independently predicts poor outcome (HR: 1.850, *p*=0.004), with performance comparable to o CICs in univariate analysis. Among the rest three heterotypic CICs, in addition to a small amount of LiT(leukocytes in tumor cells, 1.4%), two subtypes were identified as TiM (tumor cells in macrophages, 3.6%) and MiT (macrophages in tumor cells, 19.4%). Remarkably, MiT, a novel subtype of CICs independently predicting patient survival (HR: 1.7, *p*=0.013), synergistically increased TiT´s prognostic performance when jointly used (HR: 2.005, *p*=0.001). Thus, the presence of MiT, demonstrating macrophage cannibalism by tumor cells, likely surrogates enhanced malignancy and predicts even worse outcomes of PDAC patients. Together, we presented the first subtype-based prognostic analysis for CICs in human PDAC tumors, demonstrating CICs a valuable prognostic factor that were profoundly affected by its subtype composition with macrophage cannibalism (MiT) an important weighting factor, which may be a reference for other type of tumors.

P0758

Mercuric chloride modulates the proportion of immune cells in peritoneal cavity

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Little is known about mercury-mediated immunotoxic effects on the local tissues. Here, we investigated the effects of mercury on peritoneal cavity immune cells. BALB/c mice were intraperitoneally injected with mercury chloride at a single dose of 5mg/kg. At different time-points (i.e., 3h, 12h, 1d, 2d, 3d, 5d, 7d and 9d) post injection, peritoneal cavity was flushed with PBS to collect peritoneal lavage fluid and immune cells. By using flow cytometer, we determined cell number, apoptosis rate and cell proportion. Mercury decreased $CD45^+$ cell number while increased the apoptosis (Annexin-V^+PI^+) rate, in a time-dependent manner. The immune cells of peritoneal cavity constitute 11% of T cells, 68% of B cells, and 0.4% of neutrophils. Mercury dramatically modulated this cell proportion. At 3h, the percentage of T and B cells decreased to 1.8% and 25.8%, while neutrophils increased to 54%. At 1d, T cells recovered to 11%, B cells decreased to 6.6%, and neutrophils were 47%. At 5d, T cells increased to 31%, B cells were 9.4%, and neutrophils decreased to 3.9%. These changes lasted until 9d. The protein concentration of the peritoneal lavage fluid increased between 3h and 2d, and then gradually decreased. Our results indicate that mercury chloride can cause local inflammatory reactions in the abdominal cavity and alter the proportion of immune cells in the peritoneal cavity.
Fundings: National Natural Science Foundation of China (81460503), Jilin Provincial Science & Technology Department (20150101207ZC), Education Department of Jilin Province (JIJIAOKEHEZI2015No.3), Health and Family Planning Commission of Jilin Province (2014Z089).

DOI: 10.1002/eji.201970400

P0759

The EMT transcription factor Zeb1 regulates activation induced cell death in T cells and TLR7/9 signaling in plasmacytoid dendritic cells by repressing E-cadherin expression

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Zeb1 and Zeb2, two members of the family of Zinc-Finger E homeobox-binding transcription factors, are major players to drive epithelial to mesenchymal transition. It has been reported that Zeb2 plays important role in development or differentiation of several types of immune cells, including plasmacytoid dendritic cells (pDCs), conventional dendritic cells (cDC1), tissue resident macrophages, and also B cells and $CD8^+$effector T cells. However, the role of Zeb1 in immune cells remains poorly defined. For this end, we generated mice lacking *Zeb1* specifically in $CD11c^+$ cells or T cells.
We observed that mice with *Zeb1*deficiency in T cells exhibited dramatic reduction in T cell activation as well as B cell responses after LCMV infection. Further *in vitro*and *in vivo*analysis revealed that Zeb1 deletion actually did not affect activation and proliferation of both $CD4^+$and $CD8^+$ T cells but promoted their apoptosis. Mechanistically, upregulation of E-cadherin expression caused by *Zeb1*deficiency in T cells increase their susceptibility to death receptor-mediated activation induced cell death.
On the other hand, we found that mice with Zeb1 deficiency in dendritic cells developed spontaneous fatal inflammatory diseases after 3 months. Moreover, deficiency of Zeb1 resulted in aberrant changes in TLR7/9 signaling in plasmacytoid dendritic cells but not in conventional dendritic cells. Our data suggested that Zeb1 might differentially regulate TLR7/9 signaling pathway through down-regulation of E-cadherin.

P0760

MicroRNA-539 inhibits the progression of Wilms' tumor through downregulation of JAG1 and Notch1/3

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Background: Previous studies demonstrated that miR-539 play an important role in the carcinogenesis of some cancers. The aim of the present study was to determine the role of miR-539 in the pathogenesis of Wilms' Tumor (WT).
Methods: The expression level of miR-539 was measured by qRT-PCR in 42 WT tissues and SK-NEP-1 cell line. Protein expression of genes (E-cadherin, N-cadherin, Vimentin, Notch 1, Notch 3 and JAG1) was assessed by Western blot. The function of miR-539 was investigated in SK-NEP-1 cells by MTT and Transwell assays. The relationship between miR-539 and JAG1 was verified by a dual luciferase assay in SK-NEP-1 cells.
Results: The expression level of miR-539 was significantly decreased in WT tissues. Downregulation of miR-539 was closely related to NWTS-5 stage, lymph node metastasis and histological type of WT patients. Furthermore, low miR-539 expression was associated with a shorter overall survival rate in WT patients. *In vitro*, overexpression of miR-539 suppressed proliferation, migration and invasion of SK-NEP-1 cells. In addition, JAG1 was a direct target of miR-539. MiR-539 inhibited the development of WT by inhibiting JAG1-Notch1/3 expressing and blocking EMT.
Conclusion: MiR-539 inhibited the progression of WT through downregulation of JAG1 and Notch1/3.

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DOI: 10.1002/eji.201970400

P0761

The application of peripheral blood neutrophil extracellular traps (NETs) in the monitoring of gout disease activity

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For a long time, there is no accurate, stable and feasible index to evaluate the severity of gout. Recent studies have shown that polymorphonuclear neutrophils activation after release into the extracellular neutrophil extracellular traps may be involved in the acute gouty arthritis to chalkstone formed a series of pathological mechanism.
Methods: Clinical collected 56 cases of gout in patients with acute exacerbation (G) and 25 healthy volunteers (HC) (peripheral venous blood samples were collected after centrifugation of plasma), general (including age, gender and body mass index) and routine laboratory tests (including blood glucose, triglycerides and low density lipoprotein cholesterol). The content of NET-DNA in plasma samples was determined by PicoGreen® assay fluorescence spectrophotometry. The content of neutrophil elastase in plasma samples was detected by enzyme linked immunosorbent assay (ELISA). The statistical software is used to analyze the above data.
Result: The content of NETs in the peripheral blood of gout patients is positively related to the activity and severity of the disease during the acute attack, and can be used to evaluate and monitor the gout.
Keywords: Gout, Neutrophils, Neutrophils extracellular traps, Monosodium urate

P0762

Effect of rapamycin-mTOR pathway-induced autophagy on inhibition of chondrocyte apoptosis

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Objective: To investigate the possibility of rapamycin (RAPA)-induced autophagy in chondrocytes inhibiting the progression of osteoarthritis (OA).
Methods: In vitro culture of chondrocytes from femoral neck fractures for hip arthroplasty and total knee arthroplasty in patients with advanced OA, induced apoptosis with 1x10^-6mol/L DEX, were divided into BSA group, DEX-BSA group and RAPA+DEX-BSA group respectively. The activity was detected by MTT. Autophagy levels were detected by GFP-RFP-LC3 adenovirus, Western blot and qRT-PCR. The concentrations of IL-1 and TNF-α were measured by qRT-PCR and ELISA. Apoptosis was detected by flow cytometry and TUNEL.
Results: In normal or OA chondrocytes, Western blot showed that the totalLC3B (P< 0.001), LC3B-II (P< 0.001), Beclin1 (P< 0.01) and ULK1 (P< 0.001) expression in the RAPA+DEX-BSA group were higher than those in the DEX-BSA group. qRT-PCR showed increased expression of LC3 (P< 0.05) and ULK1 (P< 0.01) in RAPA+DEX-BSA group compared with DEX-BSA, TNF-α (P< 0.01) and IL-1 (P < 0.05) decreased either in normal or OA chondrocytes. ELISA showed that TNF-α (P< 0.001) and IL-1 (P< 0.01) decreased in the RAPA+DEX-BSA group compared with DEX-BSA, regardless of normal or OA chondrocytes. Flow cytometry showed that the apoptotic rate of the RAPA+DEX-BSA group was lower in the normal (P< 0.01) and OA chondrocytes (P< 0.001) than in the DEX-BSA group.
Conclusion: RAPA induced autophagy in chondrocytes, inhibiting the expression of TNF-α and IL-1, inhibiting the apoptotic pathway and inhibiting the OA-like damage of DEX on chondrocytes.

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DOI: 10.1002/eji.201970400

P0763

Gasdermin D stabilized by Caspase1-dependent deubiquitination involves in the pathogenesis of non-alcoholic steatohepatitis in mice

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Objective: Pyroptosis mediated by Gasdermin D (Gsdmd) has been shown to be involved in the pathogenesis of non-alcoholic steatohepatitis (NASH). Here, we aim to illustrate the exact mechanisms underlying Gsdmd regulation and its role in NASH.
Design: The mRNA, protein and ubiquitination level of Gsdmd were determined in the liver of Methionine-choline deficient (MCD) diet induced NASH mice. Hepatocellular Gasdermin D (Gsdmd) and Caspase1 were knocked down by AAV-saCas9 mediated CRISPR (clustered regularly insterspaced short palindromic repeat) respectively to evaluate their biological significance in the NASH. The impact of Caspase1 silencing on the protein level and ubiquitination level of Gsdmd was further explored.
Results: Firstly, we identified that the levels of full-length and N-terminal Gsdmd protein was greatly upregulated in the liver of NASH mice. Then, we found that Caspase1 protein levels and cleavage were increased, whereas Caspase11 protein levels remained unchanged. We also found that the dramatically upregulated protein levels of Gsdmd was not mediated by elevation of its mRNA levels but by the decrease of ubiquitination levels of Gsdmd, especially K48-linked-polyubiquitination levels. Furthermore, we found that knockdown of hepatocellular Gsdmd via AAV mediated CRISPR/SaCas9 system could ameliorate pathological manifestations. In addition, knockdown of hepatocellular Caspase1 could also lead to amelioration of steatohepatitis. More intriguingly, knockdown of Caspase1 downregulated the protein levels of Gsdmd while increased the ubiquitination levels of Gsdmd, which indicated that the deubiquitination of Gsdmd was dependent on Caspase1.
Conclusion: Gsdmd was stabilized by Caspase1-dependent deubiquitination and was involved in the pathogenesis of NASH in mice.

P0764

Caspase-dependent apoptosis induction via viral protein ORF4 of porcine circovirus 2 binding to mitochondrial adenine nucleotide translocase 3

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Previews studies considered the pathogenesis of PCV2 were relevant to various kinds of factors, among them apopotosis caused by virus play a key role in pathogenic mechanism. ORF4 was once regared as a viral protein involved in apoptosis, but how it affected apoptosis was still unknown. In the present study, we report the molecular mechanism of apoptotic induction by viral protein ORF4. Apoptosis detection revealed that not only is the activity of caspases 3 and 9 is increased in PCV2-infected and ORF4-transfected cells, but also cytochrome c release from the mitochondria to the cytosol is upregulated. Subsequently, ORF4 colocalization with adenine nucleotide translocase 3 (ANT3) was observed using structured illumination microscopy. Moreover, co-immunoprecipitation and pulldown analyses confirmed that ORF4 interacts directly with mitochondrial ANT3. Binding domain analysis further confirmed that N-terminal residues 1 to 30 of ORF4, comprising a mitochondrial targeting signal, are essential for the interaction with ANT3. Knockdown of *ANT3* markedly inhibited the apoptotic induction of both ORF4 and PCV2, indicating that ANT3 plays an important role in ORF4-induced apoptosis during PCV2 infection. Taken together, these data indicated that ORF4 is a

DOI: 10.1002/eji.201970400

mitochondrial targeting protein that induces apoptosis by interacting with ANT3 through the mitochondrial pathway.

P0765

Activated fibroblast facilitated cardiac inflammatory resolution through inducing CCR2$^+$M1 selective apoptosis and conversion

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Macrophages are key regulators of tissue injury and wound healing. After tissue injury, infiltrated monocytes/macrophages undergo phenotypic and functional changes and play their roles in inflammatory damage. The acute inflammation will become chronic and the damage organ can't be remodeling if the M1 macrophage can't vanish in time. Therefore, it is crucial to clarify the functional shift of M1 and M2 during tissue injury. Our results that angiotensin II (ANG II) induced cardiac fibroblasts transdifferentiation into cardiac myofibroblast (MFB). In MFB and macrophage co-culture system, firstly MFB promoted macrophage polarization into M1 phenotype and then induced the polarized M1 selective apoptosis. M1 selective apoptosis was associated with TNF/TNFR1 axis and independent on NO produced by itself. Surprisingly, co-culturing for 36h, the survival CCR2$^+$M1 phenotype conversion into M2, which was dependent on leptin produced by MFB or polarized macrophage via PI3K or Akt pathway. *In vivo*, CCR2$^+$CD45.2$^+$ monocytes/macrophages were adoptively transferred into CD45.1$^+$ mice suffered from viral myocarditis (VMC). During inflammatory resolution stage, CD45.2$^+$CX3CR1$^+$M2 were detected in cardiac tissue. Our data highlighted a novel mechanism about the resolution of cardiac inflammation: under cardiac injury, transdifferentiation of cardiac fibroblast into myofibroblast induced cardiac infiltrated M1 macrophage selective apoptosis and conversion of M1/M2.

P0766

Metformin inhibited the proliferation of TM3 testicular cells via activating AMPK pathway

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Objective: To investigate the role and molecular mechanism of metformin in mouse testicular development.
Methods: The effects of metformin on the proliferation,apoptosis and cell cycle of TM3 cells were detected by MTT assay, Annexin V-FITC/PI staining and flow cytometry. Real-time PCR and Western blot were used to analyze the expression of cell cycle protein and AMPK（phospho172）to evaluate the effect of metformin on TM3 cells.
Results: MTT assay showed that metformin significantly inhibited the proliferation of TM3 cells in vitro. Annexin V-FITC/PI staining showed that metformin did not induce apoptosis of cells. Flow cytometry showed that metformin could induce TM3 cell cycle arrest at G2/M phase. Real-time PCR showed that the expression of cyclin B1 and cyclin D1 were down-regulated in metformin treated TM3 cells. Western blot showed that the phosphorylation of AMPK at Thr172 was significantly increased in metformin treated TM3 cells.
Conclusion: Metformin significantly inhibits the proliferation of TM3 cells in vitro and induces the TM3 cell cycle arrest in G2/M phase, activating AMPK signal pathway, and exerting inhibitory effect on TM3 cells in vitro.

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DOI: 10.1002/eji.201970400

P0767

Efferocytosis mediated anti-inflammatory responses are restricted at the site of peripheral nerve injury

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It is well known that peripheral nerve injury (PNI) facilitates recruitment of macrophages in the injured nerves and elicits neuropathic pain in rodents. Generally, macrophages are classified into two types (i.e. M1-like macrophages and M2-like macrophages) and M2-like macrophages participate in the resolution of inflammation. Previous reports have shown that suppression of M1-like macrophages in the injured nerves attenuates neuropathic pain. However, the roles of M2-like macrophages in the injured nerves are less studied. By flowcytometry and immunohistochemistry, we observed IL-1β expressing M1-like macrophages and CD206 expressing M2-like macrophages at the site of PNI and found that M2-like macrophages phagocytosed apoptotic neutrophils (efferocytosis). Next, we analyzed gene and protein expression levels of efferocytosis-related molecules in macrophages at the site of PNI. Although gene expression levels of *Mer* and *Axl* in macrophages at the site of PNI were comparable with peritoneal macrophages, the surface protein expression levels of Mer and Axl were detected in peritoneal macrophages but not macrophages at the site of PNI. Given that Mer and Axl signaling are involved in not only efferocytosis but also attenuation of inflammation through the inhibition of pro-inflammatory mediator expression, the anti-inflammatory effect of M2-like macrophages may be restricted by post-transcriptional regulation of Mer and Axl in the injured nerves. Further studies are ongoing to elucidate the mechanism of restriction of Mer and Axl expression on the surface of M2-like macrophages in the injured nerves and to examine whether rescuing the expression of Mer and Axl suppress inflammation and attenuate neuropathic pain.

P1360

Knockout of Usp9y gene in mouse TM3 leydig cells caused cell cycle arrest and inhibited cell proliferation

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The Azoospermia factor (AZF) region on the Y chromosome has been considered closely related to spermatogenesis. Ubiquitin specific peptidase 9, Y-linked (Usp9y) gene, located in the AZF region, plays a important role in spermatogenesis. Our previous study found that the Usp9y gene is mainly expressed in mouse Leydig cells. Here we established the Usp9y knockout TM3 mouse Leydig cell line using the Crispr-Cas9 technique. Furthermore, we studied the function of Usp9y to clarify its role in spermatogenesis based on the Usp9y knockout cell model. The cell proliferation and cell cycle were examined by Cell Counter Kit-8 (CCK-8) and flow cytometry, respectively. The expression of Cyclin B1 and Caspase-3 were detected by real-time PCR and western blot, respectively. As a result, the proliferation of Usp9y knockout TM3 cells was inhibited and cell cycle was arrested in G1 phase. The expression of Ccnb1 was increased in the Usp9y knockout cells, and the expression of apoptosis protein Caspase-3 was significantly increased. In a summary, Usp9y knockout resulted in the TM3 cell cycle arrest and cell proliferation inhibition, and induced apoptosis.

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DOI: 10.1002/eji.201970400

P1361

Interaction between usp9y and Nna1 and its mechanism of action in spermatogenesis

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Objective: To investigate the relationship between usp9y and the mutant gene Nna1, which causes the infertility phenotype of this mouse, and the mechanism of its action in spermatogenesis.
Methods: The eukaryotic expression vector pEGFP-usp9y-(nt1558-1955) was constructed by molecular cloning and transfected into HEK293T cells to detect its expression. pEGFP-usp9y-(nt1558-1955) and pCMV-Myc-Nna1 were co-transfected. The interaction between usp9y and Nna1 protein was analyzed by immunoprecipitation in HEK293T cells. The expression of Usp9y gene, spermatogenesis related genes and key signaling pathways in pcd mice was studied by single gene expression profiling.
Results: Usp9y and Nna1 could form an immunoprecipitate complex; the expression of Nna1 was down-regulated in the testis of pcd3J mutant mice, and the expression of usp9y was significantly increased.
Conclusions: There is a certain interaction between the two, which may be involved in spermatogenesis in mice, but the mode of action is not clear; NF-κB cell signaling pathway may be involved in spermatogenesis in pcd mice.

P1362

Liver-specific deletion of *Eva1a/Tmem166* aggravates acute liver injury by impairing autophagy

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Acute liver failure (ALF) is an inflammation-mediated hepatocellular injury process associated with cellular autophagy. However, the mechanism by which autophagy regulates ALF remains undefined. *EVA1A(eva-1 homolog A)/TMEM166(transmembrane protein 166)* is a programmed death-related gene first reported by our team. Previous studies have shown that EVA1A can induce autophagy and apoptosis in tumor cells. Molecular mechanism studies show that EVA1A interacts with ATG16L1 and promotes autophagosome formation. Herein, we demonstrated that Eva1a can protect mice from ALF induced by D-galactosamine (GalN) /lipopolysaccharide (LPS) via autophagy. Our findings indicate that a hepatocyte-specific deletion of *Eva1a* aggravated hepatic injury in ALF mice, as evidenced by increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), myeloperoxidase (MPO), and inflammatory cytokines (e.g., TNFα and IL-6), which was associated with disordered liver architecture exhibited by *Eva1a*$^{-/-}$ mouse livers with ALF. Moreover, we found that the decreased autophagy in *Eva1a*$^{-/-}$ mouse liver resulted in the substantial accumulation of swollen mitochondria in ALF, resulting in a lack of ATP generation, and consequently hepatocyte apoptosis or death. The administration of Adeno-Associated Virus Eva1a (AAV-Eva1a) or antophagy-inducer rapamycin increased autophagy and provided protection against liver injury in *Eva1a* $^{-/-}$ mice with ALF, suggesting that defective autophagy is a significant mechanism of ALF in mice. Collectively, for the first time, we have demonstrated that Eva1a-mediated autophagy ameliorated liver injury in mice with ALF by attenuating inflammatory responses and apoptosis, indicating a potential therapeutic application for ALF.

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DOI: 10.1002/eji.201970400

P1363

Isolation and purification of active antimicrobial peptides from *Hermetia illucens* L., and its effects on CNE2 cells

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Active antimicrobial peptide HI-3 was isolated and purified from the 5th instar larvae of *Hermetia illucens* L., and its effects on proliferation, apoptosis and migration of nasopharyngeal carcinoma (CNE2) cells were investigated. Furthermore, expressions of hTERT in CNE2 cells were also studied *in vitro* to elucidate mechanism involved in the action of HI-3 on CNE2 cells. Results showed that three fractions (HI-1, HI-2, HI-3) were isolated., However, only HI-3 showed antibacterial activities, and could effectively inhibit the proliferation of CNE2 cells *in vitro* in a dose- and time- dependent manner. In addition, HI-3 could increase the membrane permeability of CNE2 cells. Apoptosis of CNE2 cells was observed after 160 µg/ml of HI-3 treatment, and the early apoptosis rate up to 27.59±1.14%. However, HUV-C were not significantly affected. HI-3 could significantly reduce the migration ability of CNE2 cells, and their migration rates were 24.43±0.47%, 61.51±0.04% and 80 ± 1.46% at 12 h, 24 h and 48 h, respectively, which were all significantly higher than that of controls. On the other hand, the telomerase activity, and the expressions of hTERT in HI-3 treatment were all significantly lower than of control. Results indicated that HI-3 could inhibit the proliferation of CNE2 cells and induce the apoptosis of CNE2 cells by inhibiting the expression of hTERT and down-regulating telomerase activity in CNE2 cells, while no obvious effect was occurred on HUV-C. It inferred that HI-3 is a potential anti-tumor drug with low toxicity and side effects.

P1364

Gasdermin E is required for induction of pyroptosis and severe disease during EV71 infection

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Pyroptosis is an inflammatory form of programmed cell death, which is executed by gasdermin-N-domain of gasdermin family proteins to form the pore in the plasma membrane. Pyroptosis emerges as a host defense against invasive pathogenic microbial infection. However, the role of pyroptosis in the pathogenesis of EV71 infection remains unclear. In the current study, we find that EV71 infects HeLa, RD and SK-N-SH cells, resulting in cell pyroptosis which is characterized by morphological changes, cell membrane swelling and plasma membrane rupture. The expression of GSDME decreases gradually with the prolongation of infection time, while the cleavage band of GSDME is detected by Western blot after EV71 infection. Further analysis show that the cleavage is not related to 3C and 2A proteases of EV71. However, the activation of caspase-3 is essential for the cleavage of GSDME. Importantly, the disease induced by EV71 in GSDME knockout mice is alleviated compared with in wild type mice. Thus, our results revealed that GSDME is required for EV71-induced pyroptosis and severed disease during EV71 infection.

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DOI: 10.1002/eji.201970400

P1365

Therapeutic effect of Schistosoma japonicum cystatin on cardiac dysfunction in septic mice

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Background: Sepsis remains one of the leading causes of mortality in surgical patients. Echocardiographic studies suggest that 45% of patients with prolonged septic shock develop myocardial depression. *Schistosoma japonicum* cystatin (Sj-Cys) is a cysteine protease inhibitor whether reduce cardiac dysfunction induced by sepsis was investigated.
Methods: Sepsis was induced in BALB/c mice using cecal ligation and puncture (CLP), followed by intraperitoneal injection of the dose of recombinant Sj-Cys (rSj-Cys). The survival rates of mice and the left ventricular systolic and diastolic functions were examined. The mechanism was investigated by examining cytokines in mice sera and in culture of cardiomyocytes stimulated by lipopolysaccharides (LPS).
Results: r*Sj*-Cys treatment provided significant therapeutic effects on CLP-induced sepsis in mice demonstrated with increased survival rates, alleviated overall disease severity and tissue injury of myocardial. The efficacy was associated with up-regualted IL-10 and TGF-β1 cytokines and reduced pro-inflammatory cytokines TNF-α, IL-6. *In vitro* assay with H9c2 cell also showed that r*Sj*-Cys inhibited the release of pro-inflammatory cytokines. MyD88 and NF-kB expression in LPS-induced cardiomyocytes of r*Sj*-Cys-treated was reduced.
Conclusions: The results suggest the potential of r*Sj*-Cys as a promising therapeutic agent on cardiac dysfunction induced by sepsis. The immunological mechanism may involve the down-regulation of pro-inflammatory cytokines and up-regulation of regulatory cytokines possibly via down-regulation of the TLR adaptor-transducer MyD88/ NF-kB pathway.
Keywords: Cystatin, Sepsis, Cardiac dysfunction

P1367

EBV-regulated cyclophilin A contributes to proliferation promotion and apoptosis inhibition of nasopharyngeal carcinoma through activating AKT1/mTOR signaling

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Cyclophilin A (CYPA) is an immune-modulatory protein, which was found to be upregulated in nasopharyngeal carcinoma (NPC) in our laboratory. CYPA is usually hijacked by viruses to favor viral functions, but its role in Epstein-Barr virus (EBV)-associated NPC remains unknown. In the present approach, we showed that the EBV-encoded oncoprotein, latent membrane protein 1 (LMP1), promotes CYPA expression through the activation of nuclear factor kappa B (NF-κB). Simultaneously, CYPA is a direct suppression target of EBV-encoded miR-BART21-3p. This dual regulation implied the elegance of EBV in maintaining cellular homeostasis during cancer progression. Moreover, the knockdown or knockout of CYPA inhibited the proliferation and promoted the apoptosis of NPC cells. CYPA was further verified to bind to AKT1, and thus activating AKT1/mTOR (p70S6k) pathway in NPC. It is noticeably that knockout of CYPA could inactivated AKT1 completely. In addition, the knockout of CYPA reduced the size and volume of xenografted tumors in nude mice remarkably. This study reveals that EBV contributes to NPC progression as a causative agent via regulating the cellular CYPA/AKT/mTOR axis. The results represent a mechanism of immune-modulatory protein-mediated carcinogenesis that utilized by a tumor virus. As a novel regulator of AKT/mTOR pathway, CYPA could serve as a potential therapeutic target for EBV-associated NPC.

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DOI: 10.1002/eji.201970400

P1368

The role and mechanism of RPL39 in Preeclampsia

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Preeclampsia(PE) is the leading cause of maternal and perinatal mortality and morbidity. The underlying mechanism is still not completely elucidated. Ribosomal Protein L39 (RPL39) plays important roles in biological function of many cells. However, there are no research of its function in human trophoblast cells. We examined the expression of RPL39 in placental tissues, found that RPL39 significantly decreased in placental tissues of PE patients compared with that in control group by RT-PCR, western blotting and immunohistochemistry. Meanwhile, we generated RPL39 loss of function model in Bewo cells by using lentivirus systems. Scratch-wound assays, cell cycle assays, EDU and cell apoptosis assays were used to examine the function of RPL39 in cells model. We demonstrated that knockdown of RPL39 dramatically decreased the cell migration, significantly arrest G2/M phase cells and increase G0/G1 phase cells. However, the S phase cells and apotosis are not influenced in RPL39 knockdown cells. In order to explore the role RPL39 in the pathogenesis of PE, we analyzed the correlation of the expression RPL39 and the clinical pathological features in PE specimens by SPSS20.0. The results showed that the expression of RPL39 were correlated with blood pressure, gestational weeks and the weight of fetal in patients. This study was the first time to explore the role of RPL39 in PE. It will help us to better understand the pathogenesis of PE.
This work was supported by Major Science and Technology Program of Hainan Province (No. ZDKJ2017007)

P1369

NMAAP1 activates calcium-related signaling pathways to promote macrophage to polarize to M1 type

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The novel macrophage activation-associated protein 1 (NMAAP1) is a molecule specifically expressed in BCG-activated macrophages (BAM), which shares similar sequences with DANGER, a newly discovered triphosphate receptor-binding protein which can bind IP3R to regulate cell differentiation. Whether NMAAP1 plays a role in regulating macrophage differentiation to the M1 type and exerting antitumoral functions is not clear. Our study found that overexpression NMAAP1 in RAW264.7 cells induced macrophage polarization to the M1 type, knockdown NMAAP1 in RAW264.7cells induced macrophage polarization to the M2 type and maintains M1 Phenotype even in the presence of IL-4, a stronger inducer of the M2 type through qRTPCR and ELISA. Additionally, NMAAP1 activates key molecules in the PKC-dependent Raf/MEK/ERK and Ca2+/CaM/CaMKII signaling pathways through Western-blotting. Activation of PKC (Thr638/641), ERK1/2 (Thr202/Tyr204) and CaMKII (Thr286) are involved in the regulation of cell differentiation. Therefore, NMAAP1 activates the PKC-dependent Raf/MEK/ERK and Ca^{2+}/CaM/CaMKII signaling pathways. These results showed ***NMAAP1 could promote macrophage to polarize to the M1 type and prevent macrophage shift from M1 to M2 by modulating calcium-related signaling pathways.***

DOI: 10.1002/eji.201970400

P1370

Effects of hUMSCs transplantation on blood flow and locomotor recovery in nude mouse model with hind limb ischemia

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Hind limb ischemia (HLI) is common peripheral vascular disease, possibly treatable with stem cells. Nude mouse HLI model was established by ligating the left common femoral artery whilst the right served as normal control. hUMSCs (1×106 cells) were injected intramuscularly 3 days post-operation. Ischemic/normal hindlimb blood flow ratio was measured using Laser Doppler Perfusion Imaging and locomotor function was assessed by blinded researchers using BMS score. Blood flow of hind limbs decreased by 60% after common femoral artery ligation, which proved the model was successful. Ischemia caused damage to skin of hind limbs for most HLI models 3 days after operation. BMS score increased from 3.2 on day 2 post transplantation (ptn) to 7 on day 6 ptn, and blood flow gradually recovered from 40.00% after operation to 68.33%, 78.60% and 85.00% at 2, 4 and 6ptn respectively. While, M1/M2 ration of macrophage increased on day 2 and 4, and on day 6, the ratio reversed. In conclusion, hUMSCs have a good effect on blood flow and motor function recovery of HLI.

P1371

The AP-1 transcription factor, Fra1, regulates apoptosis in the acute promyelocytic leukemia (APL) cells

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Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) associated with the accumulation of fusion gene of PML/RARA. All-trans retinoic acid (ATRA) has been used in APL patients for inducing leukemic cell differentiation through the PML-RARα degradation. However, it has been known to have a limitation in therapeutic efficacy due to the resistance to ATRA. Fos-related antigen 1 (Fra-1), a member of the activating protein 1 (AP-1) family, has been reported to regulate the differentiation of osteoclasts, macrophages and adipocytes. AP-1 family members are known to control the ATRA-induced differentiation in the APL, but the correlation with Fra-1 is poorly understood. In this study, we investigated the effect of ATRA on Fra-1 expression in human APL cell line. Fra-1 expression was significantly downregulated by ATRA treatment. Inhibition of Fra-1 markedly affected the expression of differentiation-related genes, such as C/EBPα and PU.1. Nevertheless, knockdown of Fra-1 did not change differentiation of APL. Interestingly, Fra-1 overexpression induced an increase of CD38 negative cells that are known to be reduced by ATRA and related to drug resistance. We found that ATRA induced CD38 expression through the inhibition of the Fra-1 expression on HL-60 cells. Actually, knockdown of Fra-1 significantly enhanced paclitaxel-induced apoptosis in APL cells. These results indicate that the transcription factor Fra-1 might negatively control apoptosis and positively regulate drug resistance in HL-60 cells. In summary, these findings suggest that Fra-1 could be a therapeutic target for combination therapy that affects drug resistance and suppresses apoptosis in APL cells.

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DOI: 10.1002/eji.201970400

P1372

Helicobacter pylori-mediated gastric cancer: at the crossroads between hypoxia and inflammation

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Helicobacter pylori (*Hp*)-induced gastric cancer (GC) is a complex representation of host-pathogen interactions and inflammatory responses. *Hp* infection creates hypoxia or the growing gastric tumor itself creates hypoxic microenvironment due to insufficient or inefficient blood supply. Hypoxia promotes oncogenic transformation, causes infiltration of immune cells and is a distinctive common feature in infected gastric epithelium. We identified several mechanisms by which *Hp* promote GC progression by preventing apoptosis, induce metastasis by modulating gastric epithelial cell (GEC) signaling events. Here, we attempted to identify how *Hp*-induced hypoxia alters macrophage behaviour in GC. For this, AGS, MKN45 and NCI-N87 cells were infected and immunoblotting, FACS assay, immunofluorescence microscopy, chromatin immunoprecipitation, DNA-binding assay and luciferase assays were performed. Human PBMC-derived macrophages were used to study GEC-macrophage interactions. Biopsy samples collected from healthy individuals and organoids derived from those tissues were used for further validation. We identified that macrophage and GECs cross-talk and modulate the GC microenvironment depending on the extent of infection.
Keywords: *H. pylori*, gastric cancer, macrophage, apoptosis, hypoxia and inflammation
Financial support: DAE, Govt. of India

P1952

5-aminolaevulinic acid (ALA), enhances heme oxygenase (HO)-1 expression and attenuates tubulointerstitial fibrosis and renal apoptosis in chronic cyclosporine nephropathy

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Background: Cyclosporine-A (CsA) is an immunosuppressant indicated for various immunological diseases; however, it can induce chronic kidney injury. Oxidative stress and apoptosis play a crucial role in CsA-induced nephrotoxicity. The present study evaluated the protective effect of combining 5-aminolaevulinic acid with iron (5-ALA/SFC), a precursor of heme synthesis, to enhance HO-1 activity against CsA-induced chronic nephrotoxicity.
Methods: Mice were divided into three groups: the control group (using olive oil as a vehicle), CsA-only group, and CsA+5-ALA/SFC group. After 28 days, the mice were sacrificed, and blood and kidney samples were collected. In addition to histological and biochemical examination, the mRNA expression of proinflammatory and profibrotic cytokines was assessed.
Results: Renal function in the 5-ALA/SFC treatment group as assessed by the serum creatinine and serum urea nitrogen levels was superior to that of the CsA-only treatment group, demonstrating that 5-ALA/SFC significantly attenuated CsA-induced kidney tissue inflammation, fibrosis, apoptosis, and tubular atrophy, as well as reducing the mRNA level of TNF-α, IL-6, TGF-β1, and iNOS while increasing HO-1.

DOI: 10.1002/eji.201970400

Conclusion: The activity of 5-ALA/SFC has important implications for clarifying the mechanism of HO-1 activity in CsA-induced nephrotoxicity and may provide a favorable basis for clinical therapy.

P1953

Inhibition of NET formation by polydatin protects against collagen-induced arthritis

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Background: Rheumatoid arthritis (RA) is a systematic inflammatory and autoimmune disease with high disability. Increasing evidence has demonstrated that NET formation plays a significant role in the pathogenesis and progression of RA. In this study, we aimed to investigate the effects of polydatin (PD) on NET formation and its effects on disease activity in a collagen-induced arthritis (CIA) mouse model.
Methods: In the presence of PD or vehicle, neutrophils isolated from RA patients or mice were treated with phorbol 12-myristate 13-acetate (PMA) for 4 hours and NET formation investigated. In in vivo experiments, PD was administered (45mg/kg per day) intraperitoneally to collagen-induced arthritis (CIA) mice. The incidence and severity of collagen-induced arthritis were assessed and NET deposition tested.
Results: In vitro, PD significantly suppressed NET formation of neutrophils from RA patients. Consistently, decreased NETs were found in PD treated bone marrow-derived neutrophils from mice. In CIA mouse model, PD treatment delayed the onset of arthritis and attenuated arthritis severity. Compared with vehicle-treated CIA mice, the deposition of NETs in ankle joints was also reduced in PD-treated CIA mice.
Conclusion: In this study, we found that PD treatment markedly inhibited NET formation and protected CIA mice from the development of arthritis. These findings suggest that inhibition of NET formation by PD may serve as a novel mechanism for the treatment of RA.

P1954

NLRP3 inflammasome activation contributes to collagen-induced arthritis via inducing synoviocyte pyroptosis and inflammatory cytokine secretion

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Background: Aberrant activation of NLRP3 inflammasome plays a vital role in the pathogenesis of rheumatoid arthritis (RA). Here, we investigated the effect of NLRP3 inflammasome activation mediated synoviocyte pyroptosis on collagen-induced arthritis (CIA).
Methods: The expression of activated caspase-1 and GSDMD were detected in synovial tissues from CIA mice and RA patients. Collagen-induced arthritis was induced in DBA/1J mice, and then CIA mice were treated with vehicle and MCC950. The incidence and severity of arthritis, the expression of activated caspase-1 and GSDMD in synovial tissues, the serum levels of inflammatory cytokines and the ratio of T cell subsets were tested. In vitro, peripheral blood mononuclear cells (PBMCs) from RA patients stimulated with LPS+ATP were treated with MCC950, and serum levels of inflammatory cytokines examined.
Results: Synovial tissues from CIA mice and RA patients showed higher expression of activated

DOI: 10.1002/eji.201970400

caspase-1 and GSDMD. Interestingly, MCC950 treatment significantly reduced the expression of activated caspase-1 and GSDMD in the synoviocyte and decreased the serum levels of inflammatory cytokines in CIA mice. MCC950 also reduced the ratio of Th1/Th2, but increased the ratio of Treg/Th17 in the blood from CIA mice. As expected, MCC950 treatment decreased the incidence and attenuated the severity of arthritis in CIA mice. In vitro, MCC950 reduced serum levels of IL-1βin LPS+ATP stimulated PBMCs from RA patients.
Conclusions: MCC950 markedly inhibited synoviocyte pyroptosis mediated by NLRP3 inflammasome activation, decreased subsequent inflammatory cytokine secretion and protected against collagen-induced arthritis. This suggests that NLRP3 inflammasome activation mediated synoviocyte pyroptosis contributes to RA pathogenesis.

P1955

The roles and underlying mechanisms of TMEM106A in innate immune response

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Pattern recognition receptors such as Toll like receptors (TLRs) play an important role in the host defense against invading microbial pathogens. Its activation must be precisely regulated, as inappropriate activation or over-activation of TLR signaling pathways may result in inflammatory disorders such as septic shock or autoimmune diseases. Transmembrane protein 106A (TMEM106A) is a cell death- related molecule reported by our research group. It is a type II transmembrane protein, which can inhibit the growth of gastric cancer and liver cancer cells. It abundantly expressed on the surface of mouse macrophages. The antibody against Tmem106a could activate macrophages and promote the secretion of pro-inflammatory cytokines. Our recent studies demonstrated that *Tmem106a* knockout mice were more sensitive than wildtype mice for LPS, CpG and Poly(I:C) inoculation. *Tmem106a*$^{-/-}$ peritoneal macrophages significantly increased the levels of inflammatory factors, such as interleukin-6, tumor necrosis factor-α after LPS, CpG and Poly(I:C) stimulation. Similar results were obtained in *Tmem106a knockdown* RAW264.7 cell lines. Further studies indicated that *Tmem106a* knockout significantly upregulated the expression of CD80 in LPS-induced macrophages, but failed to affect the CD206 level in IL4-stimulated macrophages. These results indicated that *Tmem106a* knockout could activate macrophages and polarize them into M1-like macrophages. Taken together, our studies indicate that TMEM106A may play a negative role in the innate immune process.

P1956

Phosphotase PPM1L prevents excessive inflammatory responses and cardiac dysfunction after myocardial infarction by inhibiting IKKb activation

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Although the inflammatory response triggered by damage-associated molecular patterns (DAMPs) in the infarcted heart tissue after acute myocardial infarction (MI) contributes to cardiac repair, unrestrained inflammation induces excessive matrix degradation and myocardial fibrosis, leading to the development of adverse remodeling and cardiac dysfunction, while the molecular mechanisms that fine tune inflammation post-MI need to be fully elucidated. Protein phosphatase Mg^{2+}/Mn^{2+} dependent 1L (PPM1L), is a member of the serine/threonine phosphatase family. It is originally identified as a negative regulator of stress-activated protein kinase signaling and showed to be involved in the

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DOI: 10.1002/eji.201970400

regulation of ceramide trafficking from Endoplasmic Reticulum to Golgi apparatus. However, the role of PPM1L in myocardial infarction remains unknown. Here, **we found that PPM1L transgenic mice exhibited the reduced infarct size, attenuated myocardial fibrosis and improved cardiac function. PPM1L transgenic mice showed significant lower levels of inflammatory cytokines in cardiac tissue**. Associated with the release of DAMPs in cardiac tissue after MI, macrophages from PPM1L transgenic mice produced less inflammatory cytokine induced by DAMPs, consistently, PPM1L-silenced macrophages showed higher level of inflammatory cytokine production. Mechanically, PPM1L overexpression selectively inhibited the activation of NF-kB signaling in cardiac tissue post-MI and DAMP-triggered macrophages. **PPM1L directly bound IKKb, then inhibited its phosphorylation and activation, leading to the impaired NF-kB signaling activation and suppressed inflammatory cytokine production post-MI**. Thus, our data demonstrate that PPM1L prevents post-MI excessive inflammation and cardiac dysfunction, which sheds new light on the protective regulatory mechanism underlying MI.

P1957

The effect and mechanism of KLF4/KLF7 in FFA-induced adipocyte inflammation

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Objective: Current research has reported that obesity is a chronic inflammatory state, which is closely related with excessive accumulation of free fatty acid(FFA), while the specific mechanism that high level of FFA causes inflammation is not very clear. Thus, our research intended to investigate the role and possible molecular mechanism of Krüppel-like factor 4/Krüppel-like factor 7 (KLF4/KLF7) in the inflammatory signaling pathway activated by FFA.
Methods: qRT-PCR and Western blot were used to detect the mRNA and protein expression levels of KLF4/KLF7, and key inflammation-related factors. ELISA was used to detect the release level of inflammatory cytokines. The triglyceride (TG) and glucose (GLU) testing cassettes were used to detect the TG and GLU levels in culture medium. The CHIP and luciferase reporter assay were used to determine whether KLF4/KLF7 had a transcriptional activation effect on inflammation-related factors.
Results: In the omental tissue of obese individuals (OB), we found that KLF4 expression level was significantly lower than those of the normal weight control (NC) group, while KLF7 expression level was significantly lower than NC group. In mature adipocytes, we found that KLF4 played an important anti-inflammatory role, and FFA promoted the development of inflammation by inhibiting TLR9/KLF4 pathway. While, KLF7 played an important pro-inflammatory role by transcriptional activation on IL-6, and high concentration of FFA promoted the development of inflammation by up-regulating TLR4/KLF7 pathway.
Conclusions: KLF4/KLF7 had opposite effects on regulating FFA-induced adipocyte inflammation.

P1958

TNF-α modulates the adherence and migration of human placenta-derived mesenchymal stromal cell through upregulating their expression of PD-L2

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It has been reported that human placenta-derived MSCs (hPMSCs) can reduce the level of TNF-α in the serum of GHVD. However, the effect of TNF-α on hPMSCs remains unclear. Here, we investigated the effect of TNF-α on the adhesion and migration of hPMSCs. We used the serum from GVHD patient and TNF-α at different concentrations (0, 10, 20 and 30 ng/ml) to stimulate hPMSCs, the

DOI: 10.1002/eji.201970400

adhesion and migration of hPMSCs were analyzed by real time cell analysis (RTCA). RTCA analysis result showed that the adhesion of hPMSCs stimulated with serum from GVHD patients was increased while the migrtion was decreased. TNF-α inhibited the adhesion as well as migration of hPMSCs. Further, in vitro results showed that blocking JAK/STAT or PD-L2 signal pathway with TNF-α/JAK inhibitor or TNF-α/anti-PD-L2mAb upregulation the adhesion and migration of hPMSCs.These experimental findings reflect that TNF-α have an effect of on adhesion and migration of the hPMSCs via JAK-STAT signal pathway and increase the expressin of PD-L2 in hPMSCs surface. The results of this study will be of benefit for the application of hPMSCs in clinical trials.
Keywords: TNF-α, human placenta mesenchymal stem cells(hpMSCs), adherence, migration, JAK-STAT signal pathway, programmed death ligand 2 (PD-L2)

P1959

IL-6 promotes the development of endometrial cancer by inhibiting miR-152

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Objective: Obesity is one of the major known risk factors for endometrial cancer, while the specific molecular mechanism is not very clear. It has been reported that obesity-induced high level of IL-6 can significantly promotes proliferation of endometrial cancer cells. IL-6 promotes the development of pancreatic cancer by inhibiting the expression of miR-152.Thus, our research intended to investigate the role and possible molecular mechanism of miR-152 in the development of endometrial cancer induced by IL-6
Methods: IL-6 recombinant protein stimulates endometrial cancer cells, simultaneous up-regulation of miR-152 expression. qRT-PCR and Western blot were used to detect the mRNA and protein expression levels of downstream target genes. Changes of biological behavior of tumor cells were detected by CCK8 assay, transwells assay and scratch assay. ELISA was used to detect the release level of IL-6.
Results: In endometrial carcinoma patients, the mRNA and protein expression levels of IL-6, CBLB, DDX6 in tumor tissue were significantly higher than the normal endometrial tissue, while the expression level of miR-152 was significantly lower than the normal endometrial tissue. In endometrial cancer cells, IL-6 can inhibit the expression of miR-152 and promote the proliferation, invasion and migration of endometrial cancer cells. miR-152 could significantly reduce the expression levels of target genes CBLB, DDX6 and the ability of proliferation, invasion and migration. Moreover, overexpression of miR-152 could convert the IL-6 effects on promoting the ability of proliferation, invasion and migration.
Conclusions: IL-6 promotes the development of endometrial cancer by inhibiting miR-152.

P1960

Mechanical ring-associated vinculin mediates entotic cell-in-cell formation

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Homotypic cell-in-cell structures (hCICs) are prevalent in a wide range of human tumors, and implicated in tumor development and progression. While adherens junction and actomyosin contraction were identified as two core elements driving hCIC formation, the molecular and structural bases coupling them remain poorly understood. Here, we reported the existence of a ring-like structure interfacing between peripheries of invading and engulfing cells, which is tightly associated

DOI: 10.1002/eji.201970400

with the formation of hCICs. The ring-like structure is a multi-molecular complex consisting of adhesive and cytoskeletal proteins known to be essential for hCICs formation, in which, vinculin (VCL), the so-called mechanical sensor, was highly enriched. The structure may serve to mediate force transmission between cells as indicated by decreased vinculin FRET signals, therefore was termed as mechanical ring (MR) marked by vinculin. The MR dynamics is likely dictating for the accomplishment of hCICs formation as vinculin depletion inhibited hCICs formation associated with compromised MR formation. Truncation analysis identified the head, but not tail, region of vinculin responsible for MR localization and regulating hCICs formation. Functionally, vinculin depletion resulted in enhanced anchorage-independently tumor cell growth associated with inhibited hCICs formation, and lower vinculin expression is correlated with poor relapse-free survival of breast cancer patients. Together, our data suggest a pivotal role of MR-associated vinculin in hCICs formation and tumor suppression.

P1961

Polydatin protects against uricacid nephropathy by inhibiting pyroptosis in the renal tubular epithelial cells

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Background: Uricacid nephropathy (UAN) is one of the best-known gout complications that concerns many patients. Pyroptosis contributes to the pathogenesis and development of many inflammatory diseases. In this study, we aim to investigate the effects of polydatin (PD) on pyroptosis in the renal tubular epithelial cells and its effects on disease development in UAN model mice.
Methods: In vitro, HK2 cell line were incubated with PD, followed by stimulated with uric acid crystal. And then, the release of LDH and the levels of IL-1β and IL-18 were examined. In vivo, UAN mouse models were established by a 2-week daily intraperitoneal injection of potassium oxazine combined with hypoxanthine. Kidney histopathology, and the expression levels of caspase-1 and GSDMD-N domain were detected. And serum uric acid, creatinine and urea nitrogen were also examined after treatment.
Results: In vivo, PD treatment resulted in reduction of serum levels of uric acid, creatinine and urea nitrogen in UAN mice. Importantly, decreased accumulation of uric acid, mitigated renal tubular disorders and reduced renal fibrosis were found in kidneys of PD treated UAN mice. In vitro, uric acid crystal could induce pyroptosis of renal tubular epithelial cells. PD significantly also inhibited renal tubular epithelial cell pyroptosis induced by uric acid crystal.
Conclusions: Overall, we found that PD treatment protected against the development of nephropathy in UAN mice perhaps via suppressing pyroptosis in the renal tubular epithelial cells. These results suggest the involvement of pyroptosis in UAN pathogenesis and reveal that targeting pyroptosis may provide a novel therapeutic avenue for UAN.

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DOI: 10.1002/eji.201970400

P1964

Phenotypic and functional analysis of macrophage subpopulation in patients with sarcoidosis, idioupathic pulmonary fibrosis and non-fibrotic interstitial lung disease

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Sarcoidosis is a granulomatous disease that mostly affects the lungs as well as Idiopathic Pulmonary Fibrosis (IPF). An early and accurate diagnosis of sarcoidosis and IPF remains challenging. In fact, patients with sarcoidosis that progresses to pulmonary fibrosis may share some similar mechanistic and morphologic aberrations with IPF. Furthermore, initial clinical manifestations may be shared with other non-fibrotic interstitial lung disease (ILD). Recently, two macrophage subpopulations have been described in the alveolar space: small (SAM) and large (LAM) alveolar macrophages (AM). Wile playing its protective functions, these tow subpopulations may operate in different ways in the initiation and maintenance of inflammation leading to the development of fibrosis.
The aim of this study was to investigate and compare several functional activities of SAM and LAM within patients with Sarcoidosis, IPF and non-fibrotic ILD. SAM and LAM were isolated from bronchoalveolar lavage (BAL) of patients with confirmed sarcoidosis (14), IPF (6) and non-fibrotic ILD (9). Activation and adhesion surface markers as well as the phagocytosis and superoxide burst activity were measured by flow cytometry.
We observed that, SAM expressed significantly higher activation and adhesion surface markers compared to LAM. HLA-DR, CD11c and CD18 expression was high on SAM and LAM of patients with Sarcoidosis and non-fibrotic ILD compared to IPF. On the other hand, CD38 revealed a higher density on SAM and LAM in sarcoidosis versus the other two groups.
Our results showed a relevant potential of the activation and adhesion markers in the perspective of differential diagnosis of sarcoidosis and IPF.

P3291

Hexokinase-voltage dependent anion channel interaction: Possible role in high glucose/palmitic acid-induced lymphocyte apoptosis

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Impaired immune function have been linked to increased infection in diabetes. One of the possible causes could be increased lymphocyte apoptosis observed in diabetes, a condition characterized by altered metabolism leading to hyperglycemia and hyperlipidemia. Therefore, we investigated lymphocytes apoptosis using mouse peripheral blood lymphocytes (PBL) exposed to high glucose (GL, 40mM)/palmitic acid (PA, 150uM) for 72h, a state which mimics hyperglycemic- and hyperlipidemic-condition respectively. Glucose uptake, activities of hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH), mitochondrial membrane potential (MMP) using flow cytometer, transmission electron microscopy study, co-immunoprecipitation (Co-IP) of HK-voltage-gated anion channel (VDAC) interaction were analysed. Results revealed decreased ($p< 0.001$) glucose uptake, HK and G6PDH activities indicating disrupted metabolic pathway. Fall in MMP ($p< 0.001$) accompanied with chromatin condensation and compromised organelles structure confirms apoptosis. Co-IP showed reduced HK-VDAC interaction with decreased HK expression ($p< 0.001$) however, no

effect was observed on VDAC expression. VDAC plays a central role in mitochondria-mediated apoptosis and its interaction with HK prevent apoptosis. Reduced HK-VDAC interaction observed here suggest their role in GL/PA-induced lymphocytes death possibly explaining the reason behind increased apoptosis in diabetes. Further studies will offer new insight on the mechanism connecting metabolic dysfunction to HK-VDAC interaction that serves as the basis of the apoptotic machinery.
Keywords: Apoptosis, diabetes, glucose, HK-VDAC interaction, palmitic acid, peripheral blood lymphocytes

P2659

Thioredoxin-1 plays important role in estrogen-meidated cardiomyocyte protection in hydrogen peroxide-induced apoptosis

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A large number of researches at home and abroad indicate that cardiovascular disease is closely related to the damage caused by oxidative stress. Oxidative stress is considered to be the main factor which induced apoptosis in numerous cardiovascular diseases. Moreover, thioredoxin is an important disulfide bond reductase which maintains intracellular redox state playing an important role in protecting cells from oxidative stress. Our purpose is to explore by what means Trx1 involves estrogen (E2) mediated cardiomyocyte protection during peroxide-induced apoptosis and its cell signal transduction pathway. MTT assay was used to observe cell viability. The mRNA and protein expressions were assayed by RT-PCR and western blot. DHE dye and Hochest 33342 staining were used to observe apoptosis and reactive oxygen species (ROS). Results showed that E_2 inhibited H_2O_2-induced cell´s injury in a dose-dependent manner. E_2 does-dependently upregulated the expressions of mRNA and protein of Trx1 in normal cardiomyocyte. Exogenous H_2O_2 may induce increased ROS generation of H9c2 cells and apoptosis. E_2 pretreatment can significantly attenuate cardiomyocyte oxidative stress and apoptosis induced by H_2O_2. E_2 can reduce JNK/p38 phosphorylation and Bax activation as well as caspase3 activation, in combination with promoting Akt activation, thereby, exert its cardioprotective effect. Exogenous H_2O_2 can imitate oxidative stress and induce ROS generation of cells. E_2 does-dependently upregulates the expressions of Trx1. Then Trx1 involves in protective effect of E_2 on peroxide-induced cardiomyocyte apoptosis via promoting the activation of anti-apoptotic PI3K/Akt signal pathway, inhibiting the activation of pro-apoptotic JNK/P38 signal pathway and mitochondria-dependent caspase cascade.

P2660

Autophagy inhibits epithelial mesenchymal transition of human bronchial cells induced with chronic arsenic

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Autophagy is a conserved process that maintains the homoeostasis of the cellular metabolism. Although the evidence of autophagy on EMT has been widely observed, the molecular mechanisms are still unclear. The aim of this study is to reveal the role of autophagy on EMT of human bronchial cells induced with chronic arsenic exposure. We found that the morphology of BEAS-2B cells induced with chronic arsenic exposure transformed from epithelial cells to mesenchymal-like cells with long spindle-shaped, and the expression level of epithelial cells marker E-Cadherin was decreased and

DOI: 10.1002/eji.201970400

mesenchymal cells marker protein Vimentin was increased, and the expression of p-ERK1/2, ZEB1 and autophagy proteins Beclin1, LC3-Ⅱ in transformed cells were upregulated compared to normal cells. Further, the E-Cadherin was upregulated with down regulation of Vimentin and the ability of cell proliferation was inhibited after p-ERK1/2 was suppressed by inhibitor U0126. The E-Cadherin was increased and Vimentin was decreased in transformed cells, colony formation was diminished by enhancing autophagic flux with rapamycin treatment. However, these results were reversed with bafilomycin A1 treatment. Overall, our findings indicated that EMT was induced in BEAS-2B cells through MEK/ERK1/2 signal pathway after exposed to arsenic chronically. Cellular migration and proliferation were promoted in transformed cells. Autophagy is a protective mechanism against epithelial mesenchymal transition of human bronchial cells induced with chronic arsenic.
Keywords: Arsenic, Autophagy, EMT, MEK/ERK1/2, BEAS-2B

P2661

The protective effects and its mechanism of EPC- microvesicles on renal microvascular endothelial cell in hypertension nephropathy rats

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The major causes of renal function impaired by hypertension are endothelial cell damaged and regenerative hypofunction. EPCs can improve regeneration function of renal cell of hypoxia residents. And MVs which release from EPCs can regulate endothelial cell survived and proliferated and protect kidney. Our previous studies found that EPC-MVs protect myocardial cell from hypertrophy, apoptosis and oxidative stress. And study found that EPC-MVs contains miR-126, and it can influence biological function of the target cell via PI3K/Akt/eNOS signaling pathway. This study confirms that EPC-MVs can protect renal microvascular endothelial cell of hypertension rats from apoptosis and oxidative stress. On the basis of the previous period, we studied the effect of EPC-MVs on renal microvascular endothelial cell impaired by MMP in vivo. Furthermore, we would verify EPC-MVs can protect the renal microvascular endothelial cell of hypertension nephropathy rats in vitro via PI3K/Akt/eNOS signaling pathway. The outcomes of this project will provide a new strategy for protecting renal function of hypertension nephropathy group by EPC-MVs.

This work was supported by grants from Hainan Key Program of Science &Technology (ZDYF2019193), National Natural Science Foundation of China (81560054).

P2662

Epigallocatechin-3-gallate inhibits the growth and increases the apoptosis of human thyroid carcinoma cells through suppression of EGFR/RAS/RAF/MEK/ERK signaling pathway

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Background: Thyroid cancer is the most common type of endocrine malignancy and the incidence rate is rapidly increasing worldwide. Epigallocatechin-3-gallate (EGCG) could suppress cancer growth and induce apoptosis in many types of cancer cells. However, the mechanism of action of EGCG on the growth of human thyroid carcinoma cells has not been fully illuminated.
Methods: Cell proliferation and viability were detected by EdU and MTS assays. Cell cycle distribution was measured by flow cytometry. Migration and invasion were evaluated by scratch and transwell

DOI: 10.1002/eji.201970400

assays. Apoptotic levels were detected by TUNEL staining and western blotting. The protein levels of EGFR/RAS/RAF/MEK/ERK signaling pathway were detected by western blotting. The in vivo results were determined by tumor xenografts in nude mice. The in vivo proliferation, tumor microvessel density, and apoptosis were detected by immunohistochemistry.
Results: EGCG inhibited the proliferation, viability, and cell cycle progression in human thyroid carcinoma cells. EGCG decreased the migration and invasion, but increased the apoptosis of human thyroid carcinoma cells. EGCG reduced the protein levels of phospho (p)-epidermal growth factor receptor (EGFR), H-RAS, p-RAF, p-MEK1/2, and p-extracellular signal-regulated protein kinase 1/2 (ERK1/2) in human thyroid carcinoma cells. EGCG inhibited the growth of human thyroid carcinoma xenografts by inducing apoptosis and down-regulating angiogenesis.
Conclusions: EGCG could reduce the growth and increase the apoptosis of human thyroid carcinoma cells through suppressing the EGFR/RAS/RAF/MEK/ERK signaling pathway. EGCG can be developed as an effective therapeutic agent for the treatment of thyroid cancer.

P2663

Hydrogen sulfide alleviates lipopolysaccharide-induced diaphragm dysfunction in rats by reducing apoptosis and inflammation through ROS/MAPKand TLR4/NF-κB signaling pathways

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Diaphragm dysfunction is an important clinical problem worldwide. Hydrogen sulfide (H2S) is involved in many physiological and pathological processes in mammals. However, the effect and mechanism of H2S in diaphragm dysfunction have not been fully elucidated. In this study, we detected that the level of H2S was decreased in lipopolysaccharide- (LPS-) treated L6 cells. Treatment with H2S increased the proliferation and viability of LPS-treated L6 cells. We found that H2S decreased reactive oxygen species- (ROS-) induced apoptosis through the mitogen-activated protein kinase (MAPK) signaling pathway in LPS-treated L6 cells. Administration of H2S alleviated LPS-induced inflammation by mediating the toll-like receptor-4 (TLR-4)/nuclear factor-kappa B (NF-κB) signaling pathway in L6 cells. Furthermore, H2S improved diaphragmatic function and structure through the reduction of inflammation and apoptosis in the diaphragm of septic rats. In conclusion, these findings indicate that H2S ameliorates LPS-induced diaphragm dysfunction in rats by reducing apoptosis and inflammation through ROS/MAPK and TLR4/NF-κB signaling pathways. Novel slow-releasing H2S donors can be designed and applied for the treatment of diaphragm dysfunction.

P2664

PEST-containing nuclear protein mediates the proliferation, migration, and invasion of human neuroblastoma cells through MAPK and PI3K/AKT/mTOR signaling pathways

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Background: PEST-containing nuclear protein (PCNP), a novel nuclear protein, is involved in cell proliferation and tumorigenesis. However, the precise mechanism of action of PCNP in the process of tumor growth has not yet been fully elucidated.
Methods: ShRNA knockdown and overexpression of PCNP were performed in human neuroblastoma cells. Tumorigenic and metastatic effects of PCNP were examined by tumor growth, migration, and invasion assays in vitro, as well as xenograft tumor assay in vivo.

Results: PCNP over-expression decreased the proliferation, migration, and invasion of human neuroblastoma cells and down-regulation of PCNP showed reverse effects. PCNP over-expression increased protein expressions of cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, and cleaved poly adenosine diphosphate-ribose polymerase, as well as ratios of B-cell lymphoma-2 (Bcl-2)-associated X protein/Bcl-2 and Bcl-2-associated death promoter/B-cell lymphoma-extra large in human neuroblastoma cells, however PCNP knockdown exhibited reverse trends. PCNP over-expression increased phosphorylations of extracellular signal-regulated protein kinase 1/2, p38, c-Jun N-terminal kinase, as well as decreased phosphorylations of phosphatidylinositol 3-kinase (PI3K), Akt, and mammalian target of rapamycin (mTOR), nevertheless PCNP knockdown exhibited opposite effects. Furthermore, PCNP over-expression significantly reduced the growth of human neuroblastoma xenograft tumors by down-regulating angiogenesis, whereas PCNP knockdown markedly promoted the growth of human neuroblastoma xenograft tumors through up-regulation of angiogenesis.
Conclusions: PCNP mediates the proliferation, migration, and invasion of human neuroblastoma cells through mitogen-activated protein kinase and PI3K/AKT/mTOR signaling pathways, implying that PCNP is a therapeutic target for patients with neuroblastoma.

P2665

Hydrogen sulfide as a novel regulatory factor in liver health and disease

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Hydrogen sulfide (H2S), a colorless gas smelling of rotten egg, has long been recognized as a toxic gas and environment pollutant. However, increasing evidence suggests that H2S acts as a novel gasotransmitter and plays important roles in a variety of physiological and pathological processes in mammals. H2S is involved in many hepatic functions, including the regulation of oxidative stress, glucose and lipid metabolism, vasculature, mitochondrial function, differentiation, and circadian rhythm. In addition, H2S contributes to the pathogenesis and treatment of a number of liver diseases, such as hepatic fibrosis, liver cirrhosis, liver cancer, hepatic ischemia/reperfusion injury, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, hepatotoxicity, and acute liver failure. In this review, the biosynthesis and metabolism of H2S in the liver are summarized and the role and mechanism of H2S in liver health and disease are further discussed.

P2666

Cancer-educated mesenchymal stem cells promote survival of disseminated cancer cells in bone marrow via the induction of PMN-MDSCs expansion

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Bone marrow mesenchymal stem cells (BMSCs) are multipotent stromal cells that can differentiate into a variety of cell types. BMSCs are chemotactically guided toward the cancer cells and contribute to the formation of a cancer microenvironment. The homing of BMSCs was affected by various factors. Disseminated tumor cells (DTCs) in bone marrow are the source of cancer metastasis and cancer relapse. DTC survival is determined by bone marrow microenvironment. Here, we aim to elucidate how cancer-educated BMSCs home systemically to bone marrow and remodel the microenvironment.

DOI: 10.1002/eji.201970400

We highlight the dynamic change by identifying different gene expression signatures in intratumoral BMSCs and in BMSCs that move back in bone marrow. Intratumoral BMSCs acquire high mobility. Intratumoral BMSCs that ultimately home to the bone marrow exhibit an immunosuppressive function. Cancer-educated BMSCs promote the survival of DTCs via expansion of MDSCs in bone marrow. These Ly6G+ MDSCs suppress proliferation of T cells. CXCL5, nitric oxide, GM-CSF produced by cancer-educated BMSCs contribute to the formation of malignant bone marrow microenvironments. Treatment with CXCL5 antibody, the iNOS inhibitor 1400w and GM-CSF antibody reduced MDSC expansion in bone marrow and promoted the efficiency of PD-L1 antibody via reactivation of the T cell response. Our study reveals that cancer-educated BMSCs are the component of the niche for DTCs in bone marrow and that they can be the target for T cell-directed immunotherapy.

P2667

Intestine epithelial exosomes inhibit LPS-induced inflammatory responses via Wnt3a/Fzd-1/β-catenin signaling in macrophage

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As we know, many types of gut bacteria emit LPS, a very commonly found toxin, which might contribute to various inflammatory disorders, even endotoxin-induced septic shock. Intestinal epithelials and macrophages play great roles in maintaining the homeostasis. However, the mechanism of intestine protect against LPS-mediated inflammation is unclear. We found that intestinal epithelial exosomes play great roles on LPS-induced inflammatory response. In LPS-induced septic shock model, mice treated by intestinal epithelial exosomes displayed that their inflammatory response was alleviated and survival time was prolonged, and their bone marrow cell derived macrophages stimulated by LPS produced lower level of IL-6 and TNF-a, expressed lower level of MHC II and CD86. Based on the previous findings, we further investigate the biological function of intestinal epithelial exosomes on LPS-induced inflammation through different levels, such as cell, molecule, animal model and pathological tissue and found that Wnt-beta catenin signal transduction pathway mediated by intestinal epithelial exosomes might crosstalk NF-kB signal pathway mediated by LPS on the macrophages, thus leading to inhibit LPS-induced inflammations. The research may provide theoretical and experimental evidences supporting the application of intestinal epithelial exosomes as a new drug for clinical control and treatment of LPS-induced inflammation in future.

P2668

Epigenetic modifier trichostatin a enhanced osteogenic differentiation of mesenchymal stem cells by inhibiting NF kappa B (p65) DNA binding and promoted periodontal repair in rats

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Aim: To evaluate whether epigenetic modifiers have beneficial effect in treating experimental periodontitis, and mechanisms in regulating cell fate of MSCs in inflammatory micro-environments.
Methods: MSCs were isolated from healthy and inflamed gingival tissues to investigated whether trichostatin A (TSA) could improve osteogenic differentiation and resolve inflammation *in vitro*. The tissue regenerative potentials were evaluated when treated with a temperature-dependent, chitosan- scaffold-encapsulated TSA, in a rat model of periodontitis.
Results: After induction with conditioned medium, TSA treatment increased the osteogenic differentiation potential of inflamed MSCs and healthy MSCs. In addition, interleukin-6 and IL-8 levels

DOI: 10.1002/eji.201970400

in supernatants were significantly decreased after TSA treatment. Moreover, TSA promoted osteogenic differentiation by inhibiting nuclear factor kappa B (p65) DNA binding in MSCs. In rats with experimental periodontitis, 8 weeks after local injections of chitosan-scaffold-encapsulated TSA, histology and micro-computed tomography showed a significant increase in alveolar bone volume and less inflammatory infiltration in treated compared to vehicle-treated rats. The concentrations of interferon-γ and IL-6 were significantly decreased in gingival crevicular fluid after TSA treatment. **Conclusions:** This study demonstrated that TSA had anti-inflammatory properties and could promote periodontal tissue repair*, which* indicated that epigenetic modifiers hold promise as potential therapeutic option for periodontal tissue repair.

P2669

Inhibin B suppresses anoikis resistance and migration through TGF-β signaling pathway in nasopharyngeal carcinoma

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Inhibin B (INHBB), a heterodimer of a common α subunit and a βB subunit, is a glycoprotein belonging to the transforming growth factor-β (TGF-β) family. In this study, we observed INHBB expression was reduced in nasopharyngeal carcinoma (NPC) tissues compared to nontumor nasopharyngeal epithelium tissues, and INHBB was associated with metastasis and clinical progress. Positive expression of INHBB in NPC predicted a better prognosis (overall survival, P = 0.038). However, the molecular mechanisms of INHBB has not been addressed in NPC. We induced anoikis-resistant cells in NPC cell lines under anchorage-independent conditions, then found Epithelial-Mesenchymal transition (EMT) markers changed, cell apoptosis decreased, cell cycle modified, and invasion strengthened in anoikis-resistant NPC cells. These anoikis-resistant NPC cells exhibited decreased expression of INHBB compared with adhesion cells. INHBB was found to influence the above-mentioned changes. In the anoikis-resistant NPC cells with INHBB over-expression, apoptotic cells increased, S phase cells weakened, vimentin, matrix metallopeptidase-9 (MMP-9), and vascular endothelial growth factor-A (VEGF-A) expression were down-regulated, E-cadherin expression was up-regulated, and vice versa in knockdown of INHBB anoikis-resistant NPC cells. Diminished INHBB expression could activate the TGF-β pathway to phosphorylate Smad2/3 and form complexes in the nucleus, which resulted in the above changes. Our results manifested for the first time that INHBB could suppress anoikis resistance and migration of NPC cells by the TGF-β signaling pathway, decrease p53 overexpression, and may serve as a potential biomarker for NPC metastasis and prognosis as well as a therapeutic application.
Keywords: anoikis, inhibin B, metastasis, nasopharyngeal carcinoma, TGF-β

DOI: 10.1002/eji.201970400

P2670

PTEN modulates neurites outgrowth and neuron apoptosis involving the PI3K/Akt/mTOR signalling pathway

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Aim: To explore the role of the PTEN/Akt/mTOR signalling pathway in neurite outgrowth and apoptosis of cortical neurons.
Materials and methods: Cortical neurons were seeded on or adjacent to chondroitin sulfate proteoglycans. The length, number and crossing behaviour of the neurites were calculated. Immunohistochemical staining and TUNEL data were analysed.
Results: Neurites with PTEN silencing exhibited significant enhancements in elongation, initiation and crossing abilities when they encountered chondroitin sulfate proteoglycans in vitro. In addition, this effect disappeared when the PTEN/Akt/mTOR signalling pathway was blocked. Neurites with PTEN silencing exhibited significant enhancements in the survival ability of the neurons.
Conclusions: This experiment proved that PTEN inhibition can promote axonal elongation and initiation, as well as the ability to cross the CSPG border, in cerebral cortical neurons. In addition, PTEN inhibition is useful for protecting the neuron from apoptosis. The PTEN/Akt/mTOR signalling pathway is an important signalling pathway.

P2671

Dual roles of BRD7 in colorectal cancer: from inflammation to cancer

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Objective: BRD7 is a member of bromodomain-containing protein family and encodes a transcriptional regulatory factor. This study aims to explore the roles and mechanism of BRD7 in the progression from inflammation to colorectal cancer (CRC).
Methods: CRC cells, LPS-stimulated MEF cells, DSS-induced acute colitis model and AOM/DSS induced CRC model with BRD7$^{-/-}$ mice were used in this study, and RT-PCR, Western Blot, IHC and series of cell biology methods were performed to investigate the function and mechanism of BRD7 involved in the switch from inflammation to CRC.
Results: The expression of IL-6, TNF-a, CXCL-1 and iNOS as well as the NF-kB(p65) nuclear translocation and the NF-kB reporter activity were significantly increased in LPS-stimulated BRD7$^{-/-}$ MEFs for 1 or 6 hours and DSS-treated BRD7$^{-/-}$ mice compared with BRD7$^{+/+}$ group, and disease activity index (DAI) was also significantly increased in DSS-induced acute colitis in BRD7$^{-/-}$ mice from 1 to 5 days. Furthermore, DAI was also increased in the early stage of 1-5 days, while markedly reduced in the late stages of AOM/DSS-induced colorectal inflammatory carcinoma model in BRD7$^{-/-}$ mice. Surprisingly, none of the BRD7$^{-/-}$ mice developed to tumor (n=20), while ~70% of the wild-type mice developed tumors (n=20). Moreover, overexpression of BRD7 promotes CRC cell proliferation, cell cycle G1/S phase progression and tumor formation in nude mice.
Conclusion: BRD7 plays an anti-inflammatory role during early acute inflammation, while it functions as a pro-inflammatory factor and oncogene in the switch from inflammation to CRC and promotes CRC progression.

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DOI: 10.1002/eji.201970400

P2672

Tumor necrosis factor-alpha upregulated PHLPP1 through activating nuclear factor-kappa B during myocardial ischemia/reperfusion

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Aims: The pleckstrin homology domain leucine-rich repeat protein phosphatase 1 (PHLPP1) specifically regulates phospho-Ser473 of protein kinase B (PKB, Akt) opposing cell survival during myocardial ischemia/reperfusion (I/R). Previous studies demonstrated PHLPP1 expression level was controlled by several mechanisms. However, the regulation mechanism of cardiac PHLPP1 expression following myocardial I/R remains unknown.
Main methods: The current study utilized the mouse model of myocardial I/R injury in vivo and the neonatal rat ventricular myocytes (NRVMs) of hypoxia/reoxygenation (H/R) injury in vitro. Expression of PHLPP1, nuclear factor-kappa B (NF-κB) and pNF-κB were determined by western blot. The expression of PHLPP1 and translocation of NF-κB was assessed by immunofluorescence. Chromatin immunoprecipitation (ChIP) assay was used to detect the binding of NF-κB to the promoter region of phlpp1 gene.
Key findings: Myocardial I/R had no effect on cardiac PHLPP1 expression following I/R (30min/2h) but decreased after 4 hours reperfusion. In vitro, H/R (4h/1h) and tumor necrosis factor-alpha (TNF-α)-stimulation resulted in upregulation of PHLPP1 in NRVMs, which was blocked with etanercept. Yet, H_2O_2-induced oxidative stress had no obvious effect on PHLPP1 expression of NRVMs at early stage but N-acetylcysteine (NAC) pretreatment increased PHLPP1 levels after 4 hours H_2O_2 stimulation. TNF-α and H/R led to both expression and transcriptional activity of NF-κB, accompany with higher expression of PHLPP1. Pyrrolidine dithiocarbamate (PDTC), a NF-κB inhibitor, prevented the response not only in TNF-α-treated cardiomyocytes but also in H/R-treated group.
Significance: These results implicated that TNF-α involved in cardiac PHLPP1 upregulation during reoxygenation, which was mediated by NF-κB transcriptional activity.

P2673

Enhanced PUMA expression induces RIP3 dependent necroptosis by activation of DAI

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PUMA is a BH3-only Bcl-2 family protein that plays an essential role in multiple cellular stress induced apoptosis. Recently, we found that PUMA is also involved in TNF-α induced necroptosis by amplification feedback loop to enhance the activation of RIP3. However, it remains unclear how PUMA expression mediates the activation of RIP3 in the process of necroptosis. In this study, we found that enforced expression of PUMA in LoVo cells by adenovirus infection triggers RIP3, MLKL activation and necroptosis in presence of apoptosis inhibitor, z-VAD. However, the PUMA overexpression induced necroptosis is independent of RIP1. We therefore investigated other RIP3 interacting proteins, and found that DAI, a Z-DNA binding protein, was activated and form a complex with RIP3 by their RHIM domain in response to PUMA overexpression induced necroptosis. Knockdown of DAI abolished the necroptosis induced by PUMA overexpression. Reversely, enhanced expression of DAI sensitized the LoVo cells to PUMA overexpression induced necroptosis, but did not take effect in RIP3

DOI: 10.1002/eji.201970400

deficient LoVo cells. Since DAI is a cytosolic DNA sensor, we also found that PUMA overexpression led to mitochondria DNA release into cytoplasm, which was bound with DAI and triggers its activation. Furthermore, Depletion of Drp1 abrogated the mtDNA release and mitochondria cleavage, as well as necroptosis induced by PUMA overexpression. As to the Dpr1 activation, our data suggested PUMA overexpression interacts with Bcl-XL to release Drp1, and promotes mitochondria translocation. Collectively, our results reveal a novel mechanism of RIP3 activation by PUMA overexpression, which is via Drp1-mtDNA-DAI signal pathway.

P2674

Pathological function of caecum epithelial cell necroptosis

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Necroptosis is an important type of regulated cell death. Although the signaling pathway involved in necroptosis is relatively clear, the exact pathological function of necroptosis remains largely unknown. Our previous results show caecum cells undergo rapid necroptosis in TNF-α-induced system inflammatory response syndrome (SIRS) model in mice, but the pathological function of this phenome in is still unclear. We further proved caecum necroptosis determinate mice survival since cecectomy increases the survival of TNF-α-treated mice in a Rip3- or Mlkl- dependent manner. We also found cecectomy does not affect TNF-α-induced bacteremia and inflammatory cytokines secretion, suggesting inflammatory responses are not direct causes of mice lethal in SIRS. Indeed, cecectomy protects mice from TNF-a-induced cardiac trauma and kidney damage, but show no influence on other organs damage (liver, intestine, lung et al). Besides, increased necroptosis (indicated by p-RIP3 staining) are observed in human acute appendicitis samples, suggest an important pathological role of necroptosis in acute appendicitis.

P2675

Mechanism of TNFAIP2 on atherosclerosis by regulating macrophage functions

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Macrophages are very important cells in AS occurrence. The immune disorder in vascular region caused by macrophage activation is an important pathological process in the formation and development of atherosclerotic plaque. Our results suggest that TNFAIP2 can promote the formation of atherosclerosis by regulating macrophages activation. We found that overexpression of TNFAIP2 protein could promote cell proliferation and inhibit apoptosis. The mRNA levels of phagocytic related genes such as CD36, SR-A, and SR-BI were upregulated. Interfering of TNFAIP2 with siRNA could decrease cell migration. TNFAIP2 regulates macrophage cell functions through enhancing phosphorylation levels of I Kappa-B, P38, ERK, AKT. As a result, the aortic root plaque area of ApoE-/- mice treated with the lentivirus of siRNA-TNFAIP2 was significantly reduced. Those data indicate that TNFAIP2 might plays important roles during the formation of atherosclerosis.

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DOI: 10.1002/eji.201970400

P2676

he mechanisms of PIM kinase inhibitor, SGI-1776 free base, in treating myeloma associated bone disease

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Background and Purpose: The PIM kinase family which includes PIM1, 2 and 3 have an essential role in cell growth and survival. In this work, we investigate the mechanisms of a PIM kinase inhibitor, SGI-1776 free base, of anti-myeloma associated osteolytic bone disease in vitro and in vivo.
Methods: CCK-8 and flow cytometry for measuring growth inhibition. The mechanisms of the drug were explored by western blot and immunofluorescence. IVIS in vivo imaging system, ELISA and micro-CT were used to surveillance in myeloma-mouse models.
Results: SGI-1776 free base induced myeloma cells apoptosis and cell cycle arrest in the G1 phase mediated by decreasing the phosphorylation of PIM kinase targets C-Myc. Also, the expression of Mcl-1, C-Myc, and cyclin D2 were reduced. Apoptosis related proteins Cleaved Caspase-3, 7, 8, 9 and PARP-1 were increasing. Importantly, SGI-1776 free base inhibited osteoclasts formation and resorption though decreasing PIM-1, NFATc1, Cathepsin K and DUSP-1. Calcium coated slides assay, TRAP staining and F-acting ring formation also proved the inhibition effect. Finally, SGI-1776 free base reduced the tumor burden of subcutaneous and disseminated myeloma murine models, prevented myeloma associated bone loss. Moreover, SGI-1776 free base could against the RANLK induced rapid bone loss in non-tumor bearing mice model directly.
Conclusion: Our investigation displays the mechanisms of SGI-1776 free base in treating myeloma associated bone disease. The drug has a dual effect of both anti-myeloma and bone protective effect. These evidence may support the further clinical use of this drug in multiple myeloma.

P2677

BRCT domain containing protein ECT2 is an essential regulator in DNA repair and genome integrity

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Defects of DNA damage response (DDR) will result in genome instability and drive carcinogenesis. Proteins carrying BRCA1 C Terminus (BRCT) domains have evolved to response and resolve DNA damage. However, it remains unknown that whether BRCT domain containing protein ECT2 plays a role in DDR. Here, we report that ECT2 is recruited to DNA damage sites in PARP1-dependent manner. We showed that ECT2 is required for efficient DSB repair and for the recruitment of Ku70 and BRCA1, the essential component of non-homologous end-joining and homologous recombination, respectively. Significantly, ECT2 deficiency disengages the DNA repair process and sensitizes cells to genotoxic insults. Our results suggest that ECT2 is a *bona fide* DNA damage response protein and indicate that ECT2 is an important genome caretaker and a critical regulator of genome stability.

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DOI: 10.1002/eji.201970400

P2678

ECT2 and USP7 shape a feedforward circuit to promote breast carcinogenesis

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A number of guanine nucleotide exchange factors (GEFs) including cell transforming factor ECT2 are believed to drive carcinogenesis through activating distinct oncogenic GTPases. Yet, whether GEF-independent activity of these factors also plays a role in tumorigenesis remains unclear. Here, we report that ECT2 is involved in breast carcinogenesis. Interestingly, we demonstrated that GEF activity-deficient ECT2 is able to alleviate ECT2 depletion associated growth defects in breast cancer cells. ECT2 physically interacts with ubiquitin-specific protease USP7 and functionally promotes USP7 intermolecular self-association, -deubiquitination and -stabilization in a GEF activity-independent manner. Reciprocally, USP7 deubiquitinates and stabilizes ECT2. By means of this, ECT2 and USP7 shape a feedforward circuit to impact on each other. Moreover, we demonstrated that this circuit promotes breast cancer cell survival through fuelling MDM2-p53 signaling pathway, and the expression level of ECT2 correlates with that of USP7 and MDM2 in breast cancer samples. Our study uncovers a GEF-independent role of ECT2 in promoting survival of breast cancer cells, provides a molecular insight for the reciprocal regulation of ECT2 and USP7, and supports the pursuit of ECT2/USP7 as potential targets for breast cancer intervention.

P2679

MiR-429 enhances sensitivity of Burkitt lymphoma cells to chemotherapy drugs via targeting Bmi-1

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Here, the aim of the present study was to investigate whether overexpression of miR-429 leds to the similar effect in Burkitt lymphoma(BL) cells. MiR-429(mimics-miR-429) and its inhibitor(inhibitor-miR-429) were transfected into BL cells Raji and Daudi;cell proliferation, migration, and invasion assays were performed. The sensitivity of Raji and Daudi cells to the first line chemotherapy agents-epirubicin and vinblastine(VCR) were assessed by the MTS method. Cell apoptosis and the intracellular accumulation of Rhodamine-123 were assayed by flow cytometry. Plasmid construction and dual-luciferase reporter assay was carried out to indicate whether Bmi-1 was miR-429 target genes in BL cells. The results showed that, miR-429 suppresses the cell growth, migration, and invasion of Raji and Daudi cells *in vitro*. Luciferase reporter assay confirmed that Bmi-1 mRNA actually had the target sites of miR-429 in Raji and Daudi cells. Furthermore, the expression of Bmi-1, p-Akt, p-gp and MRP1 proteins decreased in mimics-miR-429 transfected BL cells. Growth inhibition and apoptosis rates of miR-429 overexpressing Raji and Daudi cells were higher than those transfected with mimics-miR-429 control alone when treated with epirubicin and VCR. The IC 50 values of epirubicin and VCR were lower for miR-429-overexpressing Raji and Daudi cells than for cells transfected with mimics-miR-429 control. Moreover, the intracellular accumulation of Rhodamine-123 was higher, in miR-429 overexpressing Raji and Daudi cells.These results indicated that miR-429 enhanced the sensitivity of BL cells to chemotherapy agents by altering the expression of multidrug-resistance genes via downregulating Bmi-1/Akt signaling pathway, suggesting that treatment of BL targeting miR-429 might avoid MDR.

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DOI: 10.1002/eji.201970400

P2680

Effects of atorvastatin and/or probucol on recovery of atherosclerosis in high-fat-diet-fed apolipoprotein E-deficient mice

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Introduction: We have investigated the possible effects and mechanism of atorvastatin, and/or probucol,on the atherosclerosis development.
Methods: Apolipoprotein-E-deficient (ApoE$^{-/-}$) mice fed with the high fat diet were randomly divided into 3 groups : placebo, Atorvastatin (10 mg/ kg/d), and atorvastatin (10 mg/kg/d) plus probucol (10 mg/kg/d) groups. C57BL/6J mice were fed with normal diet as the control group . Animals were sacrificed 10 weeks after the intervention. To evaluate the experimental atherosclerosis, blood tests were used for measuring serum lipoprotein profile, Western blots for endoplasmic reticulum (ER) stress protein expression, histological staining of H&E for the plaque area, Sirius-red for the aortic media collagen, Orcein for the integrity of elastic fibers, immunohistology for macrophages, inflammatory cytokines, innate immune receptor TLR-4, transcription factor NF-κB, and atherosclerosis plaques.
Results: Compared with the control group, ApoE$^{-/-}$ mice in the placebo group showed with the significantly (p< 0.05) higher levels of serum TC, TG,LDL and ox-LDL, PERK, GRP78, CHOP, IL-1β, TNF-α and NF-κB, and also the increase in macrophages and the aortic media collagen, and the decrease in the elastic fibers (p< 0.01). Treatment with atorvastatin recovered all these features (p< 0.05) near to the levels in the control group. In addition, the combination of atorvastatin and probucol has shown the significant differences in the reduction of the plaque lesion areas and macrophages (p< 0.05).
Conclusions: Atorvastatin and/or probucol suppress ER stress , which lowers NF-κB, resulting in the recovery of atherosclerosis in the ApoE$^{-/-}$ mouse model.

P2681

Clomiphene citrate induces nuclear translocation of the TFEB transcription factor and triggers apoptosis by enhancing lysosomal membrane permeabilization

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The autophagy-lysosome pathway plays a central role in cellular homeostasis by regulating the cellular degradative machinery. The transcription factor EB (TFEB) regulates the biogenesis and function of both lysosomes and autophagosomes, and enhancement of TFEB function has emerged as an attractive therapeutic strategy for lysosome-related disorders. However, little is known about the role of TFEB activation in regulating the cellular fate. Here, we describe that clomiphene citrate (CC), a selective estrogen receptor modulator, promotes nuclear translocation of TFEB and increases lysosomal biogenesis in HeLa and MDA-MB-231 cells. Treatment with CC inhibits cell viability and causes apoptosis by increasing the release of proteases cathepsin B (CatB) and cathepsin D (CatD) from lysosomes into the cytosol. In contrast, knockdown of TFEB rescues the cells from CC-induced cell death. Furthermore, CC-induced TFEB activation also enhances the autophagy flux in HeLa cells. Knockdown of autophagy-related gene 7 (ATG7) significantly decreases the CC-induced CatB and CatD release and cell death, suggesting that autophagy contributes to the lysosomal membrane

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DOI: 10.1002/eji.201970400

permeabilization (LMP) caused by CC. Altogether, these findings have broad implications for our understanding of TFEB function and provide new insights into CC pharmacological therapy.

P2682

Lentivirus-mediated E1A-activated gene repressor (CREG) gene Inhibitory effect of photodamage on apoptosis of 661W cells and its mechanism

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The E1A-stimulated genes (CREG) are a novel transcription-related regulator, it can activate transcriptional activation of target genes by repressing adenovirus E1A and E2F, play a role in proliferation and differentiation in cells. It has been confirmed that over-expressed CREG can inhibit the apoptosis of vascular smooth muscle cells, endothelial cells and other cells, but the application and research of over-expression of CREG gene in the field of photoreceptor damage of retinal photoreceptors remains to be explored. In this study, by constructing a lentiviral expression vector, the over-expressed CREG was transfected into the retinal photoreceptor-like cell-661W cells, and the cells were induced to undergo apoptosis under light stimulation, and the photodamage was observed to induce apoptosis of the cell carrying CREG. The changes of apoptosis-related proteins and related signaling pathways were detected, the results showed that the expression levels of apoptosis proteins Caspase3/8/9 and Bax decreased and anti-apoptotic protein bcl-2 was increased after photodamage in 661W cells carrying CREG gene, and 661W cells transfected with CREG were pretreated with JNK inhibitor SP600125, p38 inhibitor SB203580 and MAPKK inhibitor PD98059. The changes of phosphorylated p38, JNK and non-phosphorylated JNK mitogen-activated protein kinase were detected the activation of the three factors was inhibited. These results demonstrate that lentivirus-mediated E1A-activated gene repressor (CREG) gene plays an important role in inhibiting apoptosis of retinal photodamaged cells also suggested that the over-expressed CREG gene can inhibit the apoptosis of photodamaged cells by regulating the expression of JNK, p-JNK and p-p38 proteins in the MAPK signaling pathway.

P2683

The effect of CREG gene-modified bone mesenchymal stem cells in retinal ganglion cells differentiation and apoptosis

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Retinal degeneration like retinitis pigmentosa, age-related macular degeneration cause a large number of apoptosis of visual cells and seriously endangers the visual function of patients. Bone Mesenchymal Stem Cells (BMSCs), which can be induced into retinal ganglion cells (RGCs), can be an ideal substitute for its self-renewal and multilineage differentiation ability. However, the apoptosis of large number of transplanted BMSCs become a major bottleneck hindering the application of stem cell transplantation technology. Cellular repressor of E1A-stimulated genes (CREG), cloned from the Hela cell cDNA library, is a new type of transcription related regulator which widely expressed in almost all mature and differentiated tissues. Previous study have demonstrated that Over-expression of CREG gene can fight against apoptosis induced by ischemia, hypoxia and other conditions through a variety of signaling pathways, and promote the differentiation of stem cells into target cells. Herein, we

DOI: 10.1002/eji.201970400

constructed the BMSCs transfected with CREG gene which can be induced into RGCs expressed neuron-like markers; and meanwhile the ability to resist apoptosis of BMSCs which CREG over-expressed increased significantly. This way which can not only accelerate the RGCs differentiation but improve the induced-RGCs against apoptosis may be an innovative approach for treatment of retinal degenerative diseases.

P2684

Study on the mechanism of LPS-induced apoptosis in normal human colonic epithelial cells

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Mucosa damage induced by LPS associated with epithelial cell apoptosis and defective of tightly protein, but the mechanism remains to be further studied. In the present study, the effect of LPS on NCM460 cell proliferation or apoptosis and the expression of ZO-1 and Occludin were measured. The results showed LPS induced apoptosis and inhibited the expression of tight junction proteins which related with mucosal barriers injury. Then apoptotic mechanisms induced by LPS were elucidated. The results showed (1) LPS induced cell apoptosis through death receptors pathway. (2) LPS was recognized by TLR4 to initiate a MyD88-dependent pathway. (3) LPS induced the secretion of TNF-α which promote apoptosis. In order to further study the transcription factors in LPS induced apoptosis, we investigated mTORC1, NF-κB and STAT1 signaling. The datas showed (1) LPS activated mTORC1 pathway, NF-κB and STAT1, (2) Rapamycin impaired LPS induced-STAT and NF-κB activation and the higher expression of MyD88/FADD/TNFR1 and the mRNA expression of FLIP, (3) Rapamycin decreased the secretion of TNF-α and the activity of Caspase 3/8 induced by LPS.
In conclusion, LPS could induce NCM460 apoptosis through MyD88 dependent pathway. LPS promoted the secretion of TNF-α by TLR4/MyD88 pathway. TNF-α induced cell apoptosis by TNFR1/FADD/Caspase 8 pathway. mTORC1 regulated apoptosis of NCM460 cells induced by LPS by mediating phosphorylation of NF-κB and STAT1, the protein synthesis and gene transcription related with apoptosis pathway. All these results provided some new ideas for understanding cellular and molecular mechanism of LPS induced apoptosis and mucosal barriers.

P2685

The endothelial cells (ECs) biology in a rat model of cerebral arteriovenous malformation

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In this study, we successfully established a rat model of arteriovenous malformation which can be used to study the biology of endothelial cells (ECs) by anastomosing the common carotid artery with the external jugular vein. The digital subtraction angiography (DSA) was used to confirm whether the anastomotic stoma was unobstructed at the 42nd day after fistula creation. We confirmed morphological similarities between the model and cerebral arteriovenous malformation(cAVM) by HE staining. Meanwhile we found mitochondria and endoplasmic reticulum swelling and lysosomes appeared in ECs by transmission electron microscopy. The findings above strongly prove that the increased flow in cAVMs results in the vascular changes, and this is a vital base for the model to be used in cAVMs research. To characterize and understand the biology of arteriovenous malformation

DOI: 10.1002/eji.201970400

endothelial cells (AVM-ECs), the endothelial cells of arterialized vein (AV-ECs) in the model were isolated and cultured successfully by pasting tissue blocks in vitro. The immunofuorescence staining was conducted to verify the ECs through vWF, a ECs' specific marker. The MTT and cell scratch test were undergone and the results revealed that the proliferation of AV-ECs is greater than endothelial cells of normal vein (NV-ECs).

P2686

The role of Sirtuin1 in ATL cells against apoptosis

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Adult T cell leukemia cells can inhibit apoptosis and immune escape. The mechanism could be associated with SIRT1, which was higher expression in ATL cells. We explored the role of SIRT1 involved in resistance of ATL cells to apoptosis.
Methods: The viability of ATL cells treated with recombinant protein FasL was detected by MTS assay. Cell apoptosis treated with FasL was analyzed by Flow cytometry. The expression of SIRT1, c-FLIP and Ku70 in vitro and in vivo was detected by immunoblotting. The interaction of SIRT1 with Ku70 was detected by co-IP.
Results: The survival rate of ATL cells treated with (>10ng/ml) FasL was higher than that of Jurkat cells. The apoptotic rate of ATL cells treated with FasL(5,10,100ng/ml) maintained below 20%, which was lower than that of control(42%~80%). In SIRT1 knockdown of ATL cells with FasL treatment, it exhibited an increase of apoptosis rate by 18±3.5%. The expression level of SIRT1, ku70 ,c-FLIP was increased in HTLV-1 infected humanized NOG mouse T cells and ATL cells. c-FLIP interacted with Ku70 to execute anti-apoptosis function. Knockdown of SIRT1 increased expression level of Acetyl-ku70, but decreased expression level of c-FLIP.
Conclusion: In ATL cells, Sirtuin1 upregulated the expression of c-FLIP to resist FasL-mediated cell apoptosis by deacetylation of ku70.
Keywords: ATL, Sirtuin1, c-FLIP, ku70, apoptosis

P2687

SHQ1 is a novel ERS response gene and inhibits tumor survival and chemoresistance via regulating ERS response in a positive feedback loop

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GRIMs (Genes associated with Retinoid and Interferon induced Mortality) are a group of genes associated with tumor cell death induced by interferon and retinoic acid. GRIM1, known as SHQ1, has a strong effect on growth suppression and cell death. However, the mechanism is still unclear. In this study, we used clinical samples of hepatocellular carcinoma, tumor-bearing mouse models and hepatoma and breast cancer cell lines to explore the role and molecular mechanism of SHQ1 in tumor. Clinical samples results showed that expression of SHQ1 was decreased in intratumoral tissues in comparison with peritumoral tissues. Tumor-bearing models showed that SHQ1 suppresses the growth of the subcutaneous tumor. Mechanistically, SHQ1 is not only responsive to ER-stress-sensors including p50ATF6 and XBP1s in the UPR pathway through a novel ERSE located on the promoter of SHQ1, but also interacts with the ER chaperone GRP78 and prevents its degradation mediated by ubiquitination, making cells more sensitive to ER stress and chemotherapy drugs. Our findings indicated that SHQ1 is a novel ER stress response gene, which served as a feedback loop to enhance

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DOI: 10.1002/eji.201970400

ER stress response through stabilizing GRP78. In a consistent ER stress situation caused by tumor microenvironment, SHQ1 plays role as a tumor suppressor.

P2688

ZCCHC4/AL133467.2/gamma H2AX nuclear complex regulates hepatocellular carcinoma chemotherapy resistance via modulating DNA damage response

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Chemotherapy resistance is one of the main setbacks restraining the treatment efficiency in hepatocellular carcinoma(HCC) patients and the nuclear messages underlying chemoresistance remain mysteries. Here we identified an RNA binding protein ZCCHC4, which mainly localized in the nuclei of HCC cells. The expression of ZCCHC4 was increased in HCC tumor tissues and high ZCCHC4 was correlated with poor prognosis of HCC patients. Consistently, ZCCHC4 promoted tumor progression in mice and contributed to short survival time. Transcriptome analysis indicated that ZCCHC4 was critical for HCC cell survival and viability. Furthermore, ZCCHC4 decreased the sensitivity of HCC cells to DNA damage chemotherapy agents *in vitro* and *in vivo*. Deeply, we found that ZCCHC4 inhibited DNA damage related signals when under oxaliplatin treatment. Interestingly, ZCCHC4 bound to DNA damage marker gamma H2AX and the binding intensity strengthened with the time of oxaliplatin treatment. Then we identified an intergenic long noncoding RNA AL133467.2 which bound to ZCCHC4 and counteracted the effect of ZCCHC4 in oxaliplatin induced DNA damage and apoptotic process. More intriguingly, ZCCHC4, AL133467.2 and gamma H2AX formed a nuclear complex in HCC cells, whose interacting module needed to be further explored. Worth mentioning, beyond HCC, ZCCHC4 was also increased in lung cancer(LC), pancreatic cancer(PC) and colon cancer(CC), and correlated with poor prognosis in LC and PC patients. ZCCHC4 also exerted apoptotic-resistant effect in LC, PC and CC cells. We demonstrate a critical role of ZCCHC4/AL133467.2/gamma H2AX nuclear complex in chemotherapy resistance and propose prognostic and therapeutic implication of ZCCHC4.

P2689

Deficient RACK1 synergizes with all-trans retinoic acid induced apoptosis in human acute promyelocytic leukemia cells

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As a classic differentiation agent, all-trans retinoic acid (ATRA) has been widely used in treatment of acute promyelocytic leukemia (APL). However, the clinical application of ATRA has strict limitations, for its severe side effect. The scaffold protein RACK1 (Receptor for activated C kinase 1) which regulating multiple signaling pathways, was proposed to contribute to the survival of leukemic progenitor. But it remains unclear whether it also involves in the oncogenic growth of APL. In the

present study, we demonstrated that down-regulating the endogenous RACK1 expression synergized with ATRA promoting the NB4 and HL-60 APL cells death without effect on cell differentiation induced by ATRA. Interestingly, knocking-down RACK1 combined with ATRA could mainly induce cells undergo apoptosis. It's extremely different with Idarubicin inducing cells necrosis, which is extensively used in the treatment of acute myelocytic leukemia (AML). Further exploration revealed that the lysosome-autophagy pathway is likely to responsible for the anti-apoptosis ability of RACK1. Taken together, our findings indicated that RACK1 was essential in maintaining the malignant features of APL, and targeting RACK1 may have promising therapeutic implications in the treatment of APL.

P2690

SGLT2 inhibitor empagliflozin negatively regulates IL-17A/IL-23 axis-mediated inflammatory responses in type 2 diabetes with NAFLD via mTOR autophagy pathway

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With aging of population and lifestyle changes, the incidence of type 2 diabetes mellitus (DM) is upgrading. Non-alcoholic fatty liver disease (NAFLD) is one complication of DM. Empagliflozin is one kind of sodium-dependent glucose transporters 2 (SGLT2) inhibitors, which reduces blood glucose levels via inhibiting glucose reabsorption. In addition to reducing blood glucose and uric acid levels, empagliflozin also could significantly inhibit liver injury. We selected high-fat diet combining with streptozotocin injection to induce DM, and treated mice were gavaged with empagliflozin. Compared with DM mice, blood glucose and ALT levels of empagliflozin-treated mice were significantly lower. The liver autophagy level of empagliflozin-treated mice was significantly higher than DM mice, and protein expression levels of AMPK/mTOR pathway were consistent with autophagy level. Inhibiting AMPK/mTOR/autophagy pathway, empagliflozin couldn't significantly reduce blood glucose and ALT activities. Compared with DM mice, The autophagy level of liver macrophages from empagliflozin-treated mice was also significantly higher. Macrophages are the predominant source of IL-23, and IL-23 is required for the production of IL-17A by T cells. The expression levels of IL-17A/IL-23 axis-related molecules from empagliflozin-treated mice was significantly lower than those from DM mice, with fewer proinflammatory M1 macrophages and Th17 cells. γδT and $CD4^+$T cells from empagliflozin-treated mice produced less IL-17A than those from DM mice. Neutralizing IL-17A and IL-23, ALT and IL-17A/IL-23 axis-related molecules levels significantly reduced. However, there was no significant changes in autophagy levels. In summary, empagliflozin could enhance liver macrophages autophagy by AMPK/mTOR pathway and inhibit IL-17A/IL-23-mediated inflammatory responses.

P2691

Visualization of perforin/gasdermin/complement-formed pores in real cell membranes using atomic force microscopy

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Different types of pores ubiquitously form in cell membranes, leading to various types of cell death that profoundly influence the fate of inflammation and the disease status. However, these pores have

DOI: 10.1002/eji.201970400

never truly been visualized to date. Atomic force microscopy (AFM), which is emerging as a powerful tool to analyze the mechanical properties of biomolecules and cells, is actually an excellent imaging platform that allows biological samples to be visualized by probing surface roughness at the level of atomic resolution. Here, membrane pore structures were clearly visualized using AFM. This visualization not only describes the aperture and depth of the pore complexes but also highlights differences among the pores formed by perforin and gasdermins in tumor cell membranes and by complement in immune cell membranes. Additionally, this type of visualization also reveals the dynamic process of pore formation, fusion, and repair.

P2692

Phagocyte respiratory burst activates macrophage erythropoietin signalling to promote acute inflammation resolution

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Inflammation resolution is an active process, the failure of which causes uncontrolled inflammation which underlies many chronic diseases, such as cardiovascular disease, arthritis and cancer. Therefore, endogenous pathways that regulate inflammation resolution are fundamental and of wide interest. Inflammation increases local oxygen demand and deplets local molecular O_2, resulting in inflammatory hypoxia that conversely affect inflammation outcome. However, mechanisms underlying inflammatory hypoxia-stimulated inflammation resolution remain elusive. **Here, we demonstrated that phagocyte respiratory burst-induced hypoxia activated macrophage erythropoietin signalling to promote acute inflammation resolution.** This signalling is activated following acute but not chronic inflammation. Pharmacological or genetical inhibition of the respiratory burst suppresses hypoxia and macrophage erythropoietin signalling. Macrophage-specific erythropoietin receptor-deficient mice and chronic granulomatous disease (CGD) mice, which lack the capacity for respiratory burst, display impaired inflammation resolution, and exogenous erythropoietin enhances this resolution in WT and CGD mice. Mechanistically, erythropoietin increases macrophage engulfment of apoptotic neutrophils via peroxisome proliferator activated receptor-γ, promotes macrophage removal of debris and enhances macrophage migration to draining lymph nodes. In addition, in diet-induced obese mice, in line with the delayed resolution of inflammation, the induction of macrophage EPO signalling was significantly reduced relative to normal mice. Exogenous EPO induced macrophage EPO signalling and promoted inflammation resolution in obese mice. Together, our results provide evidences of an endogenous pathway that regulates inflammation resolution, with important implications for treating inflammatory conditions.

P2693

Receptor-interacting protein kinase 3 mediates myeloid-derived suppressor cell-potentiated colorectal carcinogenesis

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As a central regulatory molecule for necroptosis, the role of receptor-interacting protein kinase 3 (RIPK3) in tumor immunity is largely unknown. Here, we found the significantly decreased RIPK3 in

DOI: 10.1002/eji.201970400

the microenvironment of colorectal cancer (CRC) correlated with the accumulation of myeloid-derived suppressor cells (MDSCs). Absence of RIPK3 boosted the infiltration and immunosuppressive activity of MDSCs in vitro and in vivo, and carcinogenesis is accelerated after RIPK3-deficient bone marrow cell chimerism. Mechanistically, the reduction of RIPK3 in MDSCs and colorectal cancer cells elicited NF-κB /COX-2 axis which catalyses the synthesis of prostaglandin E2 (PGE2). PGE2 exacerbated the immunosuppressive activity of MDSCs and accelerated tumor growth via increasing arginase 1 (Arg-1), blunting CD8+ T cell activation. Moreover, PGE2 negatively regulated RIPK3 via the PKA-CREB signaling pathway, forming a RIPK3-PGE2 circuit in MDSC to potentiate malignancy. Furthermore, inhibitors targeting COX-2 and EP2 blunted the immunosuppressive activity of MDSC and carcinogenesis. Patient databases also delineated the correlation of RIPK3 and COX-2 expression with colorectal cancer survival. Our findings demonstrate a novel signaling circuit involving RIPK3 and PGE2 enhances accumulation and immunosuppressive activity of MDSCs, implicating its potential as a therapeutic target in anticancer immunotherapy.

P2694

A pan-RAF inhibitor LY3009120 inhibits necroptosis by preventing phosphorylation of RIPK1 and alleviates dextran sulfate sodium (DSS)-induced colitis

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A dramatic increase in the incidence of inflammatory bowel disease (IBD) has been observed in the past 2 decades, mainly in developed countries but also in developing regions. Necroptosis has been found to play an important role in the pathogenesis of inflammatory bowel disease (IBD), suggesting its inhibitors are promising in clinic. However, clinical drugs targeting necroptosis are seriously lacking. Through screening a clinical compound library that contains 611 inhibitors, a pan-RAF inhibitor LY3009120 was found to be promising as a necroptosis inhibitor. LY3009120 inhibited necroptosis *in vitro*, and its inhibition against necroptosis was independent of its well known activity to inhibit RAF. Surprisingly, LY3009120 prevented phosphorylation of RIPK1 and subsequently phosphorylation of RIPK3 and MLKL which happened during necroptosis. *In vivo*, LY3009120 significantly alleviated DSS-induced colitis as indicated by prevention of body weight loss, colon shortening and decreased mortality. Furthermore, LY3009120 inhibited necroptosis of intestinal epithelial cells and prevented intestinal barrier function loss. Consistently, LY3009120 decreased DSS-induced colonic inflammation, as indicated by decreased infiltration of macrophages and neutrophils, and decreased colonic TNF-α, IL-6 and IL-1β level in DSS treated mice. These results indicate that an anti-cancer pan-RAF inhibitor LY3009120 is a necroptosis inhibitor and may serve as a potential therapeutic drug for colitis.

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DOI: 10.1002/eji.201970400

P2695

Autoinflammatory mutation in NLRC4 reveals a leucine-rich repeat (LRR)-LRR oligomerization interface

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Objective: Here we investigate the mechanism by which a novel mutation in the leucine-rich repeat (LRR) domain of NLRC4 (c.G1965C, p.W655C) contributes to autoinflammatory disease.
Methods: We studied 2 unrelated patients with early-onset macrophage activation syndrome harboring the same de novo mutation in NLRC4. In vitro inflammasome complex formation was quantified by using flow cytometric analysis of apoptosisassociated speck-like protein containing a caspase recruitment domain (ASC) specks. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 techniques and lentiviral transduction were used to generate THP-1 cells with either wild-type or mutant NLRC4 cDNA. Cell death and release of IL- 1b/IL-18 were quantified by using flow cytometry and ELISA, respectively.
Results: The p.W655C NLRC4 mutation caused increased ASC speck formation, caspase-1-dependent cell death, and IL-1b/IL- 18 production. ASC contributed to p.W655C NLRC4-mediated cytokine release but not cell death. Mutation of p.W655 activated the NLRC4 inflammasome complex by engaging with2 interfaces on the opposing LRR domain of the oligomer. One key set of residues (p.D1010, p.D1011, p.L1012, and p.I1015) participated in LRR-LRR oligomerization when triggered by mutant NLRC4 or type 3 secretion system effector (PrgI) stimulation of the NLRC4 inflammasome complex.
Conclusion: This is the first report of a mutation in the LRR domain of NLRC4 causing autoinflammatory disease. c.G1965C/p.W655C NLRC4 increased inflammasome activation in vitro. Data generated from various NLRC4 mutations provides evidence that the LRR-LRR interface has an important and previously unrecognized role in oligomerization of the NLRC4 inflammasome complex. (J Allergy Clin Immunol 2018;nnn:nnn-nnn.)

DOI: 10.1002/eji.201970400

P2696

The molecular mechanism underlying methionine enkephalin on the glycolysis of glioblastoma via GLYAT1/GLS/c-Myc pathway

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Glioblastoma (GBM) is the most common and deadly brain tumor. Despite aggressive treatment, GBM almost inevitable recurs. Therefore, overcoming the resistance of residual tumors against adjuvant therapy is urgently needed for GBM treatment. Warburg effect, one of the metabolic alterations, occurs in cancer cells that influence switches to glycolysis from oxidative phosphorylation, using opportunistic modes of nutrient acquisition, and increasing lipid biosynthesis. Emerging evidences have proved that glutamine metabolism is the main process in regulation the glycolysis pathway, which supply both energy production and fatty acid synthesis in cancer cells. In addition, the significant molecule of glutamine metabolism is glutaminase(GLS), which has been put forward as the future therapeutic perspectives and modulators in GBM treatment. Our research has revealed that MENK could inhibited the proliferation and migration of GBM cells, and enhance the apoptosis of GBM cells. Furthermore, the results from next generation sequencing(NGS), luciferase assay, ChIP and mass spectrometry shown that lncRNA GLYAT1 may bond with E2F1 and suppress the glycolysis of GBM cells via GLS/Myc pathway.

P2697

Expression profiling identified IL-8 as a regulator of homotypic cell-in-cell formation

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Homotypic cell-in-cell (CIC) structures forming between cancer cells were proposed to promote tumor evolution via entosis, a nonapoptotic cell death process. However, the mechanisms underlying their formation remained poorly understood. We performed a microarray analysis to identify genes associated with homotypic CIC formation. Cancer cells differing in their ability to form homotypic CIC structures were selected for the study. Association analysis identified 73 probe sets for 62 candidate genes potentially involved in CIC formation. Among them, twenty-one genes were downregulated while 41 genes were upregulated. Pathway analysis identified a gene interaction network centered on IL-8, which was upregulated in high CIC cells. Remarkably, CIC formation was significantly inhibited by IL-8 knockdown and enhanced upon recombinant IL-8 treatment, which correlated with altered cell-cell adhesion and expression of adhesive molecules such as P-cadherin and γ-catenin. Together, our work identified IL-8 as a positive regulator of homotypic CIC formation via enhancing intercellular adhesion.

P2698

Cholesterol inhibits entotic cell-in-cell formation and actomyosin contraction

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DOI: 10.1002/eji.201970400

Cell-in-cell structure is prevalent in human cancer, and associated with several specific pathophysiological phenomena. Although cell membrane adhesion molecules were found critical for cell-in-cell formation, the roles of other membrane components, such as lipids, remain to be explored. In this study, we attempted to investigate the effects of cholesterol and phospholipids on the formation of cell-in-cell structures by utilizing liposome as a vector. We found that Lipofectamine-2000, the reagent commonly used for routine transfection, could significantly reduce entotic cell-in-cell formation in a cell-specific manner, which is correlated with suppressed actomyosin contraction as indicated by reduced β-actin expression and myosin light chain phosphorylation. The influence on cell-in-cell formation was likely dictated by specific liposome components as some liposomes affected cell-in-cell formation while some others didn't. Screening on a limited number of lipids, the major components of liposome, identified phosphatidylethanolamine (PE), stearamide (SA), lysophosphatidic acid (LPA) and cholesterol (CHOL) as the inhibitors of cell-in-cell formation. Importantly, cholesterol treatment significantly inhibited myosin light chain phosphorylation, which resembles the effect of Lipofectamine-2000, suggesting cholesterol might be partially responsible for liposomes' effects on cell-in-cell formation. Together, our findings supporting a role of membrane lipids and cholesterol in cell-in-cell formation probably via regulating actomyosin contraction.
Keywords: Cell-in-cell, Cholesterol, Phospholipid, Liposomes, Lipofectamine-2000, Actomyosin

P2699

Subtype-based prognostic analysis of cell-in-cell structures in early breast cancer

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Though current pathological methods are greatly improved, they provide rather limited functional information. Cell-in-cell structures (CICs), arising from active cell-cell interaction, are functional surrogates of complicated cell behaviors within heterogeneous cancers. In light of this, we performed the first subtype-based CICs profiling in human breast cancers by “EML” multiplex staining method, and accessed their values as prognostic factors by Cox univariate, multivariate and nomogram analysis. CICs were detected in cancer specimens but not in normal tissues. Totally 5 types of CICs were identified with one homotypic subtype (91%) and 4 heterotypic subtypes (9%). Overall CICs (oCICs) significantly associated with patient overall survival (OS) (P=0.011) as an independent protective factor (HR=0.423, 95% CI, 0.227-0.785; P=0.006). Remarkably, TiT, TiM and MiT were also independent prognostic factors. Among them, higher TiT, from homotypic cannibalism between tumor cells, predicted longer patient survival (HR=0.529, 95% CI, 0.288-0.973; P=0.04) in a way similar to that of oCICs; conversely, the presence of MiT (macrophage inside tumor cell) predicted death hazard of 2.608 (95% CI, 1.344-5.063; P=0.05). Moreover, each CICs subtype tended to preferentially affect different categories of breast cancer. Furthermore, nomogram analysis indicated that CICs impacted patient outcomes in contributions comparable (for oCICs, TiT and TiM), or even superior (for MiT), to TNM stage and breast cancer subtype, and incorporating CICs improved nomogram performance. Together, we propose CICs profiling as a valuable way for prognostic analysis of breast cancer, and CICs subtypes, such as MiT, may serve as a type of novel functional markers assisting clinical practices.

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DOI: 10.1002/eji.201970400

P2700

CDKN2A inhibits formation of homotypic cell-in-cell structure

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Cell-in-cell (CIC) structures, characterized by enclosure of one or more cells within another cell, were extensively documented in human cancers. Homotypic cell-in-cell structures always along with cell death of inner cell. Although elevated CIC formation was found in cancers with CDKN2A inactivation, a causal link between them remains to be established. We reported here that inhibiting CDKN2A expression effectively promoted homotypic CIC formation, whereas ectopic overexpression of p16INK4a or p14ARF, two proteins encoded by CDKN2A gene, significantly suppressed CIC formation in MCF7 cells. The regulation of CIC formation by CDKN2A was tightly correlated with subcellular redistribution of E-cadherin, F-actin rearrangement and reduced phosphorylation of myosin light chain 2 (p-MLC2), consistent with which, CDKN2A expression imparted cells winner/outer identity in competition assay. Moreover, CIC formation negatively correlates with p16INK4a expression in human breast cancers. Thus, our work identifies CDKN2A as the first tumor suppressor whose inactivation promotes homotypic CIC formation in human cancer cells.

Immune Cell Trafficking and Homing

O026

The development and function of thymic portal endothelial cells in thymic homing and egress

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The specialized differentiation of blood vascular endothelial cells (ECs) in lymphoid tissues is believed essential for cellular trafficking. However, the nature and the differentiation programme of specialized thymic ECs controlling thymic trafficking remain poorly understood. We recently identified a specialized thymic EC population (P-selectin+Ly6C−) that was predominantly associated with the thymic gate perivascular spaces (PVSs) and featured for thymic trafficking at the transcriptome level. This subset of thymic ECs was named thymic portal endothelial cells (TPECs). Using bone marrow chimeric mice, conditional gene-deficient mice, LTβR agonistic antibody or LTβR-hIg fusion protein blockade, we found that lymphotoxin beta receptor (LTβR) signaling directly controls TPEC development and homeostasis. Furthermore, deficiency of T cells but not dendritic cells or B cells resulted in reduced TPEC population, suggesting T cells deliver the ligands (LT and LIGHT) of LTbR for signaling control of TPECs. Interestingly, in the disruption of LT/LIGHT-LTbR mediated T-EC interaction, both thymic homing of hematopoietic progenitor cells and thymic egress of mature thymocytes were significantly reduced. To investigate how TPECs could regulate both thymic homing and egress, we did single cell RNA-sequencing of thymic ECs and two new subsets of TPECs were revealed, suggesting distinct TPEC subsets may control thymic homing and egress separately.

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DOI: 10.1002/eji.201970400

O029

Eosinophils induced by radiation contribute to cytotoxic T lymphocyte recruitment and improve immunotherapy

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Recent studies have strongly suggested that the prognosis of cancer immunotherapy is largely dictated by intratumoral $CD8^+$ T cell infiltration and the nature of the individual tumor microenvironment (TME). By inflaming the TME to favor $CD8^+$ T cell immunity, radiation is now widely considered a neoadjuvant for immunomodulation. While the local role of radiation is attributed to the type I interferon-mediated TME switch, the systematic role of tumor-focused radiation in immune regulation remains to be explored. In various animal models, we observed that radiotherapy (RT) creates a niche in tumors to recruit eosinophils, and this recruitment is closely associated with enhanced $CD8^+$ T cell infiltration. Eosinophil depletion dampens $CD8^+$ T cell infiltration and diminishes the overall antitumor efficacy of radiation, which demonstrates the causative role of eosinophils in promoting T cell-based antitumor effects. This eosinophil-$CD8^+$ T cell association was also identified in our retrospective cohort studies of radiation-treated cancer patients. Furthermore, in animal models, we showed that radiation enhanced the intratumoral infiltration of transferred cytotoxic T lymphocytes (CTLs) and chimeric antigen receptor (CAR) T cells, and the pattern of enhanced CAR-T cell infiltration was also validated in a CD19-CAR-T cell-infused lymphoma patient. Taken together, our data suggest that eosinophil mobilization can be considered a mechanistically relevant biomarker for predicting radiotherapy prognosis as well as a new strategy to enhance T cell-mediated immunotherapies for cancers.

O030

Circadian expression of migratory factors establishes lineage-specific signatures that guide the homing of leukocyte subsets to tissues

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Evidence indicates that the oscillation of murine blood numbers is closely related to the lighting schedule. However, the dynamics and the mechanisms of the migration of leukocyte subsets over 24 hours are unknown. Here, we could show oscillations in murine blood leukocytes numbers was regulated by rhythmic homing, with a co-contribution of both microenvironmental and leukocyte-autonomous oscillations. We identified leukocyte and vascular bed specific surface expression oscillations of adhesion molecules and chemokine receptors. To test the relevance of these oscillations, functional blocking experiments using antibodies or functional blockers directed against pro-migratory molecules were performed. A stronger blocking effect targeting some of these molecules was found at night for leukocyte subsets. In the homing experiments, we found donor leukocytes preferentially migrated to organs at night, and that the recruitment process of different leukocyte

DOI: 10.1002/eji.201970400

subsets to various organs could be blocked by distinct inhibitors. Ablation of BAML1 in endothelial cells or leukocyte subsets disturbed the temporal expression profile of pro-migratory molecules as well as the rhythms of leukocyte homing. Furthermore, we showed these oscillatory patterns defined leukocyte trafficking in both homeostasis and inflammation and determined detectable tumor burden in blood cancer models. We also detected inverse oscillation patterns of human blood cell numbers compared to murine cells as well as of the expression levels of CXCR4 on leukocyte subsets. Together, we demonstrate that circadian genes control a rhythmic leukocyte- and tissue-specific molecular signature in the expression of pro-migratory factors, which allows for the recruitment of select leukocyte subsets at distinct times.

O032

TIM-1 glycoprotein mediates neutrophil trafficking during acute and chronic inflammatory responses

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Neutrophils are classically associated with acute inflammatory responses. However, it is now accepted that these cells may also play a role during chronic inflammatory diseases. Here we show that neutrophils express T cell immunoglobulin and mucin domain (TIM)-1, a molecule previously thought to be specific for T cell-mediated responses. We found that TIM-1 is mainly expressed intracellularly in mouse and human neutrophils, and it is rapidly upregulated on the plasma membrane after exposure to inflammatory stimuli, including fMLP, C5a and amyloid beta. Epifluorescence intravital microscopy studies showed that inhibition of TIM-1 decreases neutrophil rolling and firm adhesion in inflamed venules. We also observed a significant reduction of neutrophil extravasation in a model of acute peritonitis in TIM-1Δmucin C57BL/6J mice lacking functional TIM-1 compared to WT animals. We next studied the effect of TIM-1 blockade in 3xTg-AD mice, an animal model of Alzheimer's disease (AD) in which we have recently shown that neutrophils promote disease pathogenesis. Our data showed that TIM-1 inhibition by a blocking antibody or by genetic deficiency leads to a drastic reduction of neutrophil accumulation into the brain and a reduction of cognitive deficit and neuropathological markers of AD, including microglial activation, amyloid beta deposition and tau hyperphosphorylation. Finally, we found TIM-1 expressing activated neutrophils adhered in cerebral vessels or migrated inside the brain parenchyma in subjects with AD. Collectively, our data demonstrate that TIM-1 is a novel trafficking receptor for neutrophils during inflammatory responses and that targeting TIM-1 may represent an attractive therapeutic approach in inflammatory diseases.

P0184

Lung adenocarcinoma-intrinsic GBE1 signaling inhibits anti-tumor immunity

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Background: Changes in glycogen metabolism are emerging as an essential response among the various metabolic adaptations used by cancer cells to adjust to the conditions imposed by the tumor

DOI: 10.1002/eji.201970400

microenvironment. We aimed to clarify whether glycogen branching enzyme (GBE1) is involved in the immune regulation of the tumor microenvironment in lung adenocarcinoma (LUAD).
Methods: RNA-sequencing analysis and multiplex assay was used to determine the high level of factors in GBE1 knockdown cells. The role of GBE1 in lung adenocarcinoma was evaluated both *in vitro* and *in vivo*.
Results: We observed that the knockdown of GBE1 increased the expression of chemokines CCL5 and CXCL10 in A549 cells. CD8 expression was positively correlated with the frequency of CCL5 and CXCL10 in LUAD. The supernatants derived from GBE1 knockdown cells robustly enhanced the recruitment of $CD8^+$ T lymphocytes analyzed by transwell assay. However, the neutralizing antibodies of CCL5 or CXCL10 significantly hampered cell migration induced by shGBE1 cell supernatants. STING/IFN-I pathway was required for the effect of GBE1 knockdown on CCL5 and CXCL10 upregulation. Moreover, PD-L1 was significantly increased in shGBE1 A549 cells compared to those in control cells. Lastly, blockade of GBE1 signaling combined with anti-PD-L1 antibody significantly inhibited tumor growth *in vivo*.
Conclusions: GBE1 blockade promotes the secretions of CCL5 and CXCL10 to recruit $CD8^+$ T lymphocytes in the tumor microenvironment via the IFN-I/STING signaling pathway, accompanied by upregulation of PD-L1 in LUAD cells, suggesting that GBE1 could be a promising target in achieving tumor rejection through cancer immunotherapy in LUAD.

P0185

RNA-binding protein MINT regulates tumor angiogenesis and T cell anti-tumor immune response

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MINT is an RNA-binding protein and has been reported as a co-repressor of Notch signaling to regulate the development of peripheral B cell and early T progenitors. Herein, we show that MINT is expressed in quiescent endothelial cells (ECs) in mature vessels. Endothelial specific disruption of MINT by conditional gene knockout in adult mice retarded tumor growth in mice. Histological investigations indicated that tumor vasculature in MINT deficient mice was “normalized”, as shown by diminished tumor angiogenesis, increased pericyte coverage, improved tumor vessel perfusion, reduced vascular permeability, and consequently mitigated tumor hypoxia and metastasis. Of note, ***vessel normalization by MINT deficiency subverted immune-suppressive tumor microenvironment via promoted T-cell infiltration*** and significantly increased the level of IFN-γ and TNF-α in tumors. In FACS-sorted T lymphocytes from tumors of EC-specific MINT knockout mice, the expression of IFN-γ and TNF-α was also upregulated. Moreover, the increased recruitment of T lymphocytes likely further strengthened vessel normalization through enhanced endothelial IFN-γ response, indicating a mutually regulatory loop. In vitro, knockdown of MINT in HUVECs repressed cell proliferation and migration, consistent with reduced angiogenesis in tumors of EC-specific MINT knockout mice. This was likely mediated by reduced expression of ETS1 when MINT was knockout or knocked down in ECs, but appeared not dependent on Notch signaling. In summary, our study indicated that MINT is a critical regulator in regulating both tumor angiogenesis and T cell immune response. The MINT-mediated signaling pathway could serve as a target to interfere with both tumor angiogenesis and immunity.

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DOI: 10.1002/eji.201970400

P0186

The regulatory effects of Spondin-2 and CSF-1 in the migration and infiltration of CD8+ T cells into peripheral tissues

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The successful migration and infiltration of CD8+T cells into peripheral tissues are required for the tumor immunotherapy. However, the investigation on the underlying molecular mechanisms is limited. In this study, humanized mice constructed by human PBMC were established to investigate the regulatory mechanism of migration and infiltration of CD8+ T cells into peripheral tissues. We found the human CD8+T cells accumulating in mouse peritoneal cavity expressed high CSF-1, whereas human CD8+T cells accumulating in mouse periphery tissues expressed high Spondin-2. Further study indicated Spondin-2 might induce the migration and infiltration of human CD8+T cells into peripheral tissues via the interaction with integrin, which would be cushioned by CSF-1. This study will not only promote the illustration of the regulatory mechanism in the migration and infiltration of human CD8+T cells into peripheral tissues, but also uncover the new target in tumor immunotherapy.

P0187

Investigation of neutrophil trafficking via lung-associated lymphatic vessels in Aspergillus fumigatus-induced infection

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Conidia of airborne pathogenic fungus Aspergillus fumigatus can induce massive neutrophil recruitment to the airways upon inhalation. Lymphatic vessels are known to transport dendritic cells and lymphocytes, however, in some cases neutrophils can recruit to lymphatics.
In the present study we investigated neutrophil migration via lymphatics in the course of Aspergillus fumigatus-induced inflammation. Fluorescently labeled A. fumigatus conidia were applied to mice oropharyngeally. Conducting airways of mice were obtained at different time after conidia application. We developed the technique of simultaneous imaging of A. fumigatus conidia, neutrophils, lymphatic and blood vessels in whole-mount airway specimens of mice at different phases of inflammation using confocal microscopy.
Analysis of three-dimensional images revealed neutrohils in lymphatic vessels after single application of A. fumigatus conidia in dose of five millions per mouse. The number of neutrophils in lymphatic vessels increased significantly at acute phase of inflammation, however, it was significantly lower than the number of neutrophils in blood vessels at the same phase. While neutrophils internalized conidia in the airway mucosa, we did not observe neutrophils that ingested conidia in lymphatics. Moreover we did not detect any conidia both in blood and in lymphatic vessels both at acute and at late phases of inflammation.
Thus in the present study using three-dimensional confocal laser scanning microscopy of immunohistochemically stained whole-mount conducting airway we have identified neutrophils in lymphatic vessels after single dose A. fumigatus conidia challenge. Migrating to lymphatics neutrophils did not transport conidia to lymphatic vessels.

The study was supported by RFBR №18-315-00166

DOI: 10.1002/eji.201970400

P0768

The distribution of CM-Dil labeled gingival derived mesenchymal stem cells in SD mice

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Gingival tissue derived mesenchymal stem cells (GMSCs) had great advantages than other population of MSCs, however, the distribution of GMSCs are still unclear. This study aims to investigate the distribution of CM-Dil labeled GMSCs in SD mice. 36 SD mice were divided into two groups (♀♂ half and half): GMSCs transplantation group (n = 24) and the control group (n = 12). CM-Dil labeled GMSCs transplanted to SD mice via tail vein injection, and sacrificed 4 mice at day 1, day 5, day 10, day 20 and day 30 after transplantation, respectively. Mice·s heart, liver, spleen, lung, kidney, brain, bone, muscle, testis or ovary tissue were taken out, frozen sliced and then observed by fluorescent microscope to determine GMSCs biological distribution. The observation of fluorescence microscopy showed CM-Dil positive cells were highest in lung after 1 day transplantation, followed by liver, lymph nodes, thymus, spleen, kidney, bone marrow, peripheral blood and ovaries. The CM-Dil positive cells were slow down in lung and liver after transplantation; while they were decreased obviously in lymph node and thymus after 10 day transplantation and then remained stable. However, no CM-Dil positive cell was found in heart, brain, testis, epididymis, uterus, muscle and skin after GMSCs transplantation. Lung, liver, kidney and immune organ such as lymph node, spleen and thymus distributed more GMSCs than other organs after transplantation to the mice. GMSC has its special biological distributed characteristics, which is important for the guidance of GMSCs immunotherapy in near future.

P0769

Sgpl1 deficiency foster gamma delta T cell LN sequestration and skin pigmentation under inflammation

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Gamma delta T cell plays a critical role in mucosa innate immune system; it egresses from LN through S1P (Sphingosine-1-phosphate) gradient then enters other organs, such as skin. Sphingosine-1-phosphate lyase-1 (Sgpl1) is the only enzyme lysis S1P in mammals and essential for the sphingolipid matabolism. Sgpl1 KO mice are with lymphopenia and very sick of nephrotic syndrome, die within 4 weeks after born. We obtained a Sgpl1 point mutation strain through ENU mutagenesis screening, of which its Sgpl1 Methionine 465 is mutated to Lysine (M465K); this point mutation prolongs its survival up to 20 weeks. We confirmed lymphopenia symptom in adult (8-20 weeks) $Sgpl1^{M465K}$ mice. $Sgpl1^{M465K}$ gdT cell number in Skin is comparable with WT, however the IL17 producing gdT is largely sequestrated within LN. After skin Imiquimod administration, the lacking of IL17-gdT cell within the inflamed tissue promotes melanin production and causes hyper-pigmentation in $Sgpl1^{M465K}$. Sgpl1 deficiency impedes IL17 producing gdT egressing from LN may also explain skin hyper-pigmentation plague observed in some human Sgpl1 mutated patients.

DOI: 10.1002/eji.201970400

P0771

Instead of natural killer cells, the anti-ASGM1 antibody depletes a CD44- and LFA-1-positive CD8 T cell population and impairs immune clearance of hepatitis B virus

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Hepatitis B virus (HBV) infection causes chronic liver diseases that may progress to chronic hepatitis, liver cirrhosis, and subsequent hepatocellular carcinoma. Natural killer (NK) cells are major innate immune cells that are abundant in the liver. Previous studies demonstrated that treatment with anti-asialo GM1(ASGM1) antibody (Ab), an NK cell-depleting Ab for ablating the function of NK cells *in vivo*,led to impairment of the ability to clear HBV in a mouse animal model. In order to confirm the role of NK cells in HBV clearance, we further used NFIL3 knockout (KO) mice, which are deficient in NK cells, to evaluate the role of NK cells in anti-HBV immunity. Unexpectedly, the ability to clear HBV was not significantly impaired in NFIL3 KO mice, indicating that conventional NK cells do not play a critical role in HBV clearance. Moreover, the ability to clear HBV was abolished when NFIL3 KO mice were further treated with anti-ASGM1 Ab, implying that other non-NK ASGM1-positive immune cells mediate HBV clearance. We isolated ASGM1^{+}cells from the liver of NFIL3 KO mice and analyzed the immune phenotype of these cells. Our results demonstrated a distinct CD44^{+}LFA-1^{hi} CD8 T cells, a population of liver resident CD8 T cells, that were the major intrahepatic ASGM1-positive immune cells. Collectively, our study indicates that conventional NK cells are not essential for HBV clearance in this mouse model. Instead, ASGM1-positive liver-resident CD8 T cells which express CD44 and LFA-1 are the major effector immune cells mediating anti-HBV immunity.

P1373

Endothelial Notch signaling regulates neutrophil recruitment and transmigration via vessel remodeling and endomucin in hepatic ischemia-reperfusion injury

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Recruitment and infiltration of leukocytes are essential for inflammation. Previous studies have established that leukocyte-endothelial cell adhesion (LECA) determines leukocyte transmigration in inflammatory tissues; however, factors regulating this process remains elusive. Here we demonstrate that endothelial Notch signaling plays critical roles in leukocyte recruitment and infiltration in hepatic ischemia-reperfusion injury (HIRI). In both murine model and human biopsies, HIRI induced endothelial Notch activation. Disruption of endothelial Notch signaling by conditional knockout of transcription factor RBP-j aggravated HIRI, primarily attributed to dysfunction in liver sinusoidal endothelial cells (LSECs), leading to disordered sinusoids, reduced eNOS expression. To further investigate the role of endothelial Notch in HIRI, we ectopically activated Notch signaling by crossing CDH5-CreERT mice with Rosa26^{NICD} mice to acquire NIC^{eCA} mice. HIRI-introduced, NIC^{eCA} mice exhibited more severe liver damage, as shown by increased necrosis, apoptosis. Moreover, endothelial Notch activation promoted neutrophil adhesion and infiltration in vitro and vivo. RNA-seq analysis further revealed that endothelial Notch activation increased the expression of genes involved in leucocyte adhesion and diapedesis. In addition, endothelial Notch activation significantly downregulated the expression of endomucin (EMCN), a key molecule that inhibits infiltration of leucocytes into inflamed tissues. The Notch activation-induced enhancement of HIRI was cancelled by

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DOI: 10.1002/eji.201970400

blocking LFA-1, which mediated leukocyte adherence associated with EMCN. Therefore, we proposed that endothelial Notch signaling regulates neutrophil recruitment and transmigration via vessel remodeling and EMCN in acute inflammation such as HIRI. Our research demonstrates a previously unrecognized mechanism of leukocyte recruitment and transmigration and thus provides a potential novel therapeutic target.

P1374

Glycogen synthase kinase 3 drives thymocyte egress by terminating tonic akt signaling

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Glycogen synthase kinase 3 (GSK3) regulates diverse cellular functions including cell differentiation, proliferation, transformation, and survival, but its physiological roles remain poorly understood, largely due to the functional redundancy of its two isoforms GSK3α and GSK3β. Here we show that GSK3 plays a critical role in emigration of mature thymocytes from the thymus. Mice with T cell-specific deletion of both GSK3α and GSK3β (DKO mice) exhibited a drastic reduction in the frequency and cell numbers of peripheral T cells. GSK3-deficient thymocytes were more susceptible to apoptosis accompanied with reduced expression of Bcl-2, and exhibited impaired expression of transcription factors Foxo1 and Klf2, as well as sphingosine-1-phosphate receptor 1 ($S1P_1$), CCR7 and β7-integrin, and defective downregulation of CD69, which are all indispensable for thymocyte egress. Mechanistically, both GSK3α and GSK3β are consistently expressed in developing thymocytes, are constitutively active, and act to terminate tonic Akt signaling, thereby stabilizing Foxo1 protein and promoting Klf2 and $S1P_1$ expression. In addition, both constitutively active Foxo1 ($Foxo1^{AAA}$) and inhibition of Akt activation restored impaired egress of GSK3 DKO SP thymocytes. Our study demonstrates an essential role of GSK3 in driving thymocyte egress, establishes Akt as a major mediator of GSK3 function in this process, and reveals an unconventional mechanism of action for GSK3, which can provide a potential target in controlling leukemia, rejection of organ transplantation and autoimmune disease.

P1375

Biophysical properties and motility of human dendritic cells deteriorated by suppressive cytokines through cytoskeleton remodeling

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Dendritic cells (DCs) play a crucial role in initiating and amplifying both the innate and adaptive immune responses. Clinically, the DCs-based immunotherapy against cancer is considered one of the most promising therapies to overcome cancers, but there are still many challenges need to be overcome. The motility of DCs is especially crucial for migration of immature DCs into peripheral tissue and dynamic physical interaction between mature DCs and naive T cells in the secondary lymph node. This study focuses on the investigations of DCs at different differentiation stages and under various suppressive cytokines (VEGF, TGF-β_1 and IL-10) conditioned microenvironments from the interdisciplinary viewpoints. The biophysical characteristics of cells can reflect their relationship between structures and functions. The results showed that the DCs at different differentiation stages appear various biophysical characteristics, including the suppressive cytokines deteriorate the motilities and immune functions of DCs through derangement of biophysical characteristics and

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DOI: 10.1002/eji.201970400

reorganization of F-actin cytoskeleton, moreover, these changes are closely correlated with the expression levels of some cytoskeleton-binding proteins. It is significant for further understanding of the biological behaviors of DCs and how to enhance the clinical effectiveness of DCs-based immunotherapy against cancers.
Keyword: dendritic cells, motility, immune function, suppressive cytokines, biophysics

P1376

Unique cellular immunity resides at the human fetomaternal interface

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In humans, most lymphocytes reside in tissues and not the circulating blood. However, little is known about the structure and composition of these tissues-specific T cell repertoires. This is particularly true at the human fetomaternal interface which performs the remarkable feat of pathogen defence while maintaining immune tolerance towards an allogeneic fetus. Here, we performed a high-dimensional analysis of T cell subsets at the human decidua using TCR deep sequencing and transcriptional profiling, and contrasted these populations with corresponding maternal, paternal and cord samples. We show the decidua maintains an ordered balance of recirculating and resident T cells with subsets exhibiting tissue-specific functionality. Large clonal $CD4^+$ T cell populations reside at the decidua suggesting these populations are required for normal placental function. These data provide the first comprehensive map of T cell subsets (CD8+ naïve, CD8+ memory, TRMs, CD4+ naïve, CD4+ memory and Tregs) at the human fetomaternal interface.

P1965

NOD2 signaling controls a increase of regulatory T cells in mesenteric lymph nodes

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Crohn´s disease is a relapsing and lifelong inflammatory bowel disease. The frameshift mutation of Nucleotide-binding oligomerization domain containing protein 2 (NOD2) is the first reported and most relevant to the pathogenesis of Crohn´s disease. While, the mutations of NOD2 gene are commonly found in some healthy Caucasians**,** suggesting that body may possess protective immune responses against the absence of NOD2 function. Here, we showed that regulatory T (Treg) cells increased in the mesenteric lymph nodes (MLN) of $NOD2^{-/-}$ mice. The expression of CCL5/4/3 and their receptor CCR5 were dramatically up-regulated in MLNs. The increase of Treg cells in MLN of $NOD2^{-/-}$ mice was abolished by maraviroc, an antagonist of CCR5. Furthermore, NOD2 signal activated by MDP suppressed CCR5 expression on Treg cells via ERK and PI3K. Taken together, these results suggest that the increase of Treg cells in MLN of $NOD2^{-/-}$ mice may protect mice from NOD2 deficient-mediated dysregulation of intestinal immunity.

DOI: 10.1002/eji.201970400

P1966

RNA methyltransferase NSun2 deficiency ameliorates abdominal aortic aneurysm development by inhibiting T cell migration

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Hyperhomocysteinemia (HHcy) exaggerates abdominal aortic aneurysm (AAA) by promoting the expression of chemokines like MCP-1 and RANTES, which can recruit T cells and monocytes. We have reported that homocysteine activates the methyltransferase activity of RNA methyltransferase NOP2/Sun domain family, member 2 (NSun2) and in turn upregulates protein expression of intercellular adhesion molecule 1 (ICAM-1) by methylating ICAM-1 mRNA in vascular endothelial cells, thereby increases the adhesion of leukocytes to endothelium. However, whether NSun2 mediates the migration or adhesion of leukocytes to endothelial cells in AAA remains obscure. Here, we found that in the elastase-induced AAA model, the number of local infiltrated T cells in the vascular was significantly reduced in NSun2$^{+/-}$ mice. By exerting T cell labeling and adoptive cell transfer experiments, we found that NSun2 knockdown inhibited the chemotaxis of vascular endothelial cells and the migration of T cells. Mechanistically, NSun2 promoted the binding of endothelial autotaxin, a secretive protein, with integrin α4 in T cells by methylating integrin α4 mRNA, and thus activated the outside-in signal and FAK/Src-RhoA pathway. Therefore, NSun2-mediated T cell migration may be a new mechanism of aggravating vascular inflammation, matrix degradation and AAA development.

P2701

CCR2 signal facilitates thymic egress by priming thymocyte responses to sphingosine-1-phosphate

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The signal mediated by sphingosine-1-phosphate receptor 1 (S1P1) is essential but seemingly insufficient for thymic export of newly generated T cells. Here we reported the identification of CCR2 as an additional regulator of this process. CCR2 showed a markedly increased expression in the most mature subset of single-positive (SP) thymocytes. Its deficiency led to a reduction of recent thymic emigrants (RTE) in the periphery and a simultaneous accumulation of mature SP cells in the thymus. The CCR2 signaling promoted thymic emigration primarily through modulating the chemotactic responses to S1P1 engagement. On one hand, the chemokinesis induced by CCR2 activation endowed thymocytes with enhanced capacity to respond to S1P-induced migration. On the other hand, CCR2 signaling through Stat3 augmented FoxO1 activity, leading to increased expression of S1P1. Taken together, the present study highlights a unique and novel function of CCR2 signaling in the regulation of thymic egress.

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DOI: 10.1002/eji.201970400

Regulatory Immune Cells and Immune Regulation

O209

Acute myeloid leukemia cells express ICOS ligand to promote the expansion of regulatory T cells

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CD4+CD25+Foxp3+ regulatory T cells (Tregs) accumulate in bone marrow microenvironment in acute myeloid leukemia (AML). However, little is known about how the tumor environment including tumor cells themselves affects this process. Here we demonstrated that AML cells expressed inducible T-cell costimulator ligand (ICOSL) that can provide costimulation through ICOS for the conversion and expansion of Tregs sustaining high Foxp3 and CD25 expression as well as a suppressive function. TNF-a stimulation up-regulated the expression of ICOSL. Furthermore, both the conversion and expansion of CD4+CD25+Foxp3+ T cells and CD4+ICOS+Foxp3+ T cells were induced by co-culture with AML cells overexpressed ICOSL. CD4+CD25+ICOS+ T cells possessed stronger ability to secrete IL-10 than CD4+CD25+ICOS-T cells. The mechanism by which IL-10 promoted the proliferation of AML cells was dependent on the activation of the Akt, Erk1/2, p38, and Stat3 signaling pathways. Blockade of ICOS signaling using anti-ICOSL antibody impaired the generation of Tregs and retarded the progression of an AML mice model injected with C1498 cells. The expression of ICOSL of patient AML cells and ICOS+ Tregs were found to be predictors for overall survival and disease-free survival in patients with AML, with ICOS+ Treg cell subset being a stronger predictor than total Tregs. These results suggest that ICOSL expression by AML cells may directly drive Treg expansion as a mechanism of immune evasion and ICOS+ Treg cell frequency is a better prognostic predictor in patients with AML.

O210

Interplay of MHC class II and PAI-1 signaling modulates T cell cytokine and chemokine responses in mouse autoimmune arthritis

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Rheumatoid arthritis (RA) is a disabling, costly and progressive inflammatory disease. Certain MHC class II molecule alleles (DR4/DQ8) are known to play a critical role in RA disease pathogenesis. Recently, it was found that the plasminogen activator inhibitor-1 (PAI-1) levels are increased in the synovial fluid and serum of RA patients. PAI-1 is known to play a critical role in the fibrinolytic system. It is recently appreciated that during the cell activation and the release of cellular biological mediators, receptor collaboration is absolutely required. In the present studies, we confirmed that blocking mouse PAI-1 or MHC II (H-2Ea) can confer significant protection against arthritis in our arthritis models, a superantigen (SAg)-driven collagen-induced arthritis (mCIA) and an autoimmune arthritis induced by mycoplasma components, namely OGex and MAM. The pathogenesis which derives essentially from the host responses to initial priming of selfantigens, then challenges by MAM. We found that the increaed PAI-1 in the synovial fluid followed by the interaction with MAM/H-2Ea complex can rapidly activates Th1/Th17/T_{fh} cells. It upregulated T_{bet}, RORgt, BCL6 and ICOS expressions and also induced proinflammatory cytokines, IL-1b, IL-6, IL-12, IFNg, IL-17, IL-23, stress protein HMGB1, and chemokines CXCL2, CXCL9, CXCL10, CXCL12, CCL2 and CCL5. Our work demonstrates H-2Ea and PAI-1 can collaboratively elicit inflammation in arthritis models. Thus, identifying how PAI-1 interacting with H-2Ea/MAM leading to the activation of downstream cellular events is an important for us seeking

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DOI: 10.1002/eji.201970400

the therapeutic strategy for RA.

This work was supported by a grant from the Nora Eccles Treadwell Foundation

O211

A wave of Foxp3$^+$ regulatory T cell accumulation in neonatal liver plays unique roles in maintaining self-tolerance

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Newborn animals require a tightly-regulated local and systemic immune environment to govern the development and maturation of multiple organs/tissues even when the immune system itself is far from mature during neonatal period. The regulatory T cells (Tregs) are essential in maintaining immune tolerance/homeostasis and modulating inflammatory responses. The features of Tregs in neonatal liver under steady state are not well understood. We found a wave of thymus-derived Treg influx into the liver during the first 1-2-week age window. Depletion of these Tregs between days 7 and 11 after birth rapidly resulted in Th1-type liver inflammation, liver damage, metabolic disorder, and impaired weight gain on day 12. Compared to Tregs in the spleen, more Tregs in neonatal liver underwent MHC II-dependent activation and proliferation and acquired higher suppressive functions. The transcriptome profile of these neonatal liver Tregs showed elevated expression of T-bet and features of Tregs capable of regulating Th1 responses. Neonatal liver Tregs also expressed higher levels of PPARγ, VAT Treg up-signature genes, and a variety of genes tied to lipid metabolism, suggesting that these liver-residing Tregs adopt lipid metabolic machinery in their gene signatures. Collectively, these data indicate that the accumulation of Tregs with unique features in neonatal liver is critical to ensure self-tolerance and liver maturation.

O212

CD38 expression correlates with FoxP3, increasing the activation, proliferation, and function of regulatory T cell

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CD38 is a 42 kDa transmembrane glycoprotein expressed in several cell types including T cells. CD38 crosslinking with specific antibodies induce activation, proliferation, and protein phosphorylation. Some reports have suggested that the CD38 absence accelerates the onset of autoimmunity. Nevertheless, the mechanisms associated with CD38 in order to regulate the autoimmune process are still unknown. Some evidence reveals that CD38 is highly expressed by subsets with regulatory properties such as regulatory T (Treg) cells. In this work, we evaluated the expression and function of the CD38 receptor in the activation and proliferation of Treg cells. Our results show that Treg cells express high amounts of CD38 which strongly correlates with FoxP3 production. The CD38-/- mice have low

DOI: 10.1002/eji.201970400

percentages/absolute number of Treg cells compared to the wild type control, suggesting that CD38 exerts a function in the development of this cell population. Additionally, the CD38^{+ve} Treg cells have increased expression of CD44, CD62L, Helios, CTLA-4, PD-1, and CD69 activation and regulatory proteins, compared to the CD38^{-ve} Treg cells or CD38-/- Treg cells, indicating that CD38^{+ve} Treg cells are associated with an activation phenotype. Finally, the CD38 crosslinking was able to increase the percentage of Treg cells producing IL-10 and regulate the proliferation of activated T cells. In conclusion, we proposed that CD38 could be used as a potential marker of Treg cells, moreover, its activation is able to achieve the regulatory function of Treg cells.

O213

G-MDSC promote the stemness of colorectal cancer cells

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Cancer stem cells (CSCs) play a critical role in colorectal cancer (CRC) progression. Myeloid-derived suppressor cells (MDSCs) accumulate in CRC patients. MDSC-derived exosomes have been proposed to act as intercellular messengers. The roles of MDSCs in CRC cell stemness are unclear. Herein, we report that granulocytic MDSCs (G-MDSCs) promote colon cancer cell stemness and CRC progression in mice through exosomes and exosomal S100A9. Our results also show that hypoxia induces G-MDSCs to secrete more exosomes in a hypoxia-inducible factor 1a (HIF-1a)-dependent manner. In addition, human MDSCs enhance colon cancer cell stemness and growth via exosomal S100A9. The plasma exosomal S100A9 level is significantly increased in CRC patients. Thus, these results suggest that exosomal S100A9 from G-MDSCs promotes CRC cell stemness and growth, which may benefit the development of strategies to prevent tumor growth and dissemination.

O214

Function of protein kinase CK2 in innate and adaptive immune cells in neuroinflammation

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Immune dysregulation is critical to the pathogenesis of autoimmune diseases, including multiple sclerosis (MS). Studies in MS patients and an animal model of MS, experimental autoimmune encephalomyelitis (EAE), have highlighted the importance of both innate and adaptive immune responses in MS/EAE pathology. Antigen-presenting cells, especially dendritic cells (DCs), not only activate CD4^{+} T cells by antigen presentation, but also control T cell differentiation by expressing co-stimulatory molecules and cytokines. Our current studies demonstrate that Tamoxifen-induced global deletion of CK2a in CK2a$^{fl/fl}$ERT2Cre mice results in delayed onset and significantly slower progression of EAE, which is not observed in CK2a T cell-specific deleted CK2a$^{fl/fl}$dLckCre mice, which exhibit lower EAE clinical scores during the peak and resolution phases of disease. EAE mice with global CK2a deletion express reduced pathogenic Th1 and Th17 cells, and enhanced anti-inflammatory Treg cells. We speculate that CK2 kinase activity in DCs and T-cells is indispensable for the initiation and progression of EAE. Specifically, our data indicate that deletion of CK2a reduces the activation of DCs by suppressing the expression of CD40, TNF-a, iNOS and IL-12 and activation of AKT and p38. Furthermore, we found that activation of DCs and T cells is prevented in EAE mice with global CK2a deletion, which correlates with differential regulation of Th1, Th17 and Treg cells. Collectively, our findings establish a novel regulatory role for CK2 kinase in DCs and T cells during

DOI: 10.1002/eji.201970400

EAE initiation and progression, which may provide evidence to establish CK2 as a potential novel therapeutic target for MS treatment.

O215

Identification of nonlymphoid-tissue regulatory T cell precursors

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Regulatory T cells (Treg cells) are important mediators of peripheral tolerance, and their absence leads to catastrophic autoimmunity in men (IPEX) and mice (Scurfy). Treg cells are characterized by both expression of the hallmark transcription regulator Foxp3 and a unique epigenetic profile. Treg cells are critical to maintain self-tolerance. They modulate the functions of different immune cells, thereby affecting a variety of conditions, including autoimmunity, cancer, allergy, infection and inflammation.
In recent years it became evident that, in addition to these immunoregulatory properties, highly specialized Treg cells in non-lymphoid tissues are important to promote organ homeostasis and tissue repair. In particular, the release of the EGF-like growth factor Amphiregulin by tissue-resident Treg cells during wound repair suggested such a function. But how and where do Treg cells specialize into this tissue regenerative population found in non-lymphoid tissues?
Recently, we identified a common population of tissue-homeostasis promoting Treg cells via genome-wide epigenetic and transcriptional profiling (Delacher et al., *Nature Immunology* 2017, Delacher et al., *Nature Communications* 2019).
Now, using state-of-the-art molecular tools like scRNA and scTCR sequencing as well as ATAC profiling and other methods, we identified progenitors of nonlymphoid-tissue Treg cells and revealed their molecular path of differentiation. We identified critical transcription factor profiles and performed developmental kinetics *in-vivo*. Our study fills a critical gap in understanding nonlymphoid-tissue Treg cell differentiation and provides means to harness their potential for regenerative medicine in future applications.

O216

IL-33 via IL-17A and IL-22 significantly reduces non-alcoholic steatohepatitis that develops following drug-induced hepatitis in male but not female mice

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Non-alcoholic steatohepatitis (NASH) is more prevalent in males. Dietary triggers of NASH are well-known. Less known triggers include drugs that induce hepatitis. A form of drug-induced autoimmune hepatitis (DIH) occurs more commonly in females. Using mouse models we found that IL-33 reduces DIH. IL-33 reduces pro-inflammatory responses in a diet-induced NASH model by reducing IL-17A and IL-22. Since IL-33, IL-17A and IL-22 modulate NASH and DIH, we hypothesized that in NASH that develops following DIH, IL-33 reduces NASH via FoxP3+Treg reduction of IL-17A and IL-22. We induced DIH in male and female BALB/c and IL-33 deficient (-/-) mice with an epitope of cytochrome

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DOI: 10.1002/eji.201970400

P450 2E1 altered by a drug metabolite. We measured IL-17A and IL-22, DIH after 3 weeks and survival, and followed NASH development for 15 weeks. A p value < 0.05 was significant. Female BALB/c mice expectedly developed more severe DIH than males (N=15/group, p< 0.05). Male and female IL-33-/- mice developed similar levels of DIH. Male IL-33-/- mice had increased IL-17 and IL-22 (p< 0.05) but reduced Foxp3+Tregs (p< 0.0001) and increased mortality (p< 0.01), suggesting that IL-33 critically reduces DIH in males. After 15 weeks, BALB/c mice did not develop NASH; however 100 percent of IL-33-/- males developed NASH when compared to 25% of females (p< 0.0001) suggesting that IL-33 critically reduces NASH in males via IL-17A and IL-22. We provide one possible immunological mechanism for sex bias in NASH. Understanding mechanisms that regulate NASH could reduce the development of fibrosis or cancer.

LB087

Depletion of Treg cells induces an increased activation of T-CD4$^+$ and T-CD8$^+$ cells during colitis-associated colorectal cancer

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Colorectal Cancer (CRC) is the second most commonly diagnosed cancer in women and the third in men in North America and Europe. CAC is associated with inflammatory responses where intestinal pathology is caused by a T-cell dysregulation concluding in an imbalance between T activated (Tact) and T regulatory (Treg) cells. Treg cells are CD4$^+$Foxp3$^+$ cells that actively suppress pathological immune responses. It has been suggested a tumor-promoting function for Treg cells in colitis-associated colon cancer (CAC) but the kinetics of Treg cells during CAC development is poorly known. Thereby we used a mouse model of CAC induced by azoxymethane and three cycles of dextran sodium sulfate (DSS, 2%). We analyzed the percentage of Treg cells in spleen and mesenteric lymph nodes (MLN) in each cycle. We did not observe reduction on Treg cells in the first DSS cycle in MLN, but a significant increase in percentage was detected during the second and third cycle of DSS. A Higher percentage of Treg cells-expressing PD1, CD127 and Tim-3 during the second and third DSS cycle in spleen and MLN was observed, whereas CD25 had no changes. However, we found increasing percentages of CD4$^+$CD25$^+$Foxp3$^-$ Tact cells only in the first DSS cycle in spleen, and MLN. These results suggest that a gradually increased number of Treg cells suppress Tact cells during the second and third DSS cycle. Depletion of Treg cells with anti-CD25 PC61 antibody in the second DSS cycle increased percentages of CD4$^+$CD25$^+$Foxp3$^-$ Tact and TCD8$^+$ cells, with an impact in tumorigenesis

P0188

Modulation of intrahepatic CD8+T cell response by Salmonella flagellin

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Background: Recently, gut microbiota mediated innate immunity was regarded as important immune-regulator during HBV infection. However, as the key PAMP of gut bacterial, the role of flagellin in regulating the intrahepatic CD8+ T cell response is not clear.
Methods: C57BL/6 mice were transfected by HI of SF. Different types of liver cells, including PMHs, LSECs and KCs were isolated and co-cultured with T cells to study their immune regulatory activities. The activation of HBV-specific T cells of systemic and intrahepatic lymphocytes in response to SF

DOI: 10.1002/eji.201970400

treatment were analyzed in the acute HBV-replicative mouse model.
Results: SF induced rapid re-arrangement of the component of IHLs, resulting in prolonged T cell and macrophage infiltration in liver. PMHs responded to SF by activating the TLR5 signal pathway, and therefore mediated an enhanced IFNg production of lymphocytes. The anti-CD3 induced multiclonal and peptide-loaded DC induced specific CD8+ T cell activation and cytokine production were improved by SF-stimulated PMHs. Moreover, SF has weak effect on the HBV-specific humoral response and CD8+ T cell response in the spleen, but significantly enhanced intrahepatic HBV-specific CD8+ T cells response.
Conclusion: Our results suggest that SF play role in regulating intrahepatic CD8+ T cell response via activating the TLR5 pathway in PMHs, which may bridge the gut bacterial and liver immunity.

P0189

Therapeutic benefit of mesenchymal stem cells in pregnant rats with angiotensin receptor agonistic autoantibody-induced hypertension: implications for immunomodulation and cytoprotection

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Immunomodulation by mesenchymal stem cells (MSCs) is potentially important for maintaining peripheral tolerance. Preeclampsia may be due to maternal immune rejection of the genetically foreign fetus. This study aimed to investigate the biological function of human umbilical cord- derived mesenchymal stem cells (HU-MSCs) for the treatment of angiotensin receptor agonistic autoantibody (AT1-AA)-induced hypertension during pregnancy. HU-MSCs were isolated, cultured, and labeled in vitro. AT1-AA and HU-MSCs were administered to pregnant rats. Green fluorescent protein (GFP)-positive HU-MSCs infused in vivo were identified by immunofluorescence. Systolic blood pressure (SBP) was evaluated. The effects of HU-MSCs on fetal weight, kidney burden, and spiral artery remodeling, as well as on the expression of tumor necrosis factor α , interleukin 10 , and heme oxygenase 1 , were investigated. The SBP levels in the HU-MSCtreated pregnant hypertension rats decreased by gestational day 19. The reduction in fetal weight was largely ameliorated after HU-MSC treatment. Lesion burden in the kidney was attenuated and spiral artery remodeling was improved in HU-MSC-treated pregnant hypertension rats. However, green fluorescent protein (GFP)-labeled cells were sparingly observed in the kidney and placenta. Intravenous infusion of HU-MSCs into AT1-AA-induced rats significantly downregulated serum TNF-αlevels and upregulated IL-10 levels, concomitant with increased placenta and mesometrial triangle (MT) HO-1 expression. Taken together, intravenous infusion of HU-MSCs ameliorates AT1AA-induced pregnancy hypertension, intrauterine growth retardation, kidney impairment, and spiral artery remodeling impairment. Moreover, the potential benefits of HU-MSCs may be attributable to both an interference with the pathogenic immune response and a paracrine cytoprotective action.

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DOI: 10.1002/eji.201970400

P0190

Changes of immunological parameters and clinical application of EV71$^+$ in children with hand, foot and mouth disease

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Objective: To evaluate the clinical application of immunological parameters of children with EV71 infection.
Methods: The 144 cases of children younger than 5 years old with hand, foot and mouth disease (the HFMD group) were selected, and another 100 healthy children by clinical physical examination during the same period were randomly selected as the control group. The expression of EV71 mRNA in enteroviruses was detected by qRT-PCR, then divided the HFMD group into the EV71$^+$ and EV71$^-$ groups.
Results: The percentages of lymphocyte subsets CD3$^+$, CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ in HFMD group were lower than those in control group ($P < 0.05$), the percentages of CD19$^+$B and CD16$^+$56$^+$NK were higher than those in control group ($P < 0.05$). The concentrations of IgG and IgM in HFMD group were lower than the control group, while IgM was higher than the control group ($P < 0.05$). The percentages and absolute counts of B in EV71$^+$ group were higher than those in EV71$^-$group, while IgM was higher than those in EV71$^-$group ($P < 0.05$).
Conclusions: The cellular immunity function of the children physical with hand, foot and mouth disease are decreased, and humoural immunity are disorder. Monitoring lymphocyte subsets, immunoglobulin can provide the laboratory basis for immune status assessment of children with hand, foot and mouth disease.
Keywords: HFMD; Lymphocyte subsets; cellular immunity; humoral immunity; EV71

P0191

The IL-33-ST2-MyD88 axis promotes regulatory T cell proliferation in the murine liver

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Hepatic Foxp3$^+$ regulatory T (Treg) cells are crucial for maintaining local immune homeostasis in the liver. However, the environmental cues required for hepatic Treg cell homeostasis are unclear. In this study, we showed that the IL-33 receptor ST2 was preferentially expressed on Treg cells in the mouse liver, but it was more lowly expressed in the spleen, mesenteric lymph nodes, and blood. More importantly, we found that IL-33 promoted the proliferation of hepatic Treg cells through myeloid differentiation factor MyD88 signalling concomitant with increased CDK4 and cyclin D1 expression. These results suggested that IL-33 is a potential tissue-specific factor controlling Treg cell homeostasis via increased Treg proliferation in the liver.

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DOI: 10.1002/eji.201970400

P0192

Ligation of CD180 contributes to endotoxic shock by regulating the accumulation and immunosuppressive activity of myeloid-derived suppressor cells through STAT3

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Myeloid-derived suppressor cells (MDSCs) play an immunosuppressive role in the pathogenesis of inflammatory diseases. CD180, a TLR-like protein, can regulate the proliferation and activation of immune cells. However, the roles of CD180 in regulating the accumulation and function of MDSCs have not been investigated. Here, we found that, compared with non-treated controls, the expression of CD180 was significantly elevated in MDSCs, especially granulocytic MDSCs (G-MDSCs), from mice challenged with lipopolysaccharide (LPS). Ligation of CD180 by the anti-CD180 antibody not only blocked the expansion of MDSCs by preventing the phosphorylation of signal transducer and activator of transcription 3 (STAT3), but also reduced the immunosuppressive activity of MDSCs on M1 macrophage polarization through inhibition of Arg-1 expressionin vitro.In vivostudies showed that injection of anti-CD180 antibody significantly aggravated pathological lesions in mice challenged with LPS. Furthermore, injection of anti-CD180 antibody inhibited the accumulation of G-MDSCs in mice challenged with LPS and reduced the immunosuppressive activity of G-MDSCs on M1 macrophage polarization. Based on these findings, we conclude that ligation of CD180 contributes to the pathogenesis of endotoxic shock by inhibiting the accumulation and immunosuppressive activity of G-MDSCs, thus providing insight into the function of CD180 in inflammatory diseases.

P0193

Thermal stress strengthen Vγ9Vδ2 T cell cytotoxicity by upregulating cytokine secretion

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In recent years, thermotherapy has been increasingly used as a new treatment for tumor therapy as well. Reported works indicated that heat would enhance the immune effect function of both innate and adaptive immune system. As one of promising candidates for immunotherapy, Vγ9Vδ2 T cells has many unique biological advantages such as non-MHC restriction. Since the unique recognition and killing mechanism, Vγ9Vδ2 T cells are developing into a new strategy for tumor immunotherapy. Therefore, we are very interested to reveal how heat or thermal stress could modulate anti-tumor activity of Vγ9Vδ2 T cells.
Our work found that thermal stress could promote proliferation of Vγ9Vδ2 T cells, and significantly strengthen the anti-tumor activity of Vγ9Vδ2 T cells in vitro. Further investigation revealed that thermal stress could specifically upregulate expression of anti-tumor killer cytokines such as IFN-γ and perforin, rather than surface receptors like NKG2D. Meanwhile, we found that cytoskeleton and energy metabolism, which are closely correlated with anti-tumor cytotoxicity of Vγ9Vδ2 T cells, were augmented. In conclusion, our work provided a new, straightforward strategy for improving Vγ9Vδ2 T cell anti-tumor activity, which would benefit clinical application in the future.

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DOI: 10.1002/eji.201970400

P0194

Induced regulatory T cells ameliorated STZ-induced T1DM *via* TGF-β mediated inhibition of Tc1 cell

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Objectives: Regulatory T cells have shown potential in treating type 1 diabetes (T1DM) both in animal and clinical studies. We aimed to explore whether induced regulatory T cells (iTregs), which has an advantage under inflammatory condition, can ameliorate the progress of T1DM and further determine the underlying mechanisms.
Methods: T1DM was induced in Thy1.2$^+$ C57BL/6 mice using multiple low doses of STZ injection. 3.0 × 10^6 iTregs differentiated *in vitro* using naïve CD4$^+$ T cells isolated from Thy1.1$^+$ C57BL/6 Foxp3^{gfp} mice were given through *i.v.* injection 30 min after STZ administration. Non-fasting blood glucose level was monitored. Pancreas were harvested for HE staining, immunohistochemistry and immunofluorescence (CD8/pS6/TCF1). Tc1 cells and Tregs percentages were determined and compared on day 15 and day 30. Mouse naïve CD8$^+$ T cells were polarized to Tc1 cells with or without iTregs +/- ALK5 inhibitor, and differentiated cells percentages, mTOR activation and TCF1 expression were analyzed by flow cytometry.
Results: iTregs migrated to spleen, lymph nodes and even pancreas after adoptive transfer and showed stability *in vivo*. Infusion of iTregs significantly controlled blood glucose levels, delayed diabetes onset, ameliorated pathology scores in pancreas, and increased β cells percentages. iTregs down-regulated production of IFN-γ in CD8$^+$ T cells while up-regulated the levels of CD4$^+$ Tregs in recipient mice. iTregs inhibited Tc1 cells differentiation *in vitro* dependent on TGF-β mediated high expression of TCF1 and suppression of mTOR signaling.
Conclusions: iTregs ameliorated STZ-induced T1DM via TGF-β mediated inhibition of Tc1 Cell by maintaining high expression of TCF1 and suppression of mTOR.

P0195

Plasmacytoid dendritic cells inhibit *T. gondii* infection induced cytokine storm

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It is well known that *Toxoplasma gondii* (*T. gondii*) acute infection usually causes cytokine storm, which is characterized by local overproduction of Th1-type cytokines. However, how *T. gondii* acute infection induced cytokine storm is regulated is unclear. As plasmacytoid dendritic cells (pDCs), a subset of DCs, are essential in the elicitation of immune responses to *T. gondii* infection, we sought to investigate the role of pDCs in *T. gondii* infection induced cytokine storm. Towards this goal, BDCA2-DTR mice was used, in which pDCs can be specifically depleted by diphtheria toxin (DT) treatment. We found that *T. gondii* infection reduced the number of pDCs. And after treated with DT, BDCA2-DTR mice were extremely susceptible to *T. gondii* infection, with significantly increased serum levels of IFN-γ、IL-6 and TNF-α. In addition, depletion of pDCs also resulted in significantly increased the proportion of IFN-γ-producing CD4$^+$T cells, and exacerbated immunopathological damage caused by *T. gondii* infection. These results reveal that pDCs play a key role in negatively regulating *T. gondii* acute infection induced cytokine storm. However, the molecular mechanisms need to be further investigated.

DOI: 10.1002/eji.201970400

Kew words: pDC; cytokine storm; immune response
This work was supported by grants from the National Basic Research Program of China (2015CB943203), the National Natural Science Foundation of China (NSFC81701575), and the Natural Science Foundation of Shandong Province (ZR2015HQ033).

P0196

Glutamine metabolism controls the generation of immunosuppressive immature myeloid cells in human cancers

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Tumor associated myeloid cells are prominent components of solid tumors, serving as major immune regulators for the tumor microenvironment (TME) and insurmountable obstacles for the immune checkpoint blocking (ICB) therapy. However, it remains unclear how metabolic process regulates the generation of suppressive myeloid cells in the TME. Here, we found that hematopoietic precursor cells are enriched in the tissues of several types of human cancers and can differentiate into immature myeloid cells (IMCs). These IMCs are highly immunosuppressive, glycolytic and proliferative. Using a $CD34^+$ precursor-derived IMCs model, we found that the generation of suppressive IMCs is accompanied by increased glycolysis, but not affected by glucose deprivation due to alternative catabolism. Glutaminolysis acts as a more important catabolic pathway for the generation of IMCs regardless of glucose availability. Glutamine metabolism is indispensable for expansion, activation and suppressive capacity of IMCs. Glutamine could support the expansion of IMCs with glutamine-derived α-ketoglutarate, and promote the acquisition of suppressive capacity through the glutamate-NMDA receptor axis. Moreover, BPTES (a specific inhibitor of glutaminase GLS1) treatment synergistically enhanced the therapeutic efficacy of anti-PD-L1 treatment with delayed tumor growth in an ICB-resistant mice model. BPTES relieved ICB resistance by abrogating the expression of arginase 1, reducing PMN-MDSC/$CD8^+$ T cell ratio, rejuvenating anti-tumor immunity with increased IFN-γ^+ and granzyme B^+ T cells. Our work identified a novel regulatory mechanism of glutamine metabolism in controlling the generation of suppressive IMCs in TME.

P0197

Characteristics of regulatory T cells in leukemic hematopoietic microenvironment

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Regulatory T cells (Tregs) are important players in immune system. Accumulation of Tregs in tumor microenvironment is detected and is believed to contribute to immunosuppressive microenvironment favorable for malignant progression. However, the characteristics of Tregs in leukemic hematopoietic microenvironment (LHME) have not been well understood. In this study, we analyzed the expression of Foxp3 in myeloid leukemia patients, and studied the distribution, phenotypic and functional characteristics of Tregs in MLL-AF9 induced mouse AML model. The results show that higher

DOI: 10.1002/eji.201970400

expression of Foxp3 is detected in AML patients. Elevated level of Tregs is observed in both BM and spleen during leukemia progression in mice. AML Tregs are more activated since they express higher level of CD44 but lower level of CD62L. They also have more suppressive function since they express higher level of CTLA4, IL-10, PD-1 and perforin. Furthermore, conversion of non-Treg conventional $CD4^+$ T cells to Tregs does not contribute to Treg accumulation in LHME. CFSE and Ki67 staining assay show that AML Tregs proliferate more vigorously than healthy Tregs whereas no significant difference is observed in apoptosis of Tregs. RNA-seq analysis and RT-PCR verification reveal that AML Tregs express higher level of cell cycle-related genes. *In vitro* transwell experiments and *in vivo* recruitment experiments show that LHME is more potent to recruit Tregs. Our findings show accumulation of more activated and suppressive Tregs in LHME, which is caused by both local expansion and migration.

P0198

IL-17 blunts IL-15-driven homeostatic proliferation of memory phenotype $CD8^+$T cells via SOCS3

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Homeostatic proliferation is essential for memory $CD8^+$ T cells maintenance and long-term protection. However, the underlying mechanisms for maintaining homeostatic proliferation of memory $CD8^+$ T cells remains largely undefined. In this study, we demonstrated that IL-17A signaling curtailed the homeostatic proliferation of memory phenotype $CD44^{hi}CD8^+$ T (MPT) cells via blunting IL-15 signaling via SOCS3. Ablation of IL-17 signaling led to the increase of MPT cells, whereas persistent IL-17 signaling markedly diminished MPT cells. Mechanistically, reduced homeostatic proliferation rather than apoptosis contributed to IL-17A-induced alteration of MPT cells. IL-17A dampened IL-15-driven dephosphorylation of STAT5 via up-regulation of SOCS3 in MPT cells. Pharmacological blockade of SOCS3 rescued IL-17A-induced decrease of MPT cells. Moreover, higher frequency of IFN-γ^+MPT cells was stimulated in mice without IL-17 signaling. The investigation might enhance the understanding of homeostatic proliferation of memory $CD8^+$ T cell and help overcome infections, tumors and autoimmune diseases.

P0199

WASp is essential for immunological tolerance by promoting IL-2/ IL-2R signaling in Treg

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Approximately 42-70% of Wiskott-Aldrich syndrome (WAS) patients suffer from autoimmune diseases in their lifespan. The underlying mechanism is not fully understood by far. Regulatory T cells (Tregs) play a central role in maintaining of peripheral tolerance. However, it remains controversial whether the number and function of Tregs is affected in the absence of WAS protein. In the present study, we found that thymic Tregs were significantly reduced in WAS patients and WASp-/- mice, the active markers of PD-1/ICOS/CD103 and inhibitory markers of GITR, CTLA4 and LAG3 on Tregs were reduced. WASp-/- mice showed impaired proliferation and inhibition function. The IL-2-IL2α/β-BCL2 or IL-2-IL2α/β-pSTAT5 signaling pathway was disrupted in WASp-/- mice. Additional IL2 treatment was able to restore Tregs number and expression of functional molecules in vitro and in vivo. We also found that WASp promoted IL-2/IL2R signaling by triggering F-actin polarization and enhancing functional makers such as CD25, CTLA4, which suggest WASp is essential for immunological tolerance by promoting IL-2/ IL-2R signaling in Tregs. This study explains the mechanism for

DOI: 10.1002/eji.201970400

autoimmune manifestations associated with WASp deficiency. what's more, it can be a new therapeutic method for WAS patients.

P0200

A regulatory macrophage induced by a helminth molecule alleviate CLP-induced sepsis in mice

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Background: Immunomodulation is a common feature of chronic helminth infections and mainly attributed to the secretion of bioactive molecules, which target and modify host immune cells. **In this study, we show that the helminth immunomodulator r-*Sj*cystain, a cysteine protease inhibitor, induces a regulatory macrophage, which is sufficient to mitigate major parameters of sepsis in mice.**
Methods: Bone marrow-derived macrophage cells (BMDMs) were generated and treated with varying concentrations of r-*Sj*cystain to detect $CD206^+CD86^+$, pro-inflammatory cytokines (IL-6, TNF-α), anti-inflammatory cytokines (IL-10, TGF-β) and MyD88. For adoptive transfer, r-*Sj*cystain treated-BMDMs were labeled with CSFE and then injected intravenously 30 min after to cecal ligation and puncture (CLP) challenge. Then, observing the 96-hour survival rate of the mice after adoptive transfer and the ratio of (FIZZ1, iNOS) in the splenocytes was analyzed by flow cytometry. Blood samples were used to measure biochemical indicators (ALT, AST, BUN and Cr). The infiltration of F4/80 and CD163 macrophages into inflammatory tissues in liver, kidney and lung tissues was observed.
Results: r-*Sj*cystain induces $CD206^+$ and anti-inflammatory cytokines expression but significantly decreased MyD88 level. r-*Sj*cystain-BMDMs ameliorated the severity of the CLP-induced sepsis including reduces iNOS, ALT, AST, BUN and Cr.
Conclusions: Our results demonstrate a specific suppressive macrophage population induced by a single parasite immunomodulator, which protects against inflammatory disease.
Keywords: r-*Sj*cystain, regulatory macrophage, adoptive transfer, inflammatory disease
Supported by the academic and technical leaders for research activities of Anhui (No. 2018H174), Scientific Research Innovation Team Platform of University (No. 2016-40).

P0201

Immunoregulatory functions of immature rbcs in end-stage renal failure disease

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In recent years, studies showed $CD71^+$ $CD235a^+$ immature RBC have immune regulation characterizes.
In this study, we investigated the frequency and the immune-suppression activity of this cells in ESRF patients.
In this study, thirty ESRF patients have participated. 10 ml of heparinized blood was obtained from participants. We did Panel reactive test (PRA) by micro-cytotoxicity assay and patients were divided into two group according to PRA test: 1) positive PRA 2) negative PRA.
PBMC were isolated by centrifugation of density gradient and stain with CD71 FTC and CD235PE monoclonal antibodies. At the next step, we transfer the PBMC into two tubes with RBC lysis and without RBC lysis, stain with CFSE, active by anti-CD3, anti-CD28 antibody and cultured for 72 hours.

DOI: 10.1002/eji.201970400

In this study, we showed the frequency of immature RBC in ESRF were significantly higher in comparison with healthy control (p=0.01). Although after that we divided patients to two groups (PRA positive and PRA negative), our results showed the frequency of immature RBCs in PRA positive patients were not significantly difference with normal control (p= 0.66), but the frequency of immature RBC in PRA negative were significantly higher in comparison healthy control (p< 0.001) and PRA negative patients (p=0.01). also our results showed immature RBCs suppressed lymphocytes activation after 72 hours´ co-culture (p = 0.02).
Conclusion: According to our study, immature RBC may have immunosuppression function but the it need more study about It.

P0202

The effects of regulatory T cells in the pathogenesis of end-stage renal disease in patients with systemic lupus erythematosus

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Autoimmunity is an identified factor for development of end-stage renal disease. Treg play a fundamental role in preventing autoimmunity. This study aimed to determine whether Treg number in ESRD patients suffered from systemic lupus erythematosus (SLE) differed from those with health problems and how Treg frequency influences the cytokine profile of ESRD patients.
PBMC isolated from 26 ESRD and 10 healthy subjects were stained with anti-CD4, anti-CD25, and anti-foxP3 antibodies. Treg percentage and blood transfusion effect on Treg frequency of ESRD patients were determined by flow cytometry. Panel-reactive antibody screening was used to investigate antibodies against HLAs in the patients. The serum levels of TGF-β1, IL-4, IL-10, TNF-α, IL-17A, and IFN-γ in ESRD and healthy subjects were measured by ELISA.
ESRD patients with SLE showed a significant reduction in Treg percentage compared to other groups (p< 0.01). All women participated in the study had a reduced number of Tregs compared to men. Treg frequency in ESRD patients suffered from HLA antibodies was significantly decreased (p< 0.05). Blood transfusion induced Treg development in ESRD patients without SLE (p< 0.05), unlike the patients with SLE. ESRD patients with low Tregs indicated a reduction in TGF-β1 (p< 0.0001) and IL-4 (p< 0.05) levels and an increase in TNF-α (p< 0.01) and IL-17A (p< 0.01) productions compared to control groups. However, no change was observed in IL-10 and IFN-γ levels. There was a weak positive correlation between Treg number and patient age, while this association was significantly negative in healthy subjects (p< 0.01)

P0203

Immune paradigm and the suppressive doctrine of the major diseases of modern man

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Numerous literature data clearly indicate that the basis of the immunopathogenesis of such common human diseases as cancer, atherosclerosis, autoimmune and allergic diseases is disorders of the normal function of the immune system. In turn, the system of immunosuppressive cells, including nTreg, iTreg, subpopulations of macrophages and dendritic cells, suppressor cells of myeloid origin and others play the leading role in the immunopathogenesis of diseases. At the same time, quantitative and qualitative characteristics of suppressor cells increase in tumor diseases and decrease in atherosclerosis, autoimmune and allergic diseases. Recent years, the therapy of all

DOI: 10.1002/eji.201970400

human diseases more and more introduced by methods of molecular and cell immunotherapy, which takes a first place in the effectiveness of treatment. All this makes possible to formulate a provision on the three basic components of the new clinical medicine, including: the immune paradigm of the main diseases of modern man; immunosuppressive doctrine of the immune paradigm of the main diseases of modern man and cellular and molecular immunotherapy as the dominant immunosuppressive doctrine of the immune paradigm of the main diseases of modern man.

P0204

Never ending story - searching for surface markers exclusively expressed on Treg cells

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Since the identification of $CD4^{+}CD25^{+/hi}$ cells as Treg cells in humans in the 2001, significant progress in the characterization of Treg cells has been done, however much remains to be elucidated. Up to date, various surface markers has been suggested as Treg cell markers, however none of them is exclusively expressed on Treg cells. The vast majority of studies have shown that Treg cells are not homogeneous in phenotype and suppressive function, and indicated that a new basis for reliable delineation of human Treg cells is required.
The aim of the study was to determine the expression of surface markers present on human peripheral Treg cells and to evaluate their potential in the identification of Treg cells and their function.
For screening Treg cells, peripheral blood mononuclear cells were isolated from healthy volunteers and stained with commercially available lyophilized antibody array comprised of 371 surface markers and next the data were analyzed by flow cytometry.
Here, we investigated that Treg cells were positive for 136 among 371 checked antigens. The functional analysis of identified markers shown that the majority of them are involved in cells adhesion, TCR signaling, activation and proliferation, trafficking or belong to TNF receptor superfamily.
Moreover, Treg cells have shown expression of markers that until now were not identified on T cells.
Presented results are promising on the future better understanding of the mechanism of action Treg cells and the impact in inflammatory disease development. Moreover, might be useful in diagnosis or monitoring several inflammatory diseases.

P0205

A novel therapeutic strategy for neuroendocrine prostate cancer by targeting tumor-associated inflammation

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Tumor-associated inflammation is involved in aggressiveness and metastatic progression in several types of cancer, including prostate cancer. Here we show an inducing expression of an inflammatory response gene, prostaglandin-endoperoxide synthase 1 (PTGS1), contributes to the development of neuroendocrine prostate cancer (NEPC). NEPC is an emerging resistant phenotype prostate cancer that does not respond to androgen deprivation therapy (ADT) and often treated with chemotherapy, represents a significant therapeutic dilemma and poor prognoses. We demonstrated that ADT induces a dendritic cell-specific transcription factor zinc finger and BTB domain containing 46 (ZBTB46) expression through inhibiting the expression of the androgen-responsive SAM pointed domain containing ETS transcription factor (SPDEF), leading to increased expression of PTGS1 and

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DOI: 10.1002/eji.201970400

contributing to inflammatory response and neuroendocrine differentiation of prostate cancer cells. The single PTGS1 inhibitor treatment cannot reduce tumor growth, whereas knockdown of ZBTB46 enhances the sensitivity of PTGS1 inhibitor and restores ADT sensitivity. Overexpression of ZBTB46 disrupts the tumor-suppressive effect of this combination treatment and induces PTGS1-associated inflammatory response and neuroendocrine markers expression. Our findings provide a novel link between neuroendocrine differentiation and activation of the inflammatory response and provide new biomarkers and therapeutic targets for NEPC.

P0772

IGF-1 inhibits phagocytosis of alveolar epithelial cells in asthmatic mice

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The phagocytosis of apoptotic cells by alveolar epithelial cells helps to eliminate airway inflammation. Insulin growth factor (IGF)-1 regulates cell metabolism and proliferation and promotes cell survival and may promote the proliferation and differentiation of alveolar epithelial cells during the repair of lung injury. This study investigated the effect of IGF-1 on the phagocytic activity of alveolar epithelial cells, a nonprofessional phagocyte. **IGF-1 was elevated in lung tissue and bronchoalveolar lavage fluid from mice with ovalbumin-induced asthma**. **IGF-1 was reduced by 50% in lung tissue and by nearly 100% in the bronchoalveolar lavage fluid in the model mice by depletion of alveolar macrophages by 2-chloroadenosine**. **Interleukin (IL)-33 induced IGF-1 production in primary alveolar macrophages. IGF-1 inhibited phagocytosis of fluorescent microspheres and apoptotic cells by MLE-12 alveolar epithelial cells. Antibody blocking of IGF-1 enhanced the phagocytosis of fluorescent microspheres and apoptotic cells and significantly reduced inflammatory cell infiltration of airway and perivascular tissues**. The elevated IGF-1 in the lungs of asthma model mice was mainly produced in alveolar macrophages. The study found that IGF-1 inhibited phagocytosis by alveolar epithelial cells and that IGF-1 blockade enhanced phagocytic activity and alleviated airway inflammation. These results support study of IGF-1 as a target for the treatment of asthma.

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DOI: 10.1002/eji.201970400

P0773

Tumor-infiltrating CD39$^+$ γδTregs are novel immunosuppressive T cells in human colorectal cancer that facilitate tumor progression

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Background and aims: Tumor microenvironment promotes immune suppression through recruiting and expanding suppressive immune cells such as regulatory T cells (Tregs) to facilitate cancer progression. CD4$^+$ Tregs are considered as the major immunosuppressive T cells in many cancers including human colorectal cancer (CRC). However, this conclusion is not definitive as most of those studies did not extensively examine the whole suppressive T cell landscape. The aim of this study is to investigate different subsets of suppressive T cells within the CRC and further define their function and clinical significance.
Methods: Tumor and paired normal tissues were obtained from 109 patients with CRC who underwent surgical resection to study the phenotype, immunosuppressive activity of CD39$^+$ γδT cells and their induction mechanism in tumor microenvironment.
Results: In this study, we identify a novel CD39$^+$ γδTreg in human colorectal cancer (CRC). CD39$^+$ γδTregs are the predominant regulatory T cells and have more potent immunosuppressive activity than CD4$^+$ or CD8$^+$ Tregs via the adenosine-mediated pathway but independent of TGF-β or IL-10. They also secrete cytokines including IL-17A and GM-CSF which chemoattract myeloid-derived suppressive cells (MDSCs) thus establishing an immunosuppressive network. We further demonstrate that tumor-derived TGF-β1 induces CD39$^+$ γδT cells from paired normal colon tissues to produce more adenosine and become potent immunosuppressive T cells. Moreover, CD39$^+$ γδTreg infiltration is positively correlated with TNM stage and other unfavorable clinicopathological features.
Conclusions: CD39$^+$ γδTregs are the key player in establishment of immunosuppressive tumor microenvironment in human CRC that may be critical for tumor immunotherapy.

P0774

Protective function of interleukin 27 in colitis-associated cancer via suppression of inflammatory cytokines in intestinal epithelial cells

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Numerous studies have demonstrated that inflammation contributes to a variety of cancer formation, among them, colitis-associated cancer (CAC) represents a typical inflammation-related cancer. Interleukin 27 (IL-27) has been demonstrated to play an important role in inflammation-related disease. The effect of IL-27 in intestinal inflammation is controversial and its role in CAC is not elucidated yet. In our present study, we found that IL-27 has protective function in murine model of CAC through suppression of inflammatory cytokines in intestinal epithelial cells (IECs). IL-27Rα (WSX-1) deficiency promotes the CAC development in mice, which is driven b enhanced tumor cell proliferation, more intensive myeloid-derived suppressor cells (MDSC) accumulation in colon lamina propria and higher level of inflammatory cytokines and chemokines in IECs. The levels of IL-6, TNF-α, GM-CSF and CXCL1 triggered *in vitro* by toll-like receptor ligands are significantly upregulated in IECs from WSX-1 KO mice. Removal of commensal microorganism through antibiotic treatment in mice to eliminate TLR ligands deprives the protective function of IL-27 on CAC tumor growth. Thus, IL-27 suppresses CAC formation through an anti-inflammation mechanism targeting IECs and in turn resists the tumorigenesis. Hence, our study explained how IL-27 exerts its anti-inflammatory function on

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DOI: 10.1002/eji.201970400

epithelial cells to fight against chronic-inflammation-associated cancer, which might provide new insights on the potential therapeutic strategies for cancer.

P0775

AhR expression is essential for regulatory T cells to exert their suppressive function

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The aryl hydrocarbon receptor (AhR) is an environmental sensor that detects not only xenobiotic ligand but also physiological compounds generated by host cells. As a molecular link between the environment and the host immune system, the role of AhR in immune cells remains controversial, especially, in regulatory T cells, as both positive effects and negative effects of AhR on Treg differentiation were reported. Here, we generated mouse genetic models to ablate AhR specifically in Treg cells. We showed that AhR ablation did not affect the body weight in even aged mice, and no obvious inflammatory infiltrates in main organs were detected suggesting AhR deficiency in Treg cells may not induce grave autoimmune diseases. However, AhR abrogation in Treg cells led to decreased Foxp3 expression but promoted T-bet transcription, indicating AhR absence changed the nature of Treg cells without disturbing the immune system homeostasis. Implant tumor model in $AhR^{fl/fl}Foxp3^{cre}$mice, the AhR deficiency in Treg cell restricted tumor progress by losing their ability to downregulate IFN-γ production by NK and NKT cells. Taken together, AhR in Treg cells is essential for Treg cells to display their suppressive function.
Acknowledgements: This work was supported by grants from the National Basic Research Program of China (2015CB943203)

P0776

Carcinogen-induced proliferation of thymus-derived Treg cells is triggered by $CD11b^{+}CD301^{-}$ dermal dendritic cells

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Polyaromatic hydrocarbons (PAHs) are prevalent and potent carcinogens, and it also is an important regulator to adaptive immunity.Our present data indicated that 9,10-dimethyl-1,2-benzanthracene (DMBA), amodel PAH widely used to study tumorigenesis, induced functional thymus-derived Treg (tTreg) proliferation but not peripheral derived Tregs(pTregs) in mice draining lymph node after DMBA treatment. DMBA treatment-induced tTreg high proliferative rate was positively associated with DMBA painting area size, and it could be blocked by anti-MHC-II antibody, indicating the process of DMBA induced tTreg proliferation was conducted by antigen presenting cells. To investigate the role of conventional dendritic cells (cDCs) in DMBA treatment-induced tTreg proliferation, ZBTB46-DTR mice bone marrow reconstituted chimera mice were used. When DCs were depleted by diphtheria toxin, the proliferation of tTreg was significantly decreased.Removal of DMBA contacted skin could inhibit the proliferation of tTreg cells,suggesting that migratory DCs might play a crucial role in promoting tTreg proliferation. However, when Langerin-DTA, $Batf3^{-/-}$, or Mgl2-DTR mice, in which Langerhan scells, $CD103^{+}$dermal DCs or $CD11b^{+}CD301b^{+}$dermal DCs can be selectively depleted, respectively, were used, we failed to find any measurable alterations in tTreg proliferation.Taken together, these data suggested that DMBA-induced tTreg proliferation might be dependent on MHC-II molecules expressed

DOI: 10.1002/eji.201970400

by $CD11b^{+}CD301b^{-}$ dermal DCs.
Acknowledgements: This work was supported by grants from the National Basic Research Program of China (2015CB943203)

P0777

Human HLA-DR+CD27+ memory-type regulatory T cells show potent xenoantigen-specific suppression in vitro

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Introduction: We have previously shown that xenoantigen stimulation enhanced human Treg capacity to suppress the xenogeneic response. In this study we investigated whether xenoantigen expanded human Treg express specific cell surface markers and their potential for selection of xenoantigen-specific Treg.
Materials and Methods: Human CD4+CD25+CD127- Treg isolated from healthy donor peripheral blood mononuclear cells (PBMC) were expanded for 3 cycles with anti-CD3/CD28 beads alone or combined with irradiated porcine PBMC as polyclonally (PlTreg) or xenoantigen stimulated Treg (XnTreg), respectively. Flow cytometry was performed to determine candidate cell surface markers and consequent xenoantigen-specific Treg subset was isolated XnTreg by cell sorting. After sorting, the resulting Treg subset was assessed for their suppressive capacity by MLR.
Results: After 3 cycles of expansion, XnTreg exhibited substantially upregulated expression of Treg memory markers HLA-DR and CD27 with a larger proportion of them being $HLA\text{-}DR^{+}CD27^{+}$. The $HLA\text{-}DR^{+}CD27^{+}$ Treg subset separated by cell sorting from XnTreg demonstrated significantly enhanced potency in suppression of proliferating xenoreactive responder cells at ratios of 1:8 through to 1:64, or 1:32 and 1:64 of Treg:responder cells when compared to HLA-DR+CD27+ cell-depleted or unsorted XnTreg, respectively.
Discussion: This study demonstrated that porcine xenoantigen expanded human Treg expressed elevated levels of Treg memory markers HLA-DR and CD27 which could be used for separation of xenoantigen-specific Treg subset. The mechanism of enhanced suppression and stability of this subset are under investigation.
Conclusion: Our data suggest that human HLA-DR+CD27+ memory-type Treg are xenoantigen-specific and have potential as an effective immunotherapy in xenotransplantation.

P0778

MDSC program B cells to differentiate into $CD19^{hi}FcγRIIb^{hi}$ regulatory B cells and the underlying mechanisms

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Objective: To study whether MDSC can induce regulatory B cell generation in vitro and in vivo and the underlying mechanisms.
Methods: We sorted granulocytic and monocytic MDSC by flow cytometry and co cultured them with LPS-activated B cells, and then assayed IL-10 production through ELISA, qRT-PCR and intracellular staining. We use flow cytometry to identify the phenotype of MDSC-induced IL-10-producing B cells. To identify the B-cell subsets from which IL-10-producing $CD19^{hi}FcγRIIb^{hi}$ B cells were differentiated,

DOI: 10.1002/eji.201970400

purified splenic T1, T2, T3, FO or MZ B cells were cultured with MDSC. To determine the differentiation of $CD19^{hi}Fc\gamma RIIb^{hi}$ Bregs , we performed the experiments in transwell systems. We sorted $CD19^{hi}Fc\gamma RIIb^{hi}$ and $CD19^{low}Fc\gamma RIIb^{low}$ B cells and co-cultured them with $CD4^{+}$ T cells activated by anti-CD3/CD28, and then detect the proliferation of $CD4^{+}$T cells.
Results: Either granulocytic MDSC or monocytic MDSC could induce the generation of IL-10-producing B cells with phenotype of $CD19^{hi}Fc\gamma RIIb^{hi}$. IL-10-producing $CD19^{hi}Fc\gamma RIIb^{hi}$ B cells were derived from T1, T2, T3 and MZ B cells, but not FO B cells. $CD19^{hi}Fc\gamma RIIb^{hi}$B cells could obviously inhibit the proliferation of activated $CD4^{+}$T cells through IL-10, but had no significant effect on the activation of T cells. The transwell experiment demonstrated that IL-10 production in $CD19^{hi}Fc\gamma RIIb^{hi}$ Bregs depended on cell-cell contact.
Conclusion: Our study may provide a new manner of MDSC for negative feedback control of T-cell immune response and maintenance of immune homeostasis by, at least partially, inducing differentiation of B cells into regulatory B cells.

P0779

New advances of the tumor immunotherapy

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Tumor is a major disease that seriously endangers human health. The immune system is closely related to the occurrence and development of tumor.In addition to conventional surgery, radiotherapy and chemotherapy, tumor immunotherapy is a new type of treatment.Immunocheckpoint inhibitors represented by cytotoxic t-lymphocyte-associated antigen 4 (CTLA-4) and programmed death receptor-1 (PD-1) have achieved exciting results in the treatment of advanced melanoma, small-cell lung cancer, metastatic bladder cancer and other types of tumors.Studies in mouse solid tumor and hematopoietic tumor models have shown that anti-ctla-4 blocking antibodies can enhance the anti-tumor immune response and long-term survival immunity, and make the mature tumors fade.Clinical trials of pd-1 monoclonal antibodies in patients with advanced melanoma and lung cancer, such as nivolumab and pembrolizumab for refractory advanced melanoma in 2014 and nivolumab for advanced squamous non-small cell lung cancer (NSCLC) and renal cell carcinoma in 2015 were successful and have been approved by the FDA.Chimeric antigen receptor t-cell (Car-T) has an obvious therapeutic effect on acute leukemia and non-hodgkin lymphoma.Car-t cell therapy has a distinct targeting potential for complete clearance of tumor cells, which has been demonstrated in the treatment of acute leukemia with CD19+.Currently, a large number of studies have designed corresponding car-nk for different tumor targets, which has achieved significant efficacy in experimental studies and been verified in clinical studies.How to further improve the efficacy, increase the number of adaptive tumor diseases and control their immune-related adverse reactions becomes the focus of future research.

P0780

Interleukin-10 deficiency impairs regulatory T cell derived neuropilin-1 function and promotes Th1 and Th17 immunity

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Regulatory T cells (Tregs) expanding in peripheral lymphoid organs can produce immunosuppressive cytokines to support tumor growth. IL-10 abrogation efficiently can induce Treg formation, but dampen

DOI: 10.1002/eji.201970400

tumoral neuropilin-1 (Nrp-1) Treg signaling that simultaneously augments Th1 and Th17 immunity. These effects are associated with the plasticity and stability of Treg, and effector T cell function that could limit tumorigenesis. Within the tumor microenvironment, there appears to be the "mutual antagonism" between immunoenhancement and immunosuppression mechanisms eventually leading to decreased metastasis. On the other hand, Tumor progression is paralleled by reduction of Nrp-1-producing Tregs controlled by IL-10 and TGF-β1 levels. However, Th1, Th17 and Treg immunity are mainly regulated by IL-10 or Nrp-1, not TGF-β1 except to combine with IL10. These results emphasize the important implications for the therapeutic use of Treg that is to maintaine the number of Treg cells in a healthy and dynamic homeostatic range to prevent malignant diseases. Besides, Treg-mediated immunosuppression can be limited by reducing tumor-derived Treg Nrp-1 levels.

P0781

miRNA-210 Upregulates Indoleamine 2,3 dioxygenase Activity to Stimulate Adenocarcinoma Bone Metastasis

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As known, indoleamine 2,3 dioxygenase (IDO) expression significantly increases among adenocarcinoma patients, particularly breast, lung, prostate and pancreatic cancers bone metastasis. The experimental inhibition of IDO reduces tumour migration in the mice model and metastatic activity signals. On the other hand, miRNA-210 overexpression was noticed during breast and pancreatic cancer metastasis, especially bone metastasis. Our inhibition of miRNA-210 in the BT-474 and Panc-1 cell lines significantly inhibited the expression of IDO, since the mechanism of this process in the bone metastasis hasn't been identified yet. So, we hypothesize that miRNA-210 upregulates IDO activity to initiate and enhance tumour bone metastasis. Therefore, we investigated molecular signals and active pathways in terms of miRNA-210 and/or IDO gene knockout in the tumour cell lines. Our pre-experimental results showed that miRNA significantly upregulates the IDO pathway in the adenocarcinoma leading to initiating adenocarcinoma bone metastasis. Histological sections of mice model also presented a significant inhibition of tumor metastatic polarization under miRNA-210 depletion. In conclusion, miRNA-210 play an essential role to enhance adenocarcinoma bone metastasis by upregulating IDO pathway.

P0782

IRF8/miR-451a regulates M-MDSCs differentiation by AMPK/mTOR signal pathway during lupus development

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Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease. Myeloid-derived suppressor cells (MDSCs) have been addressed to involve in the regulation of the SLE development. However, little is known about the association between MDSC subsets and the factors that draw MDSC into abnormal expansion. In this study, we found that the percentage of M-MDSCs was increased in pristane-induced lupus mice. TLR7 signal activation and high IFN-α level could promoted M-MDSCs accumulation *in vitro*. Moreover, both AMPK agonist Metformin and two mTOR inhibitors

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DOI: 10.1002/eji.201970400

INK128 and Rapamycin inhibited the percentage of M-MDSCs in pristane-induced lupus mice as well as in the TLR7- and IFN-α-induced bone marrow (BM) differentiation into MDSCs *in vitro*. In terms of mechanism, we performed whole-genome transcriptome profiling by RNA sequencing and found that the expression level of transcription factor IRF-8 was higher in M-MDSCs isolated from pristane-induced lupus mice, compared to control mice. And IRF-8 was identified to be crucial for TLR7- and IFN-α-induced BM differentiation into MDSCs *in vitro*. Furthermore, we found IRF-8 was targeted by miR-451a in M-MDSCs differentiation. Of note, metformin-modified M-MDSCs could relieve lupus symptoms in pristane-induced lupus mice. Our findings first reveal a novel mechanism linking IRF8/miR-451a to M-MDSCs differentiation by AMPK/mTOR signal pathway during lupus development. This study may provide an important reference for SLE therapy by targeting M-MDSCs.

P0783

Heat acclimation induces up-regulation of Tregs and enhance heat tolerance of mice in vivo

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Heat acclimation is known to protect human body from heat related injure, the Physiological mechanisms inside were uncovered by lots of early studies. However,
the immunological mechanisms of Heat acclimation are not clear.
CD4+CD25+FoxP3+Treg cells are known to suppress a wide range of immune
responses through several different mechanisms. In our study, C57BL/6 mouse treated with a process of 30d-heat acclimation were used to make sure whether the heat tolerance is about to the frequency of CD4+CD25+FoxP3+Treg cells in organs,
methods besides survival-analysis, wet/dry weight ratio of organs, flow cytometry and correlation-analysis. Our result shows that after a process of 30d-heat acclimation, the heat tolerance of C57BL/6 mouse goes up. As the up-regulation of frequency of
CD4+CD25+FoxP3+Treg cells in spleens, the frequency of neutrophil reduced, which phenomenon is considered to be a protection factor to those mouse during
heat stroke. We concluded that heat acclimation may induce the production of
CD4+CD25+FoxP3+Treg cells to decrease the level of inflammation, and protect organs under heat stroke.

P0784

Eplerenone blocks Kv1.3 channels to suppress the secretion of Tregs-derived exosome and in turn shrink the proliferation of cardiac fibroblasts

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Many kinds of immune cells accelerate cardiac fibrosis by cell-cell cross talk and / or secreting cytokines, and immunomodulator may be beneficial to heart failure indirectly by regulating immune response. We found that aldosterone receptor antagonist eplererone can directly block the Kv1.3 channel, thus inhibit the activation and proliferation of regulatory T lymphocytes (Tregs) and decrease the secretion of its fibrogenic factor TGF-β to play an indirect role in inhibiting the process of myocardial fibrosis. However the half-life of TGF-β is only a few minutes, so the signal attenuation of transmission proliferation is also rapid. Exosomes, as the important biological carriers, have the important value for cytokine transferring and biological functions transmitting. We also found that Tregs-derived exosomes could promote the proliferation of co-cultured CFs ($P < 0.01$). After 48 h co-incubation of CFs with exosomes derived from Tregs and from co-incubation of the two cells, the

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DOI: 10.1002/eji.201970400

inhibition rates of CFs proliferation by 30 μM eplerenone were 36% and 59%, respectively. It can be concluded that eplerenone can inhibit myocardial fibrosis via directly blocking the Tregs Kv1.3 channel, reducing the secretion of Tregs-derived exosomes and attenuating the signal of TGF-β proliferation.

P0785

Roles of the nuclear orphan receptor Nr4a in Th/Treg differentiation and in regulation of allergic asthma pathogenesis

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CD4T cells play central roles in adaptive immunity, by driving an appropriate immune responses to invading pathogens of diverse types, which is mediated by helper T (Th) cells, as well as by suppressing excess immunity against self and non-hazardous antigens, which is mediated by regulatory T (Treg) cells. As such, the balanced activity of Th/Treg is crucial for the maintenance of immune homeostasis. In this study, we revealed a crucial roles of Nr4a family transcription factors in the regulation of Th/Treg differentiation of naive CD4T (Tnaive) cells. In vitro characterization of Nr4a-de-cient Tnaive cells revealed their augmented skewing to Th1 and Th2 cells, at the expense of iTreg cell differentiation. Transcriptome and genome-wide epigenetic analysis revealed a direct regulation of Th/Treg regulatory genes by Nr4a factors, particularly at early stages (~4 hr) of Th/Treg differentiation. Adoptive transfer of Nr4a-deficient Tnaive cells elicited airway hyperresponsiveness in recipient *CD3e*$^{-/-}$ mice, exhibiting a severe Th2 type inammation and a heightened generation of IgE. Furthermore, Nr4a-de-cient Tnaive cells were devoid of the ability to mediate oral tolerance against food-derived antigen in an experimental asthma model. Finally, to pursue the potential of Nr4a factors as therapeutic targets, we controlled Nr4a activity in the experimental asthma model. As a result, by chemically activating transgenic chimeric Nr4a2 construct at the sensitization phase, allergic asthma pathogenesis was significantly ameliorated. Collectively, our study revealed a crucial role of Nr4a factors in Th/Treg differentiation and in the suppression of pathogenesis of allergic asthma.

P0786

Suppressive effects of flavonoids on T cell activation

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Suppression of T cell activation is important for transplantation therapy, especially to avoid acute rejection. Since T cell proliferation and activation are regulated by interleukin (IL) -2, IL-2 is one of main targets for immunosuppressants.
Flavonoids are found in natural plants and have wide range of biological activities including antioxidant, anti-inflammatory, anti-carcinogenic, anti-cardiovascular, and immunosuppressive actions. We have previously demonstrated the anti-allergic effect of flavonoids was mediated via upregulation

DOI: 10.1002/eji.201970400

of enzymatic activity of heme oxygenase (HO)-1. Although flavonoids are known to suppress IL-2 production in T cells, the exact mechanisms are still unknown. In this study, we investigated the involvement of HO-1 on suppression of T cell activation induced by flavonoids.
We showed that quercetin and kaempferol suppressed the A23187-mediated expression of IL-2 in T cells. These flavonoids induced HO-1, and this induction of HO-1 was implicated in the suppression of IL-2 production. Furthermore, these flavonoids suppressed T cell proliferation through promotion of cell cycle arrest via HO-1 induction. These results suggested that HO-1 is involved in the suppressive effects of flavonoids on T cell activation and proliferation. We are now investigating the signal transduction of how flavonoids affect T cell activation.

P0787

Quercetin-induced cytoprotective action by modification of cell membrane

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Quercetin is one of the flavonoids and has a wide variety of cytoprotective effects. We have previously reported that quercetin exerted anti-allergic, anti-oxidant, and anti-fibrotic activities via heme oxygenase (HO)-1 activity. However, the mechanisms how quercetin can induce HO-1 to exhibit cytoprotective effects are poorly understood. In this study, we investigated the involvement of cell membrane changes in quercetin-induced HO-1.
We showed that quercetin changed the formation of lipid rafts, and the expression level of caveolin-1 (Cav-1), a component of caveolae, was decreased in cell membrane and increased in cytosol and nucleus after exposure to quercetin. Cav-1 and nuclear factor E2-related factor 2 (Nrf2), a transcription factor of anti-oxidant enzymes including HO-1, were interacted in cell membrane and translocated to nucleus from cell membrane after exposure to quercetin. Moreover, quercetin decreased the levels of cholesterol in cell membrane. These findings suggested that HO-1-dependent cytoprotective effects by quercetin would be mediated through the disruption of lipid rafts. The disruption of lipid rafts would translocate Cav-1 and Nrf2 from cell membrane and translocate them to nucleus, leading to HO-1 induction.

P0788

How are resident Tregs retained and maintained in the thymus?

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Foxp3+CD25+ regulatory T cells (Tregs) have emerged as a key component of the immune system critical for maintaining immune tolerance and homeostasis. Most Tregs develop in the thymus and are then released to the immune periphery. Subsequently, some Tregs are retained inside the thymus, while others recirculate back, constituting a large population of yet poorly understood thymus-resident Tregs. In order to better understand the physiological significance of thymus-resident Tregs, we first sought to molecularly characterize them by RNA sequencing using the RagGFP-FOXP3RFP reporter mouse model, which allows to distinguish them from the newly developed (GFP+) Treg cells. We found that unlike newly generated Tregs, thymus-resident Tregs are characterized by high surface expression of IL18 receptor (IL18R) and CD103. Moreover, the relative frequency of the IL18R+ CD103+ Treg subset inversely correlated with age-dependent as well as stress-induced thymic involution, suggesting that they may play an important role in the maintenance of thymic homeostasis.

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DOI: 10.1002/eji.201970400

Finally, our data demonstrate that deficiency of IL18R or CD103 results in decline of the thymus-resident Treg cells, suggesting that IL18R and CD103 are involved in Treg migration and/or retention in the thymus.

P0789

Tcf1 and Lef1 maintain the immunosuppressive function of Foxp3+ regulatory T cells

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Tcf1 and Lef1 have versatile functions in regulating T cell development and differentiation, but intrinsic requirements for these factors in regulatory T (Treg) cells remain to be unequivocally defined. To specifically address the functional requirements for Tcf1 and Lef1, we ablated both genes using the *Foxp3* locus-driven Cre recombinase. Deficiency in Tcf1 and/or Lef1 does not perturb Treg cell homeostasis. Specific ablation of Tcf1 and Lef1 in Treg cells resulted in spontaneous multi-organ autoimmunity that became more evident with age. Tcf1/Lef1-deficient Treg cells are functionally impaired, which showed reduced protection against experimentally induced colitis, indicative of diminished immuno-suppressive capacity. Unexpectedly, Tcf1 and Lef1 synergize with T-bet in regulating Treg suppressive function, and Tcf1 and Lef1 were necessary for restraining expression of cytotoxic CD8+ effector T cell-associated genes in Treg cells, including *Prdm1* and *Ifng*. Our data collectively indicate that Tcf1 and Lef1 are critical for sustaining Treg suppressive functions and preventing loss of self-tolerance.

P0790

Δ42PD-1 suppresses maturation of dendritic cells through induction of interleukin-10

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Dendritic cells (DCs) are a heterogeneous population of professional antigen presentation cells, which elaborately bridge the innate and adaptive immune response. Several DC-based vaccines have become a promising tool for treatment of viral infection and for cancer immunotherapy. Previously, we discovered a new PD-1 isoform, which consists of a 42-base pairs loss from the start of exon 2 that is equivalent to a 14 amino acid in-frame deletion. We named this new PD-1 isoform as Δ42PD-1. Δ42PD-1 is highly expressed on the surface of immature monocyte-derived dendritic cells (MoDCs). Therefore, we sought to determine the role of Δ42PD-1 in regulating DC maturation, which plays important role in T cell priming for immunotherapy. In this study, we found that Δ42PD-1 was negatively correlated with MoDC maturation after different TLRs ligations and Δ42PD-1 negative MoDCs displayed higher level of costimulatory molecules (CD80/CD86) with or without stimulation. In the MLR assay, compare to Δ42PD-1 positive MoDCs, the Δ42PD-1 negative MoDCs can induce higher proliferation and IFN-γ production of allogeneic T-cell. Mechanistically, Δ42PD-1 positive MoDCs produced large amounts of interleukin 10 (IL-10) to inhibit the NF-κB pathway by activating the STAT-3. So far, we conclude that Δ42PD-1 could suppress dendritic cell maturation via induction of IL-10, leading to loss of T cell stimulatory activity. It warrants to further study the potential ligand of Δ42PD-1 and the specific agent for Δ42PD-1 blockage.

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DOI: 10.1002/eji.201970400

P1377

Interaction of cancer cell-derived Foxp3 and tumor microenvironment in human tongue squamous cell carcinoma

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The forkhead transcription factor, Foxp3, has been proved essential for differentiation and activation of regulatory T cells (Tregs). Recently, Foxp3 expression in tumor cells (cancer cell-derived Foxp3) has gained increasing interest, but the function has yet to be confirmed. In the current investigation, we identified the interaction of cancer cell-derived Foxp3 and tumor microenvironment in human tongue squamous cell carcinoma(TSCC) by various *in vitro* methods. We detected cancer cell-derived Foxp3 was closely associated with the infiltration of Foxp3+ lymphocytes in TSCC lesions using immunohistochemical staining. The cytokines secretion (IFN-γ, TGFβ, IL-2, IL-6, IL-1β, IL-10, IL-8, IL-17, IL-23) of PBMC and differentiation of CD4+T cells were modulated by the expression of Foxp3 in TSCC, shown by ELISA and flow cytometry. As feedback, increasing TGFβ and decreasing IL-17 further up-regulated cancer cell-derived Foxp3. Furthermore, CHIP on chip assay showed that both TGFβ and IL-17 decreased the number of Foxp3-binding genes in TSCC. GO and pathway analysis suggested that, treated with TGFβ or Th17, Foxp3-binding genes were inclined to the negative regulation of TGFβ signal pathway. Taken together, this study showed cancer cell-derived Foxp3 contributed to Tregs expansion in TSCC microenvironment with positive and negative feedbacks.

P1378

CD19+IL-10+ regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4+ T cells to CD4+Foxp3+ regulatory T cells

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Objectives: Increase of regulatory T cells (Tregs) in the tumor microenvironment predicts worse survival of patients with various types of cancer including tongue squamous cell carcinoma (TSCC). Recently, the cross-talk between Tregs and regulatory B cells (Bregs) has been shown in several tumor models. However the relevance of Bregs to tumor immunity in humans remains elusive. Our objective was to investigate the distribution and function of Bregs in TSCC microenvironment.
Materials and Methods: Double staining (Bregs: IL10/CD19 and Tregs: Foxp3/CD4) was performed on tissue sections of 46 TSCC, 20 metastasis lymph nodes, and tumor adjacent normal tissue. Flow cytometry analysis was used to detect the Bregs from magnetic bead- sorted B cells after co-culture with TSCC cell lines, and Tregs from sorted $CD4^+ CD25^-$ T cells after co-culture with stimulated B cells.
Results: The immunohistochemical (IHC) results showed that the frequency of Bregs/$CD19^+$ B in TSCC (0.80%±0.08%) was significantly higher than adjacent normal tissue (0.52%±0.04% $p < 0.01$.). And the increase of Bregs in TSCC microenvironment was related to Tregs and predicts worse survival in patients. Cytological experiments indicated that frequency of Bregs increased after co-culture with TSCC cell line and that the induced B cells converted $CD4^+ CD25^-$T cells into Tregs.
Conclusion: The increased expression of Bregs in the TSCC microenvironment plays a significant role in the differentiation of resting $CD4^+$ T cells and influenced the prognosis of TSCC patients.

DOI: 10.1002/eji.201970400

P1379

A strategy of targeting B10 cell by CD19scFv-IL10R for tumor therapy

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IL-10 producing B (B10) cells, a subset of regulatory B (Breg) cells, produce IL-10 and play immunosuppressive roles in antitumor immunity. B10 cells are associated with enhanced tumor-aggressiveness and a poorer prognosis. To specifically inhibit the IL-10 secreted by B cells, we constructed the recombinant plasmid pcCD19scFv-IL10R, which contained the gene of anti-CD19 single-chain variable fragment (CD19scFv) and the extracellular domain of IL-10R1. Soluble CD19scFv-IL10R protein was identified *in vitro* and *in vivo* after the cells were transfected with pcCD19scFv-IL10R plasmid or the mice were injected with the plasmid. The fusion protein had the bispecific ability to target both IL-10 and CD19 molecules *in vitro*. Intramuscularly (*i.m.*) injecting mice with pcCD19scFv-IL-10R plasmid inhibited hepatocellular carcinoma growth *in vivo*. Mice treated with pcCD19scFv-IL-10R showed a significant reduction in B10 cells and regulatory T (Treg) cells, but an increase in the anti-tumor Th1 immune response and the cytotoxic $CD8^+$ T cell response. Thus, targeting B10 cells by CD19scFv-IL10R molecule may offer a new avenue for tumor therapy.

P1380

The interaction between DENV-2 infected HUVECs and macrophages on major inflammatory cytokines

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To study the HUVECs infected by DENV-2, Co-culturing with macrophages, and the Effects of major inflammatory cytokines were observed in two kinds of cells. Monocyte were obtained from culture flasks and macrophages were obtained by stimulation of M-CSF. After HUVECs were infected with DENV-2, the expression of NS1 in HUVECs cells was detected. And transwell was used to co-culture with Macrophages. The control group was pretreated with CYM-5442, Co-culture with macrophages. The reverse transcription levels of IL-6, IL-8, TNF-α and IL-1β in HUVECs and Macrophages were detected. The main cytokines in culture supernatants were detected. The transcription level of NS1 gene gradually increased and reached a peak at 24h. The transcriptional levels of IL-6 and IL-8 in HUVECs infected with DENV-2 were up-regulated, reached peak at 24h after infection. The expression of IL-6, IL-8, TNF-alpha and IL-1β in Macrophages infected with DENV-2 increased significantly. After pretreatment with CYM-5442, the mRNA transcription levels of IL-6 and IL-8 of the infected HUVEC were significantly decreased. DENV-2 can infect primary HUVECs, and HUVEC infected by DENV-2 can activate Macrophages promote the secretion of large amounts of IL-6, IL-8, TNF-α and IL-1β. At the same time, activated Macrophages reduce the production of inflammatory cytokines in HUCECs to a certain extent.
Keywords: DENV-2, HUVECs, Macrophages, Cytokines, Immunoregulation

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DOI: 10.1002/eji.201970400

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Passive DNA demethylation mediated by ETS-1 play a key role in stable expression of Foxp3 induced by RAPA

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Stability of Foxp3 expression is very important for the function of nTreg and iTreg. Rapamycin (RAPA) could maintain the stability of Foxp3 expression, however, the mechanism for that is unclear.
In this study, we found that RAPA could promote the stability of Foxp3 expression in iTreg induced by TGF-β/IL-2/RAPA in vitro via maintaining the demethylation state of the CpG motifs within Treg-specific demethylated region (TSDR, as known as CNS2) , but not CNS1 and CNS3. Whereas we didn't observe the significant upregulation of the ten-eleven translocation (TET) family protein (Tet1, 2 and 3) in iTreg, which are key enzymes for DNA demethylation. Instead, the DNA methyltransferases family proteins (DNMT1, 2 and 3) that mediate DNA methylation are upregulated. We further analyzed the demethylation locus of TSDR and found that the locus is a specific binding cite (ATCCG) of ETS-1 transcription factor. ChIP results confirmed this. Meanwhile, the expression of ETS-1 is upregulated by RAPA during conversion from conventional $CD4^+$ T cells to iTreg. When EST-1 was knocked down, both the demethylation of TSDR and the stable expression of Foxp3 in iTreg are reduced significantly. These results suggested that passive DNA demethylation mediated by ETS-1 is involved in stable expression of Foxp3 induced by RAPA.

P1382

Hes1 attenuates type I IFN responses via VEGF-C and WDFY1

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Induction of type I interferons (IFNs) is critical for eliciting competent immune responses during viral and bacterial infections. However, excessive IFN production often acts as an amplifier of undesirable autoimmune and inflammatory responses and has been causally linked to pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE). Therefore, comprehensive understanding of positive and negative regulatory mechanisms controlling magnitude and duration of IFN production is essential for rationally design pharmacological interventions targeting IFNs in human diseases. In this study, we found that transcription factor Hes1 suppressed production of type I IFNs and expression of IFN-stimulated genes at both basal and TLR-stimulated conditions. As a result of heightened IFN expression, Hes1-deficient mice displayed heightened IFN signature, mounted enhanced resistance against encephalomyocarditis virus infection, and exacerbated experimental lupus nephritis *in vivo*. Mechanistically, Hes1 did not suppress IFNs via direct transcriptional repression of IFN-encoding genes. Instead, Hes1 attenuated activation of TLR upstream signaling by inhibition of a TLR signaling adaptor molecule WDFY1. Genome-wide assessment of Hes1 occupancy revealed that suppression of WDFY1 was secondary to direct binding and thus enhancement of expression of VEGF-C by Hes1, making *Vegfc* a rare example of Hes1-positively regulated gene. Furthermore, the molecular connections between Hes1 and VEGF-C-WDFY1 were functionally validated *in vivo* in viral infection and experimental lupus models. In summary, these results identified

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DOI: 10.1002/eji.201970400

Hes1 as a homeostatic negative regulator of type I IFNs for the maintenance of immune balance in the context of anti-viral immunity and autoimmune diseases.

P1383

Gulingwan alleviates collagen-induced arthritis in mice by down-regulating myeloid-derived suppressor cells

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The objective of the study is to intervention mechanism of Gulingwan (GLW), a traditional Chinese medicine for tonifying kidney and strengthening the bone marrow, to affect rheumatoid arthritis (RA) bone destruction by regulating myeloid-derived suppressor cells (MDSC). In CIA model induced by DBA/1J mice, each group of the CIA mice were continuously intragastrically administrated for 30 days with high-dose GLW, medium-dose GLW, low-dose GLW, MTX, MTX combined with medium-dose GLW and ddH_2O separately. High-dose GLW, MTX, MTX combined with GLW could all reduce the arthritis score and pathological score of CIA mice. Flow cytometry showed that high-dose GLW and GLW combined with MTX intervention could significantly reduce the proportion of MDSC and its subgroups in spleen and bone marrow, and the proportion of Th17 cells in spleen of CIA mice. High-dose GLW could down-regulate the expression of RANKL on the surface of Th17 cells. Micro-CT and TRAP staining showed that high-dose GLW, MTX and MTX combined with GLW could increase the bone mineral density and bone volume fraction and reduce the number of osteoclast (OC) in the joint of CIA mice. It was found that medium-dose GLW, high-dose GLW and MTX combined with GLW can reduce the level of RANKL in plasma and increase the level of OPG. **GLW may inhibit the occurrence of bone erosion by inhibiting the amplification of MDSC and its subgroups in CIA and reducing its potential of inducing Th17 cell differentiation and its differentiation into OC.**

P1384

A critical role of PERK/ATF4 signaling in maintaining the lineage stability of natural Treg cells under prolonged hypoxia

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Regulatory T cells (Tregs) contribute dominantly to the immune suppression which maintain immune homeostasis and limit collateral damage from deleterious immune response. However, how Treg cells function and maintain their lineage stability under stressful conditions remains largely elusive. In this study, we investigate the effect of hypoxia, a common hallmark of colitis and tumor, on the Treg cells. We observed that hypoxia impaired *de novo* Treg cells differentiation from naïve T cells, but didn't destabilize the natural Treg cells in the presence of TGF-b in vitro. Interestingly, under prolonged hypoxic stress, HIF-1a expression was dismissed. In contrast, hypoxia induced the PERK/ATF4 signaling of Unfolded Protein Responses. Co-transfection of ATF4 enhanced the expression of *Foxp3*-luciferase, suggested that ATF4 could regulate Foxp3 expression directly. In addition, our preliminary data show that mouse with conditional deletion of ATF4 in Treg cells predisposed to autoimmune

DOI: 10.1002/eji.201970400

diseases, such as splenomegaly and dermatitis. The $CD4^+$ and $CD8^+$ T cells of $ATF4^{flox/flox}$_$Foxp3^{Cre}$ mouse were highly activated and proliferated while co-occurred with loss of Treg cells in vivo. Taken together, our results suggested that ATF4 might act as a HIF-1a-independent factor to maintain the lineage stability of natural Treg cells in response to prolonged hypoxia. Our study will provide a new insight into the mechanism regulating Treg function and stability under hypoxic stress.

P1385

Erythroid progenitor cell reprogrammed to MDSC-like immune suppressive cells in late-stage cancer

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Hematopoiesis is an intricately controlled developmental process that could be dysregulated during tumor progression. Our previous study identified a novel potent immunosuppressor cell type, $CD45^+$ erythroid progenitor cells ($CD45^+$EPCs) in tumor-bearing hosts. Besides having the similar immunosuppressive function as myeloid-derived suppressor cells (MDSCs). CD45+EPC's gene expression profile resembles MDSC's. We hypothesized that there might be abnormal lineage differentiation between erythroid and myeloid cells under tumor influence. In this study, we demonstrated that $CD45^+$EPCs from extramedullary erythropoiesis organs (spleen and liver), either *in vitro* or *ex vivo*, can differentiate into MDSCs, with $Ter119^+CD71^+CD45^+CD11b^+Gr\text{-}1^+$ phenotype These $CD45^+$EPCs derived MDSCs ($Ter119^+CD71^+$MDSC, D^{pos}MDSCs) widely existed and increased with tumor progression in tumor tissues and possessed greater suppressive capacity than classical MDSCs ($Ter119^-CD71^-$MDSC, D^{neg}MDSCs). Transfer of D^{pos}MDSCs significantly increased tumor growth and metastasis than transfer of D^{neg}MDSCs, either in B16F10- or LLC-bearing mouse model. Mechanically, D^{pos}MDSCs expressed higher level of immune suppressive molecules, including Arg-1, iNOS, CD38 and CD49. In cancer patients, $CD45^+$EPCs can convert into D^{pos}MDSCs to suppress immunity with the expression of MDSC related immune suppressive molecules. This study therefore identifies a new origin of MDSC and provides clues to the design of novel anti-tumor immunotherapy.

P1386

Hepatic NK cells contribute to dysfunctional CD8+T cells through regulating CD11b+ DCs in HBV persistent infection

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Immune regulation in the liver is controlled by innate immune cells that enable fine-tuned modulation of T cell immunity and tolerance. However, the regulatory role of NK cells during persistent HBV infection is still elusive. Here we show that the frequency and absolute number of IFN-γ+NK cells and IFN-γ+CD8+T cells decreased markedly in patients with CHB compared with healthy controls. Meanwhile, hepatic DX5+CD49a-NK cells secreted less IFN-γ along with reduced frequency of HBcAg-specific CD8+ T cells which is correlated with HBV persistence. However, higher IFN-γ produced by hepatic DX5+CD49a-NK cells was accompanied with HBcAg-specific CD8+ T cell activation in HBV-clearance mouse model. In addition, CD11b+DCs were less activated in HBV carrier

mice compared with that of HBV-clearance mice. More importantly, adoptive transfer of IFN-γ-sufficient NK cells of HBV-clearance mice restored CD11b+ DC activation in HBV transfected Nfil3-/- mice. By contrast, adoptive transfer of IFN-γ-sufficient DX5+CD49a$^-$NK cells from HBV-carrier mice can not restored CD11b+DC activation and donor CD8+ T cell function in HBV transfected Nfil3-/- mice. Therefore, we observed a three-way interaction, whereby dysfunctional DX5+CD49a-NK cells fail to activate CD11b+DCs and HBV-specific CD8+ T cells leading to HBV tolerance in HBV carrier mice, while NK cell derived IFN-γ and CD11b+ DC were involved in anti-HBV CD8+ T cell responses in HBV-clearance mice.

P1387

IL-17 prevents regulatory T cells-mediated suppression of CD4$^+$ T cells in psoriasis

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Regulatory T cells (Tregs) are a subset of CD4$^+$CD25$^+$ T cells which are critical in maintaining the self-tolerance. In normal conditions, Tregs prevent the aberrant activation of effector T cells (Teff) and sustain the immune homeostasis. In psoriatic patients, reduced inhibitory function of Tregs lead to the uncontrolled activation of autoreactive T cells. However, the mechanism referring to the impaired function of Tregs in psoriatic patients remains unclarified. IL-17 family cytokine, secreted by T helper cell 17 (Th17), promotes the keratinocyte hyperproliferation and drives the secretion of proinflammatory cytokines and chemokines in psoriasis. Thus, the IL-17 signaling pathway is critical in triggering the pathogenesis of psoriasis. As IL-17A is significantly upregulated in psoriatic lesions, we suspected that IL-17 might contribute to the impaired inhibitory function of Tregs in psoriatic patients. Firstly, we found that addition of IL-17 had no remarkable effect on the proliferation of Teff cells. However, the addition of IL-17 at 20 ng/ml could significantly block the suppressive function of Tregs on Teff cells. Importantly, we uncovered that IL-17-mediated suppression of the inhibitory function of Tregs were related to the reduced secretion of inhibitory cytokine TGF-β. To clarify the signaling pathway downstream of IL-17 in psoriatic Tregs, we screened the NF-κB and MAPK pathways. Moreover, we found that phosphorylation of NF-κB was increased in psoriatic Tregs, revealing an aberrant activation of NF-κB signaling pathway. Our study uncovers a previously unrecognized role for IL-17 in blocking the suppressive function of Tregs on CD4$^+$ Teff cells in psoriatic patients.

P1388

Xeno-transplantation of human umbilical cord tissue mesenchymal stem cells attenuates the development of collagen-induced arthritis in rats

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Rheumatoid arthritis (RA) is a T-cell-mediated systematic disease and is usually accompanied by bone and cartilage damage. Mesenchymal stem cells have immunomodulatory and regenerative potential thus make them a potential therapeutic agent for RA. In the present study, we have evaluated the efficacy of human mesenchymal stem cells (MSCs) in RA treatment. MSCs were isolated and cultured by explant method from human umbilical cord tissue and were characterized for

DOI: 10.1002/eji.201970400

cell surface markers (CD73+, CD90+, CD105+, CD34-, CD45-) by flowcytometry and differentiation potential.
The immunomodulatory effects of MSCs on mononuclear cells isolated from the synovial fluid and peripheral blood of the RA patients (proliferation and activation status of T cells, T reg, Th17 cells population) was studied by using flow-cytometry and cytokine bead array analysis. There was decrease in expression of activation markers- CD25, CD38 ,HLA-DR and proinflammatory cytokines- TNF- alpha, IL-1Beta and increase in anti-inflammatory cytokines.
MSCs were injected into rat with collagen induced arthritis (CIA) and were monitored for severity of joint swellings using digital caliper for 2, 4 and 6 weeks.Transplantation of hUCMSCs in CIA rats significantly ameliorated the severity of arthritis after long term treatment with stem cells. Histopathological analysis revealed pathological changes had been alleviated in rats treated with MSCs as compared to untreated CIA Rats. There was a decrease in expression of genes related to bone and cartilage damage(TRAP, Catepsin K, MMP3, MMP9) after MSC treatment. These changes suggest the potential of MSC treatment in regeneration of damaged cartilage and bones.

P1389

CD19+CD24^{hi}CD38^{hi} regulatory B cells: a potential immune predictive marker of severity and therapeutic responsiveness of HCV infection

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Background: Hepatitis C virus (HCV) infection is associated with abnormal immune responses. Since regulatory T and B cells modulate the progression of infectious and inflammatory diseases, this study aimed at examining how these cells are involved with the development and progression of HCV infection.
Methods: The frequencies and phenotypes of peripheral circulating regulatory B and T cells were characterized using flow cytometry. Both the potential association and dynamic changes of these cells with related clinical parameters were analyzed after Direct-Acting Antiviral (DAA) agent treatments.
Results: The frequencies of circulating Bregs in HCV-infected patients increased significantly, and were positively correlated with levels of sera HCV RNA load, AST and TBILI. Additionally, the increased Bregs returned to normal levels after DAA treatment. However, circulating Tregs increased markedly in patients with HCV-cirrhosis, and were significantly associated with APRI index and FIB-4 scores.
Conclusions: Increased circulating Bregs not only may be associated with poor viral eradication and liver injury but also may provide an important predictive marker of HCV disease severity and therapeutic efficacy following DAA-treatment. Tregs probably control disease status in the early stages but may, paradoxically, contribute to the progression of liver fibrosis in the late stages of HCV infection.

DOI: 10.1002/eji.201970400

P1390

Altered regulatory T cells at the maternal foetal interface in HIV infected women

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Background: The hallmark of HIV infection is progressive CD4 lymphopenia and increased HIV-specific CD8 responses. Furthermore, HIV infection has been shown to impact on the regulatory T cell (Treg) pool. In the context of pregnancy, there is very limited data on the effect of HIV exposure on these T cell phenotypes at the maternal foetal interface (MFI).
Methods: Placentas were collected at delivery from HIV-infected women (n=24), taking antiretroviral therapy (ART) and compared with HIV-uninfected controls (n=6). Cells were liberated from decidual parietalis (DP), decidua basalis (DB) membranes and villous tissue (VT) and stained with a 15 colour flow cytometry panel to identify placental Treg cells. Data was analysed in FlowJo and with R.
Results: There were lower proportions of CD4+ T cells and higher CD8+ T cells in the maternal decidua (both p< 0.0001), reflecting the maternal systemic circulation. There was a higher trend of CD4+ Tregs (defined as CD127-CD25^{Hi}FoxP3++) in placentas from HIV-infected women, however, co-expressing significantly lower highly suppressive CD39, TIGIT and CTLA4 markers (p=0.03). Using unbiased data analysis, we identified a unique CD8+ population expressing a Treg phenotype in DP, DB and foetal VT. When compared with placentas from healthy controls, the novel CD8+CD127loFoxP3+CD28+PD1+TIGIT+ population was significantly lower in DP and DB (p=0.04 and p=0.01) while CD8+CD127loFoxP3+CD28-TIGIT+ were significantly lower in VT (p=0.01).
Conclusions: Collectively, our data show that either HIV and/or ARV drugs were associated with lower proportions of highly suppressive CD4+ Treg as well as a unique population of CD8+ Treg at the MFI.

P1391

Immunoregulatory functions of TNF in steady state and during neuroinflammation

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Deregulation of TNF has been observed in numerous autoimmune diseases, including multiple sclerosis (MS). However anti-TNF therapy was not successful in clinical trials of MS. One possible explanation might be that in addition to its pathogenic features, TNF can also possess immunoregulatory functions.
Our goal was to investigate the role of TNF/TNFR2 in the maintenance of suppressive microenvironment in steady state and during neuroinflammation. For this purpose, we generated and validated doubly humanized TNF/TNFR2 mice, with the option of conditional inactivation of TNFR2. TNFR2 deficiency in Treg cells resulted in down-regulated expression of Treg signature molecules and significant exacerbation of EAE (Atretkhany et al, PNAS, 2018). Furthermore, 6-8 months old mice with TNFR2-deficiency in Treg cells acquired imbalance in T-cell compartment. In particular, we observed the accumulation of Treg cells with decreased suppressive features and accumulation of effector Th1 and Tc1 cells. Moreover, newly generated doubly humanized hTNFKI x hTNFR2KI allowed us to investigate the efficacy of systemic anti-TNF therapy vs myeloid specific inhibition of TNF with MYSTI (Efimov et al, PNAS, 2016) in experimental autoimmune encephalomyelitis (EAE).

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DOI: 10.1002/eji.201970400

Systemic anti-TNF therapy with Infliximab, but not with myeloid-specific treatment with MYSTI, increased EAE severity at late stages.
Altogether our data confirm that intrinsic TNFR2 signaling is crucial for the maintenance of Treg suppressive functions in steady state and during neuroinflammation and indicate that cell-type restricted therapy may have an advantage over systemic TNF neutralization.
This work is supported by the Russian Science Foundation grant # 19-75-30032.

P1392

Lack of NFATc1 SUMOylation prevents autoimmunity and alloreactivity

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Post-translational modification with SUMO is known to regulate the activity of transcription factors, but how SUMOylation of individual proteins might influence immunity is mostly unexplored. The NFAT transcription factors play an essential role in antigen receptor-mediated gene regulation. SUMOylation of NFATc1 represses IL-2 *in vitro,* but its role in T cell-mediated immune responses *in vivo* is not clear. To this end, we generated a novel *Nfatc1* transgenic mouse, which prevents SUMO modification of NFATc1. Avoidance of NFATc1 SUMOylation ameliorated experimental autoimmune encephalomyelitis as well as graft-versus-host disease. An elevated IL-2 production promoted Treg expansion and suppressed autoreactive or alloreactive T cells. Mechanistically, increased IL-2 secretion counteracted IL-17 and IFN-γ expression through STAT5 and Blimp-1 induction. Then, Blimp-1 repressed IL-2 itself and the as well induced, proliferation-associated survival factor Bcl2A1. Collectively, we demonstrate that prevention of NFATc1 SUMOylation fine-tunes T-cell responses towards lasting tolerance. Thus, targeting NFATc1 SUMOylation presents a novel and promising strategy to treat T cell-mediated inflammatory diseases.

P1393

Mitochondrial reactive oxygen species induced by NADPH oxidase 2 deficiency enhances Th1/Th17 immune responses through SHP1/JAK/STAT signaling

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DOI: 10.1002/eji.201970400

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Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a professional reactive oxygen species (ROS) generating enzyme. NADPH oxidase 2 (Nox2) initially identified in phagocytes is responsible for the production of ROS that kills engulfed pathogens. Chronic granulomatous disease (CGD) is a rare hereditary disorder caused by mutations in a component of NADPH complex. Therefore, CGD patients are prone to be severely susceptible to life-threatening infections from bacteria and fungi. However, paradoxically, CGD patients also suffer from autoimmune diseases caused by excessive immune reactions. Despite the co-existence of these contradictory symptoms, the molecular mechanisms are still unclear and controversial. In this study, we uncovered Nox2 deficiency in T cells promotes *in vitro* T cell proliferation and type 1 help T cell (Th1) and Th17 cell-mediated immune responses in a T cell-intrinsic manner. Furthermore, using Th1 and Th17-mediated disease model, experimental autoimmune encephalomyelitis (EAE), we clarified Nox2 deficiency enhances Th1/Th17-mediated inflammation through augmented mitochondrial reactive oxygen species (ROS). These are due to increased mitochondrial respiration capacity and mitochondrial biogenesis induced by lack of Nox2. We revealed increased mitochondrial ROS diminishes tyrosine phosphatase SHP-1 activity and this attenuated SHP1 activity by mitochondrial ROS promotes Th1/Th17 immune responses through JAK/STAT signaling. Collectively, we show enhanced T cell proliferation and Th1/Th17 differentiation in Nox2 deficient $CD4^+$ T cells via regulation of mitochondrial ROS and exaggerated EAE phenotypes through increased Th1/Th17-mediated inflammation. Therefore, our data indicate mitochondrial ROS-induced by a Nox2 deficiency in T cells is crucial for the Th1/Th17 inflammatory program.

P1394

Nanosecond electroporation causes SP2/0 cell tumor ablation and anti-tumor immune responses in BALB/c mice

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Calcium electroporation is a new modality of electrochemotherapy. It utilizes short pulsed electric fields (PEF) pulses to permeabilize cell membranes and expose them to supraphysiological doses of calcium, which leads to acute cancer cell death. We have investigated the influence of nanosecond electroporation on tumors growth and formation of anti-tumor immune response. Luciferase expressing SP2/0 myeloma cells were used to induce tumors in BALB/c mice. The tumors were treated with 12 kV/cm nanosecond range PEF with and without $CaCl_2$. Electroporation resulted in partial or complete tumor regression and significant prolongation of lifespan. However, $CaCl_2$ did not show a synergistic effect.
PEF induced development of anti-tumor immune response. The number of CD4 and CD8 T cells in spleens were increased in PEF treated mice compared to tumor-bearing and tumor free mice. The tumor growth caused increased suppressor cells (myeloid suppressor, $CD4^+CD25^+$ and Tr1 suppressor T cells) as well as $CD8^+Dx5^+$ cells in the spleens. The percentage of that cells decreased after PEF treatment. PEF treatment caused increased $CD4^+$ Tcm ($CD44^+CD62L^+$) cells in spleens and lymph nodes, decreased $CD4^+CD28^-$ cells in spleens, lymph nodes and in tumor infiltrated CD4 cells. Cytotoxic T lymphocytes obtained from mice with complete tumor remission showed better results in CTL assay compared to cells obtained from tumor free o tumor-bearing mice. PEF treatment induced formation of tumor cell specific antibodies in the blood. Our results show that tumor treatment with PEF induces anti-tumor immune response and prolongs lifespan of mice.

DOI: 10.1002/eji.201970400

P1395

TCF/LEF family transcription factors in Treg differentiation and function

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$CD4^+Foxp3^+$T regulatory (Treg) cells are key players in preventing lethal autoimmunity and deleterious tissue inflammation. To fulfill this role, Tregs undertake activation and differentiation processes and acquire diverse functional properties. However, how Treg's differentiation and functional specifications are regulated remains incompletely understood. Here we report that gradient expression of two TCF/LEF family transcription factors, TCF1 and LEF1, distinguishes Tregs into three distinct subpopulations. Treg-specific ablation of TCF1 and LEF1 renders the mice susceptible to systemic autoimmunity and aberrant activation of cellular and humoral responses. The development of T follicular regulatory (Tfr) cells is completely abolished in TCF1/LEF1-conditional knockout mice, leading to unrestrained T follicular helper (Tfh) and germinal center B cell responses. Thus, TCF1 and LEF1 act redundantly to control the maintenance and functional specification of Treg subsets to prevent autoimmunity.

P1967

Exosomes derived from MDA-MB-231 promote macrophages invasion

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In breast cancers, macrophages have been observed to accumulate significantly in the tumor microenvironment. To clarify how breast cancer cells promote the invasion of macrophages to the tumor, we firstly investigate the effect of culture supernatant of breast cancer cells on the invasion of macrophages by transwell experiment. We found the migrated macrophages increased significantly with the treatment of supernatant from MDA-MB-231 or MCF7 cells. Secondly, we purified exosomes derived from MDA-MB-231 and MCF7 cells and investigated their effects on the invasion of macrophages. Results showed that both exosomes promoted the invasion of macrophages, but migrated macrophages treated with exosomes from highly metastatic MDA-MB-231 are much more than that from MCF7 cells. Previous studies have shown SIPA1 protein is important to maintain the metastasis activity in MDA-MB-231. Macrophages treated with exosomes derived from SIPA1-knock down MDA-MB-231 (231/sh-SIPA1) showed lower invasion activity compared with those treated with MDA-MB-231 derived exosomes. On the other hand, the expression of five invasion-related proteins increased in macrophages treated with exosomes derived from MDA-MB-231 comparing with that from 231/sh-SIPA1 cells. Then, the proteins in exosomes derived from MDA-MB-231 and 231/sh-SIPA1 cells were identified by mass spectra, and 8 protein candidates related to macrophages invasion were screened out by GO enrichment analyses and KEGG analysis. indicating that high expression of SIPA1 could promote the macrophages invasion via MDA-MB-231-derived exosomes. Our study suggests that SIPA1 could regulate the expression of genes related to macrophages invasion promoting breast cancer metastasis.

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DOI: 10.1002/eji.201970400

P1968

Hepatocellular carcinoma-induced aberrant erythropoiesis promotes tumor progression

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Hepatocellular carcinoma (HCC) has become the second lethal cause of cancer death globally. Non-neoplastic cell populations in tumor microenvironment have been reported to play critical roles in cancer progression. Whether and how primary tumors drives the generation of the previously unknown non-leukocyte cell population in distant organs at the late stage of cancer, and the role of the cell population in tumor progression, remain to be further investigated. In this study, we find a dramatic increase of $CD45^{-}$cells in spleen of HCC-bearing mice. Specifically, this population expresses erythroblast-like markers characterized by the presence of erythroid and megakaryocytic markers, but not lineage markers, and is referred to as Ter-cells. Using protein phosphorylation microarrays, we found that Smad3, a key downstream molecule for the TGF-β signaling pathway, was upregulated in Ter-cells. Using microarray analysis, we found that the expression level of artemin was highly upregulated in Ter-cells. High levels of serum artemin were observed in tumor-bearing mice, while splenectomy or host Smad3 deficiency inhibited the increase of artemin, suggesting that Ter-cells are the major source of artemin. In brief, we provide robust evidence that artemin promotes HCC progression and could be a potential therapeutic target. More importantly, it is noteworthy that artemin production is predominantly regulated by an erythroid lineage subset, Ter-cells. **This study provides a novel insight into the crosstalk between tumor and hematopoiesis. And targeting Ter-cell and its joint biological factors may aid in the development of accurate diagnosis and treatment for HCC patients.**

P1970

TRAF6 directs FOXP3 localization and facilitates Treg function through K63-type ubiquitination

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Regulatory T cells (Treg) are crucial mediators of immune control. The characteristic gene expression and suppressive function of Treg depend considerably on the stable expression and activity of the transcription factor FOXP3. While transcriptional regulation of the *Foxp3* gene has been studied in depth, both the expression and function of this factor are also modulated at the protein level. However, the molecular players involved in posttranslational FOXP3 regulation are just beginning to be elucidated. Here we found TRAF6-deficient Tregs were dysfunctional in *vivo*; mice with Treg-restricted deletion of TRAF6 were resistant to implanted tumors and displayed enhanced anti-tumor immunity. We further determined that FOXP3 undergoes lysine-63 linked (K63) ubiquitination at lysine 262 mediated by the E3 ligase TRAF6. When deprived of TRAF6 activity or rendered insensitive to K63 ubiquitination, FOXP3 displayed aberrant, perinuclear accumulation and disrupted regulatory function. Thus, K63 ubiquitination by TRAF6 ensures proper localization FOXP3 and facilitates the transcription factor's gene-regulating activity in Tregs. These results implicate TRAF6 as a key posttranslational, Treg-stabilizing force that may be targeted in novel tolerance-breaking therapies.

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DOI: 10.1002/eji.201970400

P1971

Preoperative exercise therapy protects hepatic ischemia-reperfusion injury

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Introduction: Hepatic ischemia/reperfusion (I/R) injury results in an increased risk of postoperative morbidity and mortality. Preoperative exercise therapy has been shown to enhance physical capacity at the moment of hospital admission and may facilitate better recovery after surgery. However, the mechanisms behind this protection remain to be elucidated. Here we hypothesize that preoperative aerobic exercise therapy has protective effects as a result of inhibiting sterile inflammatory processes after liver I/R injury.
Methods: Eight-week-male-C57BL/6-mice were randomly divided into exercise or sedentary group. The exercise-group ran on a motorized treadmill for 60-minute/day, 5-days/week at a speed of 12.5-m/min for 4 weeks. 70%-partial liver warm I/R was performed in both groups.
Results: After I/R, compared with sedentary-mice the exercised-mice showed significantly lower ALT, increased regulatory T cells(Treg)were seen in the ischemic-liver-lobe by flow cytometry, as well as less neutrophil infiltration. Suggesting a mechanism for exercise to stimulate liver immune environment. Exercise-mice livers exhibited less Neutrophil-Extracellular-Traps(NETs) formation after I/R which we have previously reported that blocking NETs can reduce the organ damage and initiates inflammatory responses during liver I/R. Conditional media derived from Treg cells can effectively decrease the NET formation *in-vitro*. RNA-seq showed more DNase-IL3 expression in the liver of exercised-mice which can digest the NET structure, also the secretion of DNase-IL3 from exercised-mice increased, especially from the Treg.
Conclusions: These data show beneficial effects of preoperative exercise therapy in liver I/R injury, which offer a rationale for encouraging predisposed patients of HCC or other end-stage liver diseases that need liver surgery.

P1972

Inhibiting mitochondrial fission as a novel approach for induction of regulatory T cells

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Unrestrained activation of Th1 and Th17 cells and dysfunction of regulatory T cells (Tregs) is associated with autoimmunity. Inactivation of dynamin-related protein 1 (Drp1), a GTPase that regulates mitochondrial fission, is known to protect myelin from demyelination and to reduce severity of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. However, its effect on T cells is not yet known. In the present study, we investigated the effect of Mdivi-1, a small molecule inhibitor of Drp1, on experimental autoimmune encephalomyelitis. The relationship of p-Drp1 expression and T cell responses in both central nervous system and periphery were further determined. Our results show that Mdivi-1 effectively suppressed disease severity by reducing demyelination and cellular infiltration in the CNS. Mdivi-1 treatment decreased the expression of p-Drp1 (ser616) on $CD4^+$ T cells, reduced the numbers of Th1 and Th17 cells and increased $Foxp3^+$ regulatory T cells in the central nervous system. Moreover, Mdivi-1 treatment effectively inhibited IFN-γ^+, IL-17^+ and GM-CSF^+ $CD4^+$ T cells, while it induced $CD4^+$ $Foxp3^+$, $CD4^+$ IL-10^+ regulatory T cells in the spleen. Together, our results demonstrate that inhibiting mitochondrial fission of $CD4^+$ T cells is a

DOI: 10.1002/eji.201970400

novel approach for the suppression of Th1/Th17 responses and induction of Tregs.

This study was supported by the NIH (NS099594 and AI135601), USA, and the National Natural Science Foundation of China (81501198).

P1973

Expression of immunoregulatory proteins in *Brucella abortus*-infected macrophages

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Brucellosis is a chronic disease caused by *Brucella abortus,* a facultative intracellular pathogen which infects macrophages and dendritic cells. Within these cells, *B. abortus* induces an intracellular retention of proteins of the Major Histocompatibility Complex (MHC) and suppress the secretion of pro-inflammatory cytokines, such as tumor growth factor alpha (TNF-a). Furthermore, this pathogen induces in these cells, the expression of Interleukin-10 (IL-10) and cyclooxygenase-2 (COX-2), inmunoregulatory molecules which promote its replication in the host. Therefore, since *B. abortus* subvert the defensive mechanism of the host, we studied if *B. abortus* 2308 strain infected RAW 267.4 macrophages express anti-inflammatory proteins, contributing to suppress the immune response. Levels of relative gene expression of COX-2 and heme oxygenase-1 (HO-1), co-stimulatory proteins (CD80, CD86 and CD40) and Nuclear Factor-kB (NF-kB) subunit protein p65 were quantified by quantitative Polymerase Chain Reaction (qPCR). Results showed that RAW 267.4 macrophages infected with the pathogenic strain *B. abortus* 2308 during 24 h, produced an up-regulation of COX-2 and HO-1. Nevertheless, co-stimulatory proteins CD80 and CD86, and transcriptional factor p65 were down-regulated. These results demonstrated that *B. abortus* negatively modulates the expression of proteins required to activate T cells and, in turn, induce the expression of immunoregulatory proteins (such as COX-2 and HO-1). Therefore, the expression of these proteins in *B. abortus* infected macrophages could contribute to suppress the immune response due to a deficient activation of T cells, which reduces the protective response of the host against this pathogen.

Supported by Grant FONDECYT 1180122

P1974

Knocking out of CD38 induces immune regulation disruption in a lupus-prone mice mediated by regulatory T cells

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Regulatory T cells (Tregs) play a key role in maintaining the homeostasis of the immune system, through suppressing excessive immune responses and preventing autoimmune disorders. Tregs are identified by the expression of the CD3+CD4+CD25+ surface markers and the transcription factor FOXP3. CD38 is a receptor/enzyme widely expressed in immune cells, its activation induces cellular activation, proliferation, differentiation, apoptosis, and mobilization of Ca^{2+}. Several reports have attributed to CD38 a regulator role, demonstrating that the ablation of CD38 induces an autoimmune

DOI: 10.1002/eji.201970400

phenotype in old mice. Remarkably, the absence of CD38 in lupus-prone mice accelerates the development of autoimmunity however, the role of CD38 in the regulation has not been completely elucidated. The aim of this work was to analyze the development and function of Tregs from B6.MRL.Fas$^{lpr/lpr}$ lupus-prone, in the absence of CD38 gene. Our strategy was to cross B6.MRL.Fas$^{lpr/lpr}$ with B6.CD38$^{-/-}$ mice, employing a novelty breeding protocol provided by Jackson Laboratory. After the model generation, we assessed the percentage and absolute numbers of thymic and splenic Tregs. The data showed a reduction in these cells from the spleen, but an increase in the thymus. Afterward, the suppressor activity of Tregs was evaluated through the CTLA-4 expression and IL-10 production. Our results showed that B6.MRL.Fas$^{lpr/lpr}$/CD38$^{-/-}$ mice produce lowers levels of IL-10 and CTLA-4, compared to B6.MRL.Fas$^{lpr/lpr}$ control. Together, these data suggest that CD38 could help to control the autoimmunity onset in lupus-prone mice, enhancing the development and function of Tregs.

P1975

Myeloid-derived suppressor cells and their effect on other tumor-immunity related cell subsets in patients with colorectal cancer - the role of surgery

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Introduction: Myeloid-derived suppressor cells (MDSCs) in cancer patients, including those with colorectal cancer (CRC) are associated with poor prognosis. MDSCs accumulated locally in the tumor microenvironment and circulating in the blood are an important element of immunosuppression observed during cancer development. The presence of MDSCs in patients with CRC has already been well documented, however, there is still lack of data on the effect of applied therapy on their level.
Materials and Methods: Flow cytometry analysis of peripheral blood mononuclear cells from 74 patients with CRC and 28 adult healthy controls was performed. In 19 patients, the level of MDSCs was examined before and after surgical removal of the tumor. The analysis included evaluation of the blood level of MDSCs of granulocyte (Gr-MDSCs) and monocyte (Mo-MDSCs) origin (LIN-HLA-DR-CD11b+CD15+ or CD14+, respectively) and its correlation with Treg, Th17, tumour antigen-specific CD8+ T cells and DCs levels, followed by evaluation of arginase-1, LOX-1 expression and functional studies to verify the suppressive nature of MDSCs.
Results: The level of Gr-MDSCs and Mo-MDSCs was significantly higher in the blood of patients with CRC in comparison to healthy controls. Isolated MDSCs effectively suppressed proliferation of autologous T lymphocytes. Additionally, Gr-MDSC expressed LOX-1 and ARG1, whereas the level of Mo-MDSCs positively correlated with the level of Treg and plasmacytoid DCs, and negatively with HER2/neu-specific CD8+ T cells. After surgical tumor removal, the level of Mo-MDSCs was significantly higher than before the treatment, and was associated with disease recurrence.

P1976

Kv1.3 channel of Tregs: discovery of drug target based on immunomodulatory process during the development of heart failure

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DOI: 10.1002/eji.201970400

Regulatory T lymphocytes (Tregs) is an important anti-inflammatory cell, which plays an important role in inhibiting immune response and maintaining immune adaptive balance. It mainly secretes anti-inflammatory cytokine IL-10 and pro-fibrotic factor TGF-β. Immune cytokines can promote myocardial fibrosis (MF), but it has been reported that Tregs can reverse MF by secreting IL-10. This controversial attracted us to set up co-incubation system of Tregs and cardiac fibroblasts (CFs). After 48 h, it was found that Tregs significantly promoted the proliferation of co-cultured CFs by ~2.5-fold, and ECMs of CFs were secreted highlightedly. Tregs occurs to activated/ proliferation by ~60%, and the increase of TGF-β was profoundly higher than that of IL-10 whatever in extracellular (2.7- *vs* 2.2-fold) or intracellular (5.8- *vs* 1.8-fold) environments, meanwhile the Tregs Kv1.3 channels silenced through RNAi could suppress the activation/proliferation of Tregs, shrink the secretion of TGF-β primarily, that in turn restrain the proliferation of CFs. The negative regulatory process was performed in the same way with the intervention of aldosterone antagonist - eplerenone. Docking calculations indicated Eplerenone had high affinity to Kv1.3 channel due to H-bond combination, proving its direct effect on the channel. In conclusion, Eplerenone antagonizes the Kv1.3 channels directly to suppress the cross-talk between the two types of cells and may exert protection against myocardial fibrosis during CHF, so Kv1.3 channel could be a potential target for anti-HF in immunomodulatory pathway.

P1977

Characterization of uncultured adipose tissue-derived stromal/stem cells of murine steatohepatitis model for liver repairing/regenerative therapy

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Background: Non-alcoholic steatohepatitis (NASH), which is frequently associated with metabolic syndrome, is an emerging chronic inflammatory liver disease whose ultimate condition is cirrhosis. Adipose tissue derived stromal/stem (regenerative) cells (ADRCs) are considered to be useful for repairing/regenerative therapy. We characterized ADRCs from mice with progressive NASH conditions, to examine their potential usefulness for NASH therapy.
Methods: ADRCs were isolated from healthy C57BL/6J mice or from those fed with atherogenic high-fat diet (AT-HFD) for 4, 12, 26 weeks; qRT-PCR, DNA microarray and FACS analysis were performed. NASH murine models were established in mice fed with AT-HFD, then, ADRCs from healthy mice were administered to NASH mice at 24 and 26 weeks of AT-HFD, on week 28 samples collected. Immunohistochemistry (IHC), fibrosis quantification, FACS of hepatic inflammatory cells (HICs) and qRT-PCR analysis were performed.
Results: IHC of NASH mice treated with ADRCs showed reduction of ballooning and fibrosis levels (p=0.0088) in liver tissues, F4/80+ cells were also reduced (-40%) compared to control. Albumin expression levels increased in treated mice (p=0.028), FACS of HICs showed a modest reduction of T cells and Gr-1+ cells. ADRCs isolated at different stages of NASH have similar cytokines expression; only at 4 weeks of AT-HFD, but not at 12 or 26 weeks, ADRCs showed increased mesenchymal marker expressions, together with a higher cell proliferation character detected by microarray.
Conclusion: ADRCs were efficacious for NASH treatment, and ADRCs of NASH mice revealed similar character except for the very early stage NASH ones.

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DOI: 10.1002/eji.201970400

P1978

Stable inducible regulatory T cells exhibit preventive effect on experimental autoimmune encephalomyelitis (EAE) and ovalbumin-induced anaphylaxis in mouse

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Regulatory T cells (Tregs) potentially can be applied to regulate autoimmune diseases and allergy. Due to the limited number and insufficient proliferation ability of thymus-derived Tregs, inducible Tregs (iTregs) become more attractive option for immune therapy. And the stability of iTregs in vivo becomes a key point in their application. We cultured stable iTregs by modifying several conditions in standard protocol. FDG (FoxP3-DTR-GFP) mice were used to culture iTreg cells, then GFP+ cells were sorted and 4 x105 GFP+ cells were i.v. transferred to each recipient mouse. One day after cells transfer, mice were induced EAE or anaphylaxis. Compared to respective control group, stable iTreg cells treated group show obviously ameliorated EAE and anaphylaxis by evaluating clinical score in EAE model and ovalbumin-specific IgE levels and clinical score in anaphylaxis model. Our results suggest that stable iTregs could be a promising immune intervention method in autoimmune diseases and allergy.

P1979

High salt diet inhibits tumor growth by modulating anti-tumor immunity

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High salt intake could affect the immune system by shifting the immune cell balance towards a pro-inflammatory state. Since this shift of the immune balance is thought to be beneficial in anti-cancer immunity, we tested the impact of high salt diets on tumor growth in mice. High salt significantly inhibited tumor growth in independent murine tumor models. Interestingly, the depletion of myeloid-derived suppressor cells (MDSCs) reverted the inhibitory effect on tumor growth. In line with this, high salt conditions blocked murine MDSC function in vitro and similar effects were observed in human MDSCs isolated from cancer patients. Of note, high salt diet further boosted efficacy of checkpoint inhibitors in tumor-bearing mice. Thus, high salt conditions seem to inhibit tumor growth by enabling more pronounced anti-tumor immunity through the functional modulation of MDSCs. Our findings might have critical relevance for cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P1980

Effects of hASCs on human T and B cells

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Background: hASCs (*human adipose-derived stem cells*) are known to be immunomodulatory through the regulation of immune cells by mechanisms which include both direct cellular contact and release of soluble factors.
We studied the effect of hASCs on the viability and activation of T cells and B cells.
Methods: hASCs were obtained from human adipose tissue.To evaluate induction of T and B cells activation and apoptosis by hASCs, mixed leukocyte reaction (MLR) was applied. At the end of MLR cultures T cells and B cells were stained with propidium iodide and annexin V and analyzed by flow cytometry.Additionally the expression of CD69 and CD25 on T (Th, Tc) and B cells was measured. The supernatants have been collected and measurement of IFN-γ, TNF-α, IL-2, IL-4,IL-6,IL-10, IL-17, TGF-β level was performed by ELISA.
Results: There was a significant decrease in the percentage of Th cells in the late stage of apoptosis in passage 2 of hASC cells and a significant increase in the percentage of Tc cells in the same stage compared to the control. There were no significant changes in the expression of CD69 and CD25 on the surface of both lymphocyte populations.
We did not observe a significant effect of hASCs on the expression of CD69 and CD25 on B cells and B cells apoptosis.
Conclusions: We have shown that hASC may affect the viability and activation of T cells.

This work was supported by Grant no. STRATEGMED2/267976/13/NCBR/2015.

P1981

The correlation between cold-heat patternization and immune activity

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Background: The cold-heat patternization is one of the most widely used diagnostic methods in Traditional Chinese Medicine (TCM). We were to find it's relationship with immune cells including dendrite cells, helper T cells (T_H), and regulatory T cells (Tregs).
Methods: We surveyed 100 subjects with the questionnaires for cold-heat patternization, and obtained their blood samples. The samples were contained in BD Vacutainer CPT tubes and centrifuged with 2000 rpm for 20 min. The obtained human peripheral blood monocytes (hPBMC) were washed with Phosphate-buffered saline (PBS). We counted cells after resuspension with Ex vivo. To count Tregs, we diluted the blood samples into the concentration of 1 x 10^6 cells/ml. Treg expansion kits were set according to the manufacturer's protocol. We added 100 ul (1 x 105 cells) of hPBMC, 20 ul of CD3/CD28 MACSiBead, 100 ul of media, and 500 U of rhIL-2 (Pepprotech) to U-bottom 96 well plates. We counted Tregs after culturing the samples in 37℃ CO_2 incubator for 72 hrs.
Results: Heat factor scores calculated from the questionnaires had significant positive correlationships with T_H and Tregs (r=0.255 and 0.203, respectively).

DOI: 10.1002/eji.201970400

Conclusion: The cold-heat patternization in TCM seemed to reflect the immune activity. Especially, Heat pattern might be the expression of immunomopotentiation.

P1982

FKBP5 splicing concurs to treg differentiation of tconv cells

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FKBP51 (*FKBP5* gene) is an immunophilin physiologically expressed by immune cells. Thanks to its scaffold and isomerase activity supporting IKK complex assembly and function, FKBP51 plays a relevant role in NF-κB activation. Recently, we identified a splicing isoform, shorter than the canonical one, termed FKBP51s. FKBP51s acts as a PD-L1 foldase, assisting to protein maturation and expression on plasma membrane. Particularly, FKBP51s was firstly identified in TILs and PBMCs of melanoma patients. In these patients, FKBP51s marked a subset of Tregs with an increased phospho-mTOR and Ki67 expression. Logistic regression analysis suggested that FKBP51sTreg is a valuable element which predicts the probability of response to ipilimumab. Given the foregoing, we attempted to investigate *FKBP5* splicing during T lymphocyte activation and assess its role in Treg formation and suppression capability. We found that *FKBP5* splicing occurred early in the course of a suboptimal but not maximal PBMCs stimulation. The increase in FKBP51s was transient and associated with a delay in Akt and STAT5 activation. When splicing was prevented, Treg count was decreased and Tconv cell proliferation increased. Moreover, measure of FKBP51s level in Foxp3CD25^{high} Treg subset showed highest levels in PBMCs cultures under suboptimal CD3 co-stimulation. Coculture suppression assays showed that Treg silenced for FKBP51s had a significantly reduced suppressive capability, compared with non-silenced Tregs. In conclusion, our findings suggest that *FKBP5* splicing occurs under conditions of suboptimal CD3 co-stimulation of Tconv cells and concurs to their differentiation into Tregs. Manipulating *FKBP5* splicing can be a means to control tumor tolerance.

P1983

Re-programming of neutrophils modulates inflammation resolution during atherosclerosis

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Non-resolving inflammation mediated by monocytes and macrophages is known to be involved in the pathogenesis of atherosclerosis. However, the role and regulation of neutrophils during the establishment of sustained inflammation and atherosclerosis are less well understood. We observed that chronic subclinical endotoxemia, a risk factor for non-resolving low-grade inflammation, significantly exacerbated atherosclerosis in high-fat-diet (HFD) fed ApoE deficient mice accompanied by the polarization of neutrophils into a pro-inflammatory phenotype. Neutrophils primed with super low dose LPS in vitro demonstrated a similar phenotype, as reflected in elevated levels of inflammatory mediators and reduced levels of homeostatic mediators. The polarization of non-resolving inflammatory neutrophils was potentially due to the compromised peroxisome-lysosome fusion, which induced intracellular ROS accumulation and subsequent up-regulation of oxCAMKII and 5-LOX. Application of 4-phenylbutyrate (4-PBA), which enhanced peroxisome homeostasis, reduced oxCAMKII and 5-LOX levels, and also re-balanced the expression profiles of pro- and anti-inflammatory mediators. Through transfusing differentially programmed neutrophils into HFD fed mice, we directly examined the outcomes of re-programmed neutrophils during the pathogenesis of atherosclerosis. Adoptive transfer of neutrophils primed with subclinical LPS resulted in elevated atherosclerotic plaque size, increased lipid deposition and diminished plaque collagen content. In

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DOI: 10.1002/eji.201970400

contrast, the mice receiving neutrophils programmed with 4-PBA exhibited suppressed atherosclerosis development and strengthened plaque stability compared to the mice that were transferred with PBS treated neutrophils. Taken together, our data have defined novel neutrophil dynamics responsible for controlling non-resolving inflammation versus resolving homeostasis during atherosclerosis, and also provided the feasibility of employing re-programmed neutrophils to treat atherosclerosis.

P1984

HDAC2 acts as a positive regulator of peripheral Treg cell generation in skin immunity

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Immune homeostasis in peripheral tissues is partially determined by the balance of regulatory T (Treg) cells and effector T (Teff) cells. This balance is especially critical in barrier tissues such as the skin where it determines the decision between immune activation in response to pathogens and tolerance of commensals.
We hypothesize that this balance is in part regulated by histone deacetylases (HDACs). To date, multiple HDACs have been shown to influence the generation, differentiation, and function of T cells through various histone-dependent and -independent processes. In particular, we hypothesize that class 1 HDACs regulate the differentiation of inflammatory Teff cells versus peripheral Treg (pTreg) cells in response to skin antigens, as well as their function and stability in the tissue.
To test our hypothesis, we utilize a mouse model developed to study T cell-mediated skin inflammation, designated K5/TGO. This model features tetracycline-inducible expression of chicken ovalbumin (Ova) in the epidermis and allows us to follow the differentiation of adoptively transferred naïve Ova-specific $CD4^+$ OTII T cells. The ensuing T cell-dependent inflammatory dermatitis can be controlled by Treg cells.
Upon transfer of HDAC2-deficient Foxp3- OTII cells into K5/TGO-recipients we followed the induction of Foxp3 and found a positive regulatory role of HDAC2 in the pTreg cell generation and function. *In vitro* as well as *in vivo* Treg cell generation and suppression assays together with clinical outcomes and transcriptomics data will further answer open questions of Treg cell regulation by HDACs and open novel avenues exploiting HDAC-dependent regulatory pathways therapeutically.

P1985

Analysis of the role played by T regulatory cells early in life: a mouse model

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It is known that exposure to non-inherited maternal antigen (NIMA) induces a specific state of immune tolerance. The aim of our study is to identify whether NIMA-specific tol- erance is due to the propensity of perinatal T cells to develop regulatory function. We have observed that H-2d-specific Foxp3+ Tregs are specifically present in adult H-2b mice that have been exposed to H-2d NIMA from (H-2bXH-2d)F1 mothers. These Tregs were specifically able to inhibit the proliferation and IL-2 production of H-2d-specific alloreactive T cells. In order to analyse NIMA-specific Tregs in neonates, we crossed female het- erozygous for the transgene 2W1S-OVA with non-transgenic males and analyzed 2W1S- specific Tregs in offsprings with specific tetramers. Surprisingly, 2W1S-specific Tregs were not detected in 5

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DOI: 10.1002/eji.201970400

day-old newborns and represented only a small fraction of 2W1S- specific T cells in 10 days-old mice (< 1%). These cells, however, were present (15-20%) in 28 day-old adults. Moreover, injection of LPS at day 1 after birth was able to stimulate the expansion of NIMA-specific CD4+ T effector cells in NIMA-exposed mice. Together, our results do not support the idea that perinatal generation of NIMA-specific Tregs is responsible for NIMA-specific tolerance in early life. We are currently performing single- cell analysis of 2W1S-specific TcR repertoire (length of CDR3) to determine whether NIMA-specific Tregs present in NIMA-exposed adults have been generated during the neonatal period.

P2289

IL-4Rα is required by Foxp3$^+$ regulatory T cells to control tissue inflammation

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An important and continuously expanding aspect of cytokine-mediated immune control is the role they exert on immune regulation. Particularly, IL-4 has been suggested to play an important role in determining the fate of Foxp3$^+$ Treg cells. However, apparently opposing reports have emerged on the role of this cytokine on Foxp3$^+$ Treg cells. Given that better understanding of that role is required to open a new door for potentially effective therapeutic strategies, we sought to dissect the role of IL-4 under steady-state and inflammatory conditions via impairment of IL-4Rα signaling on Foxp3$^+$ Treg cells by using our mouse model where *il-4rα* gene is specifically impaired within Foxp3$^+$ Treg cells. In fact, under parasitic helminths *S. mansoni* and *N. brasiliensis*, **Foxp3^{cre} IL-4Rα$^{-/lox}$ mice were less able to control tissue inflammatory responses and effector immune cell functions resulting in aggravated liver fibro-proliferative pathology (*S. mansoni*) and heavy lung mucus production as well as lung emphysematous pathology (*N. brasiliensis*).** In support, the Foxp3$^+$ Treg compartment was significantly destabilized. In fact, Foxp3$^+$ Tregs were less represented in inflamed tissues of Foxp3^{cre} IL-4Rα$^{-/lox}$ mice during infection and expressed at a much lower level several mediators of Treg function (Foxp3, IRF4, Helios), suggesting a need of IL-4Rα mediated signaling for the optimal function of the Foxp3$^+$ Treg cell. Mechanistically, *in vitro* stimulation of Foxp3$^+$ Tregs with IL-4 considerably improved survival and promoted Foxp3 expression. **Collectively, our work newly suggests the "need" for IL-4Rα mediated signaling on Foxp3$^+$ Tregs to optimally limit tissue inflammation under inflammatory conditions.**

P2702

GSK3β promotes Foxp3$^+$ Treg stability and alleviate the development of chronic colitis

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GSK3β (glycogen synthase kinase 3 beta) is a growth signaling-sensitive kinase negatively regulated by inhibitory phosphorylation downstream of the insulin receptor, Wnt, and mTOR growth signaling pathways. Two isoforms of GSK3, GSK3α and GSK3β, have been identified that have distinct non-redundant functions: global knockout of GSK3α is phenotypically silent, whereas global knockout of GSK3β is embryonic lethal. Tregs are important regulator of immune system and play a key role in

DOI: 10.1002/eji.201970400

keeping Immune homeostasis. Foxp3$^+$ Treg is a major subsets of regulatory T cells. As we all known, Foxp3 is the vital transcription factor in regulatory T cells and play a key role in the development and function of regulatory T (Treg) cells; however, the posttranslational mechanisms regulating Foxp3 transcriptional output remain poorly defined. Previous study showed that GSK3β can facilitate the Foxp3 degradation by a proteasome-mediated protein degradation pathway and high expression of GSK3β in the Foxp3$^+$Tregs has a negative correlations with the severity of some diseases. But all of these privous data did not describe the function of GSK3β in Foxp3 $^+$ Tregs at the gene level. Our study firstly described the role of GSK3β in Foxp3$^+$ Tregs at gene level .

P2703

Wild type hepatitis B virus with e antigen promotes negative immune regulation and delays viral clearance in the mouse model

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Hepatitis B virus e antigen (HBeAg) is able to regulate host immune responses and participate in the establishment of HBV chronic infection. However, its exact role on the regulation of immune response is not fully understood and controversial. This study aimed to investigate whether HBeAg induces myeloid-derived suppressor cells (MDSCs) in HBV mouse models. We constructed a plasmid containing a mutated, replication-competent HBV genome (pHBeAg(-)) with G1896A mutation which leads to cessation of HBeAg expression. Hydrodynamic injection with pHBeAg(-) and pBS-HBV1.3 with a wild type HBV genome was performed in mice. Interestingly, mice received HI with pHBeAg(-) cleared HBV more rapidly than that with pBS-HBV1.3. Mice with pBS-HBV1.3 showed lower frequencies of HBV-specific CD4$^+$ and CD8$^+$ T cells, and effector T cells (CD44^{hi}CD62L^{low}) as well as , compared with mice with pHBeAg(-). HBV specific CD4$^+$ and CD8$^+$ T cells from mice with pBS-HBV1.3 had reduced ability of IFN-γ, IL-2, and TNF-α production. Consistently, mice with pBS-HBV1.3 showed higher frequencies of MDSCs and regulatory T cells and increased IL-10 production in the liver, compared with mice with pHBeAg(-). When the separated MDSCs co-cultured with naïve lymphocytes *in vitro*, IFN-γ production by T cells was impaired. MDSCs from mice with pBS-HBV1.3 had higher potency to inhibit T cell functions. In conclusion, wild type HBV with HBeAg triggers MDSCs and Treg cells in the liver, reducing the functionality of HBV-specific CD4$^+$ and CD8$^+$ T cells, and delays HBV clearance in mice.

P2704

Sigma receptor 1 regulates T cells by PD-L1 at the maternal-fetal interface in early pregnancy

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Background: Sigma receptor (SR) 1 has been confirmed to participate in immunomodulatory in tumor cells, cardiomyocytes and retinal Müller glial cells. However its role at the maternal-fetal interface still remains unclear.
Object: This study will demonstrate the function of SR1 in regulating T cells by PD-L1 at the maternal-fetal interface.
Method: qPCR and western blotting were used to identify the mRNA and protein expression of the

DOI: 10.1002/eji.201970400

SR1 and PD-L1. Coimmunoprecipitation was used to verify the interaction between SR1 and PD-L1. Sigma1 agonist (SA4503) and inhibitor (IPAG) were used to observe the effect of the SR1 activity and inhibition on JEG-3 cells. The cells treated by IPAG were co-cultured with Jurkat T-cells, and the effect of SR1 inhibition on T cells was evaluated by luciferase reporter assay.
Result: The expression of SR1 mRNA and protein in the pregnant villus tissues of recurrent abortion (n=10) were significantly lower than that in villus tissues of normal abortion (n=10) ($P<0.05$). Coimmunoprecipitation proved that there was an interaction between SR1 and PD-L1. Compared with the control group, SA4503 and IPAG significantly increased and decreased respectively the expression of SR1 and PD-L1 of JEG-3 cells. Luciferase report assay demonstrated that SR1 inhibitor can activate the function of co-cultured T cells.
Conclusion: SR1 can regulate the function of immune cells by PD-L1 at the maternal-fetal Interface. Activating the SR1 function in trophoblast may improve the immune tolerance of maternal-fetal interface

P2705

Effects of TRIM38 and TRIM25 on RIG-I pathway-mediated IFN-I expression

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Virus can induce IFN-I expression, which is mediated by the activation of RIG-I-like receptors (RLRs), such as RIG-I and MDA5. TRIM25 can induce the production of IFN-I and other inflammatory factors by catalyzing RIG-I. However, TRIM38 inhibits the production of IFN-I. In this work, we select RSV as a virus model to explore the interaction between TRIM38 and TRIM25 on the mechanism of IFN-I expression. The protein levels and mRNA levels were detected by RT-qPCR and Western blot. Co-transfection and co-immunoprecipitation were used to determine the relationship between TRIM25, TRIM38, and RIG-I. RSV-infected A549 cells could induce TRIM25 expression, and TRIM25 induced IFN-I production by RIG-I activation of mainly NF-κB pathway. IFN-I in turn induced the expression of TRIM25 and TRIM38. When TRIM38 coexists with TRIM25, the amount of TRIM25 combined with RIG-I decreases, and the expression level of IFN-I decreases accordingly. In conclusion, **RSV-infected A549 cells could induce expressions of TRIM25, TRIM38, and IFN-I. The expression of TRIM38 and TRIM25 is interferon-dependent. TRIM38 competes with TRIM25 for binding to RIG-I, thereby inhibiting the expression of IFN-I.**
Keywords: TRIM25, RIG-I, TRIM38, Type I interferon, Respiratory syncytial virus

P2706

Regulatory T cell derived exosome stimulates the proliferation of cardiac fibroblasts in vitro

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Local inflammation of myocardial tissue can promote development of heart failure. A variety of immune cells can accelerate the proliferation of cardiac fibroblasts (CFs) through cell-cell cross-talk and/or secretion of cytokines. Our previous studies in vitro showed that Tregs could boost the proliferation of CFs significantly. It is clear that activated Tregs secreted IL-10 and TGF-β, and we confirmed that Treg cells induce the proliferation of CFs mainly by releasing TGF-β. In order to ensure that they are not degraded rapidly and function normally, the exosomes possibly exert a vital role in the transmission of information between these two types of cells. By extracting exosomes and observing them under 50,000-fold transmission electron microscopy, we found that there were plenty of

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DOI: 10.1002/eji.201970400

exosomes at 30-150 nm in both Tregs culture medium and co-culture medium of the two kinds of cells. Co-culture of exosomes with CFs, respectively, the results showed that Tregs-derived exosomes increased CFs proliferation by 84% and the exosome derived from the co-cultured cells increased CFs proliferation by 92%. In addition, 3 times of Tregs-derived exosomes increased the proliferation of CFs by 110%, indicating that the proliferation signal transmission and/or proliferation efficacy of the exosome were saturated and did not multiply with the increase of the exosomes. We conclude that the transmission of proliferative signal between Tregs and CFs is via the exosome, which has important physiological significance for the protection and transmission of the proliferative signal with short half-life (ie. TGF-β).

P2707

Exosomal miRNA-107 induces myeloid-derived suppressor cell expansion in gastric cancer

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Recent studies showed that tumor-derived exosomes (TDEs) can induce MDSC accumulation and expansion, the mechanisms of which are largely unknown. In the present study, we showed that microRNA (miR)-107 was not only overexpressed in gastric cancer cells but also enriched in their secreted TDEs. Also, these miR-107-enriched TDEs could be taken up by HLA-DR$^-$ CD33$^+$ MDSCs, where miR-107 was able to target and suppress expression of DICER1 and PTEN genes. Dampened DICER1 expression supported expansion of MDSCs, while decreased PTEN led to activation of the PI3K pathway, resulting in increased ARG1 expression. Furthermore, gastric cancer-derived miR-107 TDEs, when dosed intravenously into mice, were also capable of inducing expansion of CD11b$^+$ Gr1$^{+/high}$ MDSCs in mouse peripheral blood and altering expression of DICER1, PTEN, ARG1, and NOS2 in the MDSCs. In conclusion, these findings demonstrate for the first time that gastric cancer-secreted exosomes are able to deliver miR-107 to the host MDSCs where they induce their expansion and activation by targeting DICER1 and PTEN genes.

P2708

Circulating and tumor-infiltrating myeloid-derived suppressor cells in gastric cancer patients can be classified into two different sub-populations

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Myeloid-derived suppressor cells(MDSCs) can express high levels of arginase1(ARG1), and suppress the function of T-cells by consuming L-arginine. While we know that the function of MDSCs can be amplified in gastric cancer(GC) , the phenotypic characteristics and distribution of MDSCs expressing ARG1 remain very unclear. In the present study, we investigated the circulating MDSCs of GC patients and found that HLA-DR$^-$CD33$^+$ARG1$^+$MDSCs accounted for ≥80% and HLA-DR$^-$ CD14$^+$ARG1$^+$MDSCs accounted for ≥10%($P < 0.01$). Further immunophenotyping of HLA-DR$^-$

DOI: 10.1002/eji.201970400

CD33$^+$MDSCs indicated that MDSCs with high expression levels of ARG1 were mainly HLA-DR$^-$ CD33$^+$CD14$^-$CD15$^-$subtype MDSCs(early-stage MDSCs:eMDSCs), accounted for ≥90%(P< 0.01). Furthermore, There were significant differences in ARG1 expression and immunophenotype when compared between tumor-infiltrating MDSCs and Circulating MDSCs. Infiltrating MDSCs with high expression levels of ARG1 were maily HLA-DR$^-$CD33$^+$CD14$^-$CD15$^-$, HLA-DR$^-$CD33$^+$CD11b$^+$CD14$^-$ CD15$^-$ eMDSCs and HLA-DR$^-$CD14$^+$M-MDSCs. This preliminary study describes the phenotypic characteristics and distribution of MDSCs expressing ARG1 in GC conditions and provides clues with which to reveal the mechanisms which allow tumors to evade the immune system.

P2709

Interleukin-1β pretreatment enhances the immunosuppressive capacities of mesenchymal stem cells via exosome-mediated transfer of miR-146a

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Mesenchymal stem cells (MSCs) are attractive candidates for treating many immune and inflammatory disorders. However, recent researches suggest that the immunomodulatory capacities of rest MSCs are benign, and even cannot suppress immune reactions unless they are first been activated. So finding strategies like the modification of MSCs with cytokines or drugs to improve their immunomodulatory capacities and therapeutic efficacies will shed light on the applications of MSCs. In this study, we pretreated human umbilical cord-derived MSCs with interleukin-1β (IL-1β) and found that βMSCs could more effectively ameliarate the symptoms of murine sepsis and increase the survival rate. Furthermore, βMSCs could more significantly induce macrophage polarization to an anti-inflammatory M2 phenotype through the paracrine activity. Intriguingly, we demonstrated that exosomes derived from βMSCs contributed to the enhanced immunosuppressive properties both in vitro and in vivo. By investigating the content of exosomes we found miR-146a, a famous anti-inflammatory miRNA, was more redundant in βMSCs-exo than in MSCs-exo. This exosomal miR-146a was transferred to macrophages, resulted in M2 polarization, and finally led to increased survival in septic mice. In contrast, inhibition of miR-146a by transfecting miR-146a inhibitors partially negated the immunosuppressive capacities of βMSCs-derived exosomes. Taken together, IL-1β pretreatment effectively enhances immunosuppressive properties of MSCs partially by exosome-mediated transfer of miR-146a. Thus, we believe that the IL-1β pretreatment strategy may provide a new modality for better therapeutic applications of MSCs in inflammatory disorders.

P2710

Acidic microenvironment regulates regulatory T cells in hepatic ischemia reperfusion injury model through PI3K-mTOR signal

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Hepatic ischemia/reperfusion (I/R) injury is a major complication of liver surgery, including liver resection, liver transplantation, and trauma surgery. Much has been learned about the inflammatory injury response induced by I/R, and regulatory T cells(Tregs) play an important role in regulating I/R injury. It is crucial to understand and consider the microenvironment which regulate Tregs during the pathogenesis of I/R injury as Tregs decreased at the early phage of I/R injury and increased later which reduced the inflammatory cytokines production and liver injury. In this study, we found that

DOI: 10.1002/eji.201970400

hypoxia leads to the formation of acidic microenvironments in liver I/R injury, pondus Hydrogenii(PH) decreased to 6.5±0.2 in mice I/R model compared with sham group(7.0±0.1) and Tregs in liver decreased according to low level of PH, while increased when PH was improved at later phase of reperfusion. Feeding with NaHCO3 improved liver function accompanied while less PH was decreased and more Treg was observed. We also checked the Treg generation with acidic microenvironment in vitro, lower PH lead to lower Treg differenation and lower Treg function in T cell expansion test. The decrease of Treg differenation could be altered with NaHCO3 addiction or higher PH culture media was added in the culture system. Mechanism study showed that proton pump inhibitor could suppress the decreasing of Tregs and PI3K-mTOR signal as well was involved in Tregs differenation caused by the change of PH. This study will provide new targets and theoretical basis for clinical alleviation and intervention of liver IR injury.

P2711

CK02P38, a novel tetrazole compounds, controlled the balance of Th17 and iTreg

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The tetrazole compounds and analogs have been reported to have the antibacterial activity in vitro; however, their function in the immune system remains widely unknown. Foxp3 (fork-head box P3), also known as the most accepted marker, is a protein involved in immune system responses, which is constitutively expressed at high levels in naturally occurring $CD4^+CD25^+$ regulatory T (nTregs) cells and induced regulatory T (iTreg) cells. Here, we found that a novel tetrazole compound (CK02P38) significantly enhanced the expression of Foxp3 and blocked the differentiation of Th17 cells by a unique drug screen model. To investigate the molecular mechanism of reprograming the differentiation of Th17 cells towards induced Treg cells, we showed that CK02P38 increased Foxp3 with TGF-β or without TGF-β to promote Treg conversion and decreased the expression of IL17. Further study was identified that CK02P38 amplified Foxp3 level through activing Stat5 pathway and inhibiting Stat3 pathway. Notably, CK02P38 pointedly induced autophagy through AKT/mTOR/P70S6K pathway. Meanwhile, CK02P38 activated ERK pathway independent of TGF-β and considerably up-regulated CLC-3, SOCS3 and Hif1, whereas CK02P38 sustained stability of Stat5 by CHX array. Moreover, CK02P38 increased Foxp3 expression and control IL17 expression in peripheral blood mononuclear cells (PBMC). Altogether, our results open a novel avenues for targeting the balance of Th17 and regulatory T cells by a small molecule screen in autoimmune diseases (AID).

P2712

Inflammatory signaling regulates hematopoietic stem cell and lymphoid cell formation

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Hematopoietic stem cells(HSCs) are defined as cells that can self-renew and provide robust, long-term multilineage engraftment of immunocompromised adults. Generating HSCs from embryonic stem cells or other cell sources has been unsuccessful. Identifying signaling pathways that regulate HSC formation and characterizing new markers that improve embryonic HSC purification will guide efforts to produce and expand HSCs ex vivo.
HSCs differentiate from hemogenic endothelium and accumulate within intra-aortic hematopoietic

DOI: 10.1002/eji.201970400

clusters, although the vast majority of cluster cells are not functional HSCs. Genetic profiling revealed enrichment in gene ontology terms associated with innate immunity and inflammatory response in HSCs and lymphoid progenitors. Mouse embryos lacking interferon(IFN) g or a signaling had fewer HSCs and lymphoid progenitors. Knockdown of the negative regulator of IFN signaling, interferon regulatory factor 2 increased HSPC production. We further demonstrated the primitive myeloid population contributes to the inflammatory response to regulate the scale of HSC and lymphoid progenitor production. In addition, we also identified CD27, which is a co-stimulatory molecule for B cell and T cell activation, is a better new marker for HSC purification. CD27 is expressed on ~5% of hematopoietic cluster cells, and it marks HSCs and Type II pre-HSCs, which are immature precursors of HSCs. Furthermore, NF-kB signaling is active in the $CD27^{+}$ type II pre-HSCs.
Collectively, these data demonstrate Inflammatory signaling is active in the developing embryo and is important for HSC formation. We will continue to explore the mechanisms by which inflammatory signaling pathways were activated and how these pathways guide HSC formation.

P2713

TGR5/cathepsin E signaling regulates macrophage migration and polarization in liver ischemia/reperfusion injury

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Bile acid-responsive G-protein-coupled receptor (TGR5) is well known for its roles in regulating innate immune activation. Although its function in the anti-inflammatory response during liver ischemia/reperfusion (IR) injury has been reported, the underlying mechanisms remain ambiguous. We generated chimeric mice through bone marrow transplantation using wild-type (WT) and TGR5 knockout ($TGR5^{-/-}$) mice to study the function of TGR5 in macrophages in a murine partial warm hepatic ischemia model. The $TGR5^{-/-}$→WT chimeric mice developed more severe liver IR injury with an enhanced proinflammatory immune response in the livers compared with the WT→WT and WT→$TGR5^{-/-}$. Upon LPS stimulation, TGR5-deficient bone marrow-derived macrophages (BMDMs) displayed increased mobility toward MCP-1 and were more likely to be polarized to the proinflammatory type (M1) compared to WT BMDMs. INT-777, a TGR5 agonist, enhanced the anti-inflammatory effect of TGR5 both in vivo and in vitro. Microarray profiling of WT and $TGR5^{-/-}$ BMDMs primed with LPS revealed that $TGR5^{-/-}$ BMDMs exhibited increased cytokine secretion and enhanced chemotactic characteristics. Cathepsin E (Cat E) was the most upregulated gene in $TGR5^{-/-}$ BMDMs, and knockdown of Cat E significantly abolished the enhanced proinflammatory response induced by TGR5 deficiency both in vivo and in vitro. Correspondingly, IR stress significantly activated TGR5 in PBMCs of patients. UDCA administration enhanced TGR5 activation and the trend of human macrophage polarization. Our results indicated that TGR5 attenuates proinflammatory immune activation by restraining macrophage migration and facilitating macrophage polarization toward the M2 phenotype via suppression of Cat E and thereby protects against liver IR injury.

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DOI: 10.1002/eji.201970400

P2714

A novel MDSC-induced PD-1$^-$PD-L1$^+$ B-cell subset in breast tumor microenvironment possesses immuno-suppressive properties

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Myeloid-derived suppressor cells (MDSCs) play a vital role in forming a tumor-favoring micro-environment to suppress T-cell immune response. In breast cancer, the function and mechanism of MDSCs on B cells remain unclear. Here we show that in 4T1 tumor-bearing mice, there is concurrent increase of splenic MDSCs and B cells when compared to normal mice. In the presence of MDSCs, the surface molecules of B cells are remolded, with checkpoint-related molecules such as PD-1 and PD-L1 changing prominently. Within the tumor environment, MDSCs owned the capacity to regulate B-cell functions including proliferation, apoptosis and the abilities to secrete antibodies and cytokines. Furthermore, MDSCs can educate normal B cells into an immune-suppressive subtype with the ability to suppress T-cell immune response. The typical regulatory B cells (Bregs) termed to be PD-1$^-$PD-L1$^+$CD19$^+$, which exert the greatest suppressive effects on T cells in comparison with the previously reported Bregs characterized as CD5$^+$CD1d$^+$CD19$^+$, CD5$^+$CD19$^+$ and interleukin (IL)-10-secreting B cells. Our results indicate that MDSCs can transform normal B cells into a Breg subtype, which extend our understanding of regulation of T-cell response and shed new light on anti-tumor immunity and immune therapy.

P2715

Effect of decitabine on MDSCs and Tregs in patients with AML

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Background: Immune system abnormalities have been shown to play a critical role in the development of acute myeloid leukemia (AML) as a pathogenesis. Both bone marrow-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) have been proposed as good candidates to inhibit the body´s anti-tumor immunity leading to the immune escape of tumor cells and causing the occurrence and development of diseases. The decitabine (DAC), as a DNA methyltransferase inhibitor, has immunomodulatory functions in addition to demethylation in the treatment of AML. In this study, we evaluated the proportions of MDSCs and regulatory T cells in the peripheral blood of AML patients, and the correlation with the treatment of decitabines was analyzed, as well as clinical efficacy.
Methods: 50 AML patients (newly diagnosed、treated with decitabines or other chemotherapy regimens) were enrolled in our study along with 14 healthy individual. The proportions of the MDSCs and Tregs were analyzed by flow cytometry.
Results: MDSCs and Tregs were highly upregulated in newly diagnosed AML patients compared to the control groups (P=0.02 and P=0.02, respectively). The proportions of the MDSCs and Tregs decreased after the treatment of decitabines (P=0.012 and P=0.004, respectively) . In decitabines' group , the higher response rate were linked with the decreased proportions of MDSCs (P= 0.031).
Conclusions: The increased MDSCs and tregs may be associated with the inhibition of body´s anti-tumor immunity. And the treatment of decitabines can decrease the numbers of MDSCs and tregs which may delay the progression of the disease in AML patients.

DOI: 10.1002/eji.201970400

P2716

CD69 enhances immunosuppressive function of regulatory T-cells and attenuates colitis by prompting IL-10 production

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Foxp3+ regulatory T cells (Tregs) can inhibit immune responses and maintain immune tolerance by secreting immunosuppressive TGF-β1 and IL-10. However, the efficiency of Tregs become the major obstacle to their use for immunotherapy. In this study, we investigated the relevance of the C-type lectin receptor CD69 to the suppressive
function. Compared to CD4+Foxp3+CD69− Tregs (CD69− Tregs), CD4+Foxp3+CD69+ Tregs (CD69+ Tregs) displayed stronger ability to maintain immune tolerance. CD69+ Tregs expressed higher levels of suppression-associated markers such as CTLA-4, ICOS, CD38 and GITR, and secreted higher levels of IL-10 but not TGF-β1. CD69+ Tregs from Il10+/+ rather than Il10−/− mice significantly inhibit the proliferation of CD4+ T cells. CD69 over-expression stimulated higher levels of IL-10 and c-Maf expression, which was compromised by silencing of STAT3 or STAT5. In addition, the direct interaction of STAT3 with the c-Maf promoter was detected in cells with CD69 over-expression. Moreover, adoptive transfer of CD69+ Tregs but not CD69−Tregs or CD69+ Tregs deficient in IL-10 dramatically prevented the development of inflammatory bowel disease (IBD) in mice. Taken together, CD69 is important to the suppressive function of Tregs by promoting IL-10 production. CD69+ Tregs have the potential to develop new therapeutic approach for autoimmune diseases like IBD.

P2717

Regulatory T cells suppress dendritic cell expansion and contribute to peripheral $CD8^+$ T cell tolerance via intercellular adhesion molecule-1

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A key event in the successful induction of peripheral $CD8^+$ T cell tolerance is the suppression of dendritic cells (DC) by regulatory T cells (Treg). ICAM-1 highly expresses on DC and plays an important role in promoting DC-mediated immunity. However, whether ICAM-1 on Treg contributes to peripheral $CD8^+$ T cell tolerance controlling remains unclear. Here we show that the expression level of ICAM-1 on Treg is significantly higher than that of on effector T cells (Teff) under steady state. ICAM-1^+ Treg are the dominant cellular source of Ki67^+ Treg. In a Treg proliferation in vivo model, induced by 7,12-dimethylbenz [a] anthracene (DMBA) painting, the proliferation of Treg can be inhibited by anti-ICAM-1 antibody treatment. These data suggested ICAM-1 may play an important role in the maintenance of Treg homeostasis and the expansion of Treg induced by DMBA. To further study the role of ICAM-1 on Treg, we crossed mice carrying loxP-flanked ICAM-1 alleles with FOXP3-Cre mice to specifically delete ICAM-1 on Treg (KO mice). We found that the number of Treg was significantly decreased in spleen and lung of KO mice. Unexpectedly, the number of DC is significantly

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DOI: 10.1002/eji.201970400

increased in KO mice. More importantly, the number of T-bet$^+$CD8$^+$ T cell is significantly increased in spleen and lung of KO mice. Thus, these data indicated that the Treg may regulate DC and contribute to CD8$^+$ T cell tolerance through ICAM-1, although the underlying mechanisms need to be further investigated.
This work was supported by grants from 973 program (2015CB943203).

P2718

Antitumor and immunomodulatory activity of water-soluble crude polysaccharide from solid fermentation of *Phellinus igniarius* and *Mulberry* leaves in H22 tumor-bearing mice

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To further clarify the potential antitumor mechanism of Traditional Chinese Medicine (TCM) from the molecular level, provide a clinical guidance and lay a foundation for the development of novel, effective antitumor TCM agents. The present study was designed to evaluate the immune effects of *Mulberry* leave fermenting *Phellinus igniarius* mycelial polysaccharide (MPFP) in vitro and the antitumor potentials of MPFP in H22 tumor-bearing mice and the underlying mechanism. The results showed that MPFP could effectively stimulate splenic lymphocyte proliferation and active RAW264.7 cells such as phagocytosis, secretion of nitric oxide in vitro. Besides, the spleen and thymus indexes of H22 tumor bearing mice were also improved by MPFP administration. Furthermore, the serum levels of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2), as well as percentages of CD4+ and CD8+ cells were markedly enhanced by MPFP. Moreover, Western blot assay showed that MPFP might significantly increase Bcl-2 protein levels and decrease caspase-3 and caspase-9 protein levels in the thymus and spleen tissues, especially for low dose of MPFP. These data collectively indicate that MPFP has both immune effects in vitro and vivo. In addition, MPFP alters the protein expression of the mitochondria in immune organs, which is another potential pathway for the antitumor activity of enhancement of host's immune system. Taken all together, these findings clearly demonstrated that MPFP has antitumor activity at least partly via improving immune functions, which may provide a basis for the development of fermented polysaccharides of anticancer TCM.

P2719

Immune modulation by ANGPTL4 in inflammatory bowel disease and colorectal cancer

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Angiopoietin-like protein 4 (ANGPTL4), a member of the angiopoietin-like molecular family, is secreted by visceral adipose tissues, macrophages, and some cancer cells. ANGPTL4 regulates glycolipid metabolism, angiogenesis, and promotes tumor growth. ANGPTL4 is also involved in local inflammation. It is not clear whether ANGPTL4 regulates activity of immune cells. We found that frequency of effector CD8$^+$ T cells in ANGPTL4$^{-/-}$ mice increased. IFN-γ secretion and cytotoxicity of CD8$^+$ T cells also increased significantly. The growth of transplanted tumors were suppressed in the ANGPTL4 KO mice. On the contrary, the recombinant ANGPTL4 protein down-regulated CD8$^+$T cell activity. Molecular mechanism studies showed that ANGPTL4 affected metabolism and biological activity of CD8$^+$T cells through the LKB1-AMPK-mTOR pathway. The frequency of macrophages also increased in ANGPTL4$^{-/-}$ mice. However, rANGPLT4 had no effects on functions of macrophages in vitro. Up-regulation of macrophages by ANGPTL4 deficiency was involved with endoplasmic reticulum

DOI: 10.1002/eji.201970400

stress induced by more fatty acid synthesis. In patients with inflammatory bowel diseases, ANGPTL4 was elevated in sera and colon tissues, and is negatively associated with $CD8^+$ T cells. DSS-induced colitis was more severe in ANGPTL4$^{-/-}$ mice. Recombinant ANGPTL4 attenuates DSS-induced colitis through suppressing $CD8^+$T cell infiltration. ANGPTL4 increased in sera and tumor tissues of colorectal cancer patients, and it was positively correlated with regulatory T cells and M2 macrophages. In conclusion, ANGPLT4 participates in the pathogenesis of inflammatory bowel disease and colorectal cancer by regulating biologic activity of immune cells. ANGPTL4 would be a potential target in the treatment of colorectal cancer.

Immunological Memory

O073

Epigenetic control during CD8+ T cell fate commitment

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Following activation by antigens, naïve $CD8^+$ T lymphocytes establish specific heritable gene expression programs that define the progression to long-lasting memory or to short-lived effector cells. While lineage specification in T cells is critical for protection, the impact of epigenetic silencing on T lymphocyte differentiation is still incompletely understood.
Here, we explore the role of heterochromatin-mediated gene expression silencing by Suv39h1, a histone H3 lysine 9 methyltransferases that plays a critical, evolutionary conserved, role in heterochromatin structure and dynamics. We show that in murine $CD8^+$ T cells activated after *Listeria monocytogenes* infection *in vivo*, Suv39h1-dependent H3K9me3 deposition controls the expression of a set of stem cell-related/memory genes. Single-cell RNA sequencing analysis reveals that the silencing in stem/memory genes selectively affects terminally differentiated effector subsets. The results also show increased proportions of $CD8^+$ T cells with central memory phenotype and the de-repression of stem cell-related genes across different *Suv39h1*-defective $CD8^+$ T cell sub-populations. In line with these observations, *Suv39h1*-defective $CD8^+$ T cells show increased memory potential, including sustained survival and increased long-term re-programming capacity, as compared to *Suv39h1*-proficient $CD8^+$ T cells.
We conclude that Suv39h1 plays a critical role in marking chromatin to silence stem/memory gene expression during $CD8^+$ effector T cell terminal differentiation. In doing so, Suv39h1/H3K9me3 would establish an epigenetic barrier on the stem/memory gene expression program, preventing the effector re-programing into memory cells. These results open new perspectives for the manipulation of epigenetic programming of T lymphocyte identity in the context of T cell-based immunotherapies.

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DOI: 10.1002/eji.201970400

O074

Using single-cell RNA-seq to lineage trace effector to memory transitions in CD4+ T cells during malaria

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Hallmark features of adaptive CD4+ T-cells include differentiation into multiple effector T helper (Th) fates and subsequent emergence of memory states. Due to the heterogeneous nature of CD4+ T-cells, determining possible developmental relationships between effector and memory states *in vivo* has remained a challenge. Previously we employed single-cell RNA-sequencing (scRNA-seq) to delineate the molecular trajectories of *Plasmodium*-specific TCR transgenic CD4+ T cells during the first week of experimental malaria. We observed a fate bifurcation as cells progressed from an intermediate, activated state to either effector Th1 or T follicular helper (Tfh) cells. My current project tests the hypothesis that CD4+ Th cells directly convert into memory CD4+ T cells during malaria, and aims to define the underlying transcriptomic processes. Tracking endogenous TCR sequences via TraCeR analysis firstly confirmed that sibling clones populated both Th1 and Tfh fates. Next, by comparing transcriptomes, and performing computational lineage tracing of the scRNA-seq data, we observed a direct transition from Th1 and Tfh effector states to memory states. This was characterised by gradual transcriptomic quiescence during the first month of infection, as well as transcriptomic coalescence of Th1 and Tfh fates, but with retention of Th1/Tfh bifurcated structure in memory cells. Finally, premature cure of infection with anti-malarial drugs altered transcriptomic pathways taken by cells towards those with better Th1 recall and germinal centre Tfh phenotypes, respectively. Thus, by examining transcriptional dynamics using scRNA-seq, I am tracing the molecular pathways taken by effector CD4+ T cells towards functional memory states.

O075

Deciphering CD4+ T cell immunodominance to Influenza virus

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Influenza viruses represent a public health concern due to their pandemic potential and to the sporadic spread of highly pathogenic strains from zoonotic hosts to humans. Several recent studies on the clonal composition of the human antibody repertoire against hemagglutinin (HA) - a main target of neutralizing antibodies against Influenza virus - have revealed that broadly neutralizing antibodies against conserved epitopes in the HA stem region can develop in the course of an immune response to infection or vaccination. Surprisingly, the repertoire of human CD4+ T helper (Th) cells against HA remains poorly defined, in spite of the fact that these cells play an important role in the induction of the antibody and cytotoxic CD8+ T cell responses.
In this study, we set out to understand the role of antigen presentation in shaping the antigen-specific human Th cell repertoire, and its contribution to the development of immunological memory following vaccination. By combining antigenic stimulation of naïve and memory T cell libraries, T cell cloning,

DOI: 10.1002/eji.201970400

peptide mapping and TCR sequencing, we provide a comprehensive description of the clonal composition of human Th cell repertoire against HA. Furthermore, using mass spectrometry-based MHC-II immunopeptidomics we define the HA-derived peptides naturally presented by monocytes-derived dendritic cells and HA-specific B cell clones.
This study can shed new light on the mechanisms underlying T cell repertoire selection, immunodominance, and formation of immunological memory, and could have important implications for vaccine design and prediction of immunization outcome.

O076

Maintenance of efficient longterm memory to systemic pathogens by resident and resting memory T lymphocytes of the bone marrow

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It is a matter of current debate, whether the bone marrow is a hub for circulating memory T lymphocytes, and/or the home of a discrete population of bone marrow-resident memory T lymphocytes. Here we provide evidence that at least 30% of $CD4^+$ and 60% of $CD8^+$ memory T lymphocytes of the bone marrow express CD69, and rest in terms of transcription and proliferation, being in the G0 phase of the cell cycle not expressing KI-67. Obviously, they are not maintained by homeostatic proliferation, but rather by contact to stromal cells of the bone marrow. They express the tissue-resident memory T (T_{RM}) cell signature genes described for T_{RM} cells of other tissues, and thus qualify as bona fide bone marrow T_{RM} cells. Their T cell receptor repertoire is significantly enriched for specificities against systemic pathogens/vaccines, in particular for childhood challenges. They are polyfunctional. It is notable that memory $CD4^+$ T cells specific for measles are maintained in the bone marrow, even in aged individuals in which they are not detectable in the blood, confirming that these cells are indeed resident. If such individuals are vaccinated again, measles-specific T_{RM} are mobilized into the blood and contribute to the secondary immune reaction. Collectively, these results demonstrate that bone marrow contains significant populations of resident memory T cells, providing efficient reactive longterm memory to systemic pathogens/vaccines, and that these cells are not maintained by homeostatic proliferation, but rather as cells resting on and maintained by stromal cells.

LB013

RNA-binding motifs of AID-cofactor hnRNP K are involved in class switch recombination and somatic hypermutation

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Activation-induced cytidine deaminase (AID), a cytidine (C) to uracil (U) editing enzyme specifically expressed in B lymphocytes, is responsible for generating antibody memory through inducing DNA breaks at the immunoglobulin heavy chain (IgH) locus during somatic hypermutation (SHM) and class switch recombination (CSR). Although the substrate of AID is unknown, many evidence support the hypothesis that AID should edit putative RNA(s). Our previous study identified that heterogeneous nuclear ribonucleoprotein K (hnRNP K) binds to AID through RNA(s) and is required for AID-induced

DOI: 10.1002/eji.201970400

DNA breaks.
Here, in order to find the responsible amino acids for AID-dependent DNA breaks, point mutations are introduced into hnRNP K' s RNA-binding motifs, GXXGs and RGGs. Surprisingly, GXXG or RGG mutant losing the interaction with AID also shows significant reduction of AID-induced DNA breaks, leading to the deficiency on inducing CSR, SHM and IgH/c-Myc chromosomal translocations. We found that nuclear localization of these mutants is much less than wild type hnRNP K, however, nuclear localizing signal mutant of hnRNP K showing cytoplasmic enrichment rescues CSR comparably to wild type hnRNP K, meaning that functional deficiency of these mutants is not due to their cytoplasmic accumulation. These phenomena suggest that hnRNP K may interact with AID and present RNA(s) to AID for editing through GXXGs and RGGs for DNA breaks. Taken together, we expect that comparing trapped RNAs by wild type hnRNP K and those by functionally deficient mutants will be a promising method to open the way for understanding the mystery about AID.

P0206

Trend of T-SPOT.TB in the effective process of early antituberculosis treatment of tuberculosis

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Objective: To study the change trend of T-SPOT.TB in the effective process of early anti-tuberculosis treatment of tuberculosis.
Methods: Through the follow-up observation of 50 cases diagnosed active tuberculosis patients who were recruited in our hospital for regular anti-tuberculosis treatment from 2016 to 2019, analysis of the dynamic changes trend of T-SPOT.TB results before and after treatment (0.5 months, 1 month, 3 months) and the changes of the body´s response level to mycobacterium tuberculosis specific antigen stimulation before and after treatment.
Results: The number of spots before ESAT-6, CFP-10 and "ESAT-6 + CFP-10" antigen-pore anti-tuberculosis treatment showed a decreasing trend compared with that after anti-tuberculosis treatment at 0.5, 1 and 3 months, respectively. The P values were all < 0.001, and the difference was statistically significant. There was no significant difference in the change trend of immune response to ESAT-6 and CFP-10 stimulation before treatment and 3 months after treatment ($\chi 2=1.954$, $P=0.162$).
Conclusion: T-SPOT.TB spots showed a downward trend in the effective process of early anti-tuberculosis treatment of tuberculosis; the changes of body response to ESAT-6 and CFP-10 were consistent before treatment and after anti-tuberculosis treatment (at the 3rd month).

P0207

Ketogenesis-generated β-hydroxybutyrate is an epigenetic regulator of $CD8^+$ T cell memory development

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Elucidation of metabolic flux and switch is fundamental for understanding of $CD8^+$ memory T (Tm) cell development. Glycogen, an intracellular carbon reservoir, has long been functionally considered as the use of energy metabolism. However, our recent study indicated that glycogen metabolism, directed by a cytosolic phosphoenolpyruvate carboxykinase (Pck1), controls the formation and maintenance of $CD8^+$ Tm cells through regulating the redox homeostasis. This unusual metabolic program raises the

DOI: 10.1002/eji.201970400

question of how Pck1 is upregulated in CD8$^+$ Tm cells. Here, we show that mitochondrial acetyl-CoA is shunted to ketogenesis, which indirectly regulates Pck1 expression. Mechanistically, ketogenesis-derived β-hydroxybutyrate (BHB) is stocked in CD8$^+$ Tm cells, which epigenetically modifies H3K9 of *FoxO1* and *PGC-1α* with β-hydroxybutyrylation so to upregulate these genes expression. As a result, FoxO1 and PGC-1α cooperatively upregulates Pck1 expression, thus directing the carbon flow along the gluconeogenic pathway to glycogen and the pentose phosphate pathway. These results unveil that the ketogenesis acts as an unusual metabolic pathway in CD8$^+$ Tm cells, linking epigenetic modification required for memory development.

P0208

Resident memory CD8 T cells persist for years in human small intestine

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Resident memory CD8 T cells (Trm) have been shown to provide effective protective responses in the small intestine (SI) in mice. However, studies of human SI CD8 Trm are still scarce. A better understanding of the generation and persistence of SI CD8 Trm cells in humans may have implications for intestinal immune-mediated diseases and vaccine development.
Analyzing normal human SI we showed that virtually all intraepithelial (IE) and the majority of lamina propria (LP) CD8 T cells expressed a Trm phenotype. By exploiting the HLA class I allotype disparity between donor and recipients in transplanted duodenum we demonstrated that most of SI CD103+ CD8 T cells survived for over 1 year in the graft, while CD103- CD8 T cells were rapidly replaced by recipient cells. In agreement with their long persistence, CD8 Trm immune repertoire was dominated by expanded clones conserved both spatially in the tissue and over time in transplanted SI. In addition, high clonal overlap was found between IE and LP CD8 Trm cells. Functionally, LP CD8 Trm cells were very potent cytokine-producers, exhibiting a polyfunctional (IFN-Y+ IL-2+ TNF-α+) cytokine profile, and efficiently expressed cytotoxic mediators after stimulation.
In summary, our data demonstrate that *bona fide* CD8 Trm cells with effective protective capabilities persist for at least a year (probably years) in the human SI. These results suggest that SI CD8 Trm cells could be targets for future oral vaccines and new therapeutic strategies in gut disorders, such as inflammatory bowel disease, and celiac disease.

P0209

Resident memory T cells in the CNS and the role of local antigen

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DOI: 10.1002/eji.201970400

Classically memory T cells have been divided into central memory - and effector memory T cells. However, more recently a third subset, named tissue-resident memory cells (Trms) have been described. Trms were originally demonstrated in barrier-tissues but subsequent studies have revealed that Trms are present in most non-lymphoid organs including the CNS. The brain has traditionally been considered an immune-privileged site, but the CNS may nevertheless be the focus of immune mediated inflammation in the case of infection and certain autoimmune diseases, e.g. multiple sclerosis. As in other tissues it has been found that acute T-cell infiltration may be followed by establishment of persistent local T-cell memory. To improve our understanding regarding the regulation of the tissue-resident memory T cells (Trms) in CNS, we devised a new model system for studying the generation of Trm cells in this site. To avoid severe disease that might act as a confounder, we used replication-deficient adenovirus for infection of the CNS. Non-replicating adenoviruses are highly immunogenic, and our studies demonstrate that intra-cerebral inoculation results in a potent T-cell response, followed by infiltration of both $CD4^+$ and $CD8^+$ T cells. Phenotypical and functional analysis of CNS infiltrating $CD8^+$ T cells indicated that these cells were bona fide Trms. Most importantly, our experiments revealed a key role for local antigen encounter in the establishment of sustained $CD8^+$ T-cell memory in the brain.

P0791

Transcription factor BCL6 is required for the generation but not maintenance of memory CD8+ T cells in acute viral infection

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The differentiation of memory CD8+ T cells is critical to the long-term cellular immunity. The transcription factor BCL6 has been reportedly important for the generation and maintenance of memory CD8+ T cells; however, using the newly established BCL6 conditional knockout mouse model, we demonstrate that BCL6 is dispensable for the maintenance of established memory CD8+ T cell pool, although BCL6 is still required for the generation of CD8+ memory precursors upon acute viral infection. In addition, BCL6 regulates the expression of TCF-1 via directly binding to the Tcf7 (gene symbol for TCF-1) allele in CD8+ effector cells and forced expression of TCF-1 restores the generation of BCL6-deficient memory precursors. Thus, our findings clarify that BCL6 is dispensable for the maintenance of memory CD8+ T cells, but functions as an important transcription factor upstream of TCF-1 to regulate the generation of memory precursors in acute viral infection.

P0792

Immune memory in the liver against malaria infection

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Immune memory is of great significance to the development and evaluation of malaria vaccine. The mechanism of long-term immune memory deficit in malaria has always been a difficult problem in the field of malaria research.Little is known about the antimalaria drug driving liver immune memory against malaria infection. In this study, we investigate the immunological mechanisms underlying the anti-parasite protective properties of atorvastatin (AVA) treatment alone and combination with Artesunate (AS) in *Plasmodium yoelii* 17XL (*P.y* 17XL) infected BALB/c mice. Here, we show that AVA treatment alone has an essential role in down-regulation of malaria pigments deposition in liver, improving liver pathological damage, and delayed the peak of parasitemia. Furthermore, AVA and AS combination treatment confer protection against *P.y* 17XL reinfection challenge, which boosts higher

DOI: 10.1002/eji.201970400

humoral IgG and IgM response and up-regulation of CD44+CD62L- effector T cells immnue response. It has been reported that IFN-γ is the key mediator of the anti-malarial mechanism. We demanstrate in AVA and AS combination treatment rodent model that liver $CD8^+$T effector lymphocytes are indispensable for the clearance of malaria infection, which mainly consists with a higher number of CD3+CD8+IFN-r+ cells. Importantly, we find that a higher number of CD3+CXCR6+ cells in the liver is associated with the development of immune memory in AVA and AS combination treatment. Thus, we show the evidences that AVA has an essential role in improving the liver pathological damage during blood stage malaria infection. The combination of AVA and AS could establish immune memory in liver against malaria infection.

P0793

WASp regulates effector-to-memory conversion and maintenance of $CD8^+$T cell memory

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Wiskott-Aldrich syndrome (WAS) is a very rare X-linked primary immunodeficiency featured by recurrent infections, micro thrombocytopenia, eczema, and a higher incidence of autoimmunity and malignancy. A defect in the T cell compartment is thought to be a major cause of immunodeficiency in patients with WAS, whether the antigen specific T memory cell is altered has not been extensively studied. Here, we examined the kinetics underlying expansion/contraction and maintenance of memory $CD8^+$T cells in WAS KO mice.The results showed that WAS protein (WASp) is not required for differentiation of effector cells; however, memory $CD8^+$T cells from WAS KO mice were hyperactive, resulting in increased cytokine production. Impaired recall responses and poor protective immunity were also observed. WASP deficiency in bone marrow chimeric mice did not disrupt clonal expansion; however, effector cells failed to survive and form memory $CD8^+$T cells. Taken together, these findings indicate that WASp is a key regulator of functional $CD8^+$ T cell memory.

P0794

Distinct subsets of human memory B cells define the strategy of the human memory response throughout life

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Immunological memory protects us from being re-infected by a previously encountered pathogen. In adults, 50% of the B cells are memory B cells (MBCs). Based on the expression of the immunoglobulin (Ig), two populations of MBCs have been described, switched and IgM. Whereas switched MBCs are mostly generated in the germinal centers (GC), we demonstrate that there are three IgM MBCs with different developmental history: innate (GC-independent), remodelled and GC-derived IgM MBCs.
In the mouse, based on the expression of surface markers, but independently of the expressed Ig isotype, different subsets of MBCs have been identified that are either more naïve- or more memory-like.
CD27 is the cell surface marker that identifies MBCs in humans. The intensity of CD27 expression changes with age. We show that, independently of the expressed Ig isotype, in infants MBCs express low levels of CD27 ($CD27^{dull}$). In the adult most MBCs are $CD27^{bright}$. We demonstrate that $CD27^{dull}$

DOI: 10.1002/eji.201970400

MBCs are T-, GC- and CD40L-independent. CD27^{bright} MBCs, in contrast, are exclusively generated in individuals with functional GCs. We demonstrate that CD27^{dull} and CD27^{bright} MBCs represent two distinct and sequential MBC developmental stages.
Thus, the most used definition of human MBCs, as either IgM or switched, may be insufficient to describe the complexity of human B-cell memory responses and functions.
For the first time we show that highly selected and specific MBCs derive from less refined MBCs precursors, thus challenging the postulate that high affinity MBCs always derive from naïve B cells recruited in the GC response.

P1396

Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity

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Innate immune memory represents an emerging area of research and is currently defined as either trained immunity or immune tolerance in innate immune cells against homo- or heterologous re-stimulation. However, key cellular and molecular mechanisms for the priming of innate immune memory at major mucosal sites remains poorly understood. The respiratory mucosa is frequently exposed to a variety of airborne viral and bacterial pathogens. By using a murine experimental model of adenoviral infection, we show here that respiratory viral infection induces long-lasting innate immune memory (trained immunity) generation in alveolar macrophages (AMs).Memory AMs are programed to express high MHC II, a defense-ready gene signature, increased glycolytic metabolism, and produce increased neutrophil chemokines upon re-stimulation. Using a multitude of approaches, we reveal that the priming, but not maintenance, of memory AMs requires the help from effector CD8 T cells that home to the lung compartments post infection. Upon direct cellular contact with AMs, T cells jump-start AM memory generation via IFN-gamma production. We further find that formation and maintenance of memory AMs are independent of circulating monocytes or any other potential bone marrow-derived progenitors. Finally, we demonstrate that memory AMs are poised for robust trained immunity against extracellular bacterial infection in the lung via rapid induction of chemokines and neutrophilia in lung tissues. Our study thus establishes a new paradigm of immunological memory formation whereby adaptive T-lymphocytes render innate memory of mucosal-associated macrophages. Such an adaptive-to-innate signaling paradigm for innate memory generation deserves further study in other immunological scenarios.

P1397

Lung migratory CD103^{+} dendritic cells restrain the generation of OVA-specific Th2 memory cells through IL-12 production

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DOI: 10.1002/eji.201970400

Memory Th2 cells play critical roles in allergic asthma, and its generation and maintenance could be regulated by several signal pathways. However, the roles of lung migratory $CD103^+$DC and $CD11b^+$DC in generation and maintenance of memory $CD4^+$ T ($CD4^+$ T_M) cells are still unclear. Our data demonstrated that OVA-specific Th2 memory cells generated in $CD103^+$ DC-deficient ($Batf3^{-/-}$) mice were significantly more than that in wild-type mice, indicating that $CD103^+$ DC somehow suppressed the generation of $CD4^+$ T_M cells. $CD4^+$ T effector cells primed in absence of $CD103^+$DC have stronger potential to generate memory cells. $CD103^+$DC deficiency enhanced CD62L expression in primed $CD4^+$ T and its accumulation in lung-draining lymph nodes (dLN). Meanwhile, OVA-pulsed $CD103^+$ DC suppressed CD62L expression in primed OT-II cells in DC-T co-culture experiments *ex vivo*. Subsequent *ex vivo* and *in vivo* experiments suggested that highly expressed IL-12 in $CD103^+$DC played pivotal roles in suppressing CD62L expression in $CD4^+$T effector cells and the generation of Th2 memory cells. In conclusion, Lung migratory $CD103^+$DC restrained the generation of allergen-specific Th2 memory cells through IL-12 production in an OVA/papain-induced murine asthma model.
Acknowledgements: This work was supported by grants from the National Basic Research Program of China (2015CB943203), the National Natural Science Foundation of China (NSFC31500715, NSFC81401328), the Natural Science Foundation of Shandong Province (ZR2015HQ021, ZR2013HL064) and the Project of medical and health technology development program in Shandong province (2018WS136).

P1398

A monoclonal lamprey antibody recognizes a tyrosine sulfation-dependent epitope of HLA-I uniquely on memory B cells and plasma cells

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Memory B cells and plasma cells are tasked with providing long lasting humoral protection to pathogens. However, despite their central role in protective immunity no definitive cell surface markers for these cells have been identified. Unlike conventional immunoglobulins, the variable lymphocyte receptor (VLR) antibodies of the evolutionarily distant sea lamprey utilize the leucine-rich repeat as basic structural unit. We hypothesized that the large evolutionary distance of jawless vertebrates and the distinct protein architecture of VLR antibodies may allow these reagents to recognize structures, which conventional antibodies may not readily recognize because of tolerogenic or structural constraints. Here, we report the isolation the monoclonal VLRB N8 antibody, which recognizes a tyrosine sulfation-dependent epitope of HLA-I uniquely on human memory B cells and plasma cells. Recognition of memory B cells and plasma cells by VLRB N8 can be induced by antigen receptor engagement and cytokine stimulation but not by CpG treatment and is independent of HLA-I expression levels. Furthermore, VLRB N8 binding to memory B cells is greatly increased in the context of the systemic lupus erythematosus (SLE) and multiple sclerosis (MS) autoimmune disorders.
Our study indicates that tyrosine sulfation may represent an as yet unexplored mechanism governing immune regulation by HLA-I in the memory B cells and plasma cells. Furthermore, our data highlight the unique advantages provided by the non-conventional VLR antibody platform in the interrogation of cell surface structures of functionally defined cell populations.

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DOI: 10.1002/eji.201970400

P1399

Mitochondrial function signature in fumarate-induced monocyte innate immune training

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Induction of innate immune training in monocytes is dependent on glycolysis, glutaminolysis, and cholesterol synthesis pathways. Trained monocytes accumulate fumarate, and fumarate itself induces training[1]. We analysed the mitochondrial function associated with fumarate-induced training.
Human monocytes were labelled with tetramethylrhodamine methyl ester, stimulated with 100 mM mono methyl-fumarate (MMF), and tracked by time-lapse confocal microscopy for mitochondrial dynamics and mitochondrial membrane potential (Dy_m) analyses; or labelled with Rhod-2, and tracked by high-speed time-lapse fluorescent microscopy or by flow cytometry, for mitochondrial calcium mobilization. Monocytes stimulated with MMF for 3 h and 24 h were analysed by transmission electron microscopy, for mitochondrial morphology and cristae analyses. In addition, RAW 264.7 cells were stimulated with MMF for 3 h and 24 h, mitochondrial proteins were obtained, and the phosphorylation profile was analysed.
Results showed that after 90 minutes of fumarate exposure, mitochondria increased their area, elongation, interconnectivity, and Dy_m, which is compatible with metabolically active mitochondria. At 3 h, mitochondria cristae were more electrodense, and the mitochondrial protein serine-phosphorylation profile of fumarate-stimulated RAW 264.7 cells was different from that of un-stimulated cells, both at 3 h and 24 h post-fumarate. Fumarate treatment of monocytes induced mitochondrial calcium uptake within minutes.
These results contribute to a “mitochondrial function signature” that will allow us to understand the role of mitochondria the induction of monocyte innate immune training.

[1]Arts et al., 2016, Cell Metabolism 24, 807-819.
Project financed by the VALIDATE Network, funded by the MRC and BBSRC (UK); EDCTP; and CONACYT (284602), Mexico.

P1986

Characterising antigen-specific CD8 T memory stem cells in human viral infections

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T memory stem cells (T_{SCM}), a rare subset of memory lymphocytes displaying a naïve-like phenotype, have enhanced self-renewal, multipotency and longevity. Successful vaccinations, such as against yellow fever virus, generate a high frequency of T_{SCM} that lasts for decades. $CD8^+$ T_{SCM} have been identified in human viral infections by using combinations of markers, such as IL-2Rβ (CD122), CXCR3 and CD95. There is no consensus on the definitive set of markers to identify T_{SCM} and limited knowledge of long-term maintenance of T_{SCM}. We aim to comprehensively identify the functional heterogeneity and molecular signatures of Ag-specific T_{SCM} in viral infections.
We have developed a flow-cytometry panel to identify T_{SCM} subsets and are currently validating these

DOI: 10.1002/eji.201970400

against functional characteristics, including proliferation, intracellular cytokine production (TNF-α, IL-2, IFN-γ), and self-renewal. $CD8^+$ $CCR7^{hi}CD45RA^{hi}CD95^{hi}$ T_{SCM} cells from healthy subjects previously exposed to Cytomegalovirus (CMV) and Influenza (Flu) virus were bulk sorted in subsets based on the expression of CD122 and CXCR3. High expression of CD122 and low expression of CXCR3 were associated with increased proliferation. The TNF-α, IL-2 and IFN-γ co-production levels were higher in $CD122^{hi}$ T_{SCM} (88.9%) and $CXCR3^{lo}$ T_{SCM} (100%), compared to $CD122^{lo}$ (77.8%) and $CXCR3^{hi}$ subsets (88.9%) respectively. $CD122^{hi}$ T_{SCM} had greater multi-potency and self-renewal than $CD122^{lo}$ T_{SCM}. These data revealed that the $CD122^{hi}$ phenotype is associated with greater 'stemness': increased proliferation, multi-potency and enhanced self-renewal ability. Antigen-specific responses and transcriptomic analyses of T_{SCM} subsets are underway to further characterize these phenotypes.

P1987

Crucial role of innate immune memory in vaccine-induced rapid protection

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Conventionally, host immune responses are subdivided into two parts: innate immune responses and adaptive immunity. Innate immune responses react rapidly and nonspecifically upon encountering stimuli and have no memory. Adaptive immune responses are slower to develop but specific and build up immunological memory. Emerging evidence has shown that innate immune cells (such as monocytes, macrophages, or natural killer cells) display long-term changes in their functional programs and have a memory characteristic after primary stimulation, leading to a stronger response against to secondary stimuli. This de facto immunological memory has been termed “trained immunity” or “innate immune memory.” Mechanistic studies have demonstrated that trained immunity is based on metabolic and epigenetic programming. Our study showed a single dose intranasal immunization of inactivated whole cell (IWC) of *Acinetobacter baumannii* could induce rapid 100% protection against lethal *A. baumannii* pneumonia in $Rag1^{-/-}$ mice at 7 d after immunization and the immune response showed memory feature. These data indicate the important role of innate immune memory in vaccine-induced rapid protection. We also explored the partial mechanism of vaccine-induced innate immune memory. Our work will help understand the mechanism of vaccine-induced rapid protection and further design the rapid effective vaccine for *A. baumannii* and other bacteria.

P1988

A single T cell epitope drives the neutralizing anti-idiotypic antibody response to natalizumab in patients with multiple sclerosis

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DOI: 10.1002/eji.201970400

Natalizumab (NZM), a humanized monoclonal IgG4 antibody to α4 integrins, is used to treat patients with relapsing-remitting multiple sclerosis (MS), but in about 9% of the cases neutralizing anti-drug antibodies (ADAs) are induced leading to therapy discontinuation. To understand the basis of the ADA response and the mechanism of ADA-mediated neutralization, we performed an in-depth analysis of the B and T cell response in two patients. By characterizing a large panel of NZM-specific monoclonal antibodies, we found that, in both patients, the response was polyclonal and targeted different epitopes of the NZM variable region. The neutralizing activity was acquired through somatic mutations and correlated with a slow dissociation rate, a finding that was supported by structural data. Interestingly, in both patients, the analysis of the CD4+ T cell response, combined with mass spectrometry-based peptidomics, revealed a single immunodominant T cell epitope in the NZM variable region. Collectively, our study identifies the basis of T-B collaboration that drives neutralizing anti-idiotypic antibodies to NZM.

P1989

The role of memory CD4 T cells in protective immunity against hepatitis C

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Generation of a robust immunological memory is critical for natural immunity against pathogens and successful vaccine design. In addition to conventional memory T subsets (central and effector memory), a rare subset of memory lymphocytes endowed with stem cell-like ability termed T memory stem cells (Tscm), have been shown to have superior functional properties, which include longevity, self-renewal capacity, and also the multipotent capacity to reconstitute the entire spectrum of memory and effector T cells. My project will be the first study to investigate the role of antigen-specific Tscm CD4 T cells in hepatitis C viral (HCV) re-infection. About 25% of individuals will spontaneously clear the virus following primary HCV infection. Although spontaneous clearance does not generally confer protection against reinfection, there is improved control of viral replication, an attenuated course of infection, and an increased likelihood of viral clearance in reinfection compared to primary infection, suggesting that prior clearance of an HCV infection results in partial protective immunity against persistent reinfection. I propose that Tscm are crucial for immune protection against HCV reinfection, which will ultimately underpin successful HCV vaccine design. The prospective HCV cohorts with longitudinally-collected samples, including rare individuals with repeated clearance and those with potential protection upon re-exposure will be used in this study. I will investigate the pattern and function of CD4 Tscm at both the bulk and HCV-specific levels using cutting-edge techniques established in the our laboratory, including sixteen-colour flow cytometry, multiplex cytokine-production, and single-cell RNA sequencing of antigen-specific T cells.

P2720

Effects of low-dose rapamycin treatment on lung tissue-resident memory CD8+ T cells of mice infected with influenza virus

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This study was performed to investigate the effect of low-dose rapamycin treatment on the formation of tissue-resident memory CD8+ T cells. C57BL/6J wild type mice were randomly divided into two groups: the control group and rapamycin treatment group. By adoptively transferred P14 cells (TCR transgenic CD8+T cells) and a recombinant influenza A virus expressing LCMV gp33-41 epitope,

DOI: 10.1002/eji.201970400

antigen specific memory CD8+ T cells were generated and compared between these two groups. In agreement with previous reports, we observed an overall enhancement of memory CD8+T cells, represented by the increase of memory precursor CD8+T cells in the blood, and the elevation of number and function of memory CD8+ T cells in the draining lymph node. More importantly, significant upregulation of tissue-resident memory CD8+ T cells was observed in rapamycin-treated group as compared to control group. These findings suggest that low-dose rapamycin treatment leads to increase of tissue resident memory CD8+ T cells through inhibition of mTOR signaling pathway and have important implications for the development of novel vaccine regimens.

P2721

A severe hemolytic disease of the newborn caused by Anti-Di^a and analysis of exon 19 of *SLC4A1* gene in the family

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Objective: Anti-Di^a is a rare but clinically significant red cell antibody which can cause hemolytic disease of the newborn (HDN). Here, we report a severe HDN caused by anti-Di^a antibody in a newborn, whose total bilirubin mounted to 16.9 mg/dL within 24 hours after birth. The mother had one prior parturition with no history of transfusion.
Methods: Direct Coomb's test was performed by routine serological method. Antibody in the serum of the newborn was identified by indirect antiglobulin test using panel cells. The titer of antibody was measured by doubling dilution. Genomic DNA was extracted from the peripheral blood by MagCore Nucleic Acid Extractor. Exon 19 of *SLC4A1* gene of the family were amplified by PCR and PCR products were sequenced by DNA analyzer.
Results: Direct Coomb's test on the neonate's red blood cells proved positive. Antibody identification indicated the existence of anti-Di^a antibody in the serum of the neonate. with the titer 128. Di^a is associated with 2561C>T in exon 19 of *SLC4A1* gene. Di^a represents Leu854 and Di^b Pro854 in band 3 protein. DNA sequencing of exon 19 of *SLC4A1* gene showed 2561C/T heterozygote (Di^a/Di^b) in both of the newborn's paternal grandparents, 2561T/T homozygote (Di^a/Di^a) in the newborn's father, 2561C/C homozygote (Di^b/Di^b) in the newborn's mother, 2561C/T heterozygote (Di^a/Di^b) in the newborn and her sister.
Conclusion: HDN may happen if pregnant women rapidly produce high-titer antibodies to the previously encountered antigens by the immunological memory.

P2722

Adjuvants alleviate vaccine-enhanced disease by regulate pulmonary immune memory induced by FI-RSV

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Respiratory syncytial virus (RSV) is the main pathogen of lower respiratory tract infection in infants and young children. However, no safe and effective vaccine is available due to the obstacle of formalin-inactivated RSV (FI-RSV) vaccine-enhanced disease. Our previous study found that FI-RSV immunization of mice with Notch signaling inhibitor L685,458 and the TLR9 ligand CpG as combined adjuvants can significantly reduce lung inflammation. The mechanism of the combined adjuvants regulating vaccine-enhanced diseases is not clear. Immune memory is the conceptual basis of vaccine development. Recently, tissue resident memory T cells (T_{RM}) and innate immune memory (trained

immunity) are found, which may play important roles in protection and inflammation induced by pathogens or vaccines. In this study, adult BalB/c mice were immunized with FI-RSV, FI-RSV+Al(OH)$_3$, or FI-RSV+L685,458+CpG, and lung T_{RM}, trained DCs, and trained macrophages were detected by flow cytometry, real time PCR, and ELISA. The lung inflammation in immunized mice following RSV challenge also was evaluated. The results showed that FI-RSV+L685,458+CpG induced higher level of lung T_{RM} , trained DCs, and trained macrophages than FI-RSV or FI-RSV+Al(OH)$_3$. Following RSV challenge, pulmonary inflammation in mice immunized by FI-RSV+L685,458+CpG was significantly suppressed, compared with those by FI-RSV or FI-RSV+Al(OH)$_3$. The results showed that the combined adjuvants promoted FI-RSV to induce pulmonary adaptive and innate immune memory, which may contribute to alleviate vaccine-enhanced diseases.

P2723

A Pck1-directed glycogen metabolic program regulates formation and maintenance of memory CD8+ T cells

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CD8+ memory T (Tm) cells are fundamental for protective immunity against infections and cancers 1-5. Metabolic activities are crucial in controlling memory T-cell homeostasis, but mechanisms linking metabolic signals to memory formation and survival remain elusive. Here we show that CD8+ Tm cells markedly upregulate cytosolic phosphoenolpyruvate carboxykinase (Pck1), the hub molecule regulating glycolysis, tricarboxylic acid cycle and gluconeogenesis, to increase glycogenesis via gluconeogenesis. The resultant glycogen is then channelled to glycogenolysis to generate glucose-6-phosphate and the subsequent pentose phosphate pathway (PPP) that generates abundant NADPH, ensuring high levels of reduced glutathione in Tm cells. Abrogation of Pck1-glycogen-PPP decreases GSH/GSSG ratios and increases levels of reactive oxygen species (ROS), leading to impairment of CD8+ Tm formation and maintenance. Importantly, this metabolic regulatory mechanism could be readily translated into more efficient T-cell immunotherapy in mouse tumour models.

Allergy

O169

Skin exposure to diisononyl phthalate aggravates allergic contact dermatitis by negative regulation of langerhans cells

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Diisononyl phthalate (DINP), a plasticizer in polyvinyl-chloride consumers, is an adjuvant in the development of allergic diseases. Langerhans cells (LCs), a subset of dendritic cells (DCs) in epithelia, are involved in the sensitization phase of allergic contact dermatitis (ACD). However, the effect of skin DINP exposure on ACD mice induced by 2,4-dinitrochlorobenzene (DNCB) needs to be determined

DOI: 10.1002/eji.201970400

and the role of LCs in DINP-related ACD remains unclear. We established DINP-exposed ACD mouse model, and focused on C57BL/6-background mice to analyze the role of LCs in ACD using huLangerin-DTA mice deficient in epidermal LCs. We found DINP aggravated ACD in both C57BL/6 and BALB/c mice. This finding was paralleled by ear thickening, eosinophilic inflammation, mast cell degranulation, expressions of TSLP and immunological cytokines in serum, ear tissue, and submaxillary lymph nodes (SMLN). We found for the first time that DINP enhanced FITC uptake by LCs in ear epidermis, and promoted DC differentiation by elevation of surface molecules for DC, including CD86, CD80, PD-L1 and PD-L2. Interestingly, ablation of LCs promoted the enhancement effect of DINP on ACD symptoms and on DNCB-induced proliferation of Th17 cells in SMLN. **In conclusion, DINP activates epidermal LCs, promotes LCs migration to SMLN, induces DC differentiation and Th17 responses, subsequently stimulates immunological cytokines, thus promotes ACD development. LCs inhibit DINP-related ACD symptoms mainly by suppressing Th17 responses, therefore present a novel treatment for DINP-related ACD.**

This work was supported by grants (2015CB943203, NSFC21507099, NSFC81401328, ZR2015PB011, ZR2013HL064, 2017WS426, 2018WS136).

O170

NLRP3 inflammasome-mediated bronchial epithelial cell pyroptosis contributes to airway inflammation in toluene diisocyanate-induced asthmatic mice

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Background: Toluene diisocyanate(TDI), the most common organic compound causing occupational asthma, has been proven to drive bronchial epithelium damage and IL-1β production. NLRP3 inflammasome-mediated pyroptosis is characterized by cell explosion and IL-1β release. In this study, we investigated whether NLRP3 inflammasome-mediated pyroptosis was involved in bronchial epithelial cell injury and airway inflammation in TDI-induced asthma.
Method: In vitro, human bronchial epithelial cells were stimulated with TDI. The cell viability and the levels of LDH and IL-1β were detected. In vivo, male BALF/c mice were sensitized and challenged with TDI to establish asthma models. MCC950, the specific inhibitor of NLRP3 inflammasome, was administered. The airway hyperresponsiveness(AHR) and airway inflammation in mice were assessed.
Results: We found TDI induced bronchial epithelial cell pyroptosis, as evidenced by the increased ratio of caspase-1$^+$PI$^+$ cells, as well as the enhanced levels of LDH and IL-1β. Importantly, MCC950 significantly blocked TDI-induced pyroptosis. In TDI-induced asthmatic mice, administration with MCC950 attenuated AHR and infiltration of airway inflammatory cells by inhibiting the activation of caspase-1, the expression and cleavage of pyroptotic executioner GSDMD in the lungs. Moreover, MCC950 decreased the levels of IL-1β in the BALF and plasma, accompanied by lower concentrations of IgE in the plasma and Th2-related cytokines in the BALF.
Conclusion: Our data suggest that bronchial epithelial pyroptosis plays a critical role in the pathogenesis of TDI-induced asthma via the activation of NLRP3 inflammasome, the expression and cleavage of GSDMD. Therefore, inhibition of NLRP3 inflammasome-mediated bronchial epithelial pyroptosis may be a therapy for TDI-induced asthma.

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DOI: 10.1002/eji.201970400

O171

Expression and characterization of recombinant Par j 1 and Par j 2 resembling the allergenic epitopes of *Parietaria judaica* pollen

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The weed wall pellitory, *Parietaria judaica,* is one the most important pollen allergen sources in the Mediterranean area causing severe symptoms of hay fever and asthma in allergic patients. We report the expression of the major *Parietaria* allergens, Par j 1 and Par j 2 which belong to the family of lipid transfer proteins, in insect cells. According to circular dichroism analysis and gel filtration, the purified allergens represented folded and monomeric proteins. Insect cell-expressed, folded Par j 2 exhibited higher IgE binding capacity and more than 100-fold higher allergenic activity than unfolded *Escherichia coli*-expressed Par j 2 as demonstrated by IgE ELISA and basophil activation testing. IgE ELISA inhibition assays show that Par j 1 and Par j 2, although resembling significant sequence identity, contain genuine and cross-reactive IgE epitopes. IgG antibodies induced by immunization with Par j 2 inhibited binding of allergic patients IgE to Par j 1 only partially. IgE inhibition experiments demonstrated that insect cell-expressed Par j 1 and Par j 2 together resembled the majority of allergenic epitopes of the *Parietaria* allergome. Therefore both molecules are necessary for molecular diagnosis and the design of vaccines for allergen-specific immunotherapy of *Parietaria* allergy

O172

Deficiency of Leukocyte-specific protein 1 (LSP1) alleviates asthma in a mouse model

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The mechanisms of asthma remain completely understood. Building on the role of LSP1 in neutrophil migration and acute lung inflammation, we used LSP1-deficient (*Lsp1*$^{-/-}$) and wild-type (WT) 129/SvJmice to test the hypothesis that LSP-1 deficiency would inhibit airway inflammation in ovalbumin (OVA) induced asthma. Immunohistochemistry and Western blot showed increased expression of LSP-1 in lungs of asthmatic mice compared to the normal mice. LSP-1 was localized in endothelium, airway epithelium, neutrophils, eosinophils, lymphocytes, and alveolar macrophages. While peripheral blood leukocyte numbers were not different between the normal and asthmatic mice as well as between WT and *Lsp1*$^{-/-}$mice, WT asthma mice had higher numbers of leukocytes in broncho-alveolar lavage, highermyeloperoxidase, and eosinophil peroxidase levels in the lungs ($P< 0.05$) compared to *Lsp1*$^{-/-}$asthma mice. The OVA-specific IgE levels in the serum of WT asthma mice was higher than that of *Lsp1*$^{-/-}$asthma mice ($P< 0.05$). The absence of the LSP1 gene reduced the levels of IL-4, IL-5, IL-6, IL-13, and KC ($P< 0.05$) but not the levels of IL-17, eotaxin-1, and total protein in broncho-alveolar lavage fluid. The physiological airway hyper-responsiveness to methacholine in *Lsp1*$^{-/-}$asthma mice reduced compared to WT asthma mice ($P< 0.05$). Histopathology showed more recruited inflammatory cells, as well as airway and blood vessel wall thickening, in the lungs of WT asthma mice than in those of *Lsp1*$^{-/-}$asthma mice. These data show that LSP1 promotes airway

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DOI: 10.1002/eji.201970400

inflammation associated with asthma in this mouse model and may serve as a therapeutic target in asthma.

O173

Thermal treatment altered allergenicity of peanut allergens via destructing their conformational structure

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Peanut allergy is considered as one of the most widespread and life-threatening type of food allergies, which usually does not vanish with age. Thermal processing is carried out to improve sensorial quality of peanut protein, which might also change its structure and allergenicity. In this research, the effects of several thermal processing on peanut allergens were investigated using extractable protein and digested defatted peanut powder from processed peanuts. The aim was to explore the mechanism that thermal processing affected the sensitization of peanut allergens. The tryptic digested peptides were analyzed with iTRAQ technology to find their change after roasted processing. Forty-one peptides involving fragments of IgE binding epitopes were found changed significantly in amount. The peptides with dramatically changed abundance were pinpointed on predicted spatial structure model to find that owing to the destruction of disulfide bonds or α-helices, protein presented a certain degree of stretched structure. These results showed that the exposure/coverage of cleavage sites induced by thermal processing would lead to further allergenicity change. When the advanced structure destroyed, the reaction sites of digestive enzymes exposed or masked. Then epitopes of the allergens would or would not be destroyed, which altered the allergenicity of peanut. In addition, the secondary and tertiary structure determined by CD and UV confirmed the structure change of the allergens, which matched their predicted structures.
Keywords: peanut, thermal treatment, structure, allergenicity

O174

WHO/IUIS allergen nomenclature submissions: characterization of allergens and IgE responses for official allergen names

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Official names for allergenic proteins causing reactions upon ingestion, inhalation, contact or injection are assigned by the WHO/IUIS Allergen Nomenclature Sub-Committee (www.allergen.org). Established in 1986, the database has 927 proteins. Allergen names consist of the first three or four letters from the genus and one or two letters from the species epithet, followed by an Arabic numeral (e.g. Ara h 2 for *Arachis hypogaea* allergen 2).
Process: Before publication, investigators are required to submit information of the source organism, amino acid and nucleotide (if known) sequences, characteristics of the protein, the clinically allergic population and exposure route. Scientific journals often require an official allergen name for the protein described in relevant studies. Allergen experts review the submission and may ask questions before providing a name within 2 to 4 weeks.

DOI: 10.1002/eji.201970400

Clinical subjects: Appropriately characterized relevant allergic subjects (de-identified) need to be included listing symptoms upon exposure. Information on clinical diagnostic methods (skin test, IgE values to source extracts, food challenges) are requested.
Protein characteristics: Purified natural and/or recombinant proteins may be submitted. Methods of amino acid sequence determination should be reported. Information about glycosylation and native or denaturation status with accepted database accession numbers should be included in the submission.
IgE test methods: Pure protein and extracts have to be tested for IgE binding (ELISA, RAST, immunoblotting). Inhibition assays may be used to demonstrate specificity. Biological activity tests are encouraged (basophil activation, skin prick tests). Negative control proteins should be included. Confidential example blots and ELISA appendices are helpful.

O175

A unique role for IgM isotype in regulating airway hyperresponsiveness during allergic asthma

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Allergic asthma is a disease driven by T helper 2 (TH2)-type cells secreting interleukin (IL-) 4, 5 and 13. It is characterised by eosinophils, airway hyperesponsiveness (AHR) and IgE secreting B cells. B cells play a role in allergic asthma in an allergen load dependent manner. IgM isotype secreted by naïve B cells is important for class switching. It is currently unclear how IgM isotype contributes in the development of allergic asthma. We investigated the role of IgM isotype in a house dust mite (HDM)-induced TH2 allergic asthma model. We sensitised wild type (wt), IgM-deficient (IgM-/-) and B cell-deficient (uMT-/-) mice with high (>10 ug) and low (< 3 ug) dose of HDM extracts. We found IgM to be essential in antibody class switching and in AHR, but not in TH2 airway inflammation or eosinophilia. IgM regulated AHR through modulating muscarinic receptor genes independent of microbiota. Transfer of wild type serum or B cells into IgM-/- mice could restore AHR and transfer of IgM-/- B cells into uMT-/- could reduce AHR. These unprecedented findings suggest that IgM has a unique function in allergic asthma and regulates AHR by interacting with non-immune genes.

O176

TrimX reprograms CD11b$^+$ DCs to promote Th2-dominant eosinophilic asthma

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Allergic asthma is a common immune disorder associated disease caused by inhaled antigens and is refractory clinically. Dendritic cells (DCs) are the major antigen-presenting cells in asthma and mediates the subsequent adaptive immune responses. TrimX, a member of the tripartite motif (TRIM)-containing protein family, is a multipotent protein and involved in different biological processes, especially in regulating gene expression. TrimX was reported to regulate the development of hematopoietic cells. However, the role of TrimX in the development and function of DCs remains unclear. In this study, we found that DC-specific deletion of TrimX (CD11c-creTrimX$^{fl/fl}$ mice) affects the homeostasis of both CD103$^+$ DCs and CD11b$^+$ DCs in lung. Furthermore, working in a mouse

DOI: 10.1002/eji.201970400

model of inflammation, we found that TrimX deletion in DCs (CD11c-creTrimX$^{fl/fl}$ mice) enhanced the Th2-donimant eosinophilic airway inflammation in HDM-induced asthma. Concurrently, more CD11b^{+} DCs migration was observed both in bronchoalveolar lavage fluid (BALF) and mediastinal lymph nodes (MLN) of CD11c-creTrimX$^{fl/fl}$ mice. We excluded the contribution of TrimX deficient AMs and CD103^{+} DCs in promoting HDM-induced asthma by using Lyz2-creTrimX$^{fl/fl}$ mice and Batf3$^{-/-}$ mice. Moreover, we found that acute antigen challenge efficiently induced more TrimX-deficient CD11b^{+} DCs migration in lung and MLN. TrimX-deficient CD11b^{+} DCs exhibited enhanced activation states both in vivo and in vitro compared with TrimX-sufficient DCs. We also found that TrimX-deficient CD11b^{+} DCs could mediate much stronger antigen-induced Th2 responses both in vivo and in vitro. Our study suggests that TrimX in CD11b^{+} DCs is a crucial component to promote Th2 immunity at barrier surface.

LB014

Treatment with benign IgE reduces asthma symptomology in a mast cell dependent manner

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Notoriously known for its involvement in type I hypersensitivities, Antigen (Ag)-specific IgE mediates a variety of mild to severe responses, including asthma. Ag-specific IgE is additionally important for immunity against gastrointestinal helminths, but these helminths also elicit production of low-affinity, poly-specific IgE. Helminth infection has been shown by several groups to be protective against asthma. The mechanisms behind these findings have included the induction of regulatory pathways, but the role of poly-specific or “benign” IgE has not been fully assessed. To test this in the absence of helminth infection, we utilized a four week model of murine asthma and injected IgE anti-DNP as our “benign” IgE twice a week. We found that treatment significantly reverses the eosinophil accumulation, airway hyperresponsiveness, and lung pathology seen with this model. Further analysis shows that the “benign” IgE treatment is only needed during the antigen challenge phase and not during sensitization phase. Using in vitro assays we were able to show that non-specific IgE can block Ag-specific IgE degranulation even after Ag-specific IgE pre-incubation. Overall, these data demonstrate a novel avenue for blockage of mast cell degranulation and a potential regulatory role for IgE.

LB051

Evaluation of chitinase-like genes expression in A549 cell line and mixed leukocyte culture treated with chitinous microparticles

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Chitinous microparticles (MPs) reduce allergic inflammation. Because of some contradictory data in this field, we examined the capacity of these MPs to stimulate A549 human airway epithelial cell and mixed leukocyte culture in order to determine the feasibility of using these glycans as an allergy therapeutic modality. Chitin and chitosan MPs were prepared by sonication and their toxicity were assessed by Neutral red uptake assay. Following cell treatment with MPs (100 µg/ml) for 48h, IL-6 and nitrite concentration in the culture supernatants were measured by ELISA and Griess assay, respectively. Then expression levels of chitinase domain-containing 1 (CHID1) and chitinase 3-like 1 (CHI3L1) genes, encoding chitinase-like proteins involving in chronic inflammation and allergic disease, were determined by Quantitative Real Time PCR. Chitin MPs resulted in up-regulation of CHI3L1 expression by 35.7-fold while chitosan MPs didn't change it significantly. Compared to the

DOI: 10.1002/eji.201970400

untreated group, production of IL-6 was significantly decreased in the chitosan MPs-treated group but chitin MPs treatment cause elevation of IL-6 level. Treating Mixed Leukocyte Culture with chitin MPs cause down regulation of CHID1 gene expression (P=0.004). This study demonstrates chitin potently changes chitinase-like genes expression but chitosan is relatively inert. This effect and inhibition of IL-6 suggest that chitosan MPs may possess more potential for therapeutic uses in allergic conditions. However, due to chitin MPs effect on mixed leukocyte culture, more studies needed on a better formulation of chitinous MPs to be exploit in treatment of human airway allergic inflammation.

LB088

CD28 signal blockade as a treatment for steroid resistant asthma

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Background: To control therapy-resistant eosinophilia, synergistic effects of CTLA4-Ig and glucocorticoid was investigated on T cell-induced asthma model.
Methods: Ovalbumin (OVA) specific murine helper T cell (Th) clones were established from splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for OVA/H-2^d. To analyze steroid responsiveness *in vitro*, Th clones were cultured with antigen presenting cells and OVA in the presence of various concentration of dexamethasone (DEX). Proliferative responses were measured by incorporation of either ^{3}H-thymidine or BrdU. For *in vivo* analysis, unprimed Balb/c mice were transferred with Th clones, challenged with OVA, and administered with DEX subcutaneously. CTLA4-Ig was administered either intravenously or intranasally. Bronchoalveolar lavage fluid (BALF) was obtained 48 hours after the challenge, and the number of infiltrating cells was differentially counted.
Results: Steroid-sensitive (SS) and -resistant (SR) clones were selected based on the effect of DEX on the proliferative responses of antigen-stimulated Th clones. Airway infiltration of eosinophils of mice transferred with SS clones were effectively inhibited by the administration of DEX. In contrast, those of mice transferred with SR clones were not significantly inhibited by DEX. Addition of CTLA4-Ig into the culture significantly suppressed the proliferation of DEX-treated SR clones *in vitro*. Administration of CTLA4-Ig significantly suppressed eosinophil infiltration of SR asthma model transferred with SR clones *in vivo*. CTLA4-Ig and DEX synergistically suppressed *in vitro* proliferation of SS clones and *in vivo* BALF eosinophilia of mice transferred with SS clones.
Conclusion: Blocking costimulatory signal mediated is a promising target to treat therapy-resistant eosinophilia.

P0210

Synchrotron radiation X-ray fluorescence analysis for novel insights into nickel-induced allergic contact dermatitis

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Nickel-induced allergic contact dermatitis is a global health problem. More detailed knowledge on the skin uptake of haptens is required.
Forty Hartley guinea pigs were divided into four groups according to the NiSO4 sensitizing

DOI: 10.1002/eji.201970400

concentration and it challenged concentration: the 5% NiSO4-group, 5%-10% (sensitization-challenge; chronic group); 10% NiSO4-group, 10%-10% ; and the positive and negative controls. The depth profile of nickel element concentration in the skin of guinea pigs was detected by synchrotron radiation micro X-ray fluorescence spectroscopy and micro X-ray absorption near edge spectroscopy.
In each section, the nickel element concentration in both the 5%and 10% NiSO4-group was higher than that in the negative control group. In the upper 300 µm section of skin for the acute group, the nickel elementconcentration was significantly higher than that in the lower section of skin. In deeper sections (>200 µm) of skin, the concentration of nickel in the acute group was approximately equal to that in the chronic group. Comparing the XANES data for the 10% NiSO4 metal salt solution, which indicates that nickel was not present in the Ni2+ ionic state but in the nickel-binding protein.
This study showed that the distribution of the nickel element concentration in ACD skin tissue was different between the acute and chronic groups. The nickel element was bound with certain proteins to form a complex in the stratum corneum.This is the first demonstration of nickel depth distribution and binding events for nickel contact allergy in vivo through SR-XRF and XANES technology.

P0211

Changes of T cell subsets and cytokines in patients with severe asthma

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Background: Asthma is a chronic airway inflammatory disease involving immune cells and cytokines, the roles of which are unclear in severe asthma (NA).
Objective: We performed a comprehensive assessment of Th1 cells, Th2 cells, Tregs, Th17 cells, MAIT cells and TNF-α, IL-1α, IL-1β, IL-4, IL-6, IL-8, IFN-γ, G-CSF, GM-CSF, IL-17A and IL-17F in severe neutrophilic asthma patients.
Methods: A total of 13 healthy individuals and 27 severe asthma patients were enrolled in this study. Refractory asthma patients were defined as those with eosinophilic asthma (EA, 22%) or NA (88%). T-cell subsets in PBMCs were analyzed by flow cytometry, and cytokines were detected by Cytometric Bead Array (CBA).
Results: Significant differences were observed in T-cell phenotypes, where the number of Th1 cells were reduced and the numbers of Th2 cells were increased in NA and EA groups, respectively, when compared with healthy controls. Th17 cells were not strongly associated with severe asthma. The frequencies of MAIT cells were strikingly reduced in severe asthma patients, especially in the NA group. This group also showed increased levels of IL-17A, IL-17F, TNF-α and IL-6 in serum and increased levels of IL-17A, IL-17F, IFN-γ, TNF-α, IL-1β, IL-6 and IL-8 in sputum. In addition, sputum IL-6 was positively correlated with TNF-α, IFN-γ, IL-17A and IL-8.
Conclusions: Our results uncovered that severe neutrophilic asthma was associated with a striking deficiency of MAIT cells and high pro-inflammatory cytokine levels of TNF-α, IL-6, IL-17A and IL-17F

P0212

CX3CR1-deficiency alleviates skin inflammation by blocking Ly6C^{hi} monocytes migration and Langerhans cell local repopulation

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DOI: 10.1002/eji.201970400

Conventional Langerhans cells (LCs) in epidermis play a critical role in maintaining skin homeostasis, Ly6C^{hi} monocyte-derived LCs (Mo-LCs) could be repopulated to inflamed skin and involved in inflammatory response. However, how to regulate Ly6C^{hi} Mo-LCs repopulation remains unclear. CX3CR1 is expressed in a variety of immune cells including monocytes and contributes to monocyte migration. Here we used CX3CR1 knockout (KO) mice in which *Cx3cr1* gene was replaced by the *EGFP* reporter gene, to investigate the role of CX3CR1 in Ly6C^{hi} monocyte migration and Mo-LCs regeneration. We found that Ly6C^{hi} Mo-LCs highly express MHCII molecule and secrete more proinflammatory factors TNFα and IL-1β, compared to conventional LCs, and in the DNFB-induced contact hypersensitivity (CHS) and UV irradiation-induced dermatitis, the number of Ly6C^{hi} Mo-LCs in the epidermis was significantly decreased in *Cx3cr*1KO mice compared to wild-type (WT) mice; furthermore, the inflammation were alleviated in *Cx3cr1*KO mice. Interestingly, the number of Ly6C^{hi} monocytes was increased in periphery blood and spleen in CX3CR1KO mice under inflammatory conditions, suggesting a potential defect in Ly6C^{hi} monocyte migration to skin. To further test this possibility, we co-transferred e450-labelled monocytes from WT mice with CFSE-labelled monocytes from *Cx3cr1KO mice* to the CD45.1 recipient mice challenged with DNFB, and found that more Ly6C^{hi} monocytes from WT mice were accumulated in the epidermis compared to that of *Cx3cr1*-deificient monocytes. Overall, our data suggest that CX3CR1-deficiency reduces Ly6C^{hi} monocyte migration ability to inflamed skin and decreased Ly6C^{hi} Mo-LCs local repopulation, which could contribute to reduced DNFB-induced CHS and UV-induced dermatitis.

P0213

IL-16 aggravates OVA-induced allergic inflammation by enhancing Th2 and Th17 cytokine production in a mouse model

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Asthma is a chronic inflammatory disease that involves a variety of cytokines and cells. Interleukin (IL)-16 is highly expressed during allergic airway inflammation. However, its specific mechanism of action remains unclear. In the present study, utilizing an animal model of ovalbumin (OVA)-induced allergic asthma with mice harboring an IL-16 gene deletion (KO), we investigated the role of IL-16 and its underlying mechanism in asthma. Increased IL-16 expression was observed during OVA-induced asthma in C57BL/6 mice. However, in IL-16 KO asthma mice, a diminished inflammatory reaction, the suppression of OVA-specific IgE levels in the serum and BALF, and decreased levels of Th2 and Th17 were observed. Our findings inferred that IL-16 promotes the allergic inflammatory reaction of the lungs, and suggest the mechanism probable be related to the up-regulation of IgE and the promotion of the production of Th2 and Th17 cytokines. This work explored the mechanism underlying the regulation of IL-16 in asthma and provides a new target for the clinical treatment of asthma.

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DOI: 10.1002/eji.201970400

P0214

Calcineurin A beta contributes to IgE-mediated mast cell activation

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Background: Mast cells are well positioned in host tissue for detecting environmental signals, including allergens, leading to activation of the high affinity IgE receptor FceRI, and initiating a signaling cascade that perpetuates the production of biologically potent mediators. Here, we have investigated the role of calcineurin A beta (CnAb) in mast cell IgE signaling-dependent functions by using bone marrow-derived mast cells (BMMCs) from CnAb-deficient mice.
Methods: BMMCs were harvested from the femurs and tibias of wild-type and CnAb-deficient mice, and were cultured in completed medium supplemental 2-ME and PGE_2. Western blotting, ELISA, EMSA were employed to examine MAPKs, cytokines, and transcription factors, respectively.
Results: By IgE activation, CnAb-deficient BMMCs showed significantly decreased protein phosphorylation of JNK kinase, p38 kinase, and IkB, and decreased transcriptional activity of NF-kB, NFAT, and AP1, resulting in suppressed TNF, LI6, IL13, and CCL1 production. Interestingly, the chemokine CCL2 and CCL3 showed enhanced production in CnAb-deficient BMMCs.
Conclusions: Our findings position CnAb as a critical regulator of IgE-CnAb-MAPKs-NFAT/AP1-cytokines and IgE- CnAb-IkB-NFkB-cytokines signal pathway in mast cell. Thus, CnAb is identified as a novel regulator in IgE-mediated mast cell activation and could serve as a potential therapeutic target for the management of allergic inflammation.

P0215

Novel almond allergens

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Almonds are one of the most likely tree nuts to trigger allergic reactions in the US. However, compared to peanut allergens, few almond allergens have been defined by WHO/IUIS allergen nomenclature subcommittee. Nevertheless, eight almond proteins have been known to be allergens or suspected of being allergens. One of these suspected allergens was believed to be the 2S albumin but recently debated to be the 7S vicilin instead. Two partial peptide sequences (with the uncertain residues likely to be cysteines) of the debated allergen matched 100% to those in the translation of a partial almond mRNA in the GenBank that is homologous to the N-terminal leader peptides of the 7S vicilin from some species. To uncover the identity of this allergen, we determined the complete sequence of the above mRNA and isolated the gene sequence of the almond vicilin. Western blot data with 18 sera of patients with clinically diagnosed almond allergies showed that almond vicilin is a food allergen. However, it is not the allergen with debated identity. The complete sequence of the latter was deduced from the isolated mRNA, and it was confirmed to be a food allergen although it is neither the 2S albumin nor the 7S vicilin. Rather, it belongs to a novel class of food allergens with antimicrobial activity. Further studies of these and additional almond allergens to be identified would enable a better understanding of the allergenicity of almond proteins.

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DOI: 10.1002/eji.201970400

P0216

The anti-inflammatory activity of regulatory cytokine IL-37 in atopic dermatitis

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Objective: The anti-inflammatory activity of IL-1 family regulatory cytokine IL-37 in atopic dermatitis (AD) was investigated.
Methods: Human eosinophils (EOS) and dermal fibroblast (HDF) co-culture was used to investigate inflammatory cytokine/chemokine secretion, while the autophagy signaling proteins were analysed by Western blot. CRISPR/Cas9 human IL37 knock-in mice were bred for MC903-induced AD model in vivo.
Results: IL-37 significantly inhibited IL-6 and CXCL8 production in co-culture and autophagy related protein expression ($p< 0.05$). No autophagy process was detected in either EOS or HDF alone culture, thereby suggesting interaction between EOS and HDF is essential for the autophagy mechanism in the resolution of allergic inflammation. Ear swelling was reduced 10.79 and 15.32% in IL-37 knock-in mice on Day 15 and 17 comparing with wild type mice, respectively. The EOS number in ear of IL-37 knock-in group was much lower than that of control group. Importantly, IL-37 significantly ameliorated the itching state, which is an intolerable AD clinical feature, with the change in gut metabolites. Adoptive transfer of T cells from IL-37 knock-in mice can reduce the AD-related inflammation in recipient mice. AD-related cytokines and chemokines expression in serum and ear tissue was much lower, while Treg cells in spleen was much higher in IL-37 knock-in group comparing with control group, thereby suggesting IL-37 can reduce allergic inflammation by increasing immunosuppressive Treg cells in vivo.
Conclusion: IL-37 could significantly ameliorate MC903-induced AD symptom by inhibiting eosinophils infiltration and allergic inflammation. It suggests that IL-37 can be a promising anti-inflammatory cytokine for AD treatment.

P0217

Bet v 1-specific adaptive immune responses in mice and man

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More than 100 million patients worldwide are allergic to the major birch pollen allergen, Bet v 1. These patients suffer from respiratory allergy to pollen from birch and related trees as well as from oral allergy syndrome caused by plant food due to IgE and T cell cross-reactivity. Since mouse models are frequently used as mimics of human disease we analyzed the adaptive immune response in BALB/c mice which were sensitized classically with Aluminum-hydroxide-adsorbed Bet v 1. Such as allergic patients, sensitized mice developed cross-reactive IgE antibodies to related pollen and food allergens and showed immediate allergic reactions as demonstrated by basophil degranulation. However, IgE antibodies of sensitized mice were directed against C-terminal sequential peptide epitopes whereas allergic patients IgE recognized only intact folded allergen. In mice IgG recognized the same sequential epitopes as IgE whereas patients IgG was also directed against sequential epitopes. The mapping of T cell epitopes using CFSE-dilution and six peptides spanning the Bet v 1 sequence

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DOI: 10.1002/eji.201970400

indicated the presence of T cell epitopes at the Bet v 1 C-terminus but also to other peptides as in allergic patients. Our results thus demonstrate profound differences regarding the antibody recognition of Bet v 1 in sensitized mice and allergic patients. Thus it is not possible to directly compare mice and patients regarding mechanisms of class-switching and antibody-mediated inflammation or treatment approaches whereas the similarities regarding T cell recognition may allow studying general mechanisms of T cell-mediated tolerance in mice.

P0218

Involvement of airway epithelial cells in dendritic cell activation leading to female-predominant Th2 cytokine production in allergic asthma

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The prevalence and severity of asthma after puberty are greater in female patients than in male patients. These disparities between the sexes are mainly due to enhanced Th2 cytokine production in female patients, yet the sex-dependent mechanisms leading to this enhanced allergic immune response remain unclear. Previously, using a mouse model of asthma, we showed that female-predominant Th2 cytokine production in asthma is attributable to enhanced CD86 expression on allergen-primed CD103$^+$dendritic cells (DCs). In the present study, we further examined the sex-related influence of airway epithelial cells, regulators of airway inflammation, on lung DC activation using an ovalbumin (OVA)-induced asthma mouse model. Compared with male mice, female mice exhibited larger numbers of eosinophils and lymphocytes in broncho-alveolar lavage (BAL) fluid. BAL fluid from female mice after OVA inhalation triggered significantly enhanced *Cd86*expression, but not *Ccr7*or *H2-Ab1*expression, in both CD11c$^+$cells from the spleens of naïve mice and bone-marrow derived DCs. Airway epithelial cells (CD31$^-$CD45$^-$Ep-CAM$^+$cells) from asthmatic female mice expressed higher levels of *Il-33*and *Ccl2*at 1 and 4 h after OVA inhalation, respectively, compared with those in male mice. In the lung, IL-33 production 16 h post-OVA inhalation was significantly higher in females than in males. Furthermore, in *in vitro*experiments, recombinant IL-33 significantly enhanced CD86 expression on CD103$^+$DCs. These results suggest that the enhanced IL-33 production from airway epithelial cells in female mice promotes the enhanced CD103$^+$DC activation, which may contribute to female-predominant Th2 cytokine production in asthma.

P0219

Sex-based difference in CD86 expression on CD103$^+$ dendritic cells promotes female-predominant Th2 cytokine production during allergic asthma

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The asthma severity is typically greater in female patients than in male patients, and larger numbers of IL-13-producing T cells are observed in peripheral blood from female patients. The mechanisms

DOI: 10.1002/eji.201970400

driving this immunological difference between the sexes remain unclear. In the present study, we aimed to clarify the role of two major subsets of dendritic cells (DCs), those displaying CD11b or CD103, in enhanced Th2 cytokine production upon allergen inhalation using an ovalbumin (OVA)-induced asthma mouse model. The numbers of CD11b^{high} DCs and CD103^{+} DCs in the lungs and bronchial lymph nodes (BLNs) at 16 to 20 hours after allergen exposure, as well as the CD86 expression and antigen uptake ability on CD103^{+} DCs, but not CD11b^{high} DCs in the BLNs, were greater in female mice than in male mice. In keeping with the larger numbers of DCs in the lungs, CCL20 production was also greater in the lungs of female mice compared to male mice. In an *ex vivo* experiment, OVA peptide-specific CD4^{+} T cells cultured with CD103^{+} DCs from female mice produced higher levels of Th2 cytokines, compared with CD4^{+} T cells cultured with CD103^{+} DCs from male mice. In *in vitro* experiments, 17β-estradiol-primed CD103^{+} bone marrow-derived DCs enhanced IL-5 production from CD4^{+} T cells in the presence of OVA, but this enhancement was completely abolished in the presence of anti-CD86 antibody. These results suggest that 17β-estradiol-oriented enhancement of CD86 expression on CD103^{+} DCs after allergen exposure promotes female-predominant Th2 cytokine production during asthma.

P0220

The course of allergic pathology depending on the exposure of cat allergens

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Background: High exposure of cat´s allergens can have both positive and negative effects on the course of allergic diseases. The mechanisms of this impact remain unclear.
The aim - to assess the impact of a high concentration of cat allergens on the peculiarities of the course of allergic pathology in children.
Patients and Methods: The study was conducted 228 children with allergic rhinitis, the average age 8.2 ± 1.9 years. The patients were divided into 2 groups: 1 group (110 children) - patients with cat in the house; Group 2 (118 children) - without pets. Specific IgE antibodies (sIgE-aB) were determined on ImmunoCap® (Thermo Fisher Scientific, Sweden) with common allergenic extracts.
Results: Bronchial Asthma (BA) was 2 times more frequently observed in children of the 1st group, Atopic Dermatitis - in the 2nd (OR 2.93, 95% CI 1.71, 5.03). In group 1, mild forms of allergopathology prevailed ($p < 0.01$), control over the course of BA was higher ($p < 0.01$).
sIgE-aB to cat were detected in all patients. The average level of sIgE-aB to rFel d 1 in group 1 was 1.6 times lower ($p < 0.05$). The level of sIgE-aB to nFel d 2 was significantly higher in patients of group 1 ($p < 0.05$)
Conclusion: The risk of asthma development is higher in children living in high cat allergens exposure. Polyvalent sensitization, sensitization to lipocalin, high concentration of sIgE-aB to uteroglobin is more frequent in children living in low allergen exposure.

P0221

Immunological and structural characterization of a new allergen from *Ascaris lumbricoides*

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Background: To analyze the impact of *Ascaris* infection on the pathogenesis of allergic diseases, it is necessary to identify its allergens. We aimed to evaluate the physicochemical and immunological features of Al16, an *A. lumbricoides* member of the SXP/RAL-2 protein family.

DOI: 10.1002/eji.201970400

Methods: Recombinant Al16 (rAl16), expressed in *E. coli,* was purified by Ni-NTA and size exclusion chromatography. Physicochemical characterization included circular dichroism and Fourier transform infrared spectroscopy. Divalent cation binding was performed by ANS probe assay. Evaluation of allergenic activity included IgE binding (ELISA) in three populations: 256 adult asthmatics (ASA), 254 children from FRAAT cohort and 298 all-age subjects from the rural Santa Catalina (SC). Skin prick tests (SPT) and CD203 based-basophil activation tests (BAT) were also done in 12 Ascaris sensitized subject. Passive cutaneous anaphylaxis (PCA) was performed in mice. Cross-reactivity with the house dust mites (HDM) *Blomia tropicalis* and *Dermatophagoides pteronyssinus* was evaluated by Western blot. Al16 identification in *A. lumbricoides* and HDM extracts was analyzed by liquid chromatography-mass spectrometry (LC-MS).
Results: rAl16 behaves as a monomer with an alpha helical folding. Its Ca^{2+} and Mg^{2+} binding was confirmed. Frequencies of sensitization were 31.9, 44.9 and 52.0% in ASA, FRAAT and SC, respectively. In SC Ascaris+ subjects, positive SPT was 50%. Positive BAT rate was 25%. rAl16 induced specific IgE production in sensitized mice and a positive PCA reaction. Al16 had not cross-reactive counterpart in HDM extracts.
Conclusions: Al16, a divalent cation binding protein, is a new allergen from *A. lumbricoides* that has no cross-reactivity with HDM.

P0795

Tetrahydrocurcumin ameliorates allergic airway inflammation by attenuating Th2 responses and suppressing the IL4Ra-Jak1-STAT6 pathway in asthmatic mice

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Background: Curcumin (Cur), which is derived from *Curcuma* species, exhibits anti-inflammatory, antioxidant, and anticancer effects. Its therapeutic effect on asthma has been confirmed, but its clinical application is limited by its low bioavailability. Tetrahydrocurcumin (THC), the active metabolite of Cur, has multiple biological functions, similar to Cur, and it showed enhanced bioavailability in tissues and plasma. However, the effect of THC on asthma has not been reported.
Objective: The current study sought to investigate the efficacy of dietary THC on allergic asthma compared to that of Cur in an animal model.
Methods: The anti-inflammatory effects of Cur and THC were evaluated in an ovalbumin-induced asthmatic mouse model. The nasal symptoms, pathological alterations of lung tissues, oxidants and antioxidants, cytokine production, T cell subsets, and IL-4Rα/Jak1/STAT6 pathway activity were assessed.
Results: Both THC and Cur had beneficial effects on asthmatic mice with regard to the nasal symptoms, the pathological changes (eosinophils and mucus hyper-production), oxidative stress (malondialdehyde), cytokine production (IL-13), Th17 and cytotoxic T cell subsets, and Th2 signalling pathway (IL-4Rα/Jak1/STAT6 axis) activity. THC was more effective than Cur in suppressing tissue eosinophilia, mucus production, and IL-4Rα/Jak1/STAT6 pathway activity. Furthermore, only THC inhibited peripheral eosinophil levels, Th2 cytokines (IL-4 and IL-5), and Th2 cell subsets and enhanced an antioxidant enzyme (glutathione).
Conclusion: The results demonstrated for the first time that THC was superior to Cur in modulating allergic asthmatic phenotypes, especially attenuating the Th2 response. THC might be a potentially effective agent for asthma treatment.
Keywords: Tetrahydrocurcumin, asthma, airway inflammation

DOI: 10.1002/eji.201970400

P0796

Necrostatin-1 effectively ameliorate inflammation of neutrophilic asthma by inhibiting net formation

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Introduction: Neutrophilic asthma is corticosteroid-resistant, and increases the burden of global health care, but the pathogenesis is unclear. Recent studies have indicated that there are a large amount of neutrophil extracellular traps (NETs) in the airways of neutrophilic asthma, suggesting that NETs may play an important role in the pathogenesis of neutrophilic asthma. In this study we investigate the role of NETs in neutrophic asthma and study whether inhibiting NET formation has therapy value.
Methods and Results : First, in vitro we demonstrated that NETs could induce human airway epithelial cells damage. And in vivo study, we also found that NETs were increased in bronchoalveolar lavage fluid in a neutrophil-dominated asthma model, and the formation of a large number of NETs exacerbated neutrophil-dominated asthma. Second, we found that Necrostatin-1, a small-molecule drug, that inhibits NET formation, could alleviate airway inflammation of neutrophil-dominated asthma. Finally, we explored that the inhibition of NET formation by Nec-1 may be related to the induction of neutrophil apoptosis.
Conclution: These findings suggest that NETs are closely related to the pathogenesis of neutrophilic asthma and involved in the progression of neutrophilic asthma. Necrostatin-1 could resolves inflammation by inhibiting the formation of NETs, suggesting that targeting NET formation is one of the important valuable targets in the treatments for neutrophilic asthma.

P0797

Exosomes derived from mesenchymal stem cells alleviated ILC2-dominated allergic airway inflammaion

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Type 2 innate lymphoid cells (ILC2s) were reported to be involved in the pathogenesis of allergic diseases. The cost of allergic airway inflammation is a significant burden to the society, promoting us to look for better treatment. We previously reported the immunomodulation of mesenchymal stem cells (MSCs) on suppressing airway inflammation. Extracellular vesicles (EVs) or exosomes are one of the key secretory products of MSCs involved in their immunomodulation. In this study, we aimed to investigate the effects of exosomes from MSCs on allergic airway inflammation and the possible mechanisms.
We isolated exosomes from human induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) media, and determined their characteristics. We induced mouse ILC2 dominated airway inflammation model by intratracheal instillation of IL-33. Compared with the model group, the intravenous injection with MSC-derived exosomes (MSCexo) suppressed the level of IL-5/13 Th2 cytokines in bronchoalveolar lavage fluid (BALF) and reduced the proportion of ILC2s in lung. Additionally, we co-cultured the peripheral blood mononuclear cells from patients with allergic rhinitis with MSCexo, and MSCexo significantly inhibited the IL-5 production in response to IL-33. However, fibroblast-derived exosomes (Fbexo) did not exhibit the similar effects. We further identified that

DOI: 10.1002/eji.201970400

MSCexo had higher levels of miR-146a-5p compared to Fbexo. After inhibiting the expression of miR-146a-5p in MSCs, the inhibitory effect of exosomes on inflammation was reversed both in mouse model and in vitro.
Our data suggest that exosomes from MSCs suppress the ILC2 dominated allergic airway inflammation, which is mediated via transfer of miR-146a.

P0798

The upregulation of PRMT1 by proinflammatory cytokines play central roles in the persistence and development of asthma

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Asthma is a chronic airway inflammatory disease, which involves in multiple inflammatory and lung constitutive cells. Protein arginine methyltransferase 1 (PRMT1) is the main enzyme catalyzing the asymmetric dimethylation on protein arginine residues, which play important roles in signal transduction, gene expression regulation, DNA repairing and mRNA splicing. In asthmatic lung tissues, the expression of PRMT1 elevates significantly. Multiple proinflammatory cytokines improve the expression of PRMT1 elevates in airway epithelium and interstitial tissues gradually with the persistence of asthma. In the early stage of allergic airway inflammation, IL-4 upregulated PRMT1 expression by stat 6 pathway in lung epithelium. And TGF-β and PDGF-BB upregulates PRMT1 expression by MAD2/3→C/EBPβ pathway and ERK-STAT1 pathway respectively in fibroblast. At the same time, PDGF-BB upregulates PRMT1 expression by ERK-STAT1 pathway in airway smooth muscles. The elevation of PRMT1 improves lung epithelial cells produce eotaxin, the chemokine of eosinophiles, which enhance the airway inflammation. The elevation of PRMT1 in fibroblast upregulates the expression of COX2, collagen-1 A1 and fibronectin. In airway smooth muscles, the increase of PRMT1 expression improve airway smooth muscle proliferation and upregulates the expression of COX2, collagen-1 A1 and fibronectin. The effects of PRMT1 upregulation in fibroblast and airway smooth muscles promote airway remodeling. These results indicates that PRMT1 is the centre of regulatory effects of multiple proinflammatory cytokines, and the upregulation of PRMT1 induces various pathophysiological process in multiple cells, which means that PRMT1 play a central role in the persistence and development of asthma.

P0799

Treatment of low molecular weight extracts of bovine spleen regulates the level and activity of histaminase in serum from allergic rhinitis mouse model

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Allergic rhinitis is a non-infectious inflammatory disease of the nasal mucosa, which is mainly released by IgE-mediated mediators after exposure to allergens, and involves a variety of immunoreactive cells

DOI: 10.1002/eji.201970400

and their secreting inflammatory factors. Among the inflammatory factors, histamine, which can induce many allergic symptoms, is a very powerful factor in allergic rhinitis. Usually, histamine is inactivated very quickly by histaminase in tissue or in serum. Oral administration of low molecular weight extracts of Bovine spleen (LMWEBS) shows a highly efficacious in the treatment of allergic rhinitis patients during its 20 years' clinical practices. But the mechanism in details of this treatment is still in detecting. Here, we establish a mouse model of allergic rhinitis and then treated it with a low molecular weight extract of the spleen. The results showed that the allergic symptoms of the mice were significantly reduced after treatment of LMWEBS, the serum histaminase activity was significantly increased, 2.88 folds higher than those from mice without the treatment of LMWEBS ($P< 0.05$). Furthermore, we also found that the intestinal flora of the mice became obvious changes, anti-allergic probiotics contents increased significantly ($P < 0.01$), and significantly correlated with the activity level of serum histaminase. Thus, it suggests that LMWEBS may be efficient in allergic rhinitis treatment by affecting the intestinal flora and the elevating the activity of serum histaminase.

P0800

Mite immunotherapy with Alutard SQ 510 induces an incomplete protective antibody response

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Objective: To investigate the mechanisms of treatment with the house dust mite (HDM) extract-based vaccine Alutard SQ 510 by monitoring the development and epitope specificity of allergen-specific IgG antibodies in sera of patients.
Methods: Serum samples were obtained from HDM allergic patients (n=26) who received Alutard SQ 510 for one year at defined time points (baseline, 7 w, 15 w, 8 m and at 1 y). Sera were tested for IgE and IgG reactivity to 15 HDM allergens using ImmunoCAP ISAC chip technology. IgG subclasses levels to the major allergens and epitopes thereof were determined in sera by ELISA. huRBL assays were performed with the relevant HDM allergens after pre-incubation with the pre and post-treatment sera.
Results: Treatment with Alutard SQ 510 induced specific IgE and IgG against the major group 1 and 2 allergens and Der p 23, but not to the other important allergens such as Der p 5, Der p 7 and Der p 21. The treatment-induced IgG antibodies inhibited allergen-induced β-hexosaminidase release from huRBL cells only for group 1 and 2 HDM allergens, to some extent for Der p 23 but not for Der p 5, 7 and 21. HDM allergic patients with IgE-reactivity to Der p 5, 7, 21 and 23 showed only little improvement of symptoms.
Conclusion: The house dust mite (HDM) extract-based vaccine Alutard SQ 510 induces an incomplete protective IgG response. Component-specific diagnosis could improve the results of AIT by identifying patients who are sensitized to allergens present in the vaccine.

DOI: 10.1002/eji.201970400

P0801

Modification of allergic inflammation in murine model of allergic conjunctivitis without CD40 signal

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Allergic conjunctivitis (AC) is an allergic ocular diseases characterized by conjunctival eosinophilic infiltration and Th2 type immune responses. AC is considered an IgE-mediated type I allergic reaction due to repeated direct contact of allergen on the conjunctival surface. In this study, we investigated the involvement of IgE-independent mechanisms of AC by using CD40 knock-out (CD40KO) mice.
Murine AC model was induced by ovalbumin (OVA) or short ragweed (SRW) pollen. WT and CD40KO mice were immunized with OVA or SRW pollen plus alum twice with an interval of two weeks. Mice were then challenged with OVA or SRW pollen via eye drops on three consecutive days. AC symptoms, eosinophil infiltration and degranulation of mast cells in the conjunctiva and antigen-specific IgE antibody in the serum were subsequently analyzed.
Clinical symptoms, including conjunctival edema, redness, and mucus secretion, were observed in both WT and CD40KO mice although CD40KO mice could not produce antigen-specific IgE antibody. Moreover, marked mast cell degranulation and the infiltration of eosinophils in conjunctiva were also observed in both WT and CD40KO mice. Based on these results, AC could be developed without antigen-specifi IgE antibody. We are now engaged in the investigation of IgE-independent mechanisms of AC development.

P0802

Interaction of eosinophils and dermal fibroblasts in fungal-infected atopic dermatitis

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Atopic dermatitis (AD) is an allergic inflammatory skin disease, histologically characterized by eosinophils infiltration in the dermal layer. This chronic and pruritus disease is often aggravated by microbial infection in the dried and impaired skin area. Colonization of cutaneous fungal communities such as *Malassezia* yeast and *Candida* yeast has been reported in AD patients. Nevertheless, the interaction between the infiltrating effector cells eosinophils, human dermal fibroblasts (HDF) and the yeast is not well-understood. Therefore, we aim at investigating the immunological mechanism underlying the fungal interaction with eosinophils in the skin of AD.
Dectin-1 agonists curdlan and heat-killed *Candida albicans* (HKCA), as well as the dectin-2 agonist furfurman, were co-cultured with the human eosinophils and HDF. Inflammatory cytokines and adhesion molecules were measured by cytometric bead-based assay and flow cytometry, respectively. Upon curdlan stimulation, the release of AD-related IL-6, CXCL8, TNF-α and CCL2 were significantly upregulated in eosinophils/HDF co-culture but not in eosinophils single culture. Adhesion molecule ICAM-1 and VCAM-1 were also expressed on both cells. IL-6 and CXCL8 were exclusively increased in the co-culture system upon HKCA stimulation. Dectin-2 was expressed on eosinophils but not on HDF. In eosinophils/HDF co-culture, the release of IL-6, CXCL8 and CCL5 were significantly increased.

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DOI: 10.1002/eji.201970400

The increase in pro-inflammatory cytokines and adhesion molecules expression highlight the pathological immune response in the dermal layer in AD. The results demonstrated the interaction between the skin and eosinophils upon fungal colonization. Further experiments are ongoing to evaluate the underlying mechanism of fungal infection in AD.

P0803

Descurainia sophia water extract attenuates eosinophil infiltration and airway inflammation in ovalbumin-induced asthmatic BALB/c mice

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Asthma is a chronic respiratory disease with high prevalence worldwide. The T-helper type 2 (Th2) cytokines play important roles in asthma. Asthma is characterized by airway hyperresponsiveness (AHR), activated Th2 cells, eosinophil infiltration, mucus hypersecretion, goblet cell proliferation and IgE production. *Descurainia sophia*(DS) is effective for treating cough, asthma and removing phlegm in the lung, but mechanisms aren't clear yet. Thus, we would like to study whether DS water extract has a potential effect on asthma and Th2-related responses. BALB/c mice were sensitized with ovalbumin (OVA). The mice were administrated intraperitoneally (IP) with different doses of DS water extract for consecutive seven days before the last OVA. The levels of OVA-specific antibodies in serum and Th2 cytokines in OVA-cultured splenocytes were determined by ELISA. The cell populations in bronchoalveolar lavage fluid (BALF) were calculated. The gene expression in the lung was detected by q-PCR. The lung tissues were stained by H&E, PAS and trichrome stain. The results showed that DS water extract reduced AHR and Th2 cytokines. DS water extract abated the percentages of eosinophils and total cell numbers in BALF. The gene expression of CCL24 which is eosinophil-attracting chemokine was significantly reduced. Based on the histological studies, the cell infiltration, mucus secretion, and collagen deposition were significantly reduced. The data showed that DS water extract can down regulate OVA-induced allergic asthma in BALB/c mice. These results suggest that DS water extract can be used as an effective therapeutic remedy for mucus secretion and airway inflammation.

P0804

Immunosuppressive Activity of Cyn-1324 on Ovalbumin-sensitized Asthmatic Mice

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T lymphocytes play an important role in several immune diseases such as asthma, which is a chronic inflammatory disorder of the airways. It has been well established that cytokines produced by T helper 2 (Th2) cells are responsible for asthmatic pathology. Th2 cells produce large amounts of cytokines such as interleukin (IL)-4, IL-5, and IL-13, which induce immunoglobulin (Ig)-E production, eosinophilic

DOI: 10.1002/eji.201970400

inflammation, mucus secretion and airway hyperresponsiveness (AHR). Thus, it might be possible that controlling the activation of Th2 cells via blocking co-stimulatory molecular, CD28 and CD80/86 to attenuate the asthmatic responses. A small molecule compound, Cyn-1324, was identified to have a stronger binding affinity with CD28 compared to CD80/86. This study aims to investigate the effect of Cyn-1324 on reducing T cell-mediated immune responses in ovalbumin (OVA)-induced asthmatic mouse model. Our results demonstrated that Cyn-1324 has the blockade ability between CD28 and CD80/86 by a competition assay. And, we examine the immunosuppress efficacy of Cyn-1324 by *in vitro*. The results show that Cyn-1324 could repress the Th2-mediated cytokines production of T cells. Among asthmatic mice model, administration of Cyn-1324 significantly reduced eosinophil infiltration and AHR to improve asthma symptoms. Cyn-1324 treatment also dramatically reduced Th2-mediated cytokines production in OVA-stimulated splenocyte culture supernatants. These results suggested that Cyn-1324 reduced allergenic responses and it might be a potential immunosuppressive agent to alleviate the allergic disease in the future.

P0805

House dust mite increases pro-Th2 cytokines, IL-25 and IL-33 via the activation of TLR1/6 signaling

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House dust mite (HDM) has been implicated in the etiology and exacerbation of atopic dermatitis (AD). Diverse factors contribute to HDM allergenicity through the activation of innate immunity. We investigated whether Dermatophagoides farinae extract (DFE) allergens mediate innate immune activation through specific Toll-like receptors (TLRs) in epidermal keratinocytes, DFE-induced murine AD model and human AD lesions. DFE activated the expression of TLR1, TLR6, interleukin (IL)-25, and IL-33 in human primary keratinocytes and HaCaT cells. Knockdown of TLR6 inhibited DFE-induced upregulation of IL-25 or IL-33. In addition, the suppression of TLR1 inhibited the release of IL-33. DFE induced the expression of IL-25 and IL-33 by upregulation of IRAK1, TAK1, IKK, and NF-kB pathways. Tlr6$^{-/-}$ mice did not show DFE-induced upregulation of IL-25 and IL-33. Furthermore, DFE-induced upregulation of IL-25 was not induced in Tlr1$^{-/-}$ mice. We also identified upregulated mRNA and protein expression of TLR1, TLR6, IL-25 and IL-33 in human AD skin lesions with high HDM sensitization. We found that DFE-induced activation of TLR1 and TLR6 may cause polarization towards a Th2 immune response via the release of IL-25 and IL-33.

P0806

Immune response to allergens with different molecular weight: polymerized versus native allergens

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Effective approaches in the constracting of low allergenic extracts for specific immunotherapy is the polymerization of allergenic molecules by glutaraldehide. It have been shown that polymerization of native allergens (NA) increases the immunogenic activity of ragweed pollen allergenic extract (RPA). Both allergens induces the synthesis of IgG-Ab and anti-ragweed specific IgE-Ab (sIgE-Ab) as well, IgE response in mice immunized by PRA increased more slowly than in mice immunized by NRA.We

DOI: 10.1002/eji.201970400

study was to compare the prossesing and induction of IL-1 by mice peritoneal macrophages (PM) and human neutrophiles (HN). PM of F1 hybrid mice (CBA[C57BL) and human neutrophiles were incubated with 10 µg (protein) of PRA and NRA. In was demonstrated that production of IL-1 was much higher in cell´s culture preincubated with PRA (p< 0.001). 4h-preincubated cells were transfered intraperitoneally to intact syngeneic mice and it have been shown that synthesis of the sIgE-Ab synthesis was more than 60% higher when PM were preincubated with PRA. Unlike NRA, the presentation of PRA was prostaglandin-dependent. Thus, preincubation of PM in the presense of prostaglandin synthetase antagonist (indomethacin) and PNA highly stimulates the synthesis of sIgE-Ab, while PM incubated with indomethacin and NRA induces sIgE-Ab synthesis at the same extent like in control group. Thus, the induction of immune response by high molecular weight allergens is more active in comparison to the native allergens. It may be one of explanations the better immunotherapetical efficacy of polymerized allergens.
The publication has been prepared with the support of the "RUDN University Program 5-100".

P0807

Olive pollen allergy and the risk of diseases development

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Olive pollens are one of the main factors causes of allergies affecting the population in industrialized countries.
We studied prevalence of olive pollen allergy and the relationship between olive pollen allergy and the existence or development of other diseases in Beni Mellal (BM) city. We conducted a cross-sectional study using a questionnaire among subjects allergy in a sample size of 400 subjects,chosen randomly from BM.
Our epidemiologic studies revealed a crucial role of olive pollen in increasing of prevalence of allergic disorders. Furthermore our results show dependence between asthma and cardiovascular diseases in people allergic to olive pollen suggesting that asthmatic people who are allergic to olive pollen are the most susceptible to develop cardiovascular disease. These findings should be explored by clinical research in the future that can help in understanding the mechanisms associated with the asthma and cardiovascular disease in people allergic to olive pollen.
KEYWORDS
Olive pollen; Allergy; Asthma; Cardiovascular disease; Beni Mellal and Morocco.

DOI: 10.1002/eji.201970400

P1400

Plasma exosomes from patients with allergic rhinitis regulate the differentiation of T helper cells

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Background: Extracellular vehicles (EVs) are involved in the pathogenesis of many diseases. Allergic rhinitis (AR) is an IgE-mediated nasal mucosal inflammatory disease. We sought to investigate the role of plasma EVs in the development of AR.
Methods: Peripheral blood was collected from healthy subjects (HS, n=6), mild AR patients (n=6) or moderate-severe AR patients (n=6). EVs were isolated from plasma by differential ultracentrifugation. The isolated EVs were characterized by transmission electron microscopy (TEM), western blot and nanoparticle tracking analysis. Human peripheral blood monocellular cells isolated from healthy donors were stimulated with HC-EVs, mild AR-EVs and moderate-severe AR-EVs. After 3 days, cells were collected to determine the levels of Th1, Th2 and Th17 by flow cytometry.
Results: The EVs were bilipid-layered particles with a diameter of 100 nm as determined by TEM. Western blot analysis showed that plasma EVs were all positive for EVs specific markers, including Alix, TSG101 and CD63. No significant difference was found among the concentrations of EVs isolated from HC, mild AR and moderate-severe AR. The level of Th2 was significantly increased by both the mild and moderate-severe AR-EVs while no effects were found on the levels of Th1/Th17.
Conclusion: Plasma EVs derived from AR patients could increase the level of Th2, demonstrating that plasma EVs played a significant role in the development of AR and could be the potential therapeutic target in the future.

P1401

Specific IgG antibodies of aquatic products in 1321 patients with food intolerance in Hainan

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Objective: To investigate the distribution characteristics of food intolerance about aquatic products in Hainan, and to provide a reference for clinical diagnosis and diet guidance for the patients.
Methods: A total of 1321 patients received IgG antibodies test by Enzyme-linked immunosorbent assay and the results were analyzed statistically.
Results: Among the 1321 patients, the main allergen was sardine, the positive rate was 23.39%, followed by scallop(22.33%) and grass carp(19.08%). There was statistical significance in most aquatic products among patients with different ages and seasons($P< 0.05$), but the gender difference was not precise.
Conclusion: Sardine, scallop and grass carp are main aquatic products allergens for food intolerance in Hainan and the detection of specific IgG antibody plays a momentous role in individualized treatment and health education for food intolerance disease.

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DOI: 10.1002/eji.201970400

P1402

Prostaglandins E2 signal mediated by receptor subtype EP2 and EP4 regulates IgE production in vivo and contributes to asthma development

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Prostaglandin E2 (PGE2) is one of the most abundantly produced prostanoids. By acting on one or more receptor subtypes [E-prostanoid (EP) 1-4], PGE2 affects multiple aspects of immune and inflammatory responses, including asthma. In B lymphocytes, previous studies have shown, PGE2, in an EP2-depedent manner, enhanced IgE production, but we found PGE2 had a negatively regulatory function regarding IgE production by EP4 receptor. And inappropriate IgE responses also constitute a mechanism for allergic diseases such as asthma. Here, we explore the mechanisms of this unexpected opposite function of PGE2 by EP2 and EP4 receptors and its effect on the development of asthma. We found EP4 played a negative role of AID, εGLT and IgE class switching in B cells stimulated with anti-CD40+IL4. Mechanistic studies demonstrated EP4-deficient B cells have decreased phosphorylation of AKT and enhanced phosphorylation of STAT6.This effect was mimicked by AKT inhibitor MK-2206. Meanwhile, we found EP4 deficiency led to enhanced PPARα, as well as reduced PPARγ expression. PPARγ antagonist T0070907 was used and down-regulated PPARγ showed an increase of p-STAT6. Then, we confirmed this corelation is direct via CO-IP experiment. Furthermore, in the ovalbumin (OVA) - induced asthma models, administration of EP4 agonist resulted in diminished serum IgE, reduced infiltration of inflammatory cells and alleviated lung pathology. These findings reveal a novel function of EP4 as a negative regulator of IgE production and highlight a role of PGE2-EP4-AKT-PPARα/PPARγ-STAT6 signalling pathway in IgE response and allergic diseases.
Keywords: PGE2; EP2; EP4; IgE; AKT; PPARγ; Asthma

P1403

Immune repertoire data provide new evidence for hygiene hypothesis in allergy

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Allergic disorders are the most common immune related diseases worldwide, and its prevalence has grown dramatically over the past few decades, especially for pre-school kids. Although the popular “hygiene hypothesis” attributes this increased prevalence to the reduced diversity of microbe exposure in early-life, more evidence is demanded to support this hypothesis. T cells can specifically recognize antigens by the T cell receptor (TCR) and differentiate into the long-term memory cell, which enables them to efficiently record the exposure history of an individual. In this study, we investigated the TCR beta chain (TCRB) repertoires of 821 allergic individuals and compared them with healthy controls. We found that the TCRB repertoire diversity decreased with aging and the TCRB diversity of allergic kids was higher than healthy kids. In contrast, allergic adults showed a reverse trend. We also found that the proportion of known pathogen-specific CDR3s (PKPSC) increased with aging and peaked in 20 years old, whereas it began to decrease after 35 years old, which implied that an individual has the best immunity in his 20-30s. The PKPSC in allergic kids was significantly decreased compared to healthy kids; however, for allergic adults, no difference was observed. These characteristics showed potential in predicting the risk of allergy for kids, and the identified allergen associated T cells could be used to help the diagnose of allergic disorders. Our results suggested that the diverse environmental exposure during early-life is critical for the induction of immune tolerance, which might prevent the development of allergy.

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DOI: 10.1002/eji.201970400

P1404

The pathogenesis of IL-6 trans-signaling in allergic airway inflammation and IL6R gene polymorphisms analysis in asthmatic patients

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IL-6/STAT3 signaling plays a crucial role in the pathogenesis of allergic inflammation, during this process, the soluble form of IL-6R mediated trans-signaling is an important approach for STAT3 activation. Since human genetic background strongly influences the susceptibility of asthma, we hypothesis that IL6R gene polymorphisms may influence the IL-6 trans-signaling activation. A cohort of asthmatic patients and healthy control individuals were genotyped and analyzed for serum IL-6, sIL-6R levels, meanwhile, two important polymorphic sites were selected for genotyping: rs4845617 in the promoter region and rs2228145 encoding IL-6R splicing site in the exon 9. Increased expression of IL-6, IgE and sIL-6R in plasma, the expression of PD-1 and STAT3 activation in PBMC were observed in asthmatic patients; the frequency of Treg in asthmatic patients was reverse correlated with the circulating sIL-6R level. We also found that allele A of rs2228145 was a significant predisposition allele of asthma. In the in vivo study, administration of soluble gp130, a natural antagonist of IL-6 transsignaling pathway, could alleviate the OVA induced asthmatic airway inflammation. Therefore, the expression of sIL-6R were directly regulated by the IL-6R gene polymorphic, especially in the rs2228145 encoding IL-6R splicing site in the exon 9, the increased expression of sIL-6R in the asthmatic patients may inhibit the Treg function. The IL-6/sIL-6R signaling pathway seems playing an essential role in the pathogenesis of asthma.

P1405

Analysis of the eosinophil-rich cell infiltrate in colonic polyps of patients with food allergy

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Juvenile polyps (JP) lead to rectal bleeding and diarrhea in children. We characterized the allergic inflammatory cell infiltrate of the polyps in patients with food allergy and the origin of the high frequency of eosinophils.
Polyps were removed in 20 patients, along with a biopsy of the normal surrounding tissue (SCT). The production of CCL26 and RANTES were assessed by qPCR and confocal microscopy. Additionally, Caco-2 and HMEC-1 cell lines were stimulated with IFN-g (10ng/ml) or IL-13 (10ng/ml) and the activation of STAT-3 and STAT-6, and expression of chemokines were analyzed by immunoblotting and qPCR, respectively.
We found a significant increase of eosinophils and eotaxin-3 expression in polyps compared to SCT ($p < .05$), which was mainly produced in the epithelial compartment. Lamina propria and endothelial cells of polyp tissue were also positive for CCL26 staining. The SCT showed a basal expression. Caco-2 and HMEC-1 cells stimulated with IL-13 rendered higher levels of eotaxin-3 transcripts compared to cells incubated with medium or IFN-g ($p < .02$). The IL-13-dependent induction of CCL26 secretion triggered the STAT-3 and STAT-6 pathway activation.
In conclusion, we found that the epithelial compartment and endothelium were the main sources for eotaxin-3 secretion, being IL-13 responsible for STAT-3 and STAT-6 activation. These findings may shed light on understanding the etiology of the eosinophil homing to the gut of patients with food

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DOI: 10.1002/eji.201970400

allergy and may be critical to study the IL-13/STAT-3/6/CCL26 axis during the novel therapy with biologics (Dupilumab or anti-IL4Ralpha) for patients with eczema and food allergy.

P1406

MiR-135-5p-p62 axis regulates autophagic flux, tumorigenic and metastatic potential mediated by allergic inflammation

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Background: Dysregulation of autophagy and inflammasome activity contributes to the development of auto-inflammatory diseases. Autophagy plays a crucial role in degranulation of mast cells. Scaffolding adaptor protein P62/SQSTM1 is an autophagy receptor that acts as a link between ubiquitination and autophagy machineries. Overexpression of p62 increases expression levels of proinflammatory cytokines, such as TNFa, CXCL10, and CCL2. Effect of p62 on allergic inflammation in conjunction with autophagic flux has not been reported previously.
Methods: In this study, we investigated autophagic flux in antigen stimulated RBL2H3 cells by western blots and immunofluorescence. Expression level of miR-135-5p or p62 was quantified with qRT-PCR. To measure tumorigenic or metastatic potential, mouse melanoma B16F1 cells, after induction of passive systemic anaphylaxis, were injected into the BALB/c mouse.
Results: *In vitro* allergic inflammation was accompanied by an increased autophagic flux in RBL2H3 cells. P62, increased by antigen stimulation, mediated *in vitro* allergic inflammation, passive cutaneous anaphylaxis, and passive systemic anaphylaxis. P62 mediated cellular interactions during allergic inflammation. It also mediated tumorigenic and metastatic potential of cancer cells enhanced by PSA. TargetScan analysis predicted that miR-135-5p was a negative regulator of p62. Luciferase activity assay showed that miR-135-5p directly regulated p62.MiR-135-5p mimic negatively regulated features of allergic inflammation and inhibited tumorigenic and metastatic potential of cancer cells enhanced by PSA. MiR-135-5p mimic also inhibited cellular interactions during allergic inflammation.
Conclusion: miR-135-5p-p62 axis regulates allergic inflammation in conjunction with autophagic flux, cellular interactions, and allergic inflammation promoted enhanced tumorigenic and metastatic potential of cancer cells.

P1407

SOCS1 modulates allergic reaction mediated-metastatic potential in cancer cells through miR-122-SOCS1 feedback loop

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The regulatory role of suppressor of cytokine signaling 1 (SOCS1) in inflammation has been reported. However, its role in allergic inflammation has not been previously reported. SOCS1 mediated in vitro and in vivo allergic inflammation. Histone deacetylase-3 (HDAC3), a mediator of allergic inflammation, interacted with SOCS1, and miR-384 inhibitor, a positive regulator of HDAC3, induced features of allergic inflammation in an SOCS1-dependent manner. miRNA array analysis showed that the expression of miR-122 was decreased by antigen-stimulation. TargetScan analysis predicted the binding of miR-122 to the 3′-UTR of SOCS1. miR-122 inhibitor induced in vitro and in vivo allergic features in SOCS1-dependent manner. SOCS1 was necessary for allergic inflammation-promoted enhanced tumorigenic and metastatic potential of cancer cells. SOCS1 and miR-122 regulated cellular interactions involving cancer cells, mast cells and macrophages during allergic inflammation. SOCS1

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DOI: 10.1002/eji.201970400

mimetic peptide, D-T-H-F-R-T-F-R-S-H-S-D-Y-R-R-I, inhibited in vitro and in vivo allergic inflammation, allergic inflammation-promoted enhanced tumorigenic and metastatic potential of cancer cells, and cellular interactions during allergic inflammation. Janus kinase 2 (JAK2) exhibited binding to SOCS1 mimetic peptide and mediated allergic inflammation. Transforming growth factor- Δ1 (TGF-Δ1) was decreased during allergic inflammation and showed an anti-allergic effect. SOCS1 and JAK2 regulated the production of anti-allergic TGF-Δ1. Taken together, our results show that miR-122-SOCS1 feedback loop can be employed as a target for the development of anti-allergic and anti-cancer drugs.

P1408

Human AdMSCs regulate expression of MIP-2 and attenuate atopic dermatitis

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The objective of this study was to investigate the effect of human adipose tissue-derived mesenchymal stem cells (AdMSCs) on atopic dermatitis (AD) in the BALB/c mouse model. The AdMSCs attenuated clinical symptoms associated with AD, decreased numbers of degranulated mast cells (MCs), IgE level, amount of histamine released, and prostaglandin E2 level. Atopic dermatitis increased the expression levels of cytokines/chemokines, such as interleukin-5 (IL-5), macrophage inflammatory protein-1ß (MIP-1ß), MIP-2, chemokine (C-C motif) ligand 5 (CCL5), and IL-17, in BALB/c mouse. The AdMSCs showed decreased expression levels of these cytokines in the mouse model of AD. In vivo downregulation of MIP-2 attenuated the clinical symptoms associated with AD. Atopic dermatitis increased the expression levels of hallmarks of allergic inflammation, induced interactions of Fc+RIb with histone deacetylase 3 (HDAC3) and Lyn, increased ß-hexosaminidase activity, increased serum IgE level, and increased the amount of histamine released in an MIP-2-dependent manner.

P1409

Can calcitriol, PUFAs and probiotics prevent excessive inflammation in patients with severe atopic dermatitis (AD), MBL deficits and high levels of Immunoglobulin E (IgE)?

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In this case rapport wil be presented 10 years old girl with severe AD and elevated IgE levels. She is followed in the outpatient pediatrics clinic from the neonatal period, at 6 months of age, she had elevated IgE>5000, recurrent bacterial, viral and fungal infections, severe AD defined by the Scoring of AD (SCORAD) and the Eczema Area and Severity Index (EASI), severe food and environmental allergies, asthma, extensive cutaneous vasculitis, eosinophilia, neutropenia, thrombocytosis, increased IgG levels, low absolute lymphocyte counts, decreased B cellsconcentration, normal proportions and concentrations of T-, K/NK- lymphocytes, normal exposure of adhesions molecules and Tcell proliferation by polyclonal stimulation, normal value of somatic mutation, nice response of virgin Tcell. There was not confirmed HIES type 1 (there is not found mutations in signal transducer and activator of transcription 3 (*STAT3*) gene) and SCID (normal thymus function). DOCK8 deficiency are not been examinated. She has had decreased C1q concentration and MBL levels, reduced complement activity via the MBL pathway. In age at 9 months started with restrictiondiet, on the basis of evidence of allergen sensitization and conformed clinically relevant allergy and get continuty treatment with calcitriol, LGG probiotics and intermittent PUFA. Now she has normal lymphocyte-, especially nice levels of eosynophylocyte-, and thrombocyte-counts, reduction in IgE level to 1500. Normal proportions and concentrations of T and B / NK lymphocytes. Normal complement

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DOI: 10.1002/eji.201970400

functionality. Clinical benefit, without signs of acute infections normal skin without lesions, normal growth, introduction of normal diet, normal everyday activities and psychosocial functioning.

P1410

Significant role of TSLP receptor on CD4 T cells in chronic allergic dermatitis

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Thymic stromal lymphopoietin (TSLP) is a key cytokine that initiates and promotes allergic inflammation both in humans and mice. It is well known that TSLP is important in initial step of inflammation by stimulating DC to promote Th2 differentiation of naive T cells. However, TSLP is abundantly produced in the late phase of inflammation as well, therefore, we focused on the function of TSLP in chronic Th2-type inflammation. By establishing a novel chronic allergic skin inflammation model with repetitive challenges of hapten after sensitization, we demonstrated that T cell-specific deletion of TSLPR resulted in near complete ablation of ear swelling and infiltration of CD4 T cells and eosinophils. Of note, TSLPR deletion on T cells did not affect acute inflammation. Transfer of antigen-sensitized wild-type CD4T cells, but not of TSLPR deficient CD4T cells, increased skin inflammation upon challenge. Furthermore, production of IL-4 from TSLPR deficient CD4T cells in inflamed ear lesions was markedly diminished, demonstrating that TSLP-dependent IL-4 production from CD4T cells was critical for the exacerbation of skin inflammation. Similar results were obtained in Th2-type allergic skin inflammation model using MC903. Collectively, these results indicate that TSLP acts directly to CD4 T cells to elicit pathogenesis of Th2 cells, thereby having a critical role in exacerbation of skin inflammation in the chronic phase.

P1411

Defining the panel of clinically relevant cat allergens for immunotherapy

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Background: Patients suffering from allergy to cat suffer from severe respiratory symptoms such as asthma. Besides the major cat allergen Fel d 1 seven more allergens are described but their clinical relevance has not been studied in depth.
Methods: Fel d 1, 2, 3, 4, 7 and 8 were expressed in *Escherichia coli* and purified. Fel d 6 containing the same epitope as Fel d 5, Alpha-Gal was purified as natural allergen. Sera were obtained from 65 cat allergic patients whose clinical symptoms were recorded and 4 non-allergic control subjects. IgE levels to each of the cat allergens molecules were quantified by ImmunoCAP technology. For this purpose purified cat allergens were biotinylated and coupled to Streptavidin ImmunoCAPs
Results: For cat allergic patients the following frequencies of IgE recognition were obtained for each allergen molecule: Fel d 1: 95,1% (n=59), Fel d 2: 20,9% (n=13), Fel d 3: 38,7% (n=24), Fel d 4: 48,3% (n=30), Fel d 5-6, 8: 11,29% (n=7), Fel d 7: 50% (n=31). Patients who also suffered from atopic eczema in addition to respiratory allergy showed a broader sensitization to the cat allergens than patients suffering only from respiratory allergy.

DOI: 10.1002/eji.201970400

Conclusion: Sensitization to Fel d 1 is the most frequently recognized cat allergen but Fel d 3, Fel d 4 and Fel d 7 seem to be also of clinical relevance and may be considered as components in a vaccine for cat allergy.

P1412

Quantification of allergen-specific IgE in patients with birch and cat allergy by ELISA

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Background: The measurement of allergen-specific IgE (asIgE) levels is important for allergy diagnosis. Quantitative assays such as the ImmunoCAP technology can only be run on special instruments, consume rather large volumes of serum and are expensive.
Methods: To establish a standard for the measurement of human asIgE we have expressed and purified a human monoclonal IgE antibody specific for the major birch pollen allergen, rBet v 1 (IgEmoAb) and the corresponding allergen. Using IgEmoAb and rBet v 1 we established by ELISA a standard curve allowing to measure and quantify asIgE. Using a set of sera from clinically well-characterized birch pollen and cat allergic patients Bet v 1- and Fel d 1-specific IgE levels were quantified with the IgE ELISA and compared with quantitative IgE ImmunoCAP measurements and results obtained by ImmunoCAP ISAC chip
Results: We have established a standard curve for quantification of asIgE with a linear range covering approximately 6 ng/ml to 240 ng/ml. Allergen-specific IgE levels determined by the quantitative IgE ELISA correlated well with quantitative IgE levels determined by ImmunoCAP and ImmunoCAP ISAC. The quantitative IgE ELISA can be performed with simple ELISA equipment and requires less than half of the amount of serum required for ImmunoCAP measurements.
Conclusion: We have established a simple quantitative IgE ELISA which allows robust quantification of asIgE in the nanogram/ml range.

P1990

LPS inactivation by a host lipase sensitizes lung epithelial cells for allergic asthma

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Allergic asthma is a chronic inflammatory disease primarily mediated by Th2 immune mechanisms. Numerous studies have suggested that early-life exposure to lipopolysaccharide (LPS) is negatively associated with allergic asthma. One proposed mechanism invokes desensitization of lung epithelial cells by LPS. We report here that acyloxyacyl hydrolase (AOAH), a host lipase that degrades and inactivates LPS, renders mice more susceptible to house dust mite (HDM)-induced allergic asthma. Lung epithelial cells from Aoah$^{-/-}$ mice are refractory to HDM stimulation, decreasing dendritic cell activation and Th2 responses. Antibiotic treatment that diminished commensal LPS-producing bacteria

DOI: 10.1002/eji.201970400

normalized Aoah$^{-/-}$ responses to HDM, while giving LPS intrarectally ameliorated asthma. Aoah$^{-/-}$ mouse feces, plasma and lungs contained more bioactive LPS than did those of Aoah$^{+/+}$ mice. By inactivating commensal LPS, AOAH thus prevents desensitization of lung epithelial cells. An enzyme that prevents severe lung inflammation/injury in Gram-negative bacterial pneumonia has the seemingly paradoxical effect of predisposing to a Th2-mediated airway disease.

P1991

The amino acid sensor GCN2 controls Th9 cell differentiation and allergic airway inflammation

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T helper 9 (Th9) cells have emerged as important mediators of allergic airway inflammation. There is evidence that general control nonderepressible 2 (GCN2) affects the immune response under some stress conditions. However, whether GCN2 regulates CD4$^+$ T cell differentiation during allergic inflammation remains unknown. To clarify the regulatory roles of GCN2 in CD4$^+$ T cell differentiation and its significance in allergic airway inflammation. The intrinsic effects of GCN2 in the differentiation of Th cell subsets were detected using the in vitro induction system. GCN2 knockout (GCN2KO) mice, OVA-induced allergic airway inflammation and adoptive transfer mouse models were used to determine the significance of GCN2 in *in vivo* Th9 differentiation and allergic airway inflammation. RNA-Seq, real-time PCR, Western blots and other molecular approaches were used to identify the molecular mechanisms relevant to the regulation of GCN2 in TH9 cell differentiation. GCN2 deficiency significantly inhibited the differentiation of Th9 cells but not Th1, Th2, and Treg cells. GCN2KO mice and recombination activity gene-2 knockout (Rag2KO) mice that received adoptively transferred sorted GCN2-deficient CD4$^+$ T cells exhibited reduced Th9 differentiation and less severe allergic airway inflammation. Furthermore, the isolated GCN2-deficient Th9 cells also mediated less severe allergic airway inflammation upon adoptive transfer. Mechanistically, GCN2 deficiency inhibits Th9 cell differentiation through a hypoxia-inducible factor 1α (HIF1α)-dependent glycolytic pathway. Our results reveal a novel role of GCN2 in Th9 cell differentiation. Our findings indicate that new strategies to inhibit GCN2 activity may provide novel approaches to attenuate allergic airway inflammation.

P1992

The crystal structure of the major olive allergen Ole e 1 and its use for structure-based epitope mapping

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DOI: 10.1002/eji.201970400

Background: Olive tree pollen is one of the most prevalent allergen sources in the Mediterranean area. Ole e 1 is the major allergen of this source and is recognized by up to 96% of atopic individuals with olive tree pollinosis. To date there is little structural information on this important allergen. The only protein structure of a Ole e 1 family member is that of Pla l 1 (Stemeseder et al., 2017), which exhibits low cross-reactivity to Ole e 1.
Methods: Recombinant Ole e 1 was expressed in E. coli and purified as a natively folded protein and compared to natural Ole e 1. The rOle e 1 was crystallized and its structure solved by X-ray crystallography. The server-based program SPADE was used for IgE-epitope mapping (Dall'Antonia and Keller, 2019).
Results: rOle e 1 and nOle e 1 exhibit very similar CD-spectra and unfolding characteristics. Furthermore both proteins form stable dimers in solution. The 3D crystal structure of rOle e 1 exhibits a central 6-stranded β-barrel, resembling the fold of Pla l 1. The C-terminus, which is missing in Pla l 1, forms the dimerization interface. Structure-based epitope mapping yields putative cross-reactive epitopes close to the dimerization interface.
Conclusion: The 3D structure of rOle e 1, which represents the prototypic member of the Ole e 1-like family shows that these allergens most likely act as dimers in solution and that the C-terminal domain is essential for dimer formation. IgE-epitope mapping results may be deployed in novel diagnostic and therapeutic strategies.

P1993

Screening of nanobody specific for peanut major allergen Ara h 3 by phage display

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Peanut allergy is a major health problem worldwide. Detection of food allergens is a critical aspect of food safety. The VHH domain of single chain antibody from camelids, also known as nanobody, showed its advantages in the development of biosensors because of its high stability, small molecular size, and ease of production. However, no nanobody specific to peanut allergens has been developed. In this study, we constructed a library with random triplets (NNK) in its CDR regions of a camel nanobody backbone. We screened the library with peanut allergy Ara h 3 and obtained several candidate nanobodies. One of the promising nanobodies, NA16 was further biochemical characterization by gel filtration, Isothermal titration calorimetry (ITC), co-crystallization, and western blot in terms of its interaction with Ara h 3. NA16 specifically binds to peanut major allergy Ara h 3 with a dissociation constant of 400 nM. The nanobody NA16 may have application in the development of biosensors for peanut allergens detection.

P1994

Expression, purification and characterization of arginine kinase from house dust mites, Der p 20, a potential cross-reactive allergen

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DOI: 10.1002/eji.201970400

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Arginine kinases (AK), represent a family of pan-allergens with potential cross-reactivity in many organisms such as shrimp, octopus, mites, moth or cockroach. Der p 20, the AK of the house dust mite (HDM) species *Dermatophagoides pteronyssinus*, one of the most common allergen sources, has not yet been characterized in depth.
The cDNA of Der p 20 was ligated into pET17b and expressed in E.coli BL21 (DE3), including a C-terminal hexahistidine-tag. Expression levels and localization of the target protein within the host cells were determined by SDS-PAGE. The protein was purified under native conditions by nickel affinity chromatography and analyzed by CD-spectroscopy and size-exclusion chromatography to determine secondary structure and oligomerization, respectively. IgE-recognition frequencies were determined by ELISA in sera from 98 patients suffering from HDM-induced allergic rhinoconjunctivitis and mild asthma. Cross-reactivity between AKs from different organisms was studied by immunblotting. Recombinant Der p 20 was purified as properly folded and mostly monomeric protein. Twenty-six percent of patients showed IgE-reactivity against recombinant Der p 20. Patients with IgE reactivity to Der p 20 were more frequently sensitized to Der p 10, i.e., tropomyosin, and reported breathing-problems compared to Der p 20- negative patients. rDer p 20 inhibited IgE-binding to AK in extracts from shrimp (*L. vannamei*) suggesting that AK may be a biomarker for cross-reactivity between shrimp and HDM.

Supported by grants F4602, F4603 and F4605 of the Austrian Science Fund (FWF).

P1995

Newly generated monoclonal antibodies bind specifically to glycosylated form of human acidic mammalian chitinase

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Human acidic mammalian chitinase (huAMCase), a 50 kDa protein containing a 30 kDa N-terminal catalytic domain that can hydrolyze chitin, belongs to family 18 glycosyl hydrolases. High abundant of this enzyme was found in gastrointestinal tract. In lung, interleukin-13 was proposed to play a key role as primary effector that induces secretion of huAMCase by airway epithelial cells and macrophages. The secreted huAMCase activates elevation of monocyte chemoattractant protein 1 (MCP-1) and eotaxin-1, which induce the recruitment of eosinophils, T cells, and macrophages to site of inflammation in lung epithelial cells. However, the mechanisms underlying huAMCase mediated cells recruitment and its surface receptor have not been identified. In this study, the monoclonal antibodies to huAMCase were raised using standard hybridroma technique. Four specific monoclones, namely 4G1-D9, 4G1-E5, 6E5-C2 and 6E5-C9 that highly specific to only huAMCase were successfully generated. All the clones belong to IgG1 isotype and react with huAMCase endogenously expressed in HeLa cells and monocytic cell lines: THP1 and U937. Interestingly, Western blotting showed that clones 6E5-C2 and 6E5-C9 could detect the 70 kDa protein, which was reduced to 50 kDa by elimination of N-linked glycan using tunicamycin, indicating the glycosylated form of huAMCase. The obtained antibodies may be suitable for development of a sensitive detection of huAMCase that will help to predict the progress of the allergic disease.
Keywords: huAMCase, monoclonal antibody, glycosylated form
This work was supported financially by the National Research Council of Thailand [grant no. SUT-102-57-36-25] and Suranaree University of Technology, Thailand.

DOI: 10.1002/eji.201970400

P1996

Inhibitory NKT cells that suppress allergic airway hyperreactivity

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Infection of suckling mice with influenza virus expands a $CD4^-CD8^-$ double negative (DN) Natural Killer T (NKT) cell subpopulation that protects the mice as adults against allergen-induced airway hyperreactivity (AHR). We examined the characteristics of this NKT cell subpopulation and found that this suppressive NKT subset expressed CD38 but not CD4 and produced IFN-γ, but not IL-17, IL-4 or IL-13. The subset inhibited the development of airway hyperreactivity (AHR) through contact-dependent suppressive activity against helper CD4 T cells. The NKT subset expanded in the lungs of neonatal mice after infection with influenza, but also after treatment of neonatal mice with a Th1-biasing α-GalCer analogue, Nu-α-GalCer. These results suggest that early/neonatal exposure with infection or with antigenic challenge can affect subsequent lung immunity by altering the profile of cells residing in the lung, and that some subsets of NKT cells can have direct inhibitory activity against $CD4^+$ T cells in mouse models of allergic asthma. Importantly, our results also suggest a potential therapy for young children that might provide protection against the development of asthma.

P1997

PRMT1 contributes to the development allergic rhinitis through inducing epithelial-derived cytokines

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Allergic rhinitis is an inflammation of the nasal mucosa, induced by exposure to allergens such as pollen, animals, eggs and house dust mite, after sensitization lead to an immunoglobulin E (IgE)-mediated inflammatory response that can result in chronic disease. Protein arginine methyltransferases (PRMTs), which catalyze arginine methylation, are known to regulate a variety of biological functions. However, many studies have shown that the overexpression of these enzymes is often associated with various cancers, but the role of PRMTs has not been established in AR progression and signaling pathway yet. In this study, we examined the association between allergic rhinitis development and PRMT1 expression. In nasal tissue of AR patients and AR-induced mice, PRMT1 gene expression was significantly increased compared to healthy. Immunohistochemistry staining revealed that PRMT1 was upregulated in AR-induced mice nasal tissue. In vitro, RPMI 2650 cells, human nasal epithelial cell line, were treated with HDM and/or IL-4, and showed that PRMT1 highly induced by real-time PCR and western blot. Immunofluorescent analysis showed that PRMT1

DOI: 10.1002/eji.201970400

levels were upregulated in nucleus. In addition AR-induced PRMT1 haploinsufficiency ($PRMT1^{+/-}$) mice indicated that inflammation alleviation, significant decrease HDM-specific immunoglobulins in serum, and reduction of Th cytokines in nasal lavage (NAL) fluid compared to AR-induced wild type mice. These results indicate that PRMT1 is involved in AR disease progression and inflammation development.

P1998

SOX18, PROX1, and COUP-TFII are involved in asthma pathogenesis

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Background: Asthma characterized by airway hyperresponsiveness, increased inflammatory cells, and fibrosis and angiogenesis. SRY-related HMG-box 18 (SOX18), PROX1, and COUP-TFII is an important transcription factor involved in the development of cardiovascular and lymphatic vessels during embryonic development and wound-healing processes.
Objective: In this study we aimed to elucidate the role of SOX18, PROX1, and COUP-TFII in the pathogenesis of bronchial asthma.
Methods: Using an established mouse model of ovalbumin (OVA)-induced chronic allergic asthma, we investigated whether SOX18 is involved in pathogenesis of asthma. Airway hyperresponsiveness (AHR) was measured and bronchoalveolar lavage fluid was collected, lung tissue was processed for protein and RNA, and hematoxylin and eosin stain. Collagen was measured by trichrome stain and sircol assay. SOX18 level checked in lung human microvascular endothelial cells (HMVEC-L) and normal human bronchial epithelial (NHBE) cells treated with house dust mite (HDM). Moreover, we observed SOX18 levels in blood from asthmatic patients between stable and exacerbated state.
Results: The chronic asthma mice showed that SOX18, PROX1, COUP-TFII, mucous gland hyperplasia and collagen deposition in lung tissue were significantly increased after OVA challenge. SOX18 protein in HMVEC-L and NHBE cells was increased following HDM treatment. PROX1 and COUP-TFII protein increased in NHBE cells following HDM treatment. SOX18 in blood from exacerbated asthmatics was increased compared with those from stable asthmatics.
Conclusion: These results suggesting that SOX18, PROX1, and COUP-TFII be involved in asthma pathogenesis.
Key Words: SOX18, bronchial asthma, signal pathway

P1999

Increased ratio of regulatory T cells/t lymphocyte in esophageal tissue of patients with eosinophilic esophagitis in comparison to gastroesophageal reflux disease and control groups

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Introduction: Eosinophilic Esophagitis (EOE), is a primarily polygenic allergic disorder, involving mechanisms that fall between pure IgE-mediated and delayed Th2 responses.
There is an eosinophil-rich inflammation in the absence of known causes for eosinophilia. There is a strong correlation between allergy and EOE. Regulatory T cells may have an important role in allergies and eosinophilia. There are limited data on the association of Treg and EOE. In this study, we enumerated and compared Tregs in patient with EOE, GERD and normal controls.
Objectives: Any pediatric patient who was evaluated by endoscopy due to persistent vomiting, persistent abdominal pain, dysphagia, or failure to thrive was included in the

DOI: 10.1002/eji.201970400

study. Our population was divided into 3 groups: EOE(n=10), GERD(n=10), control group(n=8). We studied FOXP3 and CD3 markers by immunohistochemistry assay. Tregs were identified as FOXP3+, CD3+ cells and Tcells considered as CD3+ cells.
Results: Tregs, and Tcells were counted in HPF (×400) for 28 patients and the average of 10 HPF was recorded. the number of Treg in esophageal tissue of patient with EOE(mean 10.90 cells/HPF) was significantly more than the GERD(mean 2.77 cells/HPF) and control groups(mean 0.37 cells/HPF) (P value < 0.001).
Conclusion: There is an increase in number of esophageal regulatory T cells of EOE patient in comparison to GERD and control groups.

P2000

Obesity is associated with allergic responses and behavioural changes

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Along with allergic diseases, obesity is a serious health problem that is remarkedly increasing in prevalence worldwide. Obesity has been recognized as an important risk factor for enhanced allergic diseases, such as asthma. However, the causal link between obesity and allergy, as well as their impact on behaviour, has not been fully elucidated.
4-5-week-old C57BL/*6* mice have been fed on high-fat- (HFD) or standard chow diet (SD) for 9 weeks followed by immunization and challenge with ovalbumin. Metabolic parameters: glucose, leptin, amyloid P and insulin were measured in serum. Immunological analyses consisted of allergen-specific antibody detection in sera, characterization of immune cell populations and cytokines in bronchoalveolar lavage, lung, bronchial lymphoid nodes, spleen, and intestine. Airway hyperresponsiveness was assessed by plethysmography. Behavioural changes were analysed by using tail suspension-, forced swim-, light/dark- box and fear conditioning tests.
Compared to SD, HFD feeding resulted in hyperglycaemia and increased body weight in C57BL/*6* mice. Levels of leptin and insulin were higher in obese compared to lean mice, while amyloid P was higher in allergic mice. Sensitization and challenge with OVA led to increased levels of allergen-specific antibodies and Th2 cytokines in both HFD and SD fed mice. Interestingly, not only HFD feeding but also the induction of allergy was associated with changes in behaviour.
Further evaluation of the impact of HFD on immunological and behavioural parameters is ongoing. The understanding of crosstalk: obesity - allergy - behaviour, may lead to the identification of novel approaches to combat morbidities associated with Westernized lifestyle.

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DOI: 10.1002/eji.201970400

LB124

mRNA sequencing of *A.sieversiana* pollen and efficacy and safety evaluation of PUMCH® allergen extracts of *A.sieversiana* pollen

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Purpose: To study the protein and mRNA sequence of *Artemisia sieversiana* pollen sensitizing allergen and evaluate the efficacy and safety of PUMCH® allergen extracts of *A.sieversiana* pollen used for skin prick test (SPT) in the diagnosis of *A.sieversiana* pollen allergy.
Methods: mRNA sequencing of *A.sieversiana* pollen was performed on Illumina II High-throughput Sequencing Platform and PE150 Sequencing Strategy. 1026 subjects were prescribed *A.sieversiana* SPT and specific IgE (sIgE) test and diagnosed as allergic diseases before, using PUMCH® allergen extracts of *A.sieversiana* pollen. Receiver operating characteristic curve (ROC) analysis was performed according to the results of sIgE detection. The accuracy of the injection was evaluated under different cutoff values. Adverse events after SPT were recorded to evaluate the safety.
Results: Seven new pollen sensitizing allergen of *A.sieversiana* were sequenced, which were highly similar to known *A.sieversiana* major allergenic proteins. Moreover, there were 1 026 and 992 subjects in save set (SS) and full analysis set (FAS), respectively. The area under the curve (AUC) of FAS and PPS were 0.901 and 0.901, respectively in ROC analysis. According to ROC of PPS, the best and 95% specificity diagnostic cutoff values of *A.sieversiana* pollen SPT mean wheal diameter (MWD) were 5.25 mm and 5.75 mm, respectively. Only eight subjects showed adverse events. No severe adverse event occurred.
Conclusions: SPT was highly recommended in clinical diagnosis of *A.sieversiana* pollen allergy using PUMCH® allergen extracts of *A.sieversiana* pollen because of its good accuracy and safety.
Keywords: *Artemisia sieversiana* pollen; allergy; skin prick test; high-throughput sequencing

P2724

The AhR-PAH axis disturbs Th17/Treg balance through metabolic and epigenetic mechanisms in a model of asthma

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Epidemiological evidence has indicated that the exposure of ambient air particulate matter 2.5 (PM2.5) and its associated PAHs aggravates asthma. However, the underlying mechanisms have not been fully delineated. Here, we report that PM2.5 and one of its major components, PAH, impaired anti-inflammatory Treg differentiation, in an （aryl hydrocarbon receptors ）AhR-dependent manner, but promoted pro-inflammatory Th17 differentiation, thereby aggravating asthma. Mechanistically, PM2.5 and a prominent PAH associated with ambient PM2.5, Indeno[1,2,3-cd]pyrene (IP), up-regulated Got1 expression through AhR, which results in accumulation of a metabolite, R-2-HG. Accumulated R-2-HG, in turn, inhibited TET2 activity, resulting in DNA CpG hypermethylation in the Foxp3 locus, thereby impairing Treg differentiation. In contrast, exposure to PM2.5 or IP enhanced glycolysis of CD4+ T cells and promoted Th17 differentiation through up-regulating HIF-1α expression via AhR. Further, selective inhibition of Got1 with an inhibitor, AOA, ameliorated asthma by reprograming the differentiation of Th17 to Treg cells. Our findings provided novel insights into the molecular mechanism through which exposure to PM2.5 and PAH, aggravated asthma severity and provided new therapeutic strategy for asthma treatment.

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DOI: 10.1002/eji.201970400

P2725

Immunologically confirm the dapsone hypersensitivity syndrome by in vivo and in vitro tests

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Background: Diagnosis of the dapsone hypersensitivity syndrome (DHS) is difficult, and the utility of in vivo patch testing and in vitro enzyme-linked immunospot (ELISpot) and lymphocyte transformation test (LTT) in DHS remains ill-defined.
Objectives: The aim of the study was to prove whether in vivo and in vitro tests are promising for the diagnosis of DHS.
Patients and methods: 16 patients with DHS and 6 healthy controls were recruited. In vivo patch testing was performed with dapsone at a range of concentrations. Kinetics of multiple cytokines in supernatant of peripheral blood mononuclear cells (PBMCs) stimulated by dapsone or dapsone hydroxylamine were measured using electrochemiluminescense (ECL) on a platform from MesoScale Discovery (MSD). The release of drug specific cytokines (IFN-γ and IL-5) was measured using a novel modified ELISpot. The proliferation of T cells to drugs in vitro was measured using LTT.
Results: Cytokine profiles analyses showed that IFN-γ, IL-5 and IL-13 production stimulated by drugs arise at 4 days and peak at 8 days in patients, which was absent in control subjects. In 16 patients with DHS, 7 (43.75%), 12 (75%) and 10 (62.5%) patients were positive on patch testing, IFN-γ/IL-5 ELISpot and LTT, respectively. When combination of in vivo and in vitro tests, the sensitivity increased to 93.75% (15/16) with the specifity of 100%.
Conclusion: We demonstrate cytokine profiles serve as a promising tool for exploring the biomarkers of DHS, and combination of in vivo and in vitro tests could increase the sensitivity in diagnosing DHS.

P2726

Conformational and immunological changes of bovine allergen β-lactoglobulin after aggregation by metal ions

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β-lactoglobulin is the major allergen in cow's milk and about 82% of milk allergy patients are sensitive to β-lactoglobulin. The conformation and allergenicity of β-lactoglobulin are closely related to its exposed epitopes which will be changed after aggregation in different condition such as temperature, time, and pH value; however, little information is available on the metal irons to alter conformation and thus change the allergenicity of β-lactoglobulin. In this study, we investigate the structure and allergenicity of β-lactoglobulin aggregates by Mg^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+} treatment. Physiochemical properties of β-lactoglobulin aggregations were monitored by reducing and non-reducing SDS-PAGE, Differential scanning calorimetry, Fourier transform infrared spectroscopy and fluorescence intensity spectra. It showed that the dimer treated by Mg^{2+}, Zn^{2+}, Ca^{2+} conditions was assembled by disulfide bind, but β-lactoglobulin dimer treated by Cu^{2+} was linked by disulfide bind and non-disulfide bond. In addition, Mg^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+} could protect secondary structure of β-lactoglobulin and change the protein conformation. Moreover, a mouse model was set up to evaluate the allergenicity of different form (aggregation and dimer) β-lactoglobulin induced by Cu^{2+}. The results showed that the special IgG, IgG_1 and mMCP-1 levels in mice serum sensitized by β-lactoglobulin aggregates, β-

lactoglobulin dimer were higher than non-ions β-lactoglobulin. Histamine level in plasma and the levels of IL-4, IL-5, IL-13 in serum from mice sensitized by β-lactoglobulin aggregates and β-lactoglobulin dimer decreased significantly and IFN-γ increased by β-lactoglobulin dimer incitation. It is suggested that the sensitization of β-lactoglobulin reduced after the Cu^{2+} treatment.

P2727

Early-life LPS exposure inhibits the development of OVA-induced allergic airway inflammation in BALB/c mice by attenuating CCL20 and GM-CSF production from airway epithelial cells

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Background: It has long been appreciated that microbial exposure in early-life reduces the risk of developing allergic diseases. However, the underlying mechanisms remain elusive.
Objective: To investigate the effects of early-life LPS exposure on the development of OVA-induced allergic airway inflammation in BALB/c mice.
Methods: Neonatal BALB/c mice (< 1 week of age) were exposed to 100 ng LPS (i.n.) every other day for 2 weeks. Three weeks later, these mice were sensitized with OVA-Alum on days 0 and 7, then challenged with OVA aerosols on days 14-19. The airway inflammation was analyzed 24 hours after the final OVA challenge. The infiltration of inflammatory cells in the lung were analyzed by FACS and the levels of cytokines in the BALF were analyzed by ELISA.
Results: Early-life LPS exposure significantly decreased airway eosinophilia, goblet cell hyperplasia, Th2 cytokines (IL-4/IL-5/IL-13) production in the BALF and the levels of IgE and IgG1 in the serum. Compared with OVA/OVA group, LPS exposure resulted in lower levels of chemokines (CCL-20 and GM-CSF) in the BALF, accompanied with decreased number of CD11b+cDC2s.
Conclusion: Our results demonstrated that early-life LPS exposure significantly attenuates OVA induced allergic airway inflammation. The underlying mechanisms are related to reduced production of chemokines (CCL-20 and GM-CSF) from epithelial cells, which in turn decreases the migration of cDC2s, the most important antigen presenting cells in OVA-induced allergic asthma model.
Keywords: LPS, early-life, allergic airway inflammation, CCL-20/ GM-CSF

P2728

Detection of basophils-bound s-IgE/t-IgE to predict the severity of clinical symptoms in allergic rhinitis patients sensitized to house dust mite

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Background: Allergic rhinitis (AR) is a common allergic disease, characterized by IgE-mediated inflammation reaction. As the hallmark of allergic reaction, allergen-specific serum IgE (s-IgE) is routinely measured for diagnosis. However, the impact of serum s-IgE levels on symptom severity is still a matter of debate. This study aimed to establish a method to detect basophils-bound s-IgE/t-IgE and evaluate the correlation between serum s-IgE/t-IgE, basophils-bound s-IgE/t-IgE, FcεRI on basophils, basophil activation and AR symptom severity.
Methods: Serum s-IgE and t-IgE were measured by allergen specific IgE(sIgE) antibody quantative detection kit. The basophils-bound s-IgE and t-IgE were labeled by Der p-biotin-streptavidin-FITC and anti-human IgE-FITC, respectively, and detected by flow cytometry. FcεRI expressed on basophils and

DOI: 10.1002/eji.201970400

basophil activation was also measured by anti-FcεRI antibody and anti-CD63. AR symptom severity was assessed by visual analogue scale (VAS).
Results: From Nov 2016 to Jun 2017, a total of 44 AR patients sensitized to house dust mite were enrolled in our study. A moderate correlation exists between the serum s-IgE and basophils s-IgE (R^2=0.43, P=0.000), serum t-IgE and basophils t-IgE (R^2=0.48, P=0.000), respectively. Basophils-bound IgE has a superior correlation with clinic symptoms than serum IgE, however less than FcεRI level and basophil activation.
Conclusion: We successfully established the method to detect the IgE on basophils and explored the possibility of serum IgE, basophils-bound IgE, FcεRI on basophils and basophil activation as a reliable biomarker for symptom severity in AR patients.

P2729

Plasmacytoid dendritic cell deficiency in neonates enhances allergic airway inflammation via reduced production of IFN-α

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Background: Allergic asthma often originates in childhood, affecting around 5-10% of school-aged children. Immune responses at early age exhibit a Th2 bias, but the precise mechanism remain elusive. Plasmacytoid dendritic cells (pDCs) which play a regulatory role in allergic asthma were shown to be deficient in neonatal mice.
Objective: To elucidate how pDCs deficiency contributes to the development of allergic airway responses in neonates.
Methods: Neonatal and adult C57BL/6 mice were sensitized with OVA-Alum on days 0 and 7, then challenged with OVA aerosols on days 14-19. The allergic airway inflammation was analyzed 24 hours after the final OVA challenge.
Results: The deficiency of pDCs renders neonates more susceptible to severe allergic airway inflammation than adult mice in OVA-induced asthma model. Adoptive transfer of pDCs or administration of IFN-a to neonatal mice prevented the development of allergic inflammation in wildtype but not IFNAR1$^{-/-}$ mice. In turn, adult mice developed more severe allergic inflammation when pDCs were depleted. The protective effects of pDCs were mediated through the negative regulation of pDCs/IFN-α on the secretion of epithelial cell-derived CCL20, GM-CSF and IL-33, which in turn, impaired recruitment of cDC2 and ILC2 to the airways. In asthmatics, the percentage of pDCs and the level of IFN-α were lower in children than in adult patients.
Conclusion: These results identify that the impairment of pDCs/epithelial cell crosstalk in neonates is a susceptibility factor for allergic asthma.
Keywords: Allergic asthma, Plasmacytoid dendritic cells, Neonate, Airway epithelial cells, IFN-α

P2730

1, 25(OH)$_2$VD$_3$ supplementation reduces production of the endogenous danger signals in neonatal mouse allergic asthma model

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Background: There has been increasing interest in the possible link between vitamin D deficiency and childhood asthma. However, the role of vitamin D in childhood allergic asthma and the underlying

DOI: 10.1002/eji.201970400

mechanisms remain elusive.
Objective: To elucidate the role of 1,25$(OH)_2VD_3$(VD), the biologically activated form of vitamin D, on airway inflammation in neonatal allergic asthma model.
Methods: Neonatal and adult BALB/c or C57BL/6 mice were sensitized with OVA-Alum on days 0 and 7, then challenged with OVA aerosols on days 14-19, VD was administrated *i.p*. either during sensitization phase or challenge phase. The airway inflammation was analyzed 24 hours after the final OVA challenge.
Results: The effects of VD treatment varied depending on not only the dose and the timing of VD exposure but also the age and the genetic background of the mice. At a suitable dose, airway eosinophilia, goblet cell metaplasia, mucus production, Th2 cytokines in the BALF and the level of IgE in the serum were inhibited when VD was given in both age groups. In B6 neonatal model, VD treatment during challenge phase resulted in significantly lower level of IL-33, accompanied with reduced numbers of ILC2s. VD treatment during sensitization phase resulted in significantly lower level of uric acid in the peritoneal lavage.
Conclusion: Our results demonstrated that VD supplementation during sensitization phase or challenge phase significantly attenuates OVA induced allergic airway inflammation in neonatal mice. The underlying mechanisms are related to reduced production of endogenous danger signals.
Keywords: 1,25$(OH)_2VD_3$, allergic airway inflammation, IL-33, Uric acid.

P2731

HMGB1-induced ILC2s activate dendritic cells by producing IL-9 in mice asthma

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Asthma is a heterogeneous chronic inflammatory disease due to immune disorder, in which the activated T cells are predominantly of the T-helper 2 (Th2) cytokine phenotype. Previous reports have shown that Group 2 innate lymphoid cells (ILC2s) and IL-9 are associated with Th2-mediated immune inflammatory injury in asthma. However, the relationship between ILC2s and IL-9 and the immunopathological mechanism involved in asthma remain unclear. In this study, we analyzed the effect of HMGB1 on the activity of ILC2s in view of the high expression of HMGB1 in asthma, detected the levels of cytokines including IL-9 secreted by ILC2s and its effect on DCs in vitro. Our data showed that the HMGB1 activated ILC2s and promoted the production of IL-9, which could be inhibited by HMGB1 A-box. HMGB1-stimulated ILC2s promoted BMDCs activation by co-culturing with them, but this effect was lost in the presence of anti-IL-9. Simultaneously, BMDCs activated by IL-9 contributed to the progress in differentiation of naïve T cells into Th2 cells. Further in vivo experiments in asthmatic mice indicated that IL-9-activated BMDCs could aggravate the pathological changes of asthma. In conclusion, HMGB1-induced ILC2s activate dendritic cells by producing IL-9 which can promote the differentiation of Th2 cells thus exacerbating asthma.

P2732

Inhibition of food allergy in murine model by delivery of modified T cell epitopes

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DOI: 10.1002/eji.201970400

Background and objective: CD4+ T cell epitopes may offer substantial advantages over treatment with native allergen because of the reduced potential for cross-linking IgE bound to the surface of mast cells and basophils. This study aims to alleviate intestinal allergy in a mouse model with a recombinant protein consisting of scFv DEC and OVA T cell epitope.
Methods: In this study we prepared a recombinant protein composed of scFv DEC and OVA T cell epitope, investigated its effect on induction of tolerogenic dendritic cells and addressed its mechanism of inhibition upon OVA-induced food allergy in mice.
Results: We screened an advantage OVA T cell epitope-IC-15, and prepared a recombinant protein scFv DEC-IC(sDI) with immunogenicity. sDI showed good binding ability with DEC205+DCs. sDI pulsed DCs inhibited the proliferation of OVA-specific CD4+ T cells in vitro and in vivo. Treatment with sDI before sensitization and challenge alleviated the inflammation in the intestine, and down regulated the production of OVA-specific -IgE, -IgG1, and -IgG2a, and reduced the infiltration of eosinophils in the intestinal mucosa.
Conclusion: This study provides evidence that the recombinant protein sDI can inhibit intestinal allergy by induction of tolerogenic dendritic cells.
Keywords: food allergy; ovalbumin; T cell epitope; DEC205; dendritic cells

P2733

Allergen specific immunotherapy (SIT) restores the frequency and function of epidermal I-A$^+$ inflammatory dendritic cells in atopic dermatitis mice

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Allergen specific immunotherapy (SIT) can change the T-cell pattern to rescue atopic dermatitis (AD). Langerhans cells and the large amounts of inflammatory dendritic epidermal cells in AD skin are important regulators of T-cell activity. Reports on if SIT could work on cutaneous dendritic cells to slow down the disease are lacking. Because of the shortage of biopsy specimen from SIT patients, we evaluated the influence of SIT on dendritic cell subsets in a BALB/c mouse model. AD was induced with 1-Fluoro-2,4-dinitrobenzene and extracts of dust mite. Subcutaneous immunotherapy was carried out by Novo-Helisen-Depot (NHD; Allergopharma GmbH & Co.KG, Germany). SIT relieved the AD symptoms. Compared with the AD group, we observed a decreased frequency of I-A$^+$ inflammatory dendritic cells (1.487 ± 0.35 % vs 2.26 ± 0.33%, p=0.1623) and I-A$^+$ Langerhans cells (2.89 ± 0.27% vs 3.82 ± 0.34%, p=0.0501) in epidermis of SIT group. The costimulatory molecule CD86 was also down regulated in epidermal I-A$^+$Langerin$^+$ cells (16.13 ± 2.13% vs 32.38 ± 4.38%, p=0.0036) from SIT mouse. And expression of I-A^{high} in CD11c$^+$Langerin$^+$ cells in draining lymph node of SIT mouse was also decreased (18.8 ± 0.38% vs 24.8 ± 1.01%, p=0.0051). Frequency of chemokine receptor CCR2 and CXCR4 on epidermal I-A$^+$Langerin$^+$ cells in SIT group was close to the untreated normal group, while it was decreased in AD group. In this study, we demonstrate that SIT may help re-establish a more balanced immune response in part by restoring the dendritic cell capacity in skin.

P2734

T_H22 cells from Han Chinese patients with atopic dermatitis exhibit high expression of ICOS

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Atopic dermatitis (AD) is a heterogeneous disease, characterized by excess TH22 activation in Asian AD. Inducible T cell co-stimulator (ICOS) is crucial for T-cell activation and differentiation. The role of ICOS in AD and its effect on TH22 T cells remain unclear. We quantified ICOS and ICOSL expression

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DOI: 10.1002/eji.201970400

in Han Chinese AD patients and healthy controls (HC). We assessed the proliferation and the production of the TH22 chemokines CCR4 and CCR10 by ICOSL-stimulated AD PBMCs as well as their effects on keratinocyte filaggrin production. Finally, we explored the link between ICOS-expressing TH22 cells and disease activity and IgE levels in our AD patients. Our AD patients showed higher levels of ICOS-expressing TH22 cells as well as ICOSL-expressing CD19+ B cells and CD14+ monocytes as compared to HC. ICOSL increased the proliferation and the expression of CCR4 and CCR10, and of IL-22 in AD PBMCs. ICOSL-treatment also significantly increased the down-regulation of filaggrin expression by keratinocytes co-cultured with PBMCs from AD patients. Finally, blood levels of ICOS+ TH22 cells and ICOSL+ B cells in this AD cohort were correlated with disease activity as assessed by SCORAD and with total IgE levels. In Han Chinese AD patients, circulating TH22 cells, serum levels of IL-22, and IL-22-positive cells in lesional skin were all markedly increased. Our findings demonstrate that ICOS/ICOSL expression and effects are linked to TH22 skewing and the pathogenesis of Han Chinese AD, which suggests ICOSL and ICOS as well as TH22 cells and IL-22 as new and promising therapeutic targets.

P2735

Raf kinase inhibitor protein negatively regulates FceRImediated mast cell activation and allergic response

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The signaling cascades triggered by the cross-linkage of immunoglobulin E (IgE) with its high-affinity receptor (FceRI) on mast cells contribute to multiple allergic disorders, such as asthma, rhinitis, and atopic dermatitis. Restraint of intracellular signals for mast cell activation is essential to restore homeostasis. In this study, we found that Raf kinase inhibitor protein (RKIP) negatively regulated mast cell activation. RKIP-deficient mast cells showed greater IgE−FceRI-mediated activation than wild-type mast cells. Consistently, RKIP deficiency in mast cells rendered mice more sensitive to IgE−FceRI-mediated allergic responses and ovalbumin-induced airway inflammation. Mechanistically, RKIP interacts with the p85 subunit of PI3K, prevents it from binding to GRB2-associated binding protein 2 (Gab2), and eventually inhibits the activation of the PI3K/Akt/NF-κB complex and its downstream signaling. Furthermore, the expression of RKIP was significantly downregulated in the peripheral blood of asthma patients and in the IgE−FceRI-stimulated mast cells. Collectively, our findings not only suggest that RKIP plays an important role in controlling mast cell mediated allergic responses but also provide insight into therapeutic targets for mast cell-related allergic diseases.

Autoimmune Disease

O001

Understanding the mechanisms of systemic lupus erythematosus through comprehensive analyses of genetic, transcriptomic, and epigenetic data

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Systemic lupus erythematosus (SLE) is a prototype autoimmune disease with extreme clinical and mechanistic heterogeneity. Genetics play vital roles in the disease pathogenesis and there are significant differences between different major ethnicities in disease prevalence, average onset age,

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DOI: 10.1002/eji.201970400

and severity. Understanding the disease mechanisms and the mechanisms underlying individual differences is crucial for developing targeted treatment of the disease and precision medicine in SLE in terms of diagnosis, treatment and prognosis of the disease. In a recent effort to identify susceptibility genes for SLE, we have assembled the largest sample collection on Han Chinese. Through genome-wide association studies, we identified 40 new susceptibility loci for SLE reaching genome-wide significance. Interestingly, a number of these loci showed clear specificity for Chinese populations despite the fact that there is adequate power in Europeans cohorts. The shared loci between Chinese and European ethnicities for SLE showed close correlation in their selection signature measured by iHS(the integrated haplotype Score), while the loci with ethnic specificity demonstrated little correlation in their selection signature. The differences in susceptibility loci suggest that there might be differences in the disease mechanisms between major ethnicities. We present results on a comprehensive analysis of the susceptibility genes by integrating data on eQTL, gene expression profiles, cell specific open chromatin and histone markers for enhancers, and differential DNA methylation in SLE patients compared to that of controls. Analyses of multi-omics data allow us to have a better understanding of disease mechanisms and the mechanisms of genetic associations.

O002

SUMO-defective c-Maf preferentially transactivates Il21 to exacerbate autoimmune diabetes in NOD mice

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The conjugation of small ubiquitin-like modifier (SUMO) to specific lysine residues of cellular proteins (SUMOylation) is a dynamic and reversible posttranslational modification and is involved in the development of several inflammatory diseases, but the physiological significance of SUMO-modulated transcription factor c-Maf in autoimmune diabetes is not completely understood. Here, we report that an age-dependent attenuation of c-Maf SUMOylation in $CD4^+$ T cells is positively correlated with the IL-21-mediated diabetogenic process in NOD mice. Using two strains of T cell-specific transgenic NOD mice overexpressing wild-type c-Maf (Tg-WTc) or SUMOylation site-mutated c-Maf (Tg-KRc), we demonstrated that Tg-KRc mice developed diabetes more rapidly than Tg-WTc mice in a $CD4^+$ T cell-autonomous manner. Moreover, SUMO-defective c-Maf preferentially transactivated *Il21* to promote the development of $CD4^+$ T cells with an extrafollicular helper T (Tefh) cell phenotype and expand the numbers of granzyme B-producing effector/memory $CD8^+$ T cells. Furthermore, SUMO-defective c-Maf selectively inhibited recruitment of Daxx/HDAC2 to the *Il21* promoter and enhanced CREB-binding protein (CBP)/p300-mediated histone acetylation. Using pharmacological interference with CBP/p300 as a therapeutic strategy, we illustrated that CBP30 treatment ameliorated c-Maf-mediated/IL-21-based pathogenesis in autoimmune diabetes. Taken together, our results show that the SUMOylation status of c-Maf has a stronger regulatory effect on IL-21 than the c-Maf level of expression through an epigenetic mechanism. These findings provide new insights into how SUMOylation modulates the pathogenesis of autoimmune diabetes in a T cell-restricted and single transcription factor-based manner.

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DOI: 10.1002/eji.201970400

O003

Identification of a pathogenic plasma cell subset in the development of lupus nephritis

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by anti-nuclear autoantibodies secretion, immune-complex formation and multiple organ damages. Autoreactive plasma cells (PCs) are the major source of anti-nuclear autoantibodies and play pivotal roles in local autoimmune inflammation during renal damages, yet the phenotypic characteristics of autoreactive PCs have remained unknown. In this study, PC subsets in peripheral blood and renal tissue of SLE patients and lupus mouse models were examined and within the compartment of $CD20^-$ $CD38^+CD27^+CD138^+$ PCs, a marked expansion of $TLR4^+CXCR4^+$ PC subsets in peripheral blood and damaged kidney were found with the potent production of anti-dsDNA IgG, which were associated with severe renal damages in SLE patients and lupus mice. Adoptive transfer of sorting-purified $TLR4^+CXCR4^+$ PCs from lupus mice led to autoantibody production and glomerulonephritis development in *Rag2*-deficient recipients. High expression of Blimp-1 and low expression of Ki-67 were observed in $TLR4^+CXCR4^+$ PC subsets. Moreover, longer lifespans of $TLR4^+CXCR4^+$ PCs were also revealed by BrdU incorporation compared with $TLR4^-CXCR4^-$ PC counterparts, indicating that $TLR4^+CXCR4^+$ PC subsets were long-lived plasma cells (LLPCs). In culture, $TLR4^+CXCR4^+$ PCs from lupus mice and SLE patients treated with TLR4 inhibitor showed reduced anti-dsDNA IgG secretion and accelerated cell death. *In vivo* administration of TLR4 inhibitor in lupus mice could significantly attenuate autoantibody production and renal damages as well. These findings demonstrate a pathogenic role of autoreactive $TLR4^+CXCR4^+$ PCs in the development of lupus nephritis and may provide new therapeutic strategies for the treatment of SLE.

O004

Autoimmune hepatitis in mice is driven by activation of autoreactive CD4 T cells in the liver and formation of ectopic lymphoid tissue

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The mechanisms behind the loss of tolerance to liver antigens in autoimmune hepatitis (AIH) are not clear. We generated a mouse model of AIH that is characterized by hepatocellular expression of an MHC class II-restricted CD4 T cell epitope of lymphocytic choriomeningitis virus (GP61-80) under control of the albumin promoter, and by abundance of cognate CD4 T cells recognizing GP61-80. In these mice, autoreactive T cells were not deleted in the thymus, due to antigen ignorance, but accumulated in the liver. Accordingly, these mice spontaneously developed hepatitis with typical disease features of human AIH, including elevated IgG (4.0 vs. 0.9 mg/ml, p=0.0159), elevated serum transaminases (593.3 vs. 60.8 U/l, p=0.0325), antinuclear autoantibodies in 76% of the mice (titer>1:80, p=0.005), and lymphocytic periportal infiltrates with interface hepatitis (mHAI score 14/18, p=0.0079). AIH development was facilitated by pathogenic maturation of autoreactive CD4 T cells towards IFNg and TNF co-producers (38.3% vs. 4.2% of hepatic CD4), which seemed to occur within ectopic lymphoid structures in hepatic portal fields that transiently formed during the preclinical disease stage, showing the typical segregation of T and B cell zones, dendritic cell networks and high endothelial venules. In advanced disease, these lymphoid structures had disaggregated and merged into large, dispersed infiltrates. Our findings indicate that AIH pathogenesis is driven locally in the liver by pathogenic maturation of previously ignorant autoreactive CD4 T cells. Transient formation of

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DOI: 10.1002/eji.201970400

ectopic lymphoid tissue in the portal fields may be a relevant feature of early pathogenesis also in human AIH.

O005

Molecular analysis of the anti-citrullinated protein B-cell response indicates aberrant selection processes during autoreactive B-cell development

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Anti-citrullinated protein antibodies (ACPA) hallmark the most disease-specific autoimmune response in Rheumatoid Arthritis (RA). Despite the high prevalence of these antibodies in RA patients, the mechanism by which ACPA-producing B cells escape immune checkpoints is enigmatic. B-cell receptor (BCR) analysis and single-cell mRNA (scRNA)-sequencing were performed on ACPA-expressing B cells isolated from peripheral blood of patients with RA, to elucidate the molecular basis for the aberrant selection processes during autoreactive B-cell development.
Intriguingly, 82% of ACPA-IgG, 67% of ACPA-Igκ and 47% of ACPA-Igλ contained ≥1 *N*-glycosylation sites in the variable domain, compared to less than 15% in a healthy donor (HD) BCR repertoire. The distribution of these sites in ACPA-IgG and ACPA-Igκ was similar, with most sites located in framework region (FR)3 (42% and 49%, respectively). In contrast, only 7% of all *N*-glycosylation sites in ACPA-Igλ were located in FR3, while 65% were located in FR1. Computational modelling of the germinal center reaction (CLONE algorithm) using germline counterparts of ACPA-IgG heavy chain sequences generated a pattern of *N*-glycosylation sites comparable to HD IgG. Together, these data indicate that *N*-glycosylation sites in ACPA-IgG might be involved in the selection and survival of ACPA-expressing B cells. In addition, transcriptome analysis based on scRNA-sequencing showed differential expression of several genes, which might play a role in B-cell selection and subsequent development of this autoreactive B cell response.
In conclusion, BCR sequence and gene expression analysis by scRNA-sequencing revealed novel insights in the selection processes by which autoreactive, ACPA-expressing B cells escape immunological checkpoints.

O006

CD39 identifies a new subpopulation of $CD8^+CD103^+$ induced T regulatory cells that display a potent therapeutic effect on lupus nephritis

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DOI: 10.1002/eji.201970400

Many of patients with systemic lupus erythematosus (SLE) suffer from lupus nephritis, one of the severe complications of SLE. We previously reported that CD8+CD103+ T regulatory cells induced *ex-vivo* with TGF-β and IL-2 (iTregs) inhibited T cells and B cells responses to ameliorate excessive autoimmune inflammation for treating autoimmune diseases. However, the molecular mechanism(s) underlying the role of CD8+CD103+ iTregs is unclear. Here, we identified that CD39, which is highly expressed on CD8+ iTregs, crucially contributes to the immunosuppressive role of the CD8+CD103+ iTregs. We showed that adoptive transfer of CD8+CD103+ iTregs significantly relieves the chronic graft-*versus*-host disease model of lupus nephritis and CD39 inhibitor mostly abolished the functional activities of these CD8+ Tregs *in vitro* and *in vivo*. CD39+ cells sorted from CD8+CD103+ iTregs were more effective in treating lupus nephritis than CD39- partner cells *in vivo*. In addition, we found that percentages of circulating CD8+CD103+CD39+ T cells were significantly lower in patients with SLE than in healthy controls. Furthermore, human CD8+ induced Treg cells also displayed increased CD103 and CD39 expression, and CD39 was also involved in the suppressive function of human CD8+ iTregs. Thus, our data implicate a crucial role of CD39 in CD8+CD103+ iTregs in treating lupus nephritis, and CD39 could be a new phenotypic biomarker for the identification of highly qualified CD8+ Tregs. This subpopulation may have therapeutic potential in patients with SLE nephritis and other autoimmune diseases.

O007

Increases in Eomes-expressing Th cells in secondary progressive multiple sclerosis (SPMS) reveal patients at risk of increased disability

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Secondary progressive multiple sclerosis (SPMS) is a chronic neuroimmflammatory disease that develops in many patients following relapsing/remitting multiple sclerosis (RRMS). Whilst RRMS is recognized as an autoimmune disease of the central nervous system, the pathogenesis of SPMS is poorly understood, lacks effective therapies, and the clinical boundary for diagnosis is unclear. There is a growing acceptance that immune components play an important role in SPMS pathogenesis, rejecting the concept that SPMS is driven only by neurodegenerative mechanisms.
Using a novel mouse model, we previously reported that the cytotoxic-like CD4+ T cells expressing the transcription factor Eomes (Eomes+ Th cells) are associated with chronic CNS autoimmune disease (Raveney *et al*. Nat. Commun. 2015).
Our analysis reveals that Eomes^{+}Th cells are increased in the blood of patients with SPMS, but not RRMS. Importantly, using flow cytometry on SPMS post-mortem tissue samples, we have observed for the first time a very high proportion of Eomes+ Th cells amongst brain-infiltrating Th cells, strongly linking these unusual cells with pathogenic processes in this disease.
Strikingly, analysis of clinical features revealed that of high proportions of Eomes^{+}Th cells with particular immunophenotypes in SPMS patients was linked to actively progressing disease (p=0.0013, OR=5.7) and highly predicative of patients at risk of worsening symptoms within 1 year (PPV=0.793; NPV=0.778; FDR=0.179).. As SPMS diagnosis currently lacks effective biomarkers, our studies into Eomes+ Th cells and their functional phenotype may provide much-needed prognostic monitoring SPMS activity as well as new therapeutic targets for SPMS treatment.

DOI: 10.1002/eji.201970400

O008

IL-17 induces salivary gland secretory dysfunction *via* modulating calcium influx in primary Sjögren's syndrome

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Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by secretory dysfunction and inflammation in salivary glands (SG). Currently, the pathogenesis of SS is still unclear. Although previous studies indicated the involvement of IL-17 in SS pathogenesis, how IL-17 affects SG hypofunction remains largely unclear. In this study, we explored the roles of IL-17 in SG secretory dysfunction and the underlying mechanisms during SS development.
IL-17-producing cells were identified in labial gland biopsies from primary SS patients and the numbers were negatively correlated with saliva flow rates. Using murine experimental SS (ESS) model, we observed ameliorated disease development in IL-17 knockout (KO) and IL-17 receptor C (IL-17RC) KO mice compared with wild type (WT) controls. Moreover, WT ESS mice displayed markedly increased numbers of IL-17-producing γδT cells and Th17 cells in SG tissues and draining lymph nodes when compared with naïve mice. Notably, the chimeric mice with IL-17RC deficiency restricted in non-hematopoietic cells exhibited improved saliva secretion and ameliorated tissue inflammation when compared with WT controls. Acini from ESS mice showed decreased acetylcholine-induced calcium movement whereas IL-17 diminished the acetylcholine-induced store-operated calcium entry (SOCE) in cultured primary SG epithelial cells. Moreover, IL-17 treatment reduced expression of transient receptor potential channel 1 (TRPC1), the key ion channel for calcium influx in SOCE. Furthermore, retrograde cannulation of IL-17-neutralizing antibody into SG improved saliva secretion in ESS mice. Collectively, the results revealed the pathophysiological roles of IL-17 in SG secretory dysfunction, indicating that IL-17 might be a promising target for treating SS.

O041

Anti-polygalacturonic acid antibody (PGA-Ab) induces bone destruction in rheumatoid arthritis by promoting osteoclastogenesis mediated via integrin b5

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic erosive arthritis. Local or systemic bone destruction, which is almost exclusively mediated by increased osteoclastic activity, often leads to disability and severely reduced quality of life. Therefore, it is important to understand mechanisms regulating the generation of osteoclasts. In this study, we identified a new autoantibody, anti-polygalacturonic acid antibody (PGA-Ab), which may play a key role in osteoclastogenesis. PGA-Ab levels were significantly higher in sera of active and early RA patients than healthy people or other types of arthritis patients. The sensitivity and specificity of PGA-Ab in RA diagnosis were even better than anti-cyclic peptide containing citrulline (anti-CCP) and rheumatoid factor, and PGA-Ab was positively correlated with bone resorption index (cathepsin k and Tartrate Resistant (ACP5)). More importantly, PGA-Ab could directly induce arthritis and reduce bone mineral density of femoral trabecula in animals significantly. *In vitro* osteoclasts were cultured from peripheral blood mononuclear cells (PBMC), PGA-Ab treatment increased mature osteoclast numbers and cathepsin k or metalloproteinases releasing, AP-1 were also activated. By immunoprecipitation and protein spectrum sequencing (IP-MS), integrin beta5 (ITGB5) were identified as receptor of PGA-Ab on osteoclasts.

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DOI: 10.1002/eji.201970400

Knockdown of ITGB5 eliminated the binding of PGA-Ab with osteoclast and reduced PGA-Ab-induced osteoclastogenesis. Taken together, PGA-Ab is a new RA-related autoantibody, which has potential diagnostic value in RA and has pathogenic effect on arthritis bone erosion by inducing osteoclast differentiation through receptor integrin beta 5.

O042

Micromanaging lupus nephritis: miR-17 modulates T_{FH} development and regulatory T cell activity

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T follicular helper (T_{FH}) cell provide crucial growth signals to germinal center (GC) B cells supporting antibody production. Tight control of T_{FH} numbers maintains self-tolerance. Regulatory T (Treg) cells play a critical role in maintaining self-tolerance and controlling the magnitude of physiologic immune response. The Treg transcription factor forkhead box P3 (Foxp3) works in concert with other co-regulator molecules to determine suppressive phenotype of Treg. Compiling evidence show that aberrant T_{FH} , GC responses and deficiencies of Treg are associated with systemic lupus erythematous and autoantibody production. We induce pristane nephropathy on T cell specific miR-17-92 knockout (miR-17-92$^{-/-}$) mice. Mir17-92 CD4 T cell specific deficiency mitigates pristane induced-lupus nephropathy in mice. The mice showed less T_{FH} cells, less GC B cells and lower autoantibody formation. Consistent with the reduction in autoantibody production, histological analysis revealed a lower mean renal histopathology score and less compliment deposition. We further demonstrate that the miR-17 regulates T_{FH} development by targeting Akt pathway. Moreover, miR17-92 mitigate the suppression function of Tregs by targeting Foxp3 co-regulators. Ectopic expression of miR-17 downmodulates the suppression functions of Tregs and provides Tregs with partial effector activity via de-repression of cytokine genes. In addition, Tregs from patient with lupus nephritis and lupus mice both showed increased miR-17 expression. Our studies suggest that miR-17 modulates lupus activity through critical regulation in T_{FH} and Treg, unveiling the future therapeutic potential of microRNA manipulation in lupus nephritis.

O043

TLR-stimulated IRAKM activates caspase-8 inflammasome in microglia and promotes neuroinflammation

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DOI: 10.1002/eji.201970400

NLRP3 inflammasome plays a critical spatiotemporal role in the pathogenesis of experimental autoimmune encephalomyelitis (EAE). This study reports a mechanistic insight into noncanonical NLRP3 inflammasome activation in microglia for the effector stage of EAE. Microglia-specific deficiency of ASC (apoptosis-associated speck-like protein containing a C-terminal caspase-activation and recruitment [CARD] domain) attenuated T cell expansion and neutrophil recruitment during EAE pathogenesis. Mechanistically, TLR stimulation led to IRAKM-caspase-8-ASC complex formation, resulting in the activation of caspase-8 and IL-1β release in microglia. Noncanonical inflammasome-derived IL-1β produced by microglia in the CNS helped to expand the microglia population in an autocrine manner and amplified the production of inflammatory cytokines/chemokines. Furthermore, active caspase-8 was markedly increased in the microglia in the brain tissue from patients with multiple sclerosis. Taken together, our study suggests that microglia-derived IL-1β via noncanonical caspase-8-dependent inflammasome is necessary for microglia to exert their pathogenic role during CNS inflammation.

O044

Shift of autoimmunity by invalidation of the ICOS pathway in NOD mice : from diabetes to myositis and role of oxydative stress in target tissue

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Autoimmune-prone NOD mice represent an invaluable model of type 1 diabetes (T1D). Inducible T cell co-stimulator (ICOS) is involved in induction of helper T cell responses, T-dependent antibody responses and germinal center reaction. We investigated the consequences of ICOS invalidation on the autoimmune manifestations in NOD mice and studied myositis pathogenesis in this model. Neither *Icos*$^{-/-}$ nor *Icosl*$^{-/-}$ NOD mice developed T1D. In contrast, myositis spontaneously occurred by 28 wks in both lines with decreased grip strength, impaired cadence and death around 40 wks. Pathological muscle analysis revealed necrotic myofibers and important inflammatory infiltrates (CD4^{+} T cells, macrophages). Muscle lesions yielded T2 hypersignals in MRI that correlated with histopathology and regressed under steroid therapy. CD4^{+} T cells were Th1 biased. Myositis developed in CD8- but not CD4-deficient mice. Disease was adoptively transferred to NOD.*scid* recipients by *Icosl*$^{-/-}$ CD4^{+} T cells. Activating IL-2/anti-IL-2 complexes exacerbated myopathy. Oxydative stress was present in muscle, as attested by transcriptomic and proteomic analyses, histo-enzymology, reactive oxygen species production and evaluation of mitochondrial function. Abnormalities in mitochondria were found by electron microscopy. Anti-oxydant therapy significantly ameliorated disease. Similar oxidative stress was found in biopsies from patients with dermatomyositis.
The ICOS pathway is indispensable for T1D development in NOD mice. ICOS/ICOSL deficiency shifts autoimmunity from pancreas to muscle. Myositides are severe diseases leading to bedridden state and possibly death. This work establishes *Icos*$^{-/-}$ and *Icosl*$^{-/-}$ NOD mice as a unique paradigm of myositis. Inflammation causes oxidative stress in muscle, prompting to evaluate anti-oxydant therapy in myositides.

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DOI: 10.1002/eji.201970400

O045

Inhibition of peptidyl arginase deiminase 4 limits neutrophil extracellular trap formation and inflammation in experimental anti MPO-ANCA vasculitis

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A dysregulation of neutrophil extracellular trap (NETs) may be associated with the pathogenesis of myeloperoxidase (MPO) anti-neutrophil cytoplasmic antibody (ANCA) vasculitis. Peptidyl-arginine-deiminase-4 (PAD4) is an enzyme that facilitates generation of NETs. This study investigates the contribution of NETs in the pathogenesis of experimental MPO-ANCA associated glomerulonephritis (GN) by using PAD4 KO mice in an experimental murine model of MPO-ANCA GN. PAD4 KO mice had significant NET abrogation and were protected from glomerular injury and recruitment of inflammatory leukocytes: CD4 T cells, neutrophils and macrophages, and glomerular MPO (the autoantigen in this disease) (all, $P < 0.05$ compared to WT). We investigated the therapeutic possibility of 2 inhibitors; BB-Cl amidine (BB-CLA) a second generation pan PAD inhibitor and GSK484 a specific PAD4 inhibitor, both which inhibit the enzymatic activity of PAD4 and subsequent NET formation. BB-CLA treatment reduced glomeruli NET accumulation, segmental necrosis (marker of glomerular injury) and leukocyte infiltration with CD4 T Cells, macrophages and neutrophils significantly reduced (all, $P< 0.05$ compared to the vehicle treated group). BB-Cl-amidine treated mice had increased numbers of CD4+ FoxP3+ T regulatory cells from lymph nodes compared to the vehicle treated group ($P= 0.0012$). PAD4 inhibition with GSK484 resulted in a significant reduction in the number of glomeruli with NETs and MPO deposition was significantly reduced ($P< 0.05$). Segmental necrosis and leukocyte recruitment to the glomeruli was significantly reduced (CD4 T cells, Neutrophils and macrophages all $P< 0.05$ compared to vehicle). In conclusion, PAD4 inhibition may be of potential therapeutic benefit in MPO-ANCA vasculitis.

O046

B cells inhibit bone formation in rheumatoid arthritis by suppressing osteoblast differentiation

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The function of B cells in osteoblast (OB) dysfunction in rheumatoid arthritis (RA) has not been well-studied. Here we show that B cells are enriched in the subchondral and endosteal bone marrow (BM) areas adjacent to osteocalcin$^+$ OBs in two murine RA models: collagen-induced arthritis and the TNF-transgenic mice. Compared to their BM counterparts, subchondral BM B cells in RA mice express high levels of OB inhibitors, CCL3 and TNF, and inhibit OB differentiation by activating ERK and NF-kB signaling pathways. The inhibitory effect of RA B cells on OB differentiation is blocked by CCL3 and TNF neutralization. B cell depletion attenuates bone erosion and OB inhibition in RA mice. Deletion of CCL3 and TNF in RA B cells completely rescues OB function *in vivo*. B cells from RA patients express CCL3 and TNF and inhibit OB differentiation, with these effects ameliorated by CCL3 and TNF neutralization. Thus, B cells inhibit bone formation in RA by producing multiple OB inhibitors.

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DOI: 10.1002/eji.201970400

O047

Serum inflammatory biomarkers in neuromyelitis optica and multiple sclerosis

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Objective: Neuromyelitis optica spectrum disorder (NMOSD) is a rare and severe autoimmune demyelinating disorder that selectively influences optic nerves and the spinal cord. Detection of an autoantibody against aquaporin-4 (AQP4) helps to distinguish NMOSD from multiple sclerosis (MS), but its serum level in ~20%-40% of NMOSD patients is undetectable. Therefore, the pathogenesis in these patients is still unclear. The aim of this study was to clarify whether serum-secreted proteins could distinguish NMOSD from MS, as well as differentiate the AQP4-seropositive and seronegative NMOSD patients from each other.
Methods: Antibody microarrays screened the expression levels of 200 secreted proteins in serum from 41 NMOSD (32 cases with AQP4-seropositive and 9 cases with AQP4-seronegative), 12 MS patients and 34 healthy controls.
Results: Our study identified several serum-specific biomarkers in NMOSD patients compared with healthy controls and MS patients. Using Weighted Correlation Network Analysis (WGCNA), we mapped disease-specific molecular networks and assessed the correlations of NMOSD-specific biomarkers with AQP4 autoantibody titers and optic nerve degeneration. In addition, 23 cytokines were differentially expressed between AQP4 seronegative and seropositive NMOSD patients, with an FDR < 0.05 and absolute logFC > 0.263. Interestingly, AQP4 seronegative NMOSD patients and MS patients had 10 cytokines with significantly different expression levels in serum.
Conclusions: Our findings provide potential immunomodulatory targets, such as inflammatory mediators and growth factors, in the pathogenesis of demyelinating disorders.

O048

Senescent progenitor cell depletion as a potential therapy for hyposalivation in primary Sjögren's syndrome?

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Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized partly by salivary gland (SG) hypofunction and lymphocytic infiltration. We have previously shown that salivary gland progenitor cells (SGPCs) in pSS are likely to be senescent, which likely underlies hyposalivation. SGPCs in their niches (e.g. basal layer of the striated duct, BSD) maintain SG homeostasis by proliferation and differentiation. Here we further characterize the extent of SGPC senescence in pSS.

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DOI: 10.1002/eji.201970400

It is shown that the proportion of BSD cells expressing the senescence marker p16 significantly increases in incomplete pSS (an earlier stage of pSS) and pSS patients, compared to non-pSS sicca control patients. A trend for enhanced p16 mRNA expression is observed in incomplete pSS patients, and significantly increases in pSS patients. Correlation analysis and Bland-Altman plot agreement show significant correlation and agreement between $p16^+$ BSD cells and stimulated parotid saliva, unstimulated whole saliva, leukocytic infiltration and the ACR-EULAR pSS disease score. Incomplete pSS and pSS SGs both show enhanced senescence-associated secretory phenotype (SASP) gene expression. Senolytics can be used to rejuvenate senescent cell cultures. Preliminary data suggests an three-fold improvement in organoid formation efficiency (OFE) of SGPCs-grown-organoids (SGOs) from incomplete pSS patients treated with the senolytics agent Navitoclax. OFE of pSS organoids did not improve following Navitoclax. These findings suggest that SGPC senescence contributes to pSS pathology, that p16 may represent a promising biomarker for SG damage in pSS, and the retransplantation of autologous senolytics treated SGOs may represent therapeutic option for recovery of hyposalivation in earlier pSS patients.

O145

Increased GITRL impairs the function of myeloid-derived suppressor cells and exacerbates primary Sjögren's syndrome

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Although the expansion of myeloid-derived suppressor cells (MDSCs) has been reported in autoimmune disorders, it is largely unclear how MDSCs contribute to the development of primary Sjögren's syndrome (pSS). In this study, we found significantly increased MDSCs with gradually diminished suppressive capacity during disease development in mice with experimental SS (ESS). The ligand for glucocorticoid-induced TNFR family-related protein (GITRL) was increased along ESS progression whereas the increased GITRL was found to attenuate the immunosuppressive function of MDSCs. Moreover, blocking GITR signal in MDSCs significantly restored their immunosuppressive function and ameliorated ESS progression in mice. In pSS patients, expanded MDSCs were found to express low levels of arginase. Significantly increased serum GITRL levels were closely correlated with patients with higher SS disease activity index (SSDAI). Furthermore, treatment with recombinant GITRL markedly reduced the immunosuppressive function of human MDSCs. Together, our studies have demonstrated a critical role of GITRL in modulating the suppressive function of MDSCs, which may facilitate the validation of GITRL as a therapeutic target for the treatment of pSS.

O146

IgV peptide repertoires of precipitating anti-dsDNA autoantibodies in lupus

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Anti-double-stranded (ds)DNA are prototypical autoantibodies in systemic lupus erythematosus (SLE), but little is known about their immunoglobulin variable (IgV) region composition at the level of secreted proteome. Here, we use a novel mass spectrometry-based proteomic workflow to analyse IgV subfamily expression and mutational signatures of high affinity anti-dsDNA responses. Anti-dsDNA were purified from serum precipitins reactions by agarose gel immunodiffusion between plasmid

DOI: 10.1002/eji.201970400

dsDNA and SLE serum from eight patients positive for anti-dsDNA by Farr radioimmunoassays, followed by SDS-PAGE to isolate heavy- and light-chains for in gel digests. IgV peptides were then sequenced by mass spectrometry and analysed by combined de novo sequencing and database matching. Light-chain complementarity determining region 3 (LCDR3) peptides were used as biomarkers to track serum anti-dsDNA clonotypes using quantitative multiple reaction monitoring (MRM). Anti-dsDNA proteomes were oligoclonal with shared IGHV and IGKV subfamilies expression. IgV peptide maps from eight subjects showed extensive public and random (private) amino acid replacement mutations with prominent arginine substitutions across H- and L-chains. Shared sets of LCDR3 peptides specified by arginine substitutions were sequenced from the dominantly expressed IGKV3-20 subfamily, with changes in expression levels of a clonal LCDR3 peptide by quantitative MRM paralleling the rise and fall of anti-dsDNA levels by Farr radioimmunoassays. The heavily mutated IgV peptide signatures of anti-dsDNA proteomes reflect the strong selective forces that shape humoral anti-dsDNA responses in germinal centres. Direct sequencing of agarose gel precipitins using microlitre volumes of stored sera streamlines the antibody sequencing workflow and is generalisable to other precipitating serum antibodies.

O147

TCR repertoire analysis reveals the differentiation of effector memory T cells into Th17 cells in rheumatoid arthritis

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The pathogenesis of rheumatoid arthritis (RA), a systemic autoimmune disease characterized by autoreactive T-cell accumulation and pro-inflammatory cytokine overproduction, is unclear. Systematically addressing T-cell receptor (TCR) repertoires of different CD4+ T-cell subsets could help understand RA pathogenesis. Here, peripheral CD4+ T cells from treatment-naïve RA patients and healthy controls were sorted into seven subsets including naïve, effector, central memory, effector memory (EMT), Th1, Th17, and regulatory T cells. T-cell receptor β chain repertoires were then analyzed by next-generation sequencing. We identified T-cell clonal expansion in EMT and Th17 cells, with highly similar TCR repertoires between them. Ex vivo experiments demonstrated the preferred differentiation from EMT to Th17 cells in RA. Moreover, TCR diversity in subsets including Th17 was negatively correlated with RA disease activity indices such as C-reactive protein and erythrocyte sedimentation rate. Thus, shared and abnormally expanded EMT and Th17 TCR repertoires might be pivotal for RA pathogenesis.

O148

Serum level of interleukin 36 in patients with multiple sclerosis

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Introduction: Multiple sclerosis is a chronic autoimmune demyelinating disorder of central nervous system with unknown origin. In MS disease, T cells are pointed to myelin antigens and it leads to myelin loss and axonal degeneration. Cytokines are important regulators of immune system and has critical roles in MS pathogenesis. Interleukin 36, a member of interleukin 1 family, has been shown having important roles in some autoimmune disorders due to its proinflammatory actions and its role in

DOI: 10.1002/eji.201970400

host immunity.
Methods and materials: In the current study, 49 relapsing remitting multiple sclerosis patients and 41 healthy individuals were recruited. IL36 measurement was performed using enzyme-linked immunosorbent assay technique.
Results: Mean age of RRMS patient and control group were 31.84 ± 6.89 and 34.27 ± 8.83 years, respectively. Serum level of IL36 were 61.91 ± 16.29 in MS patients and 42.26 ± 17.54 in healthy group ($P < 0.001$).
Conclusion: in this study for the first time, significantly higher serum level of IL36 was determined in RRMS patients comparing healthy individuals. This data may suggest important roles of this cytokine in MS pathogenesis.

O149

Urinary epidermal growth factor predicts renal prognosis in antineutrophil cytoplasmic antibody-associated vasculitis

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Introduction: The current study aimed to investigate the association between urinary epidermal growth factor (uEGF) and renal disease severity and outcomes in patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).
Methods: Intrarenal *EGF* mRNA expression was extracted from transcriptomic data of microdissected tubulointerstitial compartments of AAV patients' kidney biopsies. Urinary EGF was measured in 173 AAV patients in active stage and 143 in remission, and normalized to urine creatinine excretion (uEGF/Cr). The association between uEGF/Cr (or *EGF* mRNA) and clinical-pathological parameters was tested using linear regression analysis. The ability of uEGF/Cr to predict renal outcomes was analyzed using Cox's regression analysis.
Results: In AAV patients, intrarenal *EGF* mRNA expression was significantly associated with eGFR($\log_2$) at time of biopsy ($\beta=0.63$, $p< 0.001$). The level of uEGF/Cr was significantly higher in patients in remission than in patients with active disease, both when looking at patients with sequential measurements (2.75±1.03 vs. 2.08±0.98, $p< 0.001$) and in cross-sectional comparison. uEGF/Cr level was positively associated with eGFR($\log_2$) at time of sampling in both active and remission stage ($\beta=0.60$, $p< 0.001$; $\beta=0.74$, $p< 0.001$, respectively). Patients with resistant renal disease had significantly lower uEGF/Cr levels than responders. After adjusting for other potential predictors, uEGF/Cr was independently associated with composite endpoint of end stage renal disease (ESRD) or 30% reduction of eGFR (HR 0.61, 95% CI 0.45 to 0.83, $p=0.001$).
Conclusion: Lower uEGF/Cr levels are associated with more severe renal disease, renal resistance to treatment and higher risk of progression to composite outcome in AAV patients.

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DOI: 10.1002/eji.201970400

O150

Interferon-mediated AIM2 inflammasome dysfunction in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disorder attributed by dysregulation in both innate and adaptive immune responses. Type I interferon (IFN) is considered as the central pathogenic cytokine in driving SLE development through its pleiotropic effects on various leukocytes including monocytes. In a study comparing the transcriptome profiles of monocytes exposed to sera from healthy subjects and SLE patients, genes of the NOD-like receptor signaling pathways, including the DNA-binding molecule, AIM2, were significantly enriched. The role of AIM2 in SLE is not completely clear and here we evaluated its function as a cytosolic autoantigen senor in activating the inflammasome activity to produce active IL-1β and IL-18 via the proteolytic activity of caspase-1. Compared with healthy individuals, monocytes from SLE patients exhibited higher expression of AIM2 and other inflammasome components caspase-1 and ASC; and concordantly produced higher levels of IL-1β and IL-18 upon stimulation with dsDNA. AIM2 expression in SLE monocytes also positively correlated with the expression of IFN-sensitive genes (ISGs) MX-1, IFIT-1 and LY6E. Healthy monocytes cultured in SLE serum induced higher AIM2 transcript expression and in positive correlation with ISGs induction. Furthermore, IFNα treatment induced expression of AIM2 mRNA together with increased inflammasome-induced IL-18 and IL-1β secretion in a dose-dependent manner. These findings suggest that dysregulated IFN-mediated AIM2 inflammasome activity may play a role in SLE pathogenesis. The molecular mechanism by which IFN mediates AIM2 induction is currently under investigation.
(This project was supported by the HKRGC GRF 17123517).

O151

Characterization of calreticulin, a stromal-derived autoantigens, recognized by RA synovial recombinant monoclonal antibodies

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We previously showed that up to 40% of RA synovial recombinant monoclonal antibodies (RA-rmAbs) generated from germinal-center-like-structure (GC-LS+) RA synovium recognize citrullinated antigens contained in neutrophils-extracellular-traps (NETs). The cellular source of other potential autoantigens targeted by the majority of locally differentiated B cells remains undefined. RA fibroblast-like-synoviocytes (RA-FLS) have been implicated in the release of cit-antigens. However, whether these cells are targeted by RA-rmAbs is still unknown. Here, we aimed to define stromal-derived autoantigens from RA-FLS targeted by RA-rmAbs.
71 RA-rmAbs were screened toward RA-FLS by living-cell immunofluorescence. Western blotting was used to identify potential autoantigens from RA-FLS protein extracts. Putative candidates were validated using colocalization immunofluorescence confocal microscopy/ELISA/immunoprecipitation assay, and surface plasmon resonance on unmodified/cit-proteins. Serum immunoreactivity was tested in anti-citrullinated-peptide/protein Abs ACPA+ versus ACPA- RA patients.
14% of RA-rmAbs showed clear reactivity toward RA-FLS in immunofluorescence with no binding to NETs. One stromal-reactive RA-rmAb (RA057/11.89.1) decorated a ~58-kDa band that mass spectrometry and Western blotting identified as calreticulin (CRT). Confocal microscopy demonstrated significant cellular colocalization between anti-CRT-RA057/11.89.1 in RA-FLS. RA057/11.89.1 was able to immunoprecipitate rCRT. Deimination of CRT to cit-CRT moderately increased RA057/11.89.1

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DOI: 10.1002/eji.201970400

immunoreactivity. cit-CRT displayed increased blocking capacity compared with unmodified-CRT in competitive binding assays. Finally, anti-cit-CRT Abs were preferentially detected in ACPA+ versus ACPA- RA sera.
Here, we identified a synovial B cell-derived RA-rmAb locally differentiated within the ELS+ RA synovium reacting toward CRT, a putative novel autoantigen recently described in RA patients, suggesting that FLS-derived CRT may contribute to fuel the local autoimmune response.

O152

NFκB binds multiple juvenile idiopathic arthritis genetic susceptibility loci

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Juvenile idiopathic arthritis (JIA) genetic association studies identified 17 loci ($p< 5\times10^{-8}$) and 21 additional loci with suggestive evidence of association ($p< 1\times10^{-6}$). As the majority of these genetic associations are located in non-coding regions of the genome, we tested the hypothesis that specific transcription factors (TFs) bind DNA at multiple JIA-associated genetic loci.
We assembled a large collection of publically available datasets, including 1,544 chromatin immunoprecipitation with next generation sequencing (ChIP-Seq) datasets, and “Active Chromatin” maps generated from combinations of histone marks in 221 different cell and tissue types. We developed a simulation test called RELI (Harley *et al*., Nat. Genetics 2018) to assess the statistical significance of the number of JIA loci that intersect with each ChIP-seq dataset. Bonferroni corrected P-values (p_c) were estimated for each ChIP-seq dataset evaluated. The top RELI analysis result involves NFKB1 binding at 20/43 (46.5%) of JIA loci ($p_c< 10^{-14}$). Four of these JIA loci have a predicted creation or deletion of the NFKB1 binding motif. Furthermore, each of these four loci demonstrates allele dependent gene expression.
In conclusion, these data are consistent with common transcriptional control mechanisms operating across multiple JIA risk loci in a shared intracellular environment. Notably, most GWAS loci have small odds ratios (usually < 1.2). If there are coordinated mechanisms across loci that alter disease risk with larger effect sizes, then shared gene regulatory mechanisms such as these are important in generating disease risk.

O249

A tolerogenic artificial antigen-presenting cell durably ameliorates EAE by directly and selectively modulating myelin peptide-autoreactive CD4$^+$ and CD8$^+$ T cells

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Here, a tolerogenic artificial antigen-presening cell (TaAPC) was developed to directly and selectively modulating meylin-autoreactive CD4$^+$ and CD8$^+$ T cells in the myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide-induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6J mice. Cell-sized polylactic-co-glycolic acid microparticles (PLGA-MPs) were generated to co-couple tartget antigens (MOG_{40-54}/H-2D^b-Ig dimer, MOG_{35-55}/I-A^b multimer), regulatory molecules (anti-Fas and PD-

DOI: 10.1002/eji.201970400

L1-Fc) and "self-marker" CD47-Fc and encapsulate inhibitory cytokine (TGF-β1). Four infusions of the TaAPCs markedly and durably inhibited the EAE progression and reduced the local inflammation in CNS tissue. They circulated throughout vasculature into peripheral lymphoid tissues and various organs, but not into brain, with retention of 36 h, and exerted direct effects on T cells *in vivo* and *in vitro*. Two infusions of the TaAPCs depleted 65-79% of MOG_{35-55}-specific $CD4^+$ and 46-62% of MOG_{40-54}-specific $CD8^+$ T cells in peripheral blood, spleen and CNS tissues in an antigen-specific manner and regulatory molecule-dependent fashion; induced robust T cell apoptosis; inhibited the activation and proliferation of MOG peptide-reactive T cells; reduced MOG peptide-reactive Th1, Th17 and Tc17 cells and expanded regulatory T cells. They also inhibited IFN-γ/IL-17A secretion and elevated IL-10/TGF-β1 production in splenocytes, but not in CNS tissue. More importantly, the TaAPCs treatment did not obviously suppress the overall immune function of host. This study provides the first experimental evidence for the capability of TaAPCs to directly modulate autoreactive T cells by surface presentation of multiple ligands and paracrine release of cytokine, thus suggests a novel antigen-specific immunotherapy for the T cell-mediated autoimmune diseases.

O250

INS1007, a reversible dipeptidyl peptidase 1 inhibitor, ameliorates interferon-alpha-accelerated lupus nephritis in mice

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Background: Lupus nephritis (LN) is one of the most common and serious manifestations of systemic lupus erythematosus, and treatment options are limited. One potential strategy is to block the deleterious effects of neutrophil serine proteases (NSPs) released by hyperactivated neutrophils during LN pathogenesis. We hypothesized that INS1007, which inhibits the activity of DPP1 - a key enzyme that converts pro-NSPs to active forms, could ameliorate interferon-alpha (IFNα)-accelerated LN in mice.
Methods: Female lupus-prone NZB/W F1 mice were injected with adenovirus-expressing IFNα5; this was followed by 8 weeks of disease progression. Daily oral INS1007 dosing (0.1, 1, and 10 mg/kg, twice a day) was initiated 2 weeks after virus injection. Blood urea nitrogen (BUN) was measured to assess renal function, and total protein, albumin, and creatinine were measured in urine to evaluate kidney damage. Left kidneys were used for histopathology; right kidneys were used for flow cytometry analysis.
Results: Mice treated with INS1007 exhibited a significant, dose-dependent reduction in both BUN and albumin/creatinine ratio. INS1007 significantly delayed the progression to proteinuria greater than 750 mg/dL, decreased renal pathological sum scores, and reduced the percentages of various inflammatory cells in the kidney, including myelocytes, $Ly6C^+$ monocytes, $Ly6C^+$ macrophages, and T cells.
Conclusions: INS1007 significantly improved renal function, reduced kidney damage, and delayed the progression to severe proteinuria in mice with IFNα-accelerated LN. Additionally, INS1007 significantly decreased histopathological sum scores and inflammatory cell infiltration into the kidney. These results suggest that further evaluation of INS1007 in the treatment of LN may be warranted.

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DOI: 10.1002/eji.201970400

O251

Increased autophagy is cytoprotective against podocyte injury induced by antibody and interferon-α in lupus nephritis

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Objectives: More recent studies suggested that defects in autophagy contribute to the pathogenesis of SLE, especially in adaptive immunity. Occurrence and progression of lupus nephritis (LN) is the end result of complex interactions between regulation of immune responses and pathological process by renal resident cells, but there is still a lot of missing information for an establishment on the role of autophagy in pathogenesis of LN and as a therapy target.
Methods: Systemic and organ specific etiologies of autophagy were firstly evaluated by autophagy protein quantification in tissue homogenates in MRL$^{lpr/lpr}$ lupus prone and female C57BL mice. Analysis of gene expression was also adopted in human blood and urine sediments. Then, some key mediators of the disease were chosen to induce podocyte autophagy. Podocyte injuries including apoptosis, podocin derangement, albumin filtration, and wound healing were monitored simultaneously with autophagy steady-state and flux.
Results: Elevated LC3B in kidney homogenates and increased autophagosomes in podocyte from MRL$^{lpr/lpr}$ were observed. In humans, mRNA levels of some key autophagy genes were increased in blood and urinary sediments, and podocyte autophagosomes were observed in renal biopsies from patients with LN. Complement inactivated serum, IgG-LN and IFN-α could induce podocyte autophagy in a time and dosage dependent manner, and by reactive oxygen species production and mTORC1 inhibition, respectively. Autophagy inhibition aggravated podocyte damage whereas its inducer relieved the injury.
Conclusion: Podocyte autophagy is activated in lupus-prone mice and patients with lupus nephritis. Increased autophagy is cytoprotective against antibody and interferon-α induced podocyte injury.

O252

Proteomic profiling and clonal tracking of secreted rheumatoid factors in primary Sjögren's syndrome

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Background: Rheumatoid factors (RFs) are associated with systemic disease in primary Sjögren's syndrome (SS) and may be pathogenic as mixed cryoglobulins. Routine RF detection methods cannot resolve RF repertoires at a molecular level. Here, we perform the first proteomic and transcriptomic analysis of secreted (serum) RFs in primary SS and identify distinct heavy-chain peptide signatures for RF clonotype tracking.
Methods: Purified heavy-chains of serum RFs (IgH-RF) from 15 primary SS patients were subjected to de novo mass spectrometric sequencing. The circulating B cell Ig repertoire was determined by massively parallel sequencing of *IGH* RNA from matched PBMCs (n=7). RF-specific heavy-chain complementarity determining region 3 (HCDR3) peptides were identified by searching IgH-RF peptide sequences against the corresponding *IGH* RNA sequence libraries. HCDR3 peptides were used as biomarkers to track serum RF clonotypes using quantitative multiple reaction monitoring (MRM).

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DOI: 10.1002/eji.201970400

Results: Serum RFs were clonally restricted and composed of shared sets of IGHV1-69, 3-15, 3-7 and 3-74 subfamilies. Cryoprecipitable RFs from patients with mixed cryoglobulinemia (MC) were distinguishable from nonprecipitating RFs by a higher frequency of amino acid substitutions and identification of stereotypic HCDR3 transcripts. Potentially pathogenic RF clonotypes were detected in serum by MRM years before presentation with MC. Levels of an IGHV4-34 RF IgM decreased following immunosuppression and remission of MC.
Conclusion: Cryoprecipitable RF clonotypes linked to vasculitis in primary SS have different molecular profiles to nonprecipitating RFs, suggesting different underlying mechanisms of production. The combined omics workflow herein provides molecular biomarkers for tracking and removal of pathogenic RF clones.

O253

Identification of autoantigens specific for biliary atresia using human proteome microarrays

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Autoimmune-mediated injury of bile ducts is recognized as the primary cause to persistent cholangitis and biliary cirrhosis eventually needing liver transplant in infants with biliary atresia (BA). To date, no known biomarkers have been reported for this disease, making it difficult to diagnosis. We employed HuProt arrays which comprised of ~20,000 unique human proteins to screen BA-specific autoantigens, and a total of 255 candidate autoantigens including 105 IgG and 176 IgM were screened out with sera from 20 BA patients and 20 controls, with simultaneous IgG and IgM response to 26 proteins. A 49 differential antigens were identified based on the criteria of fold change>2 and stable expression. GO functional analysis revealed that differential antigens mainly involved in cellular development, proliferation and apoptosis, and bile ducts' formation. The prevalence of RBPJ,CNPY4,CENP6, UBQLN3, PDCD2,and SPEG in BA were significantly higher than that in controls (100% vs. 0%, P< 0.05). A focused array with these 49 BA-related antigens were constructed and validation of these candidates were performed in a much larger cohort of 80 BA patients, 80 cholestatic disease controls and 80 healthy controls. The results showed that IgM against CNPY4 and IgG to RBPJ could distinguish BA from controls with AUC of 0.78 and 0.77, respectively (P< 0.001). In conclusion, BA-specific autoantigens have been successfully screened in this study, which may be used as potential non-invasive diagnostic biomarkers for BA, but further conformation is still needed.

O254

An endocannabinoid rheostat for plasmacytoid dendritic cell function: role in autoimmunity

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Plasmacytoid dendritic cells (pDCs) are most efficient producers of type I IFNs in response to TLR activation, and are thus key players in protective immunity against infections. PDC-derived IFNa is also implicated in different autoimmune contexts as innate initiator of inflammation. Recently we reported a hitherto unknown constitutive rheostat mechanism driven by endocannabinoids that regulates IFNa induction in human pDCs. Endocannabinoids are endogenous ligands for cannabinoid receptors, which play important role in myriad physiologic processes as well as disease contexts. Several serine hydrolases regulate systemic abundance of endocannabinoids and thus play key roles in various physio-pathologic processes. One of them, alpha beta domain containing hydrolase-6 or

DOI: 10.1002/eji.201970400

ABHD6, has been implicated in systemic lupus erythematosus (SLE) in GWAS studies and we found that ABHD6 is highly expressed in human pDCs. These led us to explore the role of endocannabinoids in two major autoimmunities associated with pDC-derived IFNa induction, viz. SLE and psoriasis. Overexpression of ABHD6 on pDCs in a SLE endophenotype was associated with higher IFNa induction. In contrast in psoriasis, the cutaneous autoimmunity involving pDC-derived IFNa, we found increased plasma endocannabinoids and lower expression of ABHD6 in immune cells and lesional skin. To further explore the role of endocannabinoids in these contexts we used two established preclinical models, spontaneous lupus in the NZB/W-F1 mice and imiquimod-induced psoriasis in C57BL/6 mice. Through these studies we could discover a novel regulatory pathway in pDCs and elucidated its role in autoimmune inflammations, paving the way towards novel therapeutic targets in relevant clinical contexts.

O255

A modified peptide derived from Goodpasture autoantigen arrested and attenuated kidney injuries in experimental anti-GBM glomerulonephritis

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Our previous studies identified a nephritogenic epitope P14 ($\alpha3_{127\text{-}148}$) on human α3(IV)NC1 which could induce crescentic nephritis in WKY rats, and further characterized its core motif as $W_{136}I_{137}$_$L_{139}W_{140}$_$G_{142}F_{143}$_F_{145}. Based on the amino acid differences of the motif between α1-P14 and α3-P14, a modified peptide (m-P14) was synthesized by the substitution of I_{137} (α3) to S_{137} (α1). m-P14 was injected into P14-immunized WKY rats either on immunization (early-treatment group, from day 0) or upon disease onset (treatment group, from day 17-21).
We found that m-P14 intervention could arrest the α3-P14 induced anti-GBM glomerulonephritis in early-treatment groups and eventually hindered the crescent formation (30mg/kg m-P14 group *vs.* P14 group: 0.5±0.4 *vs.* 68.8±15.4 %; *P*=0.002; 10mg/kg m-P14 group *vs.* P14 group: 6.3±5.6 *vs.* 68.8±15.4%, *P*=0.009), and could remarkably attenuate kidney injuries in treatment group(treatment group *vs.* P14 group: 20.1±8.4 *vs.* 68.8±15.4%, *P*=0.026).
m-P14 had a comparable binding capacity to rat MHC-II molecules like α3-P14. It inhibited the binding of α3-P14 to rat MHC molecules from 16.9±0.9% to 11.4±0.9% (*P*=0.013) and abated the differentiation of splenic Th17 subset in both early-treatment groups (30mg/kg m-P14 group *vs.* P14 group: 1.3±0.1 *vs.* 2.3±0.2%, *P*=0.004; 10mg/kg m-P14 group *vs.* P14 group: 1.3±0.2*vs*. 2.3±0.2%, *P*=0.015) and treatment group (30mg/kg m-P14 group *vs.* P14 group 1.0±0.1 *vs.* 2.3±0.2%, *P*=0.002). Moreover, m-P14 could inhibit the binding between α3-P14 and antibodies and impeded intra-molecular epitope spreading of serum autoantibodies and kidney elutes, from linear peptides to conformational α3NC1.

O256

Targeting alpha1-adrenergic pathway with light to control autoimmune uveitis

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The blood-retinal barrier (BRB) dysfunction is a hallmark of posterior autoimmune uveitis. Because the structure of the BRB allows dynamic interactions between the retinal neural network and blood vessel endothelium, we assessed whether and how light-induced neural activity in the retina is related to

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DOI: 10.1002/eji.201970400

permeability of the BRB endothelium to immune cells in experimental autoimmune uveoretinitis (EAU). At the beginning of the acute phase of EAU, when pathogenic CD4+ T cells first entered into the eye, we exposed mice to photopic (PL) or mesopic (ML) light intensities. We found that EAU mice exposed to PL had reduced infiltration of CD4+ T cells, CD8+ T cells and CD11b+ cells as well as suppressed pro-inflammatory phenotype of the BRB endothelium and chemokine expressions in the retina compared to EAU mice exposed to ML. Moreover, PL in EAU mice increased norepinephrine and epinephrine levels in the eye followed by downregulation of alpha1A-adrenoceptor (alpha1A-AR). Strikingly, treatment of EAU mice exposed to ML with siRNA targeting alpha1A-AR in the retina or with prazosin, an alpha1-AR blocker, reduced retinal chemokine expressions and immune cell infiltration in the retina to levels similar to those observed in EAU mice exposed to PL. Together, our data suggest that PL-induced neural activity in the retina suppresses EAU development through down-regulation of local alpha1-AR signaling. These results hold promise for retinal stimulation by PL or alpha1-AR antagonists as possible therapeutic tools for posterior autoimmune uveitis.
This work was supported by the JSPS Postdoctoral Fellowship for Foreign Researchers, GACR18-11795Y, ProgresQ35 and PRIMUS/17/MED/7.

O281

SP600125 alleviates disease activity in lupus-prone mice by blocking secondary pyroptosis of apoptotic renal tubular epithelial cells

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Background: Increased apoptosis and defect in clearance that result in apoptotic cells to undergo secondary necrosis have been recognized as the main cause of systemic lupus erythematosus. Recent findings revealed that GSDME, a key membrane pore formation molecule in pyroptosis, played an important role in the secondary necrosis downstream of apoptotic caspase-3. In this paper, we call the GSDME mediated cell death after apoptosis as secondary pyroptosis. We aim to investigate the effects of a JNK inhibitor SP600125 on secondary pyroptosis and disease activity in lupus-prone mice.
Methods: In vitro, human tubular epithelial cell lines（HK2）were pretreated with SP600125, followed by stimulation with apoptosis inducer-TNF+CHX or SLE sera. And then the secondary pyroptosis were examined. In vivo, both pristane-induced lupus mice and MRL/lpr mice were treated with SP600125 or vehicle. The disease severity and the expression of GSDME and cleaved-caspase-3 were detected.
Results: In our study, high levels of GSDME were found in renal tubules of lupus-prone mice. TNF+CHX or SLE sera could induce HK2 cells to undergo secondary pyroptosis in a caspase-3-GSDME dependent manner. Interestingly, SP600125 significantly reduced GSDME expression and decreased the secondary pyroptosis. In vivo, SP600125 administration effectively ameliorated lupus-like features in lupus-prone mice. Importantly, SP600125 treatment reduced the expression of GSDME and cleaved caspase-3 in renal tubules of lupus mice.
Conclusion: The increased GSDME-mediated secondary pyroptosis may be one of the pathogenesis of SLE. SP600125 can regulate the secondary pyroptosis of apoptotic cells by inhibiting GSDME expression, and thus may be a potential drug for treating SLE.

DOI: 10.1002/eji.201970400

O282

Anti-CD52 treatment of EAE is independent of PD-1 signals and differentially affects the proportion of DN T cells, and newly-generated T and B cells

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Treatment of multiple sclerosis (MS) with anti-CD52 reduces relapses but often results in patients developing alternative autoimmunity, usually of the thyroid. The mechanisms and molecules involved in restoring immune tolerance post-anti-CD52 are unknown. Here, we investigated what factors might allow restoration of tolerance vs. the development of other autoimmune diseases post-anti-CD52 treatment in mice with experimental autoimmune encephalomyelitis (EAE). Mice deficient in PD-1 were used to examine anti-CD52 efficacy in a model with more severe EAE as well as to test the role of this co-inhibitor in the mechanism of anti-CD52 therapeutic activity. Disease progression was reversed by anti-CD52 in the PD-1-/- mice that otherwise had a persistently high clinical score without anti-CD52. We found that anti-CD52 increased the proportion of newly-generated T cells and reduced the proportion of newly-generated B cells in association with their higher expression of CD52. Comparing euthymic to thymectomized mice, we found that the increased proportion of newly-generated T cells was due to their rapid repopulation from the thymus. Also, the percentage of DN T cells and regulatory T cells were elevated post-anti-CD52 treatment in mice with EAE. Histological examination showed no thyroid infiltration post-anti-CD52. Our data show the efficacy of anti-CD52 in reversing an aggressive form of EAE and the differential immunomodulating effect of anti-CD52 on newly-generated vs. established B and T cells. Furthermore, these data identify DN T cells, an immunoregulatory T cell subset, as a significant hyper-repopulating subset following CD52-mediated depletion.
Funded by: Sanofi Genzyme.

O283

Mutation in HSPG2 gene can cause a familial form of monogenic systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with a strong genetic component characterized by autoantibody production. We studied fourteen individuals from five families showing features consistent with SLE or lupus nephritis. These family members were tested by NGS whole genome exon sequencing. According to the sequencing results, a lupus family with 7 diseased patients was firstly subjected to explicit and recessive inheritance patterns and linkage analysis. It was found that there were 22 linkage regions in the region with LOD higher than 1.5, involving 1, 3, 13, 14 and 18 Chromosome. A total of 586 genetic variants were found in these Chromosomes. These genes were performed rare mutation screening, and 67 of them were detected to have rare mutations; Further genetic segregation analysis of these genes revealed that only HSPG2 (p-value < 0.01 & LOD=2.1) was a potential causative gene in the family patients. The HSPG2 gene mutation was confirmed by Sanger sequencing. Furthermore, this HSPG2 gene mutation was also found in other four unrelated SLE family patients. In women with HSPH2 mutations, autoantibodies were elevated, whereas men with this gene mutation had no apparent phenotypic abnormalities. Through transcriptome sequencing studies, it was found that HSPG2 gene mutation may participate in the development of lupus nephritis through NFKB signaling pathway. These data suggested that a

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DOI: 10.1002/eji.201970400

new monogenic form of SLE could also be identified in this population, and highlight the pathogenic role of HSPG2 as a novel causative gene in SLE.

O284

Deficiency of *Socs3* in astrocytes leads to A1-like phenotype and brain-targeted EAE

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The JAK/STAT signaling pathway plays a critical role in inflammatory responses of autoimmune diseases. Dysregulation of the JAK/STAT pathway is associated with many pathological conditions, including Multiple Sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Astrocytes are the most abundant glial cells in the central nervous system (CNS) and play vital roles in modulating immune responses in the CNS and maintaining the integrity of the blood-brain barrier. Recent studies have identified two “subsets” of astrocytes: pro-inflammatory A1 subset and anti-inflammatory A2 subset.STAT3 is thought to play an important role in A2 astrocytes, however, recent studies also suggest that STAT3 activation in astrocytes is detrimental during neurodegeneration. The exact role of STAT3 signaling in astrocytes in MS/EAE is not clear. Here we demonstrate that deficiency of Suppressor Of Cytokine Signaling 3 (*Socs3*), the negative regulator of STAT3, exhibit hyper-sensitivity in response to IL-6 *in vitro*. IL-6 leads to increased production of chemokines including *Cxcl1*and *Cxcl10*. Interestingly, *Socs3*-deficient astrocytes exhibit increased A1 markers, such as *C3*. Deficiency of Socs3 in astrocytes leads to earlier onset of EAE, and moreover, mice exhibit a severe, non-resolving brain-targeted form of EAE. Mice experience ataxia and tremor with enhanced inflammation in cerebellum. Flow cytometry analysis demonstrated a significant increase of cerebellar infiltrating cells, including CD4+ T-cells, Ly6C+ monocytes, Ly6C- monocytes, microglia and neutrophils. This study demonstrates the detrimental role of STAT3 signaling in astrocytes in the pathogenesis of EAE.

O285

TIM-3 regulates the NETs-mediated dendritic cell activation in myeloperoxidase-ANCA-associated vasculitis

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T cell Mucin-3 (TIM-3) has been reported as an important regulatory molecule on T cells and plays a pivotal role in autoimmune diseases, but their impact on dendritic cells (DCs) is poorly explored. The formation of neutrophil extracellular traps (NETs) is considered as one of pathogenesis for an autoimmune disease - myeloperoxidase-antineutrophil cytoplasmic autoantibody associated vasculitis (MPO-AAV). Here we investigated the potential roles of TIM-3 on the activation of DCs induced by NETs. By examining the peripheral blood of the patients with MPO-AAV, we observed that the expression of TIM-3 on DCs was reduced, correlating with Birmingham Vasculitis Activity Score (BVAS) and the spontaneous increasing of NETs. By in vitro assay, we further found that NETs could induce the activation of DCs and the increased level of Toll-like receptor 4 on DCs. More interestingly, the blockage of TIM-3 could enhance this phenomenon. Our findings speculate that TIM-3 may

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DOI: 10.1002/eji.201970400

negatively regulate dendritic cell activation and its deficiency may contribute to the pathogenesis of MPO-AAV.

O286

An Δ133p53-like isoform drives exaggerated immune responses to promote chronic inflammation

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Chronic inflammation is a well-known contributor to autoimmune disease and cancer. The p53 tumour suppressor has been shown to regulate immune responses. As well, there are 12 p53 isoforms co-expressed with the wild type p53 in normal human tissue, of which the Δ133p53 isoform has been found to associate with inflammation in our studies. By using a mouse mutant p53, Δ122p53, which mimics human the Δ133p53, we have found that expression of Δ122p53 is associated with a marked increase in multiple serum cytokines and chemokines in aged mice. To test whether Δ122p53 promotes inflammation, we induced acute colitis in Δ122p53 and p53 null control mice. Δ122p53 mice had increased loss of the epithelial cell barrier, a marked increase in CD3 T cell infiltration, and increased expression of inflammatory chemokines CCL11 (eotaxin) and CSF2 (GM-CSF) through the muscular and sub-mucosal layer, compared to p53 null mice. These results suggest that expression of Δ122p53 drives an exaggerated immune response to establish a chronic inflammatory environment. We are currently studying the mechanisms of Δ122p53 modulation of immune responses and if expression of Δ133p53 associates with development of IBD. The findings of this study will provide significant knowledge on p53 regulation of immune responses in autoimmune disease and inflammation-induced cancer, providing potential drug targets to treat these chronic conditions.

O287

Soluble Interleukin-36 receptor antagonizes IL-36/IL-36R signaling to control skin inflammation in psoriasis

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Interleukin-36 (IL-36) family cytokines play critical roles in the pathogenesis of psoriasis. It's known that the blockage of IL-36/IL-36R signaling by IL-36 antagonist (IL-36Ra) alleviates the pathology of psoriasis. However, whether IL-36R has its own natural soluble receptor remains unknown. **Here we identified a previously unknown natural soluble IL-36R (sIL-36R) of IL-36/IL-36R signaling, and showed that sIL-36R inhibited IL-36/IL-36R signaling to reduce inflammation in psoriasis**. To confirm that sIL-36R would act as a decoy to antagonize IL-36R signaling, a competitive assay was conducted. sIL-36R as well as IL-36R bound to IL-36γ, and the addition of sIL-36R to IL-36R-overexpressed cells decreased the interaction between IL-36R and IL-36γ. Moreover, the overexpression of sIL-36R markedly inhibited p-p38 MAPK and p-p65 induced by IL-36γ. Consistently, the expression of CCL20 and CXCL1 by IL-36γ was significantly decreased in sIL-36R-overexpressed keratinocytes compared to control cells. To evaluate whether sIL-36R would inhibit inflammatory responses in imiquimod-induced psoriatic mice, sIL-36R transgenic mice were generated and subjected to the IMQ model. Compared to control mice, sIL-36R transgenic mice exhibited greatly reduced epidermal acanthosis and significantly decreased development of pustules. The numbers and percentages of CD45+ cells, including neutrophils, dendritic cell, γδT cells, were significantly reduced,

DOI: 10.1002/eji.201970400

which accompanied by reduced Ccl20, Cxcl1, Il23, and Il17a in lesional skins. Taken together, these results for the first time demonstrate that sIL36R acts as a natural decoy of IL-36R to inhibit IL-36/IL-36R signaling, and suggest that sIL36R could be a potential therapeutic target in the treat of psoriasis.

O288

Helicobacter pylori infection is associated with autoimmune conditions in Zimbabwean patients

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The bacterium *Helicobacter pylori* (*H.pylori*) colonizes the human gastric mucosa and causes peptic ulcer disease. The pathogen is also implicated in development of autoimmune diseases such as myositis. While the prevalence of autoimmune diseases has been increasing in Zimbabwe, there are no studies investigating any association between *H.pylori* infection and any autoimmune disease. The aim was to determine the association between *H.pylori* infection and the expression of autoantibodies. Laboratory records of 1500 patients that were investigated for *H.pylori* at the Asthma, Allergy and Immunology Clinic (AAIC), Zimbabwe were studied. *H.pylori* infection was confirmed using a rapid kit. ELISA was used to determine IgG and IgA antibodies to *H.pylori.* Immunoblotting was used to determine autoantibody reactivity in the infected patients. An analytical cross-sectional study was done. 495 (33%) samples had IgG and/or IgA antibodies to *H.pylori.* 55% had autoantibodies associated with systemic sclerosis, SLE or myositis. The Female to Male ratio was 3:1. The proportion of patients presenting both autoimmune conditions and *H.pylori* infection was 18%, (20-45years). Half (50%) of *H.pylori* infected patients had autoantibodies associated with systemic sclerosis, SLE (39%) and myositis (11%). The associated auto-antibodies were anti- *RP155*, anti-*Th/To*, anti-*PM-Scl 100*, anti-*Ro52*, anti-*Ku* and anti-*dsDNA*. Anti-*Th/To* and anti-*Ku* were only present in females while anti-*Jo1* and anti-*Scl 70* were not detected in any patient. A significant number of patients with autoimmune conditions have co-existing *H.pylori* infection. It remains unclear whether the response to *H.pylori* in the gastrointestinal tract pre-disposes patients to autoimmunity. This will be subject to further studies.

O329

High level of serum neopterin is associated with rapidly progressive interstitial lung disease and reduced survival in dermatomyositis

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Objectives: To analyze the serum levels of neopterin in patients with dermatomyositis (DM) in association with clinical manifestations, laboratory data and patient prognosis.
Methods: One hundred and eighty-two consecutive DM patients and 30 healthy controls were retrospectively enrolled in the study. Serum levels of neopterin were detected by using the ELISA method.
Results: Serum levels of neopterin were significantly increased in DM patients compared to healthy controls (P< 0.001). High serum neopterin levels were associated with anti-MDA5 antibody, rapidly progressive interstitial lung disease (RP-ILD), and cutaneous involvement. Longitudinal assessment of serum samples revealed that the serum neopterin levels were closely correlated with disease severity. In addition, a significant increase in serum neopterin concentration of non-survivors was observed when compared to that of survivors (P< 0.001). ROC curves showed that serum neopterin could

DOI: 10.1002/eji.201970400

distinguish non-survivors and survivors at an optimal cut-off level of 22.1 nmol/L with a sensitivity and specificity of 0.804 and 0.625 respectively (P< 0.001). Kaplan-Meier survival curves revealed that DM patients with serum neopterin > 22.1 nmol/L had a significantly higher mortality compared to patient group with serum neopterin < 22.1 nmol/L (logrank P< 0.001). Multivariate regression analysis identified high serum neopterin concentration to be an independent risk factor for poor prognosis in DM (adjusted HR=4.619, 95%CI: 2.092-10.195, P< 0.001).
Conclusions: Increased serum levels of neopterin were significantly associated with RP-ILD and reduced survival in DM patients, suggesting it as a promising biomarker in disease evaluation of DM.

O330

Proteomic analysis of biomarkers predicting response to triple therapy in patients with rheumatoid arthritis

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Objective: Patients with rheumatoid arthritis (RA) are usually treated with triple therapy methotrexate (MTX), leflunomide (LEF) and infliximab (IFX), but nearly one-third of patients with RA do not respond to triple therapy. This study aimed to identify biomarkers for predicting efficacy of triple therapy to optimize personalized treatment of RA.
Methods: All 20 enrolled patients met 2010 ACR/EULAR criteria for RA and were classified as good, moderate and non-responders (GR, MR, NR) for triple therapy. The Responders (R) were defined as the sum of GR and MR. Protein profiles of 4 responders and 4 non-responders were investigated via isobaric tags for relative and absolute quantification (iTRAQ) to find differential expressed proteins (DEPs), and DEPs with high-confidence peptides were further validated in 15 responders and 5 non-responders by parallel response monitoring (PRM).
Results: iTRAQ identified 51 DEPs between responders and non-responders (p< 0.05, fold change>±1.2). The top five up-regulated DEPs were B7Z7M2, A0A087WZR4, Q53FL1, P08254, G3V2V8, while the top five down-regulated proteins were Q6MZX9, B3KP77, P0DJI9, P0DJI8 and P02787. Targeted mass spectrometry by PRM assays identified 10 candidate biomarkers, and 3 DEPs including fibrinogen beta chain, epididymal secretory protein Li 282 and testicular tissue protein Li 70 were confirmed as predictive biomarkers.
Conclusion: This study demonstrated the feasibility of exploring biomarkers by applying iTRAQ and PRM mass spectrometry techniques, and a panel of biomarkers were identified to predict clinical response of IFX+MTX+LEF treatment in active RA patients.

O331

Identification of a novel monogenic cause of lupus in Chinese patients

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DOI: 10.1002/eji.201970400

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Systemic Lupus Erythematosus (SLE) is a chronic, heterogenous autoimmune disease with multifactorial etiology. Although SLE is considered to be a complex polygenic disease, an increasing number of studies are uncovering novel monogenic causes of lupus. Childhood-onset sporadic SLE is rare and there is evidence to suggest it is enriched in monogenic causes. At Shanghai Renji Hospital we have undertaken whole exome sequencing of 74 chidhood-onset severe SLE trios. We have identified several *de novo* and novel variants in genes encoding poorly understood proteins, that we have been taken through to functional validation in order to explore their contribution to disease pathogenesis. I will present the results obtained from sequencing of a Chinese girl suffering from severe SLE in which we identified a novel and *de novo* missense mutation in a gene not previously associated with autoimmunity. This gene encodes for a protein important in germinal center biology and implicated in chemokine-induced cell migration. We have expressed the wild type and mutant protein in cell lines and demonstrated this protein promotes cell apoptosis and plays a role in forming focal adhesions. The patient's mutation prevents adequate expression of the protein and interferes with its role in promoting cell apoptosis and important B cell signaling pathways. We have also identified a less damaging missense variant in this same gene in Chinese adult-onset SLE. Understanding how these variants affect germinal center B cell biology and B cell tolerance promises to illuminate unappreciated aspects of lupus pathogenesis and uncover novel therapeutic targets.

O332

Role of long noncoding RNA LINC01139 in regulating activation, migration and invasion of rheumatoid fibroblast-like synoviocytes

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Fibroblast-like synoviocytes (FLSs) are critical to synovial invasion and joint destruction in rheumatoid arthritis (RA). The role of long noncoding RNAs (lncRNAs) in RA is largely unknown. We found that lncRNA LINC01139, mainly expressed in the cytoplasm, was up-regulated in RA synovial tissue and RA FLS. LINC01139 expression in RA FLS could be up-regulated by stimulation of TNF-α and LPS. RACE experiments confirmed that the full-length LINC01139 was 1540nt. Bioinformatics analysis predicted that LINC01139 exhibited no protein-coding potential. We found that LINC01139 inhibition reduced the migration, invasion and the expression of MMPs and IL-1, IL-6 and IL-8, but did not affect the proliferation and apoptosis of RA FLS. In SCID model, lentivirus mediated low expression of LINC01139 attenuated *in vivo* invasion of RA FLS. Furthermore, we found that downregulation of LINC01139 leads to decreased expression of PTK6, LRRK and HIF-1α. HIF-1α inhibition results in the reduced expression of MMPs and cytokines, and migration and invasion of RA FLS. We also found that bioinformatics analysis predicted that LINC01139 may target microRNA (miR)-1262. Further study confirm that miR-1262 expression was up-regulated or down-regulated when LINC01139 was knock downed or overexpressed. Inhibition of miR-1262 enhanced the migration and invasion of RA FLS, and miR-1262 mimic weakened the migration and invasion of RA FLS. Our findings suggest that an increase in synovial LINC01139 might contribute to synovial inflammation, aggression and joint destruction in RA through regulating HIF-1α and miR-1262, targeting the lncRNA LINC01139 may have therapeutic potential in patients with RA.

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DOI: 10.1002/eji.201970400

O333

PAI-1 is required for the neutrophil infiltration and tissue injury in colitis via activation of CXCL1/CXCL5 /CXCR2 axis

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Objective: Chronic inflammation of the intestinal wall is the symbolic characteristic of Inflammatory bowel disease (IBD), including Crohn's Disease and Ulcerative Colitis. It is well documented that IBD patients express a high level of plasminogen activator inhibitor-1 (PAI-1). PAI-1 is well known to play a critical role in the fibrinolytic system. It also has recently been implicated in the pathogenesis of IBD. The underlying mechanisms remain largely unknown.
Methods and results: Sera from the IBD patients were tested for the level of PAI-1 in comparison with those from the healthy controls. In a few parallel experiments, dextran sulfate sodium (DSS)-induced colitis were also established and examined in the wild type and the PAI-1 gene knock-out (PAI-$1^{-/-}$) mice. Body weight, histology, inflammatory cellular infiltration, and colon length were assessed in these two groups of mice. Of interest, we found that PAI-1 was shown to promote an increase level of a chemokine receptor, CXCR2 by neutrophils that were present profoundly in the inflammatory intestinal tissues. The neutrophil chemoattractant chemokines CXCL1 and CXCL5 were also higher in the colon. Conversely, in PAI-1 KO mice, the neutrophil were less infiltrated and tissue injury was significantly ameliorated.
Conclusions: Our findings clearly provide a novel insight into the role of PAI-1 in promoting the inflammatory neutrophils in the gut tissues in IBD in human and DSS-induced colitis in a mouse model. Thus PAI-1 might be a promising target for the prevention and treatment of IBD.

This work was supported by a grant from the Nora Eccles Treadwell Foundation.

O334

Citrullination of glucokinase linked to autoimmune diabetes

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In type 1 diabetes (T1D), inflammation in pancreatic islets amplifies the appearance of various post-translational modifications (PTMs) on self-proteins. The loss of immune tolerance to PTMs within the stressed beta cells triggers an autoreactive T cell response and contributes to the destruction of insulin-producing beta cells in T1D. However, the pancreatic-restricted autoantigens with citrullination, one of the common inflammation-dependent PTMs targeted by the adaptive immunity during the progress of T1D development, are still not fully identified. Glucokinase is predominantly expressed in the hepatocytes, where it regulates glycogen synthesis, and in pancreatic beta cells, where it acts as a glucose sensor to initiate glycolysis and insulin signaling. Here we show glucokinase citrullination increased in inflamed human islets and as well as from non-obese diabetic (NOD) mice. However, there is no citrullinated glucokinase detected in liver from both NOD mice and age-matched C57BL/6 mice. Moreover, autoantibodies against both glucokinase and protein arginine deiminase (PAD) treated-glucokinase arise in both human T1D and murine models. Mature insulin is stored in the form of Zn-containing hexamers until secretion. Interestingly, anti-glucokinase antibodies correlated positively with anti-ZnT8, but not with other established T1D autoantibodies (insulin, GAD and IA2). By mass spectrometry, twenty citrullination modification residues were mapped in PAD-treated glucokinase. Likewise, autoreactive $CD4^+$ T cells to citrullinated glucokinase epitopes are present in

DOI: 10.1002/eji.201970400

the circulation of T1D patients. Our studies implicate the crucial enzyme glucokinase as a biomarker, providing new insights into creating autoantigens and define the impact of PTMs on the tissue pathology of T1D.

O335

Clinical significance of anti-ribosomal P protein antibodies in patients with lupus nephritis

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Background: Systemic lupus erythematosus(SLE) is a chronic inflammatory disorder affecting multiple systems of the body. Clinical features show wide variations in patients with different ethnic background. Renal involvement is a predictor of poor prognosis. Immunological workup is an integral part of SLE diagnostic criteria. Anti-ribosomal P Protein (anti-P) antibodies are highly specific for SLE. They may be present in antinuclear antibodies (ANA) negative SLE patients. Their role in lupus nephritis (LN) is under debate, some researchers found them associated with poor prognosis whereas others found favorable effect of these antibodies on renal disease.
Objective: In this study we investigated frequency of anti-P antibodies and the effect of these antibodies on renal functions in LN patients.
Methods: A total of 133 SLE patients were enrolled in this study. All patients had ANA in their sera. Anti-P antibodies along with other autoantibodies against extractable nuclear antigens (anti-Sm, anti-SS-A, anti-SS-B, anti-histones and anti-RNP) were detected by Immunoblot assay. Anti-dsDNA antibodies were detected by indirect immunofluorescence assay (IFA).
Results: We found anti-P antibodies in 10.5% LN patients. Interestingly their presence in association with anti-dsDNA was associated with improved renal functions in comparison to those who had anti-dsDNA antibodies in isolation.
Conclusions: Anti-dsDNA antibodies are directly involved in renal pathology in SLE patients. As these antibodies are nephrotoxic, concomitant occurrence of anti-P antibodies seems to offer a shielding effect on renal functions, which was evident by normal serum creatinine levels. Therefore anti-P antibodies may be considered as a good prognostic marker in these patients

O336

Cholinergic pathway protects against autoimmune diabetes through induction of muscarinic receptors

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Type I diabetes occurs as a result of immune-mediated destruction of insulin-secreting b cells in the pancreatic islets. The pancreas is known to be innervated by the vagus nerve that releases acetylcholine (ACh). ACh can bind to nicotinic and muscarinic acetylcholine receptors (nAChRs and mAChRs, respectively) which are widely expressed on pancreatic cells. We have previously demonstrated that activation of the cholinergic nervous system through the administration of a specific acetylcholinesterase inhibitor (AChEI) attenuates the development of hyperglycemia in the multiple low-dose streptozotocin (MLD-STZ) model of experimental autoimmune diabetes. This correlated with

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DOI: 10.1002/eji.201970400

a reduction in T cell infiltration into pancreatic islets, preservation of the structure and functionality of β-cells, and a reduction in proinflammatory cytokines. The present study was designed to assess the effect of mAChRs and nAChRs antagonists, atropine and mecamylamine hydrochloride, respectively, on AChEI-mediated prevention of hyperglycemia in the MLD-STZ model. Our results indicate that inhibition of nAChR did not abolish the anti-diabetic effect of AChEI. In contrast, inhibition of mAChRs largely reversed the beneficial effect of paraoxon and was associated with increased islet cell infiltration and b-cell damage. Taken together, our data suggest that muscarinic receptors are essential for the protective effect of cholinergic stimulation on autoimmune diabetes.

LB015

IgD-Fc-Ig fusion protein targeting IgD and IgDR signaling in T cells in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune disease with chronic inflammation, synovium hyperplasia, joint destruction, and T cells hyper-activation. $CD4^+$ T cells infiltration in affected joints is associate with the etiology of RA, thus, inhibition of T cell hyper-activation has been identified as a potential clinical therapy for RA. Immunoglobulin D (IgD) has been found to be over-expressed in several autoimmune diseases including RA. We have proved the existence of IgD receptor (IgDR) on T cells, B cells, fibroblast-like synoviocytes (FLSs), accompanied with higher levels of IgD and activated T cells in RA. Excessive IgD was shown to bind to IgDR on $CD4^+$ T cells in a concentration-dependent manner and stimulate the activation and proliferation of these cells by enhancing phosphorylation of the activating tyrosine residue of Lck (Tyr394) pathway. All the data indicated that blocking the binding between IgD and IgDR might become a new strategy in the treatment of RA. To confirm our hypothesis, we synthesized a fusion protein called IgD-Fc-Ig targeting IgD and IgDR. IgD-Fc-Ig could selectively block IgD binding to IgDR, suppress the abnormal proliferation and activation of T cells induced by IgD, recover the imbalance between Th17 and Treg cells, reduce inflammatory cytokines production, and downregulate phosphorylation of Lck and ZAP70. This might explain the therapeutic effect of IgD-Fc-Ig on collagen-induced arthritis (CIA) mice. Our data suggest that IgD-Fc-Ig fusion protein has the ability to regulate T cell activity in RA. Furthermore, these findings have potential implications for IgD-targeted strategies to treat IgD-associated RA.

LB016

PD-1 expression on CD8+CD28- regulatory T cells: association with rheumatoid arthritis relapse

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Study objectives: Defect in immune regulation could implicate initiation and development of rheumatoid arthritis (RA). Although regulatory T cells (Tregs) play pivotal role in the immune hemostasis, the function and phenotypes of CD8+ Treg subset in RA is still unclear. Here, we investigated the expression of PD-1 and CXCR3 on CD8+CD28- T cells in association with the IFN-γ levels in patients with RA.
Methods: A total of 39 RA patients, including 7 newly diagnosed (ND) and 32 relapsed (RL) cases and also 20 healthy donors were enrolled. Phenotypic characterization of CD8+ T cells was performed by flow cytometry, using fluorescence conjugated monoclonal antibodies. The plasma and synovial

DOI: 10.1002/eji.201970400

fluid (SF) levels of IFN-γ were also assessed by ELISA.
Results: The frequency of CD8+ Tregs had no difference in patients and controls while it was significantly higher in peripheral blood (PB) than SF of RL patients. RL patients showed higher CXCR3 and especially PD-1 expression on CD8+ Tregs. The IFN-γ concentration was elevated in SF of patients while its plasma level was significantly lower in RL subgroup than controls. A significant correlation was observed between IFN-γ concentration and the frequency of PD-1+CD8+ Treg cells in SF of RL patients.
Conclusion: Though PD-1 could induce immune suppression in effector T cells and upregulated during inflammation, its overexpression on CD8+ Tregs within inflammatory synovium may be associated with severity of disease in RA patients. Accordingly immunoregulatory molecules could comprise different effects on the onset and development of RA.

LB017

The Proliferating Cell Nuclear Antigen (PCNA) is more frequently expressed than ds-DNA in Zimbabwean SLE patients

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SLE frequently occurs and is more severe in patients of African descent. ACR and SLICC criteria identify anti-dsDNA and anti-Sm as immunological diagnostic criteria. These criteria missed numbers of African patients who otherwise fulfilled the clinical criteria. The objective was to characterize autoantibody reactivity patterns and associations with clinical manifestations of SLE in black Zimbabwean patients.
61 black patients with SLE patients and 100 controls were included. Ages ranged from 1-81 years. Serological testing used a commercial immunoblot panel which included nRNP/Sm, Sm, SS-A, Ro52, SS-B, Scl-70, Jo-1, PCNA, Nucleosomes, Ribonuclear P-Protein, AMA-M2, Histones, dsDNA, CENP-B and PM-Scl antigens.
All patients fulfilled the ACR/SLICC clinical and laboratory criteria. 97% of the 61 SLE patients had detectable autoantibodies. Only 15% of the 100 controls ($p< 0.001$) had low titre reactivity predominantly to AMA-M2. Autoantibody reactivity to PCNA (54%) and dsDNA (41%) was highest. None reacted to Scl-70, CENP-B or PM-Scl100. Anti-PCNA and anti-dsDNA reactivity were mutually exclusive ($p< 0.001$) resulting in 2 distinct groups of patients. The anti-dsDNA reactive (N=25) were 13 times more likely to present with systemic symptoms including synovitis and serositis. The anti-PCNA group (N=34), was 9 times more likely to present cutaneous symptoms. Anti-AMA-M2 reactivity was frequent.
Clinical features of SLE can be accompanied by different autoantibody signatures. Anti-dsDNA is asscoaited with systemic disease while anti-PCNA could be a marker for the cutaneous (DLE/CLE) variants of SLE. The frequent expression of AMA-M2 by the PCNA+ group warrants investigation. The inclusion of PCNA increased the SLE diagnostic yield.

DOI: 10.1002/eji.201970400

LB052

Corticosteroids therapy further reduces decreased level of immunoprotective androgen 7α, 7β, and 16α-hydroxy-metabolites in autoimmune disease IgA nephropathy

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Background: IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide characterized by increased serum level of IgA1 with galactose (Gal)-deficient O-glycans (Gd-IgA1) which are recognized by anti-glycan antibodies, leading to formation of circulating immune complexes (CIC) which after deposition to glomeruli trigger glomerular injury. In patients with active disease nonresponding to ACE inhibitors or AT II blockers, glucocorticoids (GC) are recommended. GC therapy is associated with individual dysregulation of hypothalamic-pituitary-adrenal axis and suppression of gonadal functioning which superimposes the therapeutic effects of therapeutic GC and could be used for prediction of individual GC outcome.
Methods: The relationship between the GC therapy and serum levels of Gd-IgA1, IgA-containing CIC, and their mesangioproliferative activity was analyzed as well as the steroid metabolome in the circulation of IgAN patients and disease and healthy controls. Steroids were quantified by LC-MS.
Results: GC therapy significantly reduces levels of Gd-IgA1, and CIC. Before initiation of GC therapy, IgAN patients exhibited significantly reduced activity of adrenal zona reticularis (ZR) androgens and lack of adrenal androgens and the low androgen levels. The GC therapy further reduces overall androgen deficiency including the most efficient immunoprotective Δ5 steroids 7α, 7β, and 16α-hydroxy-metabolites. Combination of GC treatment with supplementation of Δ5 steroids or their 7α, 7β, and 16α-hydroxy-metabolites is potential solution to enhance the efficacy of GC therapy in IgAN patients.

Supported by Ministry of Health, Czech Republic - conceptual development of research organization (FNOl, 0098892) and by grant CZ.02.1.01/0.0/0.0/16_025/0007397 of the Ministry of School, Youth, and Sport, Czech Republic.

LB053

The effect of methotrexate treatment on neutrophil activation and reactive oxygen species in rheumatoid arthritis

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Background: Methotrexate (MTX) is the gold-standard disease modifying anti-rheumatic drug in rheumatoid arthritis. Its effects on neutrophils are not clear.
Methods: In this cross-sectional study, patients with rheumatoid arthritis (RA) and healthy controls were included. RA patients included naïve and MTX treated patients (at least 15 mg/week, 6 months), labeled as naïve-RA and MTX-RA. Disease activity in RA was assessed using DAS28-3. Neutrophils were separated using Hetasep and density-gradient centrifugation. Reactive oxygen species (ROS) were detected by dihydrorhodamine (DHR) assay after PMA stimulation (1.62 ug/ml) on FACS. ROS

DOI: 10.1002/eji.201970400

by Luminol was detected by chemiluminescence. Activation markers CD177, CD11b and CD64 were detected by surface staining on FACS.
Results: This study included 100 RA patients, 53 (F:M= 43:10) on MTX (MTX-RA) and 47 (F:M=39:8) naïve (naïve-RA). Naïve-RA patients had significantly lower disease activity (DAS28-3) than MTX-RA (6.2, 4.9., p< 0.001). At baseline, unstimulated neutrophils of naive-RA patients showed higher level of ROS production on DHR assay (MFI) than MTX-RA patients (11049, 7301, p=0.009). Even after PMA stimulation, ROS levels by DHR (MFI) assay were higher in naive-RA than MTX-RA. However, there was no significant difference in ROS between MTX-RA and healthy controls. Naïve RA patients had higher expression of CD177 in neutrophils (MFI) compared to MTX-RA patients and healthy controls. MTX-RA and naïve-RA patients had higher levels of CD11b than healthy controls.
Conclusions: MTX treatment in RA patients was associated with reduction of ROS production and CD177 expression in neutrophils.

LB054

Synovial fluid fibroblast-like synoviocytes enhance long-term survival of EBV infected memory B cells in rheumatoid arthritis

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Although many efforts have been done in understanding the aetiology of rheumatoid arthritis (RA), the Epstein-Barr Virus (EBV) remains one of the strongest candidates to be investigated as possible environmental factors in inducing RA. Of relevance, a high percentage of RA patients are characterised by a strong immune response to EBV, with high blood DNA-viral load and circulating cross-reactive (auto)antibodies reacting against self-citrullinated antigens (ACPA) and viral proteins. EBV infection induces the activation of the growth program in B cells, leading to their differentiation into memory B cells. Therefore, this compartment has been proposed as the source of epitopes triggering the disease.
To obtain long-standing EBV-infected B cells, we setup an *in vitro* co-culture system whereby RA fibroblasts-like synoviocytes (FLS) obtained from synovial fluid were cultured with CD19+ B cells for 28 days. RA-FLS were used as scaffolding and feeding environment for the CD19+ B cells obtained from ACPA+ RA patients. CpG was added to the co-culture in order to induce plasmacells differentiation to test the presence of ACPA antibodies towards viral epitopes. At the end of the co-culture, B cells were recovered to perform molecular biology analysis to detect and quantify specific EBV-related sequences such as BAMHI-W.
Preliminary data have suggested that the recovered B cells at the end of the co-culture were EBV+. Therefore, in this work we are proposing a new method to obtain EBV+ B cells in order to develop better tools to study the complex role of the virus in RA pathogenesis.

DOI: 10.1002/eji.201970400

LB055

Serum anti-neural immunoreactivity in patients with fibromyalgia. Preliminary study

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Fibromyalgia (FM) is a non-degenerative syndrome, characterized by generalized and chronic musculoskeletal pain, which can be associated to sleep, memory and mood disorders.
In this work, we look for the presence of seric anti-neural antibodies in 15 fibromyalgia patients, diagnosed according to the clinical criteria of the American College of Rheumatology; and 14 healthy volunteers (control subjects), All participants signed informed consent. The determination of anti-neural antibodies was made by indirect immunofluorescence assay on mouse neural tissue (brain, brainstem and cerebellum); and the proteins recognized by the neuro-immunoreactive sera were identified by Western blot (Wb).
One third of the FM patients had seric anti-neural antibodies against neurons from the medial vestibular nucleus (MVN) in the brainstem, which constitutes a part of the raphe nuclei, involved in the sleep-regulating serotoninergic system. The Wb of the immunoreactive sera recognized a 45 kDa protein.
This work is the first to show the existence of seric anti-neural antibodies against to MVN neurons of mouse brainstem in 33% of FM patients. This finding could be associated to specific clinical subgroups of the disease and explain their neuroimmune dysfunction. Further studies are necessary to investigate whether the neural immunoreactivity observed is present in a larger number of FM patients and if it exerts some biological or pathophysiological effect in the development of fibromyalgia syndrome.

LB089

MHC dextramers: versatile tools for assessing antigen-specific T cell responses

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Technologically, creation of MHC tetramers has been one of the major advancements in the T cell immunology that permit analysis of antigen-specific T cells in various settings. While, MHC class I tetramers have been successfully used to analyze CD8 T cells in a variety of systems, low sensitivity of MHC class II tetramers remains a major challenge in the field. To overcome this challenge, we created a newer version of tetramers called dextramers, and demonstrated that the dextramers are at least five-fold more sensitive than the tetramers. By generating dextramers for various mouse alleles (IA^s, IA^b, IA^d, IA^k and IE^k), we successfully enumerated the frequencies of antigen-specific T cells ex vivo, in vitro, and in situ in autoimmune models such as myocarditis, encephalomyelitis, hepatitis and uveitis. The dextramers were also useful to analyze antigen-specific T helper phenotypes with certainty. Furthermore, we successfully used dextramers as screening and sorting tools to generate T cell hybridomas for various self-antigens within a short period of approximately 7 weeks. Additionally, by using dextramers for self- and foreign-antigens, we generated cross-reactive T cell hybridomas to decipher their TCR composition, including a possibility of expression of dual TCRs by cross-reactive T

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DOI: 10.1002/eji.201970400

cells, if any. We have now derived MHC class I dextramers to analyze cardiac-specific T cells in hearts from mice affected with autoimmune or infection-induced myocarditis. Overall, because of high degree of sensitivity, the dextramer reagents are proving to be excellent tools to detect antigen-specific T cells, and to determine their functionalities in various models.

LB090

The discovery of novel inhibitors for sphingosine 1-phosphate lyase with distinct mechanisms

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Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the the central nervous system that affects over 2 million individuals worldwide. To date, no effective treatment has been developed to cure the disease. Thus, the discovery of therapeutic drug has important research significance and clinical application value. In last few decades, down-regulation of the S1P signalling pathway has been shown to be therapeutic in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, making sphingosine 1-phosphate lyase (SPL) as an important pharmacological and therapeutic target for the related diseases. In the present studies, we established a high-throughput screening assay and identified NSC626120 and Butein as novel potent inhibitors of SPL-T with IC_{50} values of 20.4 microM and 66.7 microM. Moreover, NSC626120 and Butein specifically inhibit the activity of SPL in cells and tissues, and modulate the lymphocyte egress in mice. Using the commonly used experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, we found the NSC626120 and Butein treatment significantly reduce the clinical score. Together, these results demonstrate that NSC626120 and Butein are selective inhibitors for SPL *in vitro* and *in vivo*, and could be considered as drug leads for treat autoimmune diseases, e.g. multiple sclerosis.

LB091

A unique IL-21 signature characterizes lesional and circulating T-follicular helper cells in Sjögren's syndrome patients with ectopic germinal centres and MALT lymphoma

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Dysregulated germinal center (GC) responses are implicated in the pathogenesis of Sjogren's Syndrome (SS). T follicular-helper (Tfh) cells are specialized CD4+T-cells required for the generation

DOI: 10.1002/eji.201970400

of GCs reaction, that contribute to B-cell maturation and activation via IL-21 production. Tfh cell expansion and associated IL-21 production have been linked to SS pathogenesis, nevertheless their impact on the establishment of ectopic GC within ectopic lymphoid structures (ELS) in the salivary glands (SG) and the MALT lymphoma (MALT-L) evolution in SS patients has never been addressed. Flowcytometry analysis of peripheral blood mononuclear cells (40 SS,10 sicca) showed a higher IL-21 production by Tfh cells in SS patients with SG ELS, suggesting a role for an aberrant expansion of Tfh cells and IL-21 secretion in driving aberrant GC-B cell response in SS patient with ELS. This was confirmed by the micro-array profile (30 SS and 15 sicca RNA), where the Tfh cell pathway appear as one of the most up-regulated one, together with B and plasma cells in SG biopsies with ELS. Multicolor immunofluorescence/confocal microscopy identified an increased infiltration of Tfh cells (CD3+CD4+CD45RO+ICOS+PD1+BCL6+) that closely segregate with lesional IL-21 expression within SG ectopic GCs and is strongly enriched during MALT-L development.
Taken together these data support Tfh role in sustaining GC formation and malignant transformation in SS SG. Moreover, high production of IL-21 by Tfh in peripheral blood selectively identifies SS patient with ectopic GC in the SG, suggesting a role for IL-21+ Tfh cells as surrogate markers of SG histopathology in SS.

P0222

IL-17RD constitutes a second receptor for IL-17A important in psoriatic inflammation

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T helper 17 (Th17) cells and IL-17A produced by them are critical in autoinflammatory diseases, such as psoriasis. IL-17A has been shown to signal through IL-17RA/IL-17RC complex to drive inflammatory responses. However, in a psoriasis model, we found that *Il17rc* deficiency did not completely ameliorate the disease, suggesting another receptor. We further identified that IL-17RD directly bound IL-17A but not IL-17F or IL-17A/F heterodimer and formed a heterodimer with IL-17RA. IL-17A-, but not IL-17F- or IL-17A/F-, mediated gene expression was defective in *Il17rd*-deficient keratinocytes. *Il17rd* deficiency in non-hemopoietic cells attenuated imiquimod-induced psoriatic inflammation. While IL-17RC and IL-17RD differentially activated IL-17A-dependent signaling and gene expression, their compound mutation led to complete deficits in keratinocytes. Interestingly, IL-23 was found induced by IL-17A in keratinocytes, dependent on both IL-17RC and IL-17RD, suggesting feed-forward regulation of IL-23-IL-17 axis in psoriasis. Together, IL-17RD constitutes a second functional receptor for IL-17A, and together with IL-17RC, mediates the pro-inflammatory gene expression downstream of IL-17A.

DOI: 10.1002/eji.201970400

P0223

sCD83 alleviates the symptoms of experimental autoimmune uveitis by disrupting the synapse formation of DC-T cell

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A soluble form of CD83(sCD83), generated from membrane CD83 by splice variants or by shedding, inhibites T-cell activation by suppressing the function of DCs to regulate the immune response in many autoimmune disease and organ-rejection treatment. However, the role of sCD83 in experimental autoimmune uveitis (EAU) and mechanism of sCD83 on regulating DCs is still not clear. In our study, we show that the level of sCD83 rises in the serum and aqueous humor, during onset and recovery of EAU. Systemic or topical application of sCD83 alleviates the symptom of EAU and decreases the infiltrated inflammatory cells and cytokines at the inflammation site. Mechanistically, sCD83 induces tolerogenic DCs by decreasing the synaptic expression of co-stimulatory molecules and hampering the cell contact between DCs and T cells. sCD83 disruptes the F-actin rearrangements at the DC-T cell contact zone, leading to altering localization of co-stimulatory molecules and suppressing T-cell activation. Furthermore, sCD83 is proved to enter into DCs through caveolin-1 dependent endocytic path-way. And then, sCD83 can bind with Rab1a to inhibit Rab1a-GTP activation, which further influence the regulation of LRRK2 on F-actin rearrangements. This pathway is different from the sCD83/TLR4/MD2 path-way. Thus, sCD83 disruptes the synapse formation by inhibting Rab1a/LRRK2/F-actin pathway. The ability of sCD83 to modulate DC-mediated inflammation in the eye could be harnessed to develop new immunosuppressive therapeutics for autoimmune uveitis.

P0224

Evaluation of anti-CD3 therapy in animal models of arthritis

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Objective: To investigate the potential role of Anti-CD3 therapy in rheumatoid arthritis (RA) by examining the effect of Anti-CD3 in vivo in a murine model of collagen-induced arthritis (CIA).
Method: Mice developing CIA were treated with Anti-CD3 and the ensuing disease progression and immunological profile determined. The effect of Anti-CD3 on T-cell response in vivo was also ascertained.
Results:
1) Short-term administration of Anti-CD3 at the onset of the disease significantly aggregate disease severity compared with IgG group.
2) Histological examination showed that while IgG group mice developed severe cellular infiltration in the joints, synovial hyperplasia and joint erosion, this pathology was profoundly increased in Anti-CD3 treated animals.
3) Treatment of mice with Anti-CD3 also increased the amounts of serum IL-10 and TGF-beta.
4) TGF-beta expression significantly increased, and the proportion of Th17 and Treg increased, but the balance of Th17/Treg was broken, and the proportion of Th17 cells increased.
Conclusion: Although Anti-CD3 can induce IL-10 /TGF-beta expression, it fails to effectively inhibit

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DOI: 10.1002/eji.201970400

Th17 differentiation, leading to Th17/Treg ratio increased and ultimately inducing the aggravation of disease in CIA mice.

P0225

The ratio of innate-like B cells is correlated with disease activity in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiple organ and system involvements. Present evidence found that apoptotic cells significant increased in SLE patients, and they seemed to have clearance disorder in the onset and progression of SLE. Natural IgM (nIgM) plays a key role in the clearance of apoptotic cells and prevents apoptotic cells from inducing abnormal autoimmunity. Natural IgM are mainly produced by B-1 and innate-like B cells (ILBs). Human $CD19^+$ $CD27^+$ IgD^+ B cells, also termed as un-switched memory B cells, were proposed to be a kind of ILBs. However, functional features and characteristics of these cells in SLE remained poorly understood. In this study, we found that human $CD27^+$ IgD^+ B cells could produce natural antibody-like IgM. Under SLE circumstance, the frequencies of these cells were significantly decreased. Moreover, the IgM-producing capacities of these cells were also dampened. These $CD27^+$ IgD^+ B cells were negatively correlated with SLE patient disease activities and clinical manifestations. After effective therapy with disease remission in SLE, these cells could be recovered. Taken together, these results have revealed that $CD27^+$ IgD^+B cells were impaired in SLE with dysfunctional features, which might contribute to the disease perpetuation.

P0226

Synovial fibroblasts-derived exosomes overexpressing microRNA-106b inhibits chondrocyte proliferation and migration via down-regulation of PDK4 in rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA), an autoimmune disorder, affects people of all demographics, and exosome-derived microRNAs (miRs) have also been documented to play an important role in inflammatory disease. In view of this, our study is performed to investigate the roles of synovial fibroblasts-derived exosomes expressing miR-106b in RA via regulating PDK4.
Methods: The differentially expressed genes were screened by analyzing the microarray chips related to RA. The expression of PDK4 and miR-106b were determined, and their regulatory effects on RA were evaluated. Meanwhile, exosomes derived from synovial fibroblasts (SFBs) were isolated and transferred into chondrocytes to identify the role of exosomes in RA. Furthermore, the effects of SFBs-derived exosomes overexpressing miR-106b on chondrocyte proliferation and migration were assessed using lentiviral packaged antagomir miR-106b *in vivo*.
Results: It was found that PDK4 was down-regulated while miR-106b was up-regulated in RA. PDK4 was a target gene of miR-106b. When PDK4 was over-expressed in chondrocytes, the abilities of proliferation and migration of chondrocytes were enhanced and the RANKL/RANK/OPG system was inhibited. However, when the miR-106b was transferred into the chondrocytes by exosomes derived

DOI: 10.1002/eji.201970400

from SFBs, the opposite growth characteristics were identified in chondrocytes. Moreover, the investigation *in vivo* revealed that inhibition of miR-106b in SFBs improved the symptoms of RA in mice.
Conclusion: Collectively, our study demonstrates that the SFBs-derived exosomes overexpressing miR-106b aggravates RA and affected RANKL/RANK/OPG system via down-regulation of PDK4.

P0227

Protein microarray analysis identifies heat shock factor 1 as a novel autoantigen in idiopathic inflammatory myopathy

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Objectives: Our study identified a novel autoantigen, heat shock factor 1 (HSF1), by a protein microarray analysis using serum from DM patients, and further evaluated the disease specificity and clinical significance of this new autoantibody in idiopathic inflammatory myopathy (IIM).
Methods: HSF1 was identified by a human protein microarray analysis. Anti-HSF1 autoantibody was then assayed by ELISA using serum from patients with IIM (n=612), Sjögren's syndrome (SS, n=40), systemic lupus erythematosus (SLE, n=40), rheumatoid arthritis (RA, n=37), and healthy controls (n=65), and validated by immunoblot and dot blot. Immunofluorescence and western blot were used to detect HSF1 expression in myositis muscle tissues and C2C12 cells.
Results: HSF1 was identified as a novel autoantigen. Anti-HSF1 autoantibody could be detected in 10.6% IIM, 10.8% RA, 12.5% SS and 5% SLE. Anti-HSF1 autoantibody in IIM was significantly associated with anti-transcriptional intermediary factor-1γ autoantibody (P=0.046), hypergammaglobulinemia (P< 0.001), elevated C-reactive protein (P=0.039) and erythrocyte sedimentation rate (P=0.006). Meanwhile, anti-HSF1 autoantibody was found associated with cancer-associated myositis on multivariate analysis (OR 2.4 [95%CI 1.0-5.9], P=0.05), and no detectable anti-HSF1 autoantibody was found in cancer controls. Moreover, a moderate correlation between anti-HSF1 levels and myositis disease activity was found in patients without cancer. Of interest, HSF1 was highly expressed in regenerating muscle cells of myositis muscle biopsy, and enhanced expression of it was observed in the early stage of C2C12 differentiation.
Conclusions: Anti-HSF1 autoantibody may be an indicator for cancer and disease activity in IIM, and its target may be involved in the immunopathogenesis of myositis.

P0228

Therapeutic effect of adipose tissue-derived stem cells in a murine model of bleomycin-induced systemic sclerosis

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At present, the treatment of systemic sclerosis (SSc) can only alleviate the clinical symptoms and delay the progress of the disease, and cannot eradicate the disease completely. The research of mesenchymal stem cells (MSCs) in autoimmune diseases has become a hot spot. We aimed to investigate the effects and mechanisms of adipose-derived stem cells (ADSCs) on bleomycin-induced

DOI: 10.1002/eji.201970400

systemic sclerosis (SSc) in mice. In comparison to the normal control group, both Th17 and Treg cells were increased in bleomycin-induced SSc mice (BLM group; P < 0.05). After ADSC treatment, the proportion of Th17 cells decreased (P < 0.05), and the proportion of Tregs increased (P < 0.05). By enzyme-linked-immunosorbent serologic assay (ELISA), we found that the levels of IL-17, TGF-beta, IL-6, and TNF-alpha in the ADSC treatment groups were significantly reduced in comparison to the BLM group (P < 0.01). After treatment with ADSCs, tissue lesions from SSc mice were reviewed by hematoxylin and eosin (H&E) staining. Masson staining also revealed that collagen fibers were reduced in the skin and lung tissues of SSc mice. After treatment with ADSCs, the levels of collagens I, III, and V in the lung and skin tissues were significantly reduced, and the level of CD31 in the skin was upregulated in comparison to those in the BLM group by immunohistochemistry (IHC; P < 0.01). Treatment with ADSCs modulated the immune response and relieved fibrosis, inflammation, and vascular lesions in BLM mice.

P0229

Preliminary analysis of differentially expressed circrna in murine model of bleomycin-induced systemic sclerosis

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The purpose of this study is to screen differential expression of circRNA in systemic sclerosis (SSc) murine model and to analyze whether it is involved in the pathogenesis of SSc. In this study, we first constructed and sequenced circRNA libraries of the skin and lung tissues of SSc murine model and the control mice, and then compared clean reads to reference genome and predict circRNA. Then the quantitative and differential expression of circRNA was analyzed, and the genes from circRNA were annotated in NR, GO, KEGG databases. DEGseq results showed that 15916 genes were up-regulated and 19519 genes were down-regulated in the lung tissues of model mice compared with those of control mice; 21839 genes were up-regulated and 27946 genes were down-regulated in the skin tissues of model mice compared with those of control mice. The results of Go analysis and KEGG analysis showed that differentially expressed genes were involved in immune system process. Pathway functional enrichment results showed that the differentially expressed circRNA-derived genes were enriched in the mTOR signaling pathway which was participated in physiological processes such as fibrosis and autophagy. In this study, we also calculated the different microRNAs binding to circRNA in the skin and lung tissues of control mice and SSc model mice by using microRNAanda. In conclusion, circRNA which was differentially expressed in skin and lung tissues of SSc model mice was involved in the pathogenesis of SSc. The data laid a foundation for further study on the pathogenic mechanisms of SSc.

P0230

Effect of PAD4-siRNA on T-cell subsets in thymocytes of CIA mice

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Rheumatoid Arthritis is a common autoimmune disease, collagen-induced arthritis (CIA) mice is common model of RA. The study is designed to detect the effects of small interfering RNA (siRNA)

DOI: 10.1002/eji.201970400

vector of Peptidyl arginine deaminase 4 (PAD4) on Th17, Treg, Tfh and Tfr cells in thymocytes tissue with CIA mice.At the end of the experiment, mice were sacrificed, thymus tissue was taken out to culture cells, and the proportion of Th17, Treg, Tfh and Tfr cells was detected by flow cytometry. The joint of CIA mice are obvious redness and swelling, and most of the joint scores reached more than 8 points. Compared with blank group, the proportion of Th17 cells in thymus of model group and negative control group increased, the proportion of Treg cells decreased, the ratio of Th17/Treg increased(P< 0.05), after PAD4-siRNA treatment, the proportion of Treg cells increased, and the ratio of Th17/Treg decreased(P< 0.05), but the proportion of Th17 cells did not decrease significantly. There was no significant difference. There was no significant difference in the proportion of Tfh cells and Tfr cells and Tfh/Tfr between model group and blank group. After PAD4-siRNA treatment, the proportion of Tfh cells decreased, the proportion of Tfr cells increased, and the proportion of Tfh/Tfr decreased (P < 0.05). PAD4-siRNA can treat CIA mice by reducing Th17 and Tfh cells, increasing Treg and Tfr cells in thymocytes, and improving the imbalance of Th17/Tregcells and Tfh/Tfr cells.

P0231

MicroRNA-181c promotes Th17 cell differentiation and mediates experimental autoimmune encephalomyelitis

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Among T helper (Th) cell subsets differentiated from naive CD4+ T cells, IL-17-producing Th17 cells areclosely associated with the pathogenesis of autoimmune diseases, including multiple sclerosis (MS) and the MS animal model, experimental autoimmune encephalomyelitis (EAE). The modulation of Th17 differentiation offers a potential avenue for treatment. Although a series of microRNAs (miRNAs) that modulate autoimmune disease development have been reported, further studies on miRNA roles in Th17 differentiation and MS pathogenesis are still warranted. Here, we demonstrated that mice with miR-181c knockdown presented with delayed EAE and slowed disease progression, along with a decreased Th17 cell population. We also found that miR-181c was a Th17 cell-associated miRNA and that Smad7, a negative regulator of TGF-b signaling, was a potential target of miR-181c. miR-181c knockdown rendered T cells less sensitive to TGF-b-induced Smad2/3, enhancing the expression of IL-2 which has been reported to inhibit Th17 cell differentiation. Moreover, through the analysis of published miRNA expression profiles from the Gene Expression Omnibus database, increased miR-181c levels were found in peripheral blood from MS patients. Our results identified a novel miRNA that promotes Th17 cell differentiation and autoimmunity, thus miR-181c may serve as a potential treatment target in patients with MS.

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DOI: 10.1002/eji.201970400

P0232

Blocking matrix metalloproteinase-9 abrogates collagen-induced arthritis via inhibiting dendritic cell migration

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Trafficking of dendritic cells (DCs) to lymph nodes to present antigens is a crucial step in the pathogenesis of rheumatoid arthritis (RA). Matrix metalloproteinase 9 (MMP-9) is the key molecule for DC migration. Thus, blocking MMP-9 to inhibit DC migration may be a novel strategy to treat RA. In this study, we used anti-MMP-9 antibody (α-MMP-9) to treat collagen-induced arthritis (CIA) in DBA/1J mice and demonstrated that α-MMP-9 treatment significantly suppressed the development of CIA via the modulation of DC trafficking. In α-MMP-9-treated CIA mice, the number of DCs in draining LNs was obviously decreased. In vitro, α-MMP-9 and MMP-9 inhibitor (MMP-9i) restrained the migration of mature bone marrow-derived DCs (BMDCs) in matrigel in response to CCR7 ligand CCL21. In addition, blocking MMP-9 decreased T and B cell numbers in LNs of CIA mice but had no direct influence on the T cell response to collagen II by $CD4^+$ T cells purified from LNs or spleen. Besides, α-MMP-9 did not impact on the expression of MHC II, CD40, CD80, CD86 and chemokine receptors (CCR5 and CCR7) of DCs both in vivo and in vitro. Furthermore, we discovered the number of $MMP\text{-}9^{-/-}$ DCs trafficking from footpads to popliteal LNs was dramatically reduced as compared with WT DCs in both $MMP\text{-}9^{-/-}$ mice and WT mice. Taken together, these results indicated that DC-derived MMP-9 is the crucial factor for DC migration and blocking MMP-9 to inhibit DC migration may constitute a novel strategy of future therapy for RA and other similar autoimmune diseases.

P0233

Overexpression of TIM-3 in macrophages aggravates pathogenesis of pulmonary fibrosis in mice

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disorder and lacks effective treatments due to unclear mechanisms. Aberrant function of alveolar macrophages (AMs) is directly linked to pulmonary fibrosis. Here, we show T cell immunoglobulin domain and mucin domain-3 (TIM-3), a key regulator of macrophage function, aggravates pulmonary fibrosis.
Method: TIM-3 mRNA expression of IPF patients was analyzed based on the Gene Expression Omnibus (GEO) and Array Express databases. And lung pathology and pro-fibrotic molecules were assessed in a bleomycin (BLM)-induced pulmonary-fibrosis model using wild-type (WT) and TIM-3 transgenic (TIM-3-TG) mice. Macrophage cells, RAW264.7, were then applied to investigate the effect of TIM-3 on macrophages under BLM exposure *in vitro*. A macrophage adoptive-transfer experiment was finally performed to examine lung morphology and pro-fibrotic molecules.
Result: TIM-3 expression was increased both in IPF patients and in our mouse model of BLM-induced pulmonary fibrosis. TIM-3-TG mice with pulmonary fibrosis developed more-serious pathological changes in lung tissue and higher expressions of transforming growth factor- β1 (TGF-β1) and interleukin-10 (IL-10) compared with WT mice. The expressions of TGF-β1 and IL-10 were significantly decreased in RAW264.7 cells exposure to BLM after TIM-3 was knocked out. Complementing these findings, WT mice receiving adoptive macrophages from TIM-3-TG mice also had more-serious lung fibrosis and increased expression of TGF-β1 and IL-10 compared with those receiving macrophages from WT mice.

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DOI: 10.1002/eji.201970400

Conclusion: Our findings revealed that TIM-3 expressed in AMs aggravated pulmonary fibrosis. Strategies targeting TIM-3 could be potential therapy for IPF.

P0234

IL-27 elevated Tr1 and attenuates rheumatoid arthritis symptoms in CIA mouse

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Objective: To estimate the protective effects of IL-27 on rheumatoid arthritis (RA) by observing Its anti-inflammatory effect and pathological morphology of IL-27 in a mice model of type II collagen-induced arthritis (CIA).
Methods: Intervention of CIA model mice with IL-27, and then observe the disease progression of the mouse and determine the expression of related inflammatory factors to determine the effect of IL-27 to the course of CIA.
Results:
1) Compared with IgG group,the arthritis index and symptom of the CIA mice treated with IL-27 was significantly decreased,so was the symptom.
2) Histopathological analysis show differences between CIA model mice intervening by IL-27 and IgG, for exudative lesions such as edema congestion, cellular inflammatory infiltration, synovial cell hyperplasia, and the pathological scores of lung tissue were also significantly reduced in IL-27 group.
3)The expression of CD4+IL-10+Tr1 in the IL-27 treatment group was significantly higher.
4) The Th17 differentiation was inhibited while the Th1 changes were not significantly different after administrating of IL-27.
Conclusion: IL-27 can induce CD4+IL-10+Tr1 expression and inhibit Th17 differentiation to alleviate disease progression in CIA mice ,which suggested IL-27 has potential for further investigation as a candidate anti-arthritic agent in future.

P0235

A betulinic acid derivative ameliorates autoimmune diseases by modulating T-cell differentiation and cytokine balance

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Autoimmune diseases are known to be critically related with inflammation. Among them multiple sclerosis (MS) and rheumatoid arthritis (RA) are two T cell-related inflammatory diseases. $CD4^+$ T helper cells especially Th17 cells, combined with dysfunction of immune regulatory network, play key roles in them and their animal models, experimental autoimmune encephalomyelitis (EAE) and the collagen-induced arthritis (CIA). Betulinic acid (BA), a natural pentacyclic triterpenoid, has displayed moderate anti-inflammatory activities. In order to search BA derivatives with better efficacy in anti-inflammation and treating autoimmune diseases, we screened a library of BA derivatives using a strategy based on T-cell differentiation. SH479, A heterocyclic ring-fused BA derivative, was found to

DOI: 10.1002/eji.201970400

have a greater inhibitory effect on Th17 differentiation. Further investigation demonstrated that SH479 has a more prominent effect in shifting T-cell differentiation balance to Treg while inhibited Th17 and Th1 cells. In mice disease models, SH479 ameliorated both EAE and CIA. The protective effect of SH479 was accompanied by decrease of pro-inflammatory cytokines IL-17 and IFN-γ, and enhanced anti-inflammatory cytokines such as IL-10 and IL-4. Mechanism studies indicated that SH479 affected Th17 and Treg balance mainly by JAK-STAT pathways. In conclusion, our data demonstrates that SH479 has a good therapeutic potential for MS and RA through anti-inflammatory effects. This finding confirms that anti-inflammatory Chinese herbal monomers and their derivatives could provide more potential therapeutic candidates for autoimmune diseases.

P0236

The expression and clinical significance of neurotrophic factor - artemin in rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) is a common disabling chronic autoimmune disease. Artemin is a member of glial cell line-derived neurotrophic factor ligand family. Studies found that artemin is an endogenous regulator of inflammation and pain, its activation can promote inflammatory bone pain, suggesting that artemin may be involved in the occurrence and development of bone and joint diseases. However, the role of artemin in RA has not been reported.
Methods: Serum artemin was measured by competitive ELISA in 58 healthy controls (HC), 67 RA patients and 46 osteoarthritis (OA) patients. The correlation and stratified analyses were carried out with the clinical and laboratory indexes of RA patients. The expression of artemin in synovial tissues of RA patients and OA patients was detected and compared by immunohistochemistry (IHC).
Results: The serum artemin of RA patients was significantly higher than HC ($p< 0.0001$) and OA patients ($p< 0.0001$), and positively correlated with erythrocyte sedimentation rate (ESR), IgA and IgG ($p< 0.05$) . The expression of artemin in synovial tissues of RA patients was up-regulated than OA patients, but the specimen size needs to be expanded for further confirmation.
Conclusion: The expression of artemin in both serum and synovial tissues of RA patients was up-regulated. Artemin may be involved in the occurrence and development of RA, but its mechanism needs to be further studied.
Keywords: RA; Artemin; Inflammation; Synovium

P0237

Effects of Daphnetin on the autophagy signaling pathway of fibroblast-like synoviocytes in rats with Collagen-Induced Arthritis (CIA) induced by TNF-α

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Daphnetin (DAP), an active ingredient extracted from *Daphne odora*, has pharmacological effects such as anti-inflammatory, antioxidation and anti-tumor properties. The current study aims to investigate the relationship between the anti-rheumatoid effect of DAP and the inhibition of both the PI3K/AKT/mTOR and autophagy signaling pathways. DAP inhibited the proliferation of CIA-FLS in a

DOI: 10.1002/eji.201970400

dose-dependent manner, accelerated the G1/G0 phase and inhibited the S phase. DAP reduced the phosphorylation of AKT and mTOR and the expression of Beclin-1 and LC3-II/LC3-I in CIA-FLS induced by TNF-α. DAP also reduced the inflammatory response in CIA-FLS induced by TNF-α by inhibiting the cytokine expression of TNF-α, IL-6, TGF-β, IL-17, and INF-γ and promoting IL-10 expression. Overall, DAP inhibited the proliferation of CIA-FLS by downregulating the PI3K/AKT/mTOR signaling pathway and autophagy, which may be potential therapeutic drug targets for the treatment of rheumatoid arthritis.

P0238

Human epididymis protein 4: a novel biomarker for diagnosis and prediction of lupus nephritis

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Objective: The aim of this study was to investigate the diagnostic and predictive performance of human epididymis protein 4 (HE4) for lupus nephritis (LN) in patients with systemic lupus erythematosus (SLE).
Methods: This study included two cohorts. One including 209 SLE patients was used to retrospectively analyze the diagnostic performance of serum HE4 for LN, the other including 74 SLE patients was used to prospectively explore the predictive value. Serum HE4 concentrations were measured by enzyme-linked immunosorbent assay.
Results: Serum HE4 level was significantly higher in SLE patients than that in healthy controls ($p< 0.001$), especially for those with LN. ROC curves showed HE4 had significantly better diagnostic performance with 150.1 pM of optimum diagnostic cutoff value (AUC 0.878, 95%CI 0.829-0.926, sensitivity 76.8%, specificity 91.1%) for LN in SLE patients than did creatinine, urea and uric acid. During the follow-up, the predictive performance of HE4 for LN with 64.8 pM as an optimal cutoff yielded an AUC of 0.714, with a 95% confidence interval of 0.597-0.831, and a sensitivity and specificity of 81.8% and 53.3%, respectively. The Kaplan-Meier analysis revealed that LN occurred in 72% of high-HE4 patients and 33.3% of low-HE4 patients (P=0.036). Multivariate Cox regression revealed that HE4 were independently associated with the incidence of LN.
Conclusions: Serum HE4 level can be used as a novel and useful biomarker for diagnosis and prediction of LN in SLE patients.

P0239

Serum Sp17 autoantibody is a biomarker for patients with active SAPHO syndrome

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SAPHO syndrome is a group of special symptoms, namely, synovitis, acne, pustulosis, hyperostosis and osteitis, including bone and joint injury that may or may not be accompanied by skin lesions. The pathogenesis of SAPHO syndrome is unclear but is thought to be related to autoimmune responses. In this study, results showed that serum IgG and IgM concentration of active and stable SAPHO patients were significantly higher than healthy controls (HC). To profile serum IgG autoantibodies in patients with active SAPHO syndrome, protein microarrays were used. Results indicated that the concentrations of anti-UACA and anti-Sp17 antibodies were significantly higher in patients with active SAPHO syndrome than in HCs. A further validation study was conducted by means of ELISA and western blot with independent active or stable SAPHO syndrome patients, systemic lupus erythematosus (SLE) patients, and HCs. The results showed that serum anti-Sp17 autoantibody was

DOI: 10.1002/eji.201970400

specific to patients with active SAPHO syndrome, and the concentration of this autoantibody was positively correlated with the level of serum C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). Furthermore, the results showed that the concentration of serum anti-Sp17 autoantibody in patients with active SAPHO syndrome who responded to pamidronate disodium treatment declined continuously after two treatment cycles and was accompanied by the extinction of inflammation in these patients. In conclusion, serum anti-Sp17 autoantibody was related to the autoimmune response and was a specific biomarker for patients with active SAPHO syndrome. Besides, serum anti-Sp17 autoantibody can be used as a potential therapeutic target for SAPHO syndrome.

P0240

Gut microbiota translocation promotes autoimmune cholangitis

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Gut microbiota and bacterial translocation have been implicated as significant contributors to mucosal immune responses and tolerance; alteration of microbial molecules, termed pathogen-associated molecular patterns (PAMP) and bacterial translocation are associated with immune pathology. However, the mechanisms by which dysregulated gut microbiota promotes autoimmunity is unclear. We have taken advantage of a well-characterized murine model of primary biliary cholangitis, dnTGFβRII mice, and an additional unique construct, toll-like receptor 2 (TLR2)-deficient dnTGFβRII mice coined dnTGFβRIITLR2-/- mice to investigate the influences of gut microbiota on autoimmune cholangitis. Firstly, we report that dnTGFβRII mice manifest altered composition of gut microbiota and that alteration of this gut microbiota by administration of antibiotics significantly alleviates T-cell-mediated infiltration and bile duct damage. Second, toll-like receptor 2 (TLR2)-deficient dnTGFβRII mice demonstrate significant exacerbation of autoimmune cholangitis when their epithelial barrier integrity was disrupted. Further, TLR2-deficiency mediates downregulated expression of tight junction-associated protein ZO-1 leading to increased gut permeability and bacterial translocation from gut to liver; use of antibiotics reduces microbiota translocation to liver and also decreases biliary pathology. In conclusion, our data demonstrates the important role of gut microbiota and bacterial translocation in the pathogenesis of murine autoimmune cholangitis.

P0241

Tissue-resident memory CD8+ T cells acting as mediators of salivary gland damage in a murine model of Sjögren's syndrome

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Objective: Although a role for CD4+ T cells in the pathogenesis of Sjögren's syndrome (SS) has been documented, the pathogenic significance of CD8+ T cells is unclear. The aim of this study was to investigate the role of CD8+ T cells in the development of SS.
Methods: In parallel, p40−/−CD25−/− mice were used as a murine model of SS. In addition, mice with genetic knockout of CD4, CD8a, or interferon-γ (IFNγ) were crossed with p40−/−CD25−/− mice to study the pathogenic significance of specific lineage subpopulations, including functional salivary gland tests as well as histopathologic and serologic data. A CD8+ T cell-specific depletion antibody was used in this murine SS model to evaluate its potential as a therapeutic strategy.
Results: CD8+ T cells with a tissue-resident memory phenotype outnumbered CD4+ T cells in

DOI: 10.1002/eji.201970400

the labial salivary glands of patients with SS, and were primarily colocalized with salivary duct epithelial cells and acinar cells. Infiltrating CD8+ T cells with a CD69+CD103+/− tissue-resident phenotype and with a significant elevation of IFNγ production were dominant in the submandibular glands of mice in this murine SS model. **CD8a knockout and depletion of CD8+ T cells abrogated the development of SS in these mice**. Knockout of IFNγ decreased CD8+ T cell infiltration and gland destruction.
Conclusion: These data reveal the pathogenic significance of CD8+ T cells in the development and progression of SS in the salivary glands. Treatment directed against CD8+ T cells may be a rational therapy for the management of SS in human subjects.

P0242

STAT6 deficiency ameliorates severity of Graves' Disease by suppressing thyroid epithelium hyperplasia

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Transcription 6(STAT6) was reported to be related to both Th2 mediated autoimmune responses and epithelial cell development. However, the STAT6 phosphorylation status in Graves' Disease(GD)patients in unknown. In this study, we show that STAT6 phosphorylation Increased in thyroid epithelial cells from both GD patients and EAGD mice and STAT6 deficiency ameliorates GD.Futher more, we show that the activation of STAT6 in thyroid epithelial cells was IL-4-induced, and STAT6 Induced upragulated of Bcl-Xl and Cyclin D1 is crucial mechanisms for epithelial cell development. More importantly,in vitro and in vivo studies demostrated that STAT6 inhibited by AS1517499 decreased Thyroid Epithelium Hyperplasia ,results amelioration of Graves' Disease. Thus,our study reveals that STAT6 activation is increased in GD patients' thyroid epthelial, more impaortantly, STAT6 inhibition ameliorates Severity of Graves' Disease which may provide a new sight of clinical treatment of GD.

This work was supported by General Program of National Natural Science Foundation of China (31770992, 81771736) and Young Elite Scientists Sponsorship Program by CAST (YESS20160094).

P0243

Myositis-pecific autoantibodies and clinical phenotypes correlate with the aberrant expression of immune-related microRNAs in dermatomyositis

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Aims.We assessed the expressions of these five miRNAs in association with clinical phenotypes and myositis-specific autoantibody-defined subgroups of dermatomyositis (DM). Methods. The present study included 49 patients with DM and 30 healthy controls. The serum concentrations of miR-23a-3p, miR-23b-3p, miR-146a-5p, miR-146b-5p, and miR-150-5p were measured by qRT-PCR. Associations between the serum concentrations of miRNAs and DM clinical immune phenotypes were examined as well. Results. The serum concentrations of miR-23b-3p, miR-146a-5p, and miR-150-5p were significantly downregulated in DM patients, while miR-146b-5p was remarkably upregulated in DM patients compared with healthy controls (all $P< 0.05$). Similarly, the expressions of miR-23b-3p, miR-146a-5p, and miR-150-5p were significantly downregulated in PBMCs from DM patients. Further study indicated that the serum level of miR-23b-3p was significantly correlated with CK ($r = -0. 286$) and

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DOI: 10.1002/eji.201970400

miR-146a-5p was evidently correlated with CRP (r = -0.358). Significant correlations were also observed between miR-146b-5p and CRP (r = -0.347) and the ESR (r= -0.287) (all P< 0.05). In addition, the expression level of miR-146b-5p was upregulated in DM complicated by tumors compared with those without tumors (P< 0.05). Especially, miR-150-5p was significantly downregulated in DM patients with anti-MDA5 antibodies and anti-NXP2 antibodies compared with those without (P< 0.05). No significant differences were observed between the four serum microRNAs in patients with and without interstitial lung diseases (all P>0.05). Conclusion. The results suggest an association between the four immune-related microRNAs and different clinical immune-phenotypes, and this association may regulate the complexity of disease processes through multipathways in DM patients.

P0244

The RIG-I pathway is involved in peripheral T-cell lymphopenia in patients with dermatomyositis

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Objective: To determine retinoic acid-inducible gene I (RIG-I) expression levels in peripheral lymphocytes and to explore the mechanism of RIG-I in lymphopenia in patients with dermatomyositis (DM).
Methods: The mRNA and protein expression levels of the RIG-I were determined in peripheral T lymphocytes of 26 treatment-naive DM patients by q-PCR and Western blot, respectively. The associations between RIG-I expression levels and clinical characteristics were investigated. We examined the relationship between RIG-I expression and cell apoptosis by RIG-I overexpression or knockout in Jurkat cells. Cell apoptosis was assessed by flow cytometry. Fas and Caspase 3 were identified by Western blot. CCK8 colorimeter was performed to monitor cell proliferation.
Results: RIG-I expression levels were significantly higher in peripheral T lymphocytes of DM patients than those in control subjects. The expression levels of RIG-I correlated negatively with the lymphocyte count. RIG-I protein expression decreased significantly and the number of lymphocytes increased when disease was improved. In Jurkat cells, increased apoptosis and elevated expression levels of Fas and Caspase-3 protein were observed following RIG-I overexpression or RIG-I specific ligand (pppRNA) activation. Furthermore, the proliferation of Jurkat cells was markedly reduced by RIG-I overexpression or pppRNA activation. Whereas, neither the ratio of apoptotic population nor the cell viability of the RIG-I knockout clones exhibited significant changes following pppRNA activation.
Conclusion: The present study indicated that increased expression of RIG-I in peripheral T lymphocytes was significantly associated with lymphopenia in patients with DM. The RIG-I pathway played a critical role in the induction of lymphocyte apoptosis.

DOI: 10.1002/eji.201970400

P0245

C3 glomerulonephritis with monoclonal gammopathy: a case study and review of literature

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C3 glomerulonephritis (C3GN) is a rare disease characterized by C3c dominant staining on renal biopsy and caused by inherited or acquired complement alternative pathway (CAP) dysregulation, which could also be secondary to monoclonal gammopathy of undetermined significance (MGUS). Herein, we report a 76-year-old man presenting with nephritic syndrome, impaired renal function and monoclonal gammopathy (IgGλ). His renal biopsy was consistent with C3GN, with a membranoproliferative glomerulonephritis pattern. To further identify the causes, we carried out screening tests for inherited or acquired factors. As a result, anti-complement factor H (CFH) autoantibodies were detected in his plasma. Plasmapheresiswas performed and the anti-CFH autoantibodies were purified from plasma. The purified autoantibodies were proved to be monoclonal IgGλby both western blot and immunofixation electrophoresisassay. In vitro studies further showed that these purified monoclonal autoantibodies could interfere with the bio-function of CFH, which might induceCAP activation, leading to the development of C3GN. The diagnosis of monoclonal gammopathy of renal significance (MGRS) was confirmed, andthe patient rejected chemotherapy and received oral prednisone and cyclophosphamideafter 6 rounds of plasmapheresis. He responded well with decreased proteinuria, stable renal function, negative anti-CFH antibody and increasing serum C3 level. **It is the first time to prove that intact monoclonal immunoglobulin could act as anti-CFH autoantibodies and lead to C3GN by activating the CAP.**

P0246

Anti-complement factor H autoantibodies may be protective in lupus nephritis

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Anti-CFH antibodies have been reported in SLE and the epitopes seem to be different from those inatypical hemolytic uremic syndrome (aHUS). This study aims to investigate the role of anti-CFH autoantibodies in lupus nephritis.120 patients with biopsy-proven active lupus nephritis as a discovery cohort, 60 patients as a validation cohort, and 30 healthy controls were included. The prevalence of anti-CFH autoantibodies in lupus nephritis were significantly higher than that in healthy controls (8.3% (10/120) vs. 0% (0/30), $P< 0.001$), no significance in validation group (8.3% (10/120) vs. 0% 11.7% (7/60), $P=0.13$). And the IgG subclass was mainly IgG2 (7/10). The major epitopes were in the middle (8/10 in SCRs11-14) and N-terminal (7/10 in SCRs1-4) of CFH.We further randomly selected 10 age-matched female patients without anti-CFH autoantibodies, patients with positive antibodies also had lower ratio of AKI (0%(0/10) vs. 40.0%(4/10), $P=0.025$), lower levels of sreum creatinine value than those without(67.3 (35.8, 94.0)vs. 126.0 (40.9, 985.5), $P=0.023$), and higher hemoglobin value (113.8±24.63 vs. 90.0±22.53, $P=0.037$). *In vitro*study showed that the anti-CFH autoantibodiescan improve the binding between CFH and C3b and enhance the co-factor activity of factor I. **In conclusion, anti-CFH autoantibodies can be detected in lupus nephritis and mainly IgG2 subclass, levels of anti-CFH antibodies were associated with AKI, and anti-CFH autoantibodies**

DOI: 10.1002/eji.201970400

could enhance the bio-functions of CFH, which suggested that anti-CFH autoantibodies might be protective in lupus nephritis.

P0247

Peripheral CD24^{hi}CD27^{+}CD19^{+}B cells subset as a potential biomarker in naïve systemic lupus erythematosus

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B cells play critical roles in the pathogenesis of systemic lupus erythematosus (SLE). Our aim was to investigate the role of peripheral CD24^{hi}CD27^{+}CD19^{+}B cells in new-onset SLE patients.
Methods: Peripheral CD24^{hi}CD27^{+}CD19^{+}B cells were analyzed in 55 new-onset lupus and 36 healthy controls by flow cytometry. All SLE cases were treated with prednisolone and hydroxychloroquine during a 1-year follow-up. Thirteen cases were added with cyclophosphamide or mycophenolate mofetil. The CD24^{hi}CD27^{+}CD19^{+}B cells were analyzed at days 0, 7, 14 and months 1, 3, 6, 9 and 12. IL-10-producing B cell was detected in eight naïve lupus and ten healthy controls.
Results: Compared to healthy controls, the frequency and number of circulating CD24^{hi}CD27^{+}CD19^{+}B cells were significantly reduced in SLE cases (8.22±3.48 *vs*. 31.67±5.53%, P< 0.0001; 4.04±2.85 *vs*. 38.66±10.22 10^3 cells/mL, P=0.0001) before treatment; IL-10^{+}CD19^{+}B cells and IL-10^{+}CD24^{hi}CD27^{+}CD19^{+}B cells also decreased in SLE. Interestingly, primary CD24^{hi}CD27^{+}CD19^{+}B cells inversely correlated with SLE disease activity index (SLEDAI) score. Patients with arthritis and hematologic disorders had a lower number of primary CD24^{hi}CD27^{+}CD19^{+}B cells. In 48 SLE cases who finished the 1-year follow-up, the frequency and number of CD24^{hi}CD27^{+}CD19^{+}B cells increased from 8.26±3.61 to 25.51± 4.56%; 3.99±2.86 to 28.64±11.81 10^3cells/mm^3 (P< 0.0001) accompanied by a significantly decreased SLEDAI score. Of note, CD24^{hi}CD27^{+}CD19^{+}B cells decreased in some flare cases with an elevated SLEDAI score.
Conclusion: These results demonstrate that decreased CD24^{hi}CD27^{+}CD19^{+}B cells may be an immunologic aspect of new-onset SLE. CD24^{hi}CD27^{+}CD19^{+}B cells may be a useful tool to evaluate lupus activity and monitor the response to therapy.

P0248

Podocyte injury could predict prognosis in renal thrombotic microangiopathy: a clinico-pathologic study

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Our study mainly aimed to identify the role of podocyte injury in renal thrombotic microangiopathy (TMA). Foot process effacement (FPE) was quantified by foot process width (FPW) on electron microscopy(EM).The associations between podocyte injury and other clinicopatholgocial features, prognosis were analyzed. Sixty-three patients with biopsy-proven TMA were included. Median follow-up was 40 months(range:3-207 months). **The mean FPW (1090.21±636.69nm) in our TMA cohort was significantly higher than the normal.** FPW was significantly correlated with eGFR (r_s=-0.335,

DOI: 10.1002/eji.201970400

P=0.007) and proteinuria(r_s=0.255,*P*=0.047). Compared with patients in FPW< 868.66nm group, patients with higher levels of FPW presented with higher prevelance of AKD at onset (*P*=0.046) and more refractory to treatment (*P*=0.004). Levels of FPW was significantly correlated with severity of subendothelial edema on EM (r_s=0.515,*P*=0.008) and GBM double contours (r_s=0.403,*P*=0.041) in patients with FPW< 868.66nm, but were not in those with FPW≥868.66nm, in which the levels of FPW was correlated with the severity of mesangiolysis (r_s=0.351,*P*=0.033), ischemic GBM shrink (r_s=-0.393,*P*=0.016) and glomerulosclerosis (r_s=0.369,*P*=0.025). **Kaplan-Meier method showed that patients with high-grade FPE (FPW≥868.66nm) had poorer outcome(*P*< 0.001).** By multivariate Cox hazard analysis after correction of etiology, initial clinical data, therapy regimen, or other clinicopathological features, the high-grade FPE was also an independent risk factor for renal outcome. **In conclusion, podocyte injury were associated with clinico-pathological features in renal TMA and could predict the poor prognosis, suggesting the potential role of podocyte injury in the pathophysiology of renal TMA.**

P0249

Circulating anti-C3b autoantibodies in patients with lupus nephritis: a cohort study from China

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by overproduction of numerous autoantibodies. Autoantibodies against complement C3b are found in patients with lupus nephritis. To investigate the role of anti-C3b autoantibodies as a biomarker of lupus nephritis, and further elucidate whether these antibodies were involved in the pathogenesis of lupus nephritis, we analyzed the frequency of circulating anti-C3b IgG, their clinical significance and functional properties in a multicenter study of Chinese patients. The lupus nephritis cohort consisted of 134 patients with lupus nephritis as a discovery cohort, 42 patients as a validation cohort along with 135 healthy controls. Circulating anti-C3b autoantibodies were found in 47.8% of lupus nephritis patients in the discovery cohort. The result were confirmed in the validation cohort. The serum anti-C3b autoantibodies correlated with disease severity, and inhibited C3b interaction with the regulatory proteins Factor H and CR1. In conclusion, the anti-C3b autoantibodies were present in lupus nephritis with a high frequency. Our data suggest that the production of anti-C3b autoantibodies signifies a level of complement activation and can contribute to the pathological process in lupus nephritis.

P0250

Evaluation of 10 SLE susceptibility loci in Asian populations, which were initially identified in European populations

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Ten novel loci have been found to be associated with systemic lupus erythematosus (SLE) susceptibility in Europeans. To test their disease associations and genetic similarities/differences in Asians and Europeans, we genotyped the 10 novel single nucleotide polymorphisms (SNPs) and performed an association study. A Chinese cohort from Northern China was recruited as the discovery population, and three East Asian cohorts were included for independent replication. The 10 SNPs

DOI: 10.1002/eji.201970400

were genotyped using TaqMan allele discrimination assays. To prioritize the associated SNPs, different layers of the public functional data were integrated. Among the 10 SNPs, rs564799 in IL12A was shared in both ethnicities (Padjust = 5.91×10^{-4}; odds ratio = 1.22, 1.10-1.35). We also confirmed the reported polymorphism rs7726414 in TCF7 in the current study (Padjust = 4.12×10^{-8}; odds ratio = 1.46, 1.28-1.66). The directions and magnitudes of the allelic effects for most of the 10 SNPs were comparable between Europeans and Asians. However, higher risk allele frequencies and population-attributable risk percentages were observed in Asians than in Europeans. We also identified the most likely functional SNPs at each locus. In conclusion, both genetic similarities and differences across ethnicities have been observed, providing further evidence for a genetic basis of the high incidence of SLE in Asian ancestry.

P0251

Study of von Willebrand Factor in the pathogenesis of C3 glomerulopathy

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Background: Inherited complement defects leading to the dysregulation of complement alternative pathway (AP) are associated with C3 glomerulopathy (C3G). However, most patients had a negative screening, supporting the presence of undiscovered genetic disorders to the disease. To identify novel C3G-related genes, we comprehensively screen the genetic backgrounds in C3G patients and explore the possible mechanism.
Methods: Eight-six genes in complement, coagulation and endothelial pathways were sequenced in 35C3G patients .
Results: 29 variants were identified in 22(62.9%) patients, 5 of which had been reported in diseases and the remaining were classified as likely pathogenic. Unexpectedly, the most frequently mutated gene was *VWF*. Patients with *VWF*variants had significantly higher levels of urinary protein and higher ratios of crescent formation than those without variants (*P*=0.015 and *P*=0.037, respectively).VWF could bind to FH and C3b, and acting as a cofactor for factor I to cleave C3b in a dose-dependent manner. Furthermore, we selected two variants to express vWF, and found that: (1) vWF and FH couldn't co-localize in HUVECs cells; (2) In HEK293 cells**,** the intracellular expression, basal and histamine-induced secretions of vWF were significantly reduced in c.1519A>G variant (*P*=0.003, *P*=0.080 and *P*=0.059, respectively) compared with wild-type, while the vWF secretions in c.2771G>A variant were normal. The multimerizations of vWF in both variants were normal.
Conclusions: Most C3G patients carried genetic abnormalities in our cohort. In addition to complement system, variants in coagulation-related genes, especially *VWF*, could reduce vWF secretion and interfere complement activation via AP, which might be involved in the pathogenesis of C3G.

P0252

Hyper-branched polymers accelerating delivery of myelin peptides to treat experimental autoimmune encephalomyelitis

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While there has been extensive development of soluble epitope-specific peptides to induce immune tolerance for the treatment of autoimmune diseases, the clinical efficacy of soluble-peptides-based

DOI: 10.1002/eji.201970400

immunotherapy was still uncertain. Recent strategies to develop antigen carriers coupled with peptides have shown promising results in preclinical animal models. Here we developed functional amphiphilic hyperbranched (HB) polymers with different grafting degrees of hydrophobic chains as myelin antigen oligodendrocyte glycoprotein (MOG) peptide carriers and evaluated their ability to induce immune tolerance. We show that these polymers could efficiently deliver antigen peptide, and the uptake amount by bone marrow dendritic cells (BMDCs) was correlated with the hydrophobicity of polymers. We observe that these polymers have a higher ability to activate BMDCs and a higher efficacy to induce antigen-specific T cell apoptosis than soluble peptides, irrespective of hydrophobicity. We show that intravenous injection of polymer-conjugated MOG peptide, but not soluble peptide, markedly treats the clinical symptoms of experimental autoimmune encephalomyelitis in mice. Together, these results demonstrate the potential for using amphiphilic HB polymers as antigen carriers to deliver peptides for pathogenic autoreactive T cell deletion/tolerance strategies to treat autoimmune disorders.

P0253

Bach2 regulates $CD4^+CD45RA^-Foxp3^{lo}$ cytokine-secreting T cells function and promotes B cells response in system lupus erythematous

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Objective: Systemic lupus erythematous (SLE) is an autoimmune disease and imbalance of immune cells play important role in the disease development, however, $CD4^+CD45RA^-Foxp3^{lo}$ cytokine-secreting T cells (Fr.III cells) function and its effects on B cells response as well as regulation molecular mechanisms are still unclear. Therefore, we aim to dissect Bach2 regulates Fr.III cells function and promotes B cells response during the dynamic SLE development.
Methods: Fr.III cells cytokines expression ability and Bach2 expression level from activate SLE patients and controls were measured by flow cytometry (FACS). B cells proliferation, apoptosis and antibody production were measured in co-culture system with Fr.III cells. H3K27ac levels were detected at Bach2 gene loci in the Fr.III cells using ChIP-qPCR.
Results: We showed that Fr.III cells from SLE patients produced much more cytokines and showed Th1-Th17 cell phonotypes and promoted B cells proliferation, differentiated into plasmablast cells, increased IgG and IgA production, also regulated B cells cytokines production in the co-culture system. Furthermore, Fr.III cells express high level of ICOS and CD154, while express lower level of Bach2, which has negative correlation ship with SLE Disease Activity Index. Overexpression of Bach2 decreases Fr.III cells expression cytokines and reduced B cell response. We further identify a reduction of H3K27ac levels at the Bach2 gene logi in the SLE Fr.III cells, while using the TSA restored H3K27ac level.
Conclusion: Bach2 regulates Fr.III cells function and promotes B cells response in SLE patients and targeting Bach2 may provide a new target for immune intervention therapy of SLE.

DOI: 10.1002/eji.201970400

P0254

Decreased levels of serum IgM associated with increased risk of aorta calcification in patients with systemic lupus erythematosus

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Objective: Systemic lupus erythematosus(SLE) patients have a higher incidence of aorta calcification(AC), second to coronary artery calcification(CAC). AC becomes one of the significant causes for cardiovascular(CV) events. However, risk factors for AC is still unclear in SLE patients. Recently, immunoglobulin(Ig) M and IgG have drawn much more attention due to their potential roles in subclinical atherosclerosis and CV events. The present study was to investigate the association of serum IgM and IgG with risk of AC in SLE patients.
Methods: The study enrolled 641 patients with SLE. Baseline characteristics were collected. Calcification score was quantified from the multi-detector computed tomography (MDCT) scan image by Agatston Score method. The covariates in the two groups were balanced by using propensity score matching (PSM) model. Meanwhile, retrospective analysis was performed.
Results*:* In the total subjects, 148 (23.1%) patients with SLE had AC. **Levels of serum IgM in SLE patients with AC were lower than those without AC [0.850(0.460-1.280) vs. 1.000(0.665-1.385) g/l, p=0.007].** In multivariate regression analysis, we found IgM was negatively associated with AC (OR=0.676, 95%CI 0.480-0.964, p=0.026). Using PSM model, similar results were found in 2 propensity score-matched groups. Moreover, **IgM was associated with AC progression (OR=0.291, 95%CI 0.104-0.816, p=0.019) in retrospective cohort.** No significant association of serum IgG with AC was observed.
Conclusion: Our findings suggest that **decreased levels of serum IgM are associated with increased risk of AC and serum IgM might be a predictor for AC in SLE.**

P0255

Deficiency of programmed cell death 4 results in increased IL-10 expression by macrophages and thereby attenuates atherosclerosis in hyperlipidemic mice

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Programmed cell death 4 (Pdcd4) is a newly defined inhibitor of transcription and translation and a tumor suppressor. Recent studies have suggested that Pdcd4 may also be involved in some inflammatory diseases. However, its role in atherosclerosis, a chronic inflammation of the arterial wall, remains to be investigated. Here, we found that Pdcd4 deficiency in mice increased the expression of IL-10 in macrophages and decreased the expression of IL-17 in T cells in the presence of an atherosclerosis-associated stimulator in vitro and in high fat-induced atherosclerotic plaques. Importantly, knocking out Pdcd4 led to a decrease in atherosclerotic lesions in Apoe2/2 mice fed a high fat diet. This effect could be partly reversed by blocking IL-10 with a neutralizing antibody but not by the application of exogenous IL-17. Further mechanistic studies revealed that Pdcd4 negatively

DOI: 10.1002/eji.201970400

regulated the expression of IL-10 in an ERK1/2- and p38-dependent manner. These results demonstrate that Pdcd4 deficiency attenuates atherosclerosis in hyperlipidemic mice in part through the upregulation of the anti-inflammatory cytokine IL-10. This indicates that endogenous Pdcd4 promotes atherosclerosis and therefore represents a potential therapeutic target for patients with atherosclerosis.

P0256

Platelets are activated in ANCA-associated vasculitis via thrombin-PARs pathway and can activate the alternative complement pathway

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Background: In this study, we investigated the mechanism of platelet activation in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), as well as the activation of the alternative complement pathway by platelets in AAV.
Methods: CD62P and platelet-leukocyte aggregates in AAV patients were tested by flow cytometry. Platelets were stimulated by plasma from active AAV patients. The effect of the thrombin-protease-activated receptors (PARs) pathway was evaluated by blocking thrombin or PAR1 antagonists. After platelets were activated by plasma from AAV patients, Ca/Mg-Tyrode's buffer and Mg-EGTA buffer were used to measure complement activation in liquid phase and on the surface of platelets.
Results: The levels of CD62P-expressing platelets and platelet-leukocyte aggregates were significantly higher in active AAV patients than those in remission and normal controls. Platelets were activated by plasma from active AAV patients (percentage of CD62P-expressing platelets, 97.7±3% *vs.* 1±0.2%, $p < 0.0001$, compared with those incubated with healthy donor plasma), and this was inhibited by thrombin or PAR1 antagonists (percentage of CD62P-expressing platelets, 97.7±3% *vs.* 2.7±1%, $p < 0.0001$, 97.7±3% *vs.* 5±1.4%, $p < 0.0001$, respectively). Platelets activated by plasma from AAV patients could trigger complement activation *via* the alternative pathway, as demonstrated by significant elevation of C3a, C5a, and sC5b-9 and significantly more C3c and C5b-9 deposition on the surface of platelets.
Conclusion: Platelets were activated in AAV patients, and such activation was at least partially attributed to the thrombin-PARs pathway. Activated platelets triggered the alternative complement pathway in AAV.

P0257

CD30L/CD30 protects against psoriasiform skin inflammation by suppressing Th17-related cytokine production by Vγ4$^+$ γδ T cells

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DOI: 10.1002/eji.201970400

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Psoriasis is a common, autoimmune, chronic inflammatory skin disease. It has been demonstrated that cutaneous T17 cells play an important pro-inflammatory role in the pathogenesis of psoriasis, through the production of various Th17-related cytokines. Our previous studies have demonstrated that CD30L/CD30 signal plays a pivotal role in the differentiation of $CD4^+$ Th17 cells and $V\gamma6^+\gamma\delta$ T17 cells in the gut-associated lymphoid tissues of mouse. However, its effect on the pathogenesis of psoriasis is unknown. Here, we fully prove that CD30L/CD30 signaling plays a novel protective role in the development of psoriasis in mice, through selective inhibition of CCR6 expression and Th17-related cytokine synthesis in the $V\gamma4^+\gamma\delta$ T17 cell subset. Meanwhile, treatment with agonistic anti-CD30 mAb had a significant therapeutic effect on our psoriasis mouse model. Therefore, the CD30L/CD30 signaling pathway is an ideal target for antibody therapy, which may become a new approach for the immunobiological treatment of psoriasis.

P0258

Correlation between human cytomegaloviral gene-specific antibody levels and clinical features of systemic lupus erythematosus

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Background: Human cytomegalovirus (HCMV) infection is an important cause of systemic lupus erythematosus (SLE) and aggravates disease progression. However, dominant B cell epitopes encoded by human cytomegaloviral genes expressed in peripheral blood mononuclear cells (PBMCs) and the correlation between HCMV-specific antibody levels and clinical features of SLE patients have not been statistically clarified.
Methods: HCMV gene expression profiles in PBMCs of 3 SLE patients and 3 healthy controls were analyzed by mRNA transcriptome sequencing. B cell epitopes encoded by the HCMV genes were screened by immunoinformatics analysis and verified by enzyme-linked immunosorbent assay (ELISA), western blotting, and immunofluorescence. ELISA was used to detect the HCMV-specific antibody levels in sera from 119 SLE patients and 75 healthy controls, and the association with patient clinical features was characterized.
Results: Significant differences in UL29, UL32, UL82, UL105, and US3-specific antibody levels were detected between the sera of SLE patients and healthy controls. The comprehensive detection of UL32, UL105, and US3-specific antibodies can effectively distinguish between SLE patients and healthy controls (area under the curve = 0.831). UL29, UL82, and UL105 antibody levels were associated with abnormal renal function; UL32 and US3 were associated with an abnormal blood system; while UL29, UL82, and US3 were associated with an abnormal immune system in SLE patients.
Conclusion: HCMV-specific antibodies in SLE patients can act as novel diagnostic indicators and associated with the development and progression of SLE.

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DOI: 10.1002/eji.201970400

P0259

Platelets release proinflammatory microparticles in anti-neutrophil cytoplasmic antibody-associated vasculitis

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Objectives: Biological functions of platelets contributing to anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) are largely unclear. The current study aims to investigate the potential role of platelet-derived microparticles (PMPs) in AAV.
Methods: In the current study, microparticles in AAV patients were analyzed by flow cytometry, and PMPs were probed for relative levels of 640 secreted bioactive proteins from patients' platelets using antibody microarrays. Then these data were correlated with clinical and pathological parameters.
Results: PMPs were significantly increased in 69 AAV patients, predominantly myeloperoxidase-ANCA positive, in active stage compared with in remission (4406.8/µl [2135.4, 5485.0] *vs*. 549.7/µl [350, 708.5], $p < 0.0001$), and 43% of microparticles in active AAV were PMPs. Compared with 15 patients in remission, high-expressed proinflammatory molecules in the microparticles from platelets in 15 AAV patients in active stage revealed that potential functions of PMPs promoted the effect of chemotaxis, adhesion, growth and apoptosis (all the patients for array analysis were myeloperoxidase-ANCA positive). The level of PMPs had significant association with the disease activity, inflammation and renal damage.
Conclusion: PMPs may serve as inflammatory propagators through their wide production of proinflammatory cytokines in AAV, potentially providing a novel therapeutic target.

P0260

The clinical and immunologic features of patients with combined anti-GBM disease and castleman disease

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Patients with both anti-glomerular basement membrane (anti-GBM) disease and Castleman disease have been rarely reported. In this study, we report 3 patients with this combination. They had immunologic features similar to patients with classic anti-GBM disease. Sera from the 3 patients recognized the noncollagenous (NC) domain of the α3 chain of type IV collagen (α3(IV)NC1) and its 2 major epitopes, EA and EB. All 4 immunoglobulin G (IgG) subclasses against α3(IV)NC1 were detectable, with predominance of IgG1. In one patient with lymph node biopsy specimens available, sporadic plasma cells producing α3(IV)NC1-IgG were found, suggesting a causal relationship between the 2 diseases. One patient, who achieved remission with antibody clearance and normalization of serum creatinine and interleukin 6 concentrations after plasma exchange and 3 cycles of chemotherapy, experienced recurrence of anti-GBM antibodies and an increase in interleukin 6 concentration after chemotherapy discontinuation because of adverse effects, but both returned to normal after another cycle of chemotherapy. This clinical course and the pathologic findings support the hypothesis that the Castleman disease-associated tumor cells are the source of the anti-GBM autoantibodies.

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DOI: 10.1002/eji.201970400

P0261

Up-regulation of S1P1 increases inflammatory migration of γδ T17 cells in EAMG rats

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γδT17 cells can produce IL-17 which is involved in autoimmune diseases. We use flow cytometry assay to detect that the expression of Sphingosine1-phosphate receptor 1（S1P1）was up-regulated in thymic γδT cells in myasthenia gravis (MG) patients. Previous studies show that γδT17 cells need S1P1 for their emigrationfrom the lymph tissues during both homeostatic and inflammatory conditions. Lewis rats were immunized with the 97-118 peptide of recombinant human AChRα subunit to establish EAMG(experimental autoimmune myasthenia gravis) model in order to explore whether γδT17 cells increase the output from thymus and lymph nodes by expressing S1P1 in MG conditions or not. We analyzed the inflammatory chemotaxis characteristics of γδT17 cells and the effect of S1P1 upon γδT cells function. The results showed that EAMG Lewis rats not only increased the number of γδT17 cells in lymph nodes and thymus, but also up-regulated the expression of S1P1 and CCR9 in γδT17 cells. In addition, the expression of CCR2 in γδT17 cells was also increased in lymph nodes. Up-regulation of S1P1 and CCR9, CCR2 indicates the output increasement of γδT17 from both thymus and lymph nodes in EAMG rats. Furthermore, we found that S1P1 receptor signaling can also increase the expression of CCR2 and the secretion of IL-17 and other cytokines in γδT. And stimulating or blocking S1P1 can affect the inflammatory regulation of γδT cells in vitro.

P0262

Anti-calreticulin antibody - a novel autoantibody in patients with idiopathic inflammatory myopathies and its association with malignancy

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Objective: To investigate the prevalence of autoantibodies against calreticulin (anti-CRT Abs) and evaluate its association with malignancy in patients with idiopathic inflammatory myopathies (IIM).
Methods: Sera from 517 patients with IIM and 85 healthy controls (HC) were screened for anti-CRT Abs by enzyme-linked immunosorbent assay (ELISA) using recombinant CRT protein. Immunoprecipitation assay was partially performed to confirm the results of ELISA.IIM patients with cancer were identified and were divided into cancers in newly onset, remission and recurrence respectively based on the status of cancer. Associations of anti-CRT Abs levels with disease activity were investigated in cross-sectional and longitudinal analyses.
Results: Increased serum levels of anti-CRT Abs were found in IIM patients compared to HC and 17.0% of IIM patients were seropositive for anti-CRT Abs. The anti-CRT positive sera in ELISA immunoprecipitated bands at molecular weight of 60 kDa. Anti-CRT positive IIM patients presented with more occurrences of malignancy than those negative. Higher frequency of anti-CRT Abs was found in IIM patients with cancer than those without cancer. Furthermore, patients with cancer in newly onset or recurrence had higher prevalence of anti-CRT Abs compared to those of cancer in remission. The anti-CRT Abs levels positively correlated with disease activity in IIM patients without cancer by cross-sectional and longitudinal analyses.
Conclusions: We described anti-CRT Abs as a newly recognized autoantibody in IIM patients. The anti-CRT Abs might link with cancer development and progression in IIM, and anti-CRT Abs could be a novel candidate serological marker for cancer in IIM patients.

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DOI: 10.1002/eji.201970400

P0263

Activation of toll-like receptor-7 exacerbates lupus nephritis by modulating

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Background: Toll-like receptor-7(TLR7),whick recognizes viral single-stranded RNA, can trigger immune complex glomerulonephritis in experimental lupus erythematosus. However, whether it modulates dendritic cells(DCs)phenotype and regulatory T cells(Treg) function is incompletely understood.
Method: Splenocytes and bone marrow DCs were obtained from 5- and 20-week-old female $MRL^{lpr/lpr}$ mice and C57BL/6 mice. In addition, to understand the response of Treg and DCs to TLR7 ligation in vivo, 16-week-old female $MRL^{lpr/lpr}$ and C57BL/6 mice were distributed into two groups with or without intraperitoneal injections of TLR7 ligand every other day.
Results: After activation with the TLR7 ligand imiquimod in vivo and vitro, DCs from imiquimod-treated MRL/lpr mice showed an altered costimulatory profile,with decreased induction of CD80,CD86,and MHCII expression,comparing to age-matched C57BL/6 control mice. There was no significant difference in the numbers of $CD4^+CD25^+Foxp3^+$ cells after TLR7 ligation by imiquimod in $MRL^{lpr/lpr}$ and control mice. Immunostaining of kidney sections of nephritid MRL/lpr mice revealed that CD11c was expressed in the infiltrated tubulointerstitial cells, and confocal microscopic analysis of renal $CD11c^+CD80^+$,and $CD11c^+CD86^+$ cells showed an immature phenotype with low levels of CD80,CD86,and MHCII in imiquimod-treated MRL/lpr mice.There was no difference in the number of Foxp3 positive cells in kidneys between the imiquimod and vehicle-treated groups.
Conclusions: Our results suggest that activation of TLR7 exacerbated lupus nephritis by modulating the abnormally costimulatory phenotype of dendritic cells and functions of Treg in MRL/lpr mice.

P0264

IL-33 induces production of autoantibody against autologous respiratory epithelial cells: a potential mechanism for the pathogenesis of COPD

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The mechanisms underlying the chronic, progressive airways inflammation, remodelling and alveolar structural damage characteristic of human COPD remain unclear. In the present study we address the hypothesis that these changes are at least in part mediated by respiratory epithelial alarmin (IL-33)-induced production of autoantibodies against airways epithelial cells. Mice immunised with homologous, syngeneic lung tissue lysate along with IL-33 administered directly to the respiratory tract or systemically produced IgG autoantibodies binding predominantly to their own alveolar type II epithelial cells, along with increased percentages of Tfh cells and B2 B cells in their local, mediastinal lymph nodes. Consistent with its specify for respiratory epithelial cells, this autoimmune inflammation was confined principally to the lung and not other organs such as the liver and kidney. Furthermore, the serum autoantibodies produced by the mice bound not only to murine, but also human alveolar

DOI: 10.1002/eji.201970400

type II epithelial cells, suggesting specificity for common, cross-species determinants. Finally, concentrations of antibodies against both human and murine alveolar epithelial cells were significantly elevated in the serum of patients with COPD compared with those of control subjects. These data are consistent with the hypothesis that IL-33 contributes to the chronic, progressive airways obstruction, inflammation and alveolar destruction characteristic of phenotypes of COPD/emphysema through induction of autoantibodies against lung tissue, and particularly alveolar type II epithelial cells.

P0265

Loss of smad7 promotes inflammation in rheumatoid arthritis

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Objective: Smad7 is an inhibitory Smad and plays a protective role in many inflammatory diseases. However, the roles of Smad7 in rheumatoid arthritis (RA) remain unexplored, which were investigated in this study.
Methods: The activation of TGF-β/Smad signaling was examined in synovial tissues of patients with RA. The functional roles and mechanisms of Smad7 in RA were determined in a mouse model of collagen-induced arthritis (CIA) in Smad7 wild-type (WT) and knockout (KO) CD-1 mice, a strain resistant to autoimmune arthritis induction.
Results: TGF-β/Smad3 signaling was markedly activated in synovial tissues of patients with RA, which was associated with the loss of Smad7, and enhanced Th17 and Th1 immune response. The potential roles of Smad7 in RA were further investigated in a mouse model of CIA in Smad7 WT/KO CD-1 mice. As expected, Smad7-WT CD-1 mice did not develop CIA. Surprisingly, CD-1 mice with Smad7 deficiency developed severe arthritis including severe joint swelling, synovial hyperplasia, cartilage damage, massive infiltration of CD3+ T cells and F4/80+ macrophages, and upregulation of proinflammatory cytokines IL-1β, TNFα, and MCP-1. Further studies revealed that enhanced arthritis in Smad7 KO CD-1 mice was associated with increased Th1, Th2 and, importantly, Th17 over the Treg immune response with overactive TGF-β/Smad3 and proinflammatory IL-6 signaling in the joint tissues.
Conclusions: Smad7 deficiency increases the susceptibility to autoimmune arthritis in CD-1 mice. Enhanced TGF-β/Smad3-IL-6 signaling and Th17 immune response may be a mechanism through which disrupted Smad7 causes autoimmune arthritis in CD-1 mice.

P0266

IL-6 promoting $CD4^+$ T cells into $ROR\gamma t^{dim}$ stage is neccesary for their proliferation and expansion under inflammatory conditions

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IL-6 is a critical driver of acute and chronic inflammation and has been reported to act as a T cell survival factor. The influence of IL-6 on T cell homeostasis is not well resolved. We find that IL-6 signaling drives T cell expansion under inflammatory conditions but not during normal homeostasis. When co-transferring WT and IL-6Rα-deficient naïve CD4+CD45RBhi T cells (1:1 ratio) into Rag1-/- mice, accompanied with the colitis development, WT cells were 3-fold more abundant than IL-6Rα-/- T cells in the spleen and MLN 3 weeks after the initail transfer, indicating during the inflammation, IL-

DOI: 10.1002/eji.201970400

6Rα-deficient T cells are unable to effectively compete with wild type T cells. We further demonstrate IL-6 inducing T cells under special RORγt-dim stage is the key step for T cells expansion in disease condition while the RORγt-hi stage only needed for Th17 cells development. On the other side, IL-6 signaling is not required for T cell homeostasis under noninflammatory conditions and IL-6-induced inflammation promotes T cell proliferation is independent of lymphopenia. Blockade of RORγt through genetic knockout or a small molecule inhibitor leads to T cell expansion defects comparable to those in IL-6Rα-deficient T cells. Due to IL-6 level is undetectable in healthy mice and can be quickly induced under inflammatory environment, our results indicate that IL-6 induction of low-level RORγt of T cells provides the beginning signaling for CD4+T cell expansion during inflammation.

P0267

Myopenia is associated with joint damage in rheumatoid arthritis: a cross-sectional control study

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Background: The link between BMI and disease characteristics in rheumatoid arthritis (RA) remains controversial. Our study aimed to investigate the characteristics of body composition (BC) in RA patients and their associations with disease characteristics.
Methods: BC was assessed in consecutive RA patients and controls by bioelectric impedance analysis. Myopenia was defined by appendicular skeletal muscle mass index (ASMI) ≤7.0kg/m^2 in men and ≤5.7kg/m^2 in women. BMI and clinical data including disease activity, function and radiographic assessment were collected.
Results: There were 457 RA patients (49.5±13.1 years old with 82.7% women) and 1,860 control subjects (34.3±9.9 years old with 51.2% women) recruited. Comparisons of BMI and BC between RA patients and controls in age and gender stratification showed that lower BMI with 17.7% underweight, and lower ASMI with 45.1% myopenia are the main characteristics in RA patients. Compared with those without myopenia, RA patients with myopenia had significantly higher DAS28-CRP (median 3.5 vs. 3.0), higher HAQ-DI (median 0.38 vs. 0.13) with higher rate of functional limitation (HAQ-DI>1, median 24.8% vs. 7.6%), and higher mTSS (median 22.3 vs. 9.0) with more radiographic joint damage (mTSS>10, 71.8% vs. 45.8%, all P< 0.001). Multivariate logistic regression analysis showed myopenia were positively associated with functional limitation (OR=2.546, 95%CI: 1.043~6.217) and radiographic joint damage (OR=2.660, 95%CI: 1.443~4.904).
Conclusions: Myopenia is very common in RA patients which is associated with functional limitation and joint damage**.** Further research on the underlying mechanism and the effect of skeletal muscle mass improvement in RA management are worth exploring in future.

P0268

Increased circulating Tfh17 and PD-1+Tfh cells are associated with autoantibodies in Hashimoto´s thyroiditis

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DOI: 10.1002/eji.201970400

Objective: Hashimoto´s thyroiditis (HT) is characterized by autoantibodies targeting the thyroid. Abnormal CD4+CXCR5+T cell levels were previously shown to be associated with HT. However, Tfh cells consist of heterogeneous subpopulations, and which T follicular helper (Tfh) cell subpopulation participates in the pathogenesis of HT remains poorly understood.
Methods: Thirty healthy controls (HCs) and 52 HT patients were enrolled in the study. The percentages of Tfh, ICOS+Tfh, PD1+Tfh, Tfh1, Tfh2, Tfh17, effector Tfh, resting Tfh, effector memory Tfh, central memory Tfh, and naïve Tfh cells were all determined, and the associations between the percentages of these cells and thyroid function indices were also investigated.
Resutls: The percentage of Tfh cells was significantly higher in HT patients than in HCs. Examination of the Tfh cell subsets revealed that the percentages of Tfh1, Tfh2, and resting Tfh cells were significantly decreased, while those of the ICOS+Tfh, PD1+Tfh, Tfh17, and effector Tfh cells were significantly increased in HT patients. No significant differences in effector memory, central memory or naïve Tfh cell percentages were noted between the HC and HT groups. Furthermore, the percentage of PD1+Tfh cells was positively correlated with anti-thyroglobulin antibody levels. Most importantly, only Tfh17 cell percentages were positively correlated with anti-thyroglobulin and anti-thyroid peroxidase antibody levels and were negatively correlated serum free T3 and free T4 levels in HT patients.
Conclusions: Increased circulating Tfh17 cell and PD1+Tfh percentages are associated with higher autoantibody levels in HT patients, which imply that Tfh17 or PD1+Tfh cells may play a pathogenic role in the development of HT.

P0269

Loss of nuclear-localized AIM2 attenuates systemic lupus erythematosus by altering Tfh cell fate

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Absent in melanoma 2 (AIM2) is a member of the *Ifi*200 family. In innate immune cells, AIM2 is well known as a component of the inflammasome, which is engaged in caspase-1- and IL-1beta-mediated inflammatory responses via sensing cytosolic dsDNA. However, the expression and function of AIM2 in other immune cells, such as T cells, is unclear. Here we show a high expression of nucleus-localized AIM2 in $CD4^+$ T cells, especially in follicular helper T (Tfh) cells from both peripheral blood and tonsils. A uniquely high level of AIM2 expression is observed in Tfh-like cells from lupus skin lesions and circulation compared with that in samples from healthy subjects. A reduced frequency of $CD4^+$ T cells is found in lymph nodes and spleens from $CD4^{cre}AIM2^{f/f}$ mice, and AIM2 deficiency results in fewer Tfh cells in a KLH-induced Tfh cell response, indicating that AIM2 might regulate cell development and Tfh cell differentiation. In lupus mouse models, we demonstrate that AIM2 deficiency is capable of ameliorating lupus by reducing Tfh cell frequency. Mechanistically, AIM2 regulates C-Maf and STAT3, thereby modulating IL-21 production and CXCR5 and PD-1 expression. IL-21 is found to increase AIM2 expression via upregulation of TET2 enrichment on the AIM2 promoter region and downregulation of the DNA methylation level of AIM2. Our findings reveal a novel localization and inflammasome-independent function of AIM2 in CD4+ T cells, and the loss of AIM2 resulting in attenuating lupus symptoms by inhibiting Tfh cell differentiation, indicating a potential therapeutic role of AIM2 in autoimmune disorders.

DOI: 10.1002/eji.201970400

P0270

The susceptible HLA class II alleles and their presenting epitope(s) in goodpasture's disease

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Goodpasture's disease is closely associated with HLA, particularly DRB1*1501. Other susceptible or protective HLA alleles are not clearly elucidated. The presentation models of epitopes by susceptible HLA alleles are also unclear. We genotyped 140 Chinese patients and 599 controls for four-digit HLA II genes, and extracted the encoding sequences from the IMGT/HLA database. T-cell epitopes of α3(IV)NC1 were predicted and the structures of DR molecule-peptide—T-cell receptor were constructed. We confirmed DRB1*1501 (OR = 4.6, P = 5.7 x 10^{-28}) to be a risk allele for Goodpasture's disease. Arginine at position 13 (ARG13) (OR = 4.0, P = 1.0 x 10^{-17}) and proline at position 11 (PRO11) (OR = 4.0, P = 2.0 x 10^{-17}) on DRβ1, encoded by DRB1*1501, were associated with disease susceptibility. $\alpha_{134\text{-}148}$ (HGWISLWKGFSFIMF) was predicted as a T-cell epitope presented by DRB1*1501. Isoleucine$_{137}$, tryptophan$_{140}$, glycine$_{142}$, phenylalanine$_{143}$ and phenylalanine$_{145}$, were presented in peptidebinding pockets 1, 4, 6, 7 and 9 of DR2b, respectively. ARG13 in pocket 4 interacts with tryptophan140 and forms a hydrogen bond. In conclusion, we propose a mechanism for DRB1*1501 susceptibility for Goodpasture's disease through encoding ARG13 and PRO11 on MHC-DRβ1 chain and presenting T-cell epitope, $\alpha_{134\text{-}148}$, with five critical residues.

P0271

Tissue resident memory CD8(+) T cells promotes the development of autoimmune cholangitis

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Primary biliary cholangitis (PBC) is a chronic cholestatic autoimmune liver disease caused by immune mediated bile duct damage. T cells are critical but the exact T cell subset that mediate this process remains unknown. Previously we found that IL-12p40(-/-)IL-2Rα(-/-) mice developed portal inflammation, bile duct damage and liver fibrosis similar with human PBC. Using this mouse model, we found deficiency of CD8α or depletion of CD8+ T cells abrogated autoimmune cholangitis, demonstrating that CD8+ T cells are the dominant pathogenic cells. RNA-seq analysis showed that liver CD8+ T cells from IL-12p40(-/-)IL-2Rα(-/-) mice have higher expression of gene signatures associated with tissue resident memory phenotype compared with control mice. We further found increased liver CD69+CD62L-CD44+ tissue resident memory CD8+ T (Trm) cells in IL-12p40(-/-)IL-2Rα(-/-) mice, which are differentiated from effector memory T cells. RNA-seq analysis demonstrated that liver CD8+ Trm cells from IL-12p40(-/-)IL-2Rα(-/-) mice had increased expression of inflammatory cytokines, cytotoxic molecules, and TCR signaling molecules compared with liver central memory and effector memory T cells. Knockout of IFN-γ reduced liver Trm cell number and alleviated liver inflammation. Our data suggested that increased liver CD8+ Trm cells may play a pathogenic role in portal inflammation and bile duct damage in PBC.

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DOI: 10.1002/eji.201970400

P0272

M2 macrophages contribute to RA pathogenesis by producing inflammatory cytokines and differentiating to osteoclasts

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammatory synovitis with subsequent cartilage and bone destruction. M2 macrophages, well-known as anti-inflammation and wound-healing cells, were recently reported to promote inflammation in RA, while the underlying mechanism remains unclear. In this study, we found that an immune-complex formed by lactoferrin (LTF) and its IgG antibody (LTF-IC), which was elevated in RA sera, was able to elicit immediate pro-inflammatory cytokine production (TNFa, IL-1b and IL-6) from M2-polarized human macrophages through co-ligation of CD14/TLR4 and FcgRIIa (CD32a). The LTF-IC-treated M2 cells adopted surface maker expression pattern mimicking M1 cells and became functionally hyperactive to subsequent stimuli such as LPS, zymosan and IL-1b. Besides this, LTF-IC-treated M2 cells also facilitated Th17 differentiation in vitro accompanied by IL-17 production. Based on these findings we propose that IgG ICs containing TLR-agonizing autoantigens are able to directly switch human macrophages from M2 into M1-like phenotype, thereby promoting excessive inflammation in RA.
Osteoclasts play a major role in the bone resorption during RA pathogenesis and it's intriguing to find that M2 macrophages were novel osteoclast precursor cells involved in bone erosion. M2 macrophages purified from joint of collagen-induced arthritic mice showed enhanced capability to differentiate into osteoclasts and increased bone resorption function both in vitro and in vivo through RANK-RANKL signaling pathway. Taken together, these results suggest that M2 macrophages function as both a source of inflammatory cytokines like TNF-a and osteoclast precursors, collectively contributing to erosive changes in RA pathogenesis.

P0273

IFN-γ affects the output of S1P1 and CD62L positive thymocytes in myasthenia gravis patients by regulating lymphatic endothelial cells

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Myasthenia gravis is an autoimmune disease, which is usually associated with abnormal differentiation and migration of thymocytes. S1P1 and CD62L are involved in thymocytes maturation and migration; meanwhile mature thymocytes upregulating S1P1 expression egress from the thymus along the concentration gradient of sphingosine 1-phosphate (S1P) and the S1P lyase (SPL) catalyzes the irreversible degradation of S1P. We found that S1P1 and CD62L positive thymocytes increased in the thymus of MG patients by immunohistochemistry and immunofluorescence, and most of them accumulated around the blood vessels and lymphatics vessels, through which thymocytes exit to periphery. LYVE1 positive human thymus lymphatic endothelial cells (LECs) sorted by flow cytometry were seeded in EGM-2MV medium supplemented with VEGF-C. Our data indicated that the mRNA levels and protein expression of ICAM-1, VCAM-1, SPL, HLA-A, HLA-DR, CCL5 were significantly increased ($P< 0.05$) in LECs stimulated with 100ng/ml IFN-γ for 24h,48h,72h, while CCL19 and CCL21 mRNA levels were significantly decreased ($P< 0.05$). The results suggested that IFN-γ promotes thymocytes egression by inducing increased expression of ICAM-1, VCAM-1, CCL5 and SPL. Most importantly, the elevated expression of SPL in thymic LECs led to the degradation of S1P

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DOI: 10.1002/eji.201970400

in the thymus, which expanded the concentration gradient of S1P, favored the migration of thymocytes from the thymus.

P0274

Immunoregulation of PD1/PD-L1 inhibitory pathway on fetomaternal tolerance for abortion trigged by thyroid autoimmunity

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Background: Thyroid autoimmunity (TAI) with euthyroid is associated with miscarriage. But the exact mechanism remains unclear. Studies have shown that the programmed cell death-1 (PD-1)/pd-ligand 1 (PD-L1) pathway promotes regulation of T (Treg) cell development and inhibition of Th17 cell responses, and is essential for normal pregnancy. However, whether the PD-1 / PD-L1 pathway leads to a Treg / Th17 imbalance has not been fully investigated in TAI.
Methods: Established a TAI fetal loss model after murine thyroglobulin (mTg) immunized CBA/J female mice mating with Balb/c males. The frequencies of splenic Th17, Treg, PD-1 and PD-L1 were tested by flow cytometry. Interleukin-17 (IL-17), forkhead box P3 (Foxp3), orphan retinoic acid nuclear receptor (RORγt), PD-1 and PD-L1 mRNA levels were tested by real-time PCR.
Results: Compared with the control, in mTg group the number of $CD4^+CD25^+Foxp3^+$ T lymphocyte in the placenta and spleen were significantly reduced ($P< 0.05$), $CD4^+IL\text{-}17^+$ T-cell subsets and expression of RORγt and IL-17 mRNA in the placenta were increased ($P< 0.05$), the ratio of Treg/IL-17 in the placenta and spleen was decreased ($P < 0.05$). The expression of PD-1 and PD-L1 in mice immunized with mTg in $CD4^+$ T group decreased the subset of cells in the placenta and spleen including Tregs.
Conclusions: Our findings suggested that the role of PD-1/PD-L1 pathway in an isolated TgAb positive mouse abortion model might lead to a peripheral Treg/Th17 imbalanceand break down the balance of the fetomaternal and peripheral tolerance, Ultimately lead to the fetal loss.

P0275

Impaired interactions between γδ T cells and dendritic cells at the tissue level leading to autoimmune skin disease

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Skin harbors T-cells including skin-resident γδ T-cells called dendritic epidermal T-cells (DETC), and at least three subsets of dendritic cells (DC), including Langerhans cells (LC) that reside in the epidermis, and langerin-expressing dermal DC that reside in the dermis (LangdDC). Here, we investigated the roles of DETCs and skin-DCs in the pathogenesis of lupus dermatitis using the MRL model. We applied fluorophores to their skin to track in vivo migration of skin-DC. To be able to track skin-DC in steady state, we generated langerin-driven eGFP knock-in MRL mice. We used in situ assays to identify potential sites of regulation of skin-DC migration. We treated MRL mice with a glycolipid that ameliorates lupus dermatitis and determined its effect on skin-DC migration, and investigated mechanisms of skin-DC migration using TCR $\gamma\delta^{-/-}$ mice. Finally, to directly test the role of skin-DCs in lupus, we used DTR knock-in MRL mice to conditionally deplete LC and/or LangdDC. Results show that DETCs directly modulated LC migration via direct cell-to-cell interaction. Lupus-prone mice had reduced DETCs and reduced LC-migration; both these defects were corrected by

glycolipid-treatment. Finally, conditional ablation of LC accelerated cutaneous lupus; this effect was abrogated when both LC and LangdDC were ablated together. LC-depletion or glycolipid treatment did not affect anti-DNA antibodies or lupus nephritis. Thus, skin DETCs regulate the migration of skin-DC subsets, which is impaired in lupus leading to autoimmune dermatitis. Such specialized local regulation of autoimmunity at the tissue level can be targeted to develop tissue-specific treatment without affecting systemic immunity.

P0276

Characterization of the linkage between the spectrum of T cell diabetogenicity and intrinsic TCR signaling by CD5 expression

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Before entering into peripheral tissues, thymocytes expressing diverse T cell receptors (TCR repertoire) must productively contact to self-antigens to mature (positive selection). Past studies have revealed that positive selection also enhances effective mature T cell responses to later encountering foreign antigens. However, whether deregulation of this process has a role in predisposing developing T cells to become auto-reactive T cells in autoimmune diseases is unknown. Previous studies have shown that CD5 expression level reflects the strength of TCR-self antigens interaction and the intrinsic difference in this interaction referred by CD5 level may affect later peripheral adaptive immunity response. Our study showed that previous Ptpn22-mediated attenuation of diabetogenicity in Ptpn22/NOD mice was not observed in Ptpn22/NOD8.3 transgenic mice. We hypothesize that T cells from NOD8.3 TCR transgenic mice are intrinsically high in TCR signaling, therefore are more diabetogenic to cause diabetes. We extracted $CD8^+$ T cells from NOD8.3 TCR transgenic mice and indeed found that these cells, in addition to more pathogenically causing diabetes, they were also high in CD5 level, suggesting that intrinsic difference in TCR signaling may overcome the original alleviating effect of Ptpn22 on diabetes observed in Ptpn22/NOD mice. Collectively, our results have the implication in linking the causes of autoimmune diseases back to the positive selection by TCR-self antigens interaction in the thymocytes of developmental stage.

P0277

Thyroid autoantibodies in hypothyroidism patients with type 2 diabetes mellitus in a tertiary care hospital of Bangladesh

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Background: Several reports showed a high occurrence of hypothyroidism among people with Type 2 diabetes Mellitus (T2DM). Many studies showed that T2DM is associated with auto-antibodies. Therefore, T2DM may be associated with hypothyroidism with increased auto-antibodies.
Objective: To estimate the level of thyroid auto-antibodies in T2DM patients with hypothyroidism.
Methods: In this cross-sectional comparative study, a total number of 113 subjects (60 hypothyroidism patients with T2DM, 41 non diabetic hypothyroidism patients and 12 non-diabetic normal subjects without hypothyroidism) were selected by random sampling technique following inclusion and exclusion criteria from BIRDEM OPD. The serum FT_4, TSH, thyroid peroxidase (anti-

DOI: 10.1002/eji.201970400

TPO) antibody and thyroglobulin (anti-TG) antibody were estimated by using chemiluminescent microparticle immunoassay (CMIA) method.
Results: Among the T2DM hypothyroid patients, anti-TPO antibody and anti-TG antibody were found positive in 41.7% and 40% cases respectively. Within the non-diabetic hypothyroid patients, the findings were 51.2% and 58.5% respectively. High titer of anti-TPO antibody (≥500 IU/ml) found prevalent (28.3%) among T2DM hypothyroid group than non-diabetic hypothyroid (24.5%) group. The difference of BMI was found statistically significant (p=.0043) in all three groups and the difference of positive and negative anti-TPO antibody among different BMI class was also found statistically significant (p=0.043). Pearson's correlation coefficient (r=0.396; p< 0.05) analysis showed positive correlation between anti-TPO and anti-TG antibody, which was statistically significant.
Conclusion: High frequencies of anti-TPO and anti-TG antibody were found among non-diabetic hypothyroid patient group than diabetic hypothyroid patient group. But high titer anti-TPO antibody positive patients were more common among diabetic hypothyroid patient group.

P0278

The influence of psoriasis and metabolic syndrome on the level of systemic inflammation and oxidative stress

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Aim: To assess independent influence of psoriasis and metabolic syndrome on the level of systemic inflammation and oxidative stress.
Methods: Clinical examination, Biochemical tests, Enzyme Linked Immunosorbent Assay (ELISA), Enzyme Immunoassay (EIA).
Statistics: Group of persons with psoriasis divided into subgroups of persons with and without metabolic syndrome (n=42). Control group (volunteers) divided into subgroups of persons with and without metabolic syndrome (n=80). Anderson-Darling normality test, Wilcoxon test, Bartlett test of variances homogeneity, Two-tail test, Wilcoxon rank sum test.
Assessed markers: Psoriasis Area Severity Index (PASI) score (marker of systemic inflammation). Peripheral blood samples: calprotectin (marker of systemic inflammation), 8-hydroxy-2´-deoxyguanosine, 8-hydroxyguanosine, and 8-hydroxyguanine (markers of oxidative stress), waist circumference, fasting glucose, triglycerides, HDL cholesterol and blood pressure (markers of metabolic syndrome).
Results and conclusion: Increased level of systemic inflammation (calprotectin; p< 0.001) and oxidative stress (p< 0.001) and relationship between PASI score and oxidative stress (rho=0.44, p< 0.01) in the whole group of persons with psoriasis compared to the whole control group. Increased level of calprotectin (p< 0.001) and oxidative stress (p< 0.01) in the subgroup of persons with psoriasis and metabolic syndrome (compared to the subgroup of controls with metabolic syndrome). Both, psoriasis and metabolic syndrome significantly increased the level of systemic inflammation and oxidative stress.

Supported by projects PROGRES Q40-09, Q40-10, Q40-11 and SVV-260397/2017

Keywords: Psoriasis; Metabolic syndrome; Systemic inflammation; Oxidative stress; Calprotectin

DOI: 10.1002/eji.201970400

P0279

C-reactive protein, Chemerin, Fetuin-A and Osteopontin as possible predictors of cardiovascular risks in persons with psoriasis vulgaris

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Aim: To contribute to understanding the participation of selected markers in pathophysiology of psoriasis and assess their suitability for the detection of early stages of cardiovascular diseases in persons with psoriasis.
Methods: Clinical examination, Immuno-nephelometry, Enzyme Linked Immunosorbent Assay (ELISA), multiplexed microsphere-based XMAP Luminex technology.
Statistics: Group of persons with psoriasis - patients (n=28) and control group (n=22). Statistical processing: R-software version 3.3.2, using the “nortest”, and “ggplot2” packages, Anderson-Darling normality test, Pearson's or Spearman's rank order correlation tests and Student's t test or Wilcoxon rank-sum test.
Assessed markers: Psoriasis Area Severity Index (PASI) score. Peripheral blood: C-reactive protein (CRP), Chemerin, Fetuin-A and Osteopontin.
Results and conclusions: In the patients (compared to the control group): increased level of CRP ($P< 0.001$), chemerin ($P< 0.05$), and osteopontin ($P< 0.05$) and decreased level of fetuin-A ($P< 0.05$). In the patients: significant relationships between CRP and Fetuin A (Spearman's rho=0.530, $P< 0.01$), between CRP and Chemerin (Spearman's rho=0.543, $P< 0.01$) and between CRP and age (Spearman's rho=0.590; P = 0.000); significant negative relationship between Osteopontin and Fetuin A (Pearson's r=-0.415, P< 0.05) and between Chemerin and PASI score (Spearman's rho=-0.424, P< 0.05). The results suggest that all studied markers exhibit their own role in the pathophysiology of psoriasis. All selected indicators seem to be appropriate for the detection of early stages of cardiovascular diseases in persons with psoriasis.

Supported by projects PROGRES Q40-09, Q40-10, Q40-11 and SVV-260397/2017

Keywords: Psoriasis; Cardiovascular risks; C-reactive protein; Chemerin; Fetuin-A; Osteopontin

P0280

Study of antigen presentation and antigen-specific immunomodulation in multiple sclerosis by T cell receptor-like antibodies

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The inhibition of inflammatory process during autoimmune diseases, such as Multiple Sclerosis (MS), is frequently achieved by non-specific down regulation of the immune system. Thus, targeted and

DOI: 10.1002/eji.201970400

specific inhibition of autoreactive T cells is highly desirable as a treatment for autoimmune diseases. We suggest that during inflammatory processes in MS, APCs can present several myelin derived epitopes simultaneously and consequently activate a variety of autoreactive T cells. We therefore suggest that targeting one of these epitopes with a T cell receptor-like (TCRL) antibody (Ab) could result in the elimination of specific APCs, thus preventing epitope spreading, decrease myelin-specific T cells activation and most importantly decrease disease exacerbation. To that end, we have isolated and characterized TCRL Abs that are directed against MOG(35-55) or MBP(85-99)/ HLA-DR2 epitopes. The potential mode of action of these antigen-specific TCRL Abs can be exerted via two mechanisms: (i) blocking the interactions between pathogenic T cells and their peptide-MHC ligands presented on APCs; and (ii) Specifically eliminate APCs presenting MS-associated autoantigens. The isolated TCRL Abs exhibit specific inhibition of T cell proliferation *in vitro* and *in vivo*. Moreover, treatment with a TCRL Ab of established EAE in HLA-DR2 Tg mice model significantly attenuate disease progression and abolish EAE associated symptoms. Additionally, administration of the TCRL prevents EAE development in these mice. These data demonstrate that targeting the core driving force of the immune response, the TCR-MHC axis, in an Ag-specific manner may lead to a new approach for immunotherapy of autoimmune diseases and other inflammatory disorders.

P0281

Pancreatic nerve electrostimulation inhibits recent onset autoimmune diabetes

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Type-1 diabetes (T1D) is an autoimmune disease that results from the destruction of insulin-producing pancreatic β-cells by autoreactive T lymphocytes following their activation in pancreatic draining lymph nodes (pLNs). The sympathetic nervous system inhibits inflammation via binding of noradrenaline or adrenaline to β_2-adrenergic receptors (β_2ARs) expressed on immune cells. Since LNs are innervated by sympathetic nerves, we hypothesized that pancreatic nerve electrical stimulation (PNES) may inhibit local inflammation and T1D progression. We have identified an adrenergic pancreatic nerve projecting to the pLN but not to the pancreatic islets that, upon electrostimulation, released noradrenaline in the pLNs. We then developed a minimally invasive surgical procedure for long-term implantation of micro-cuffs electrodes onto the nerve that projects to pLNs. When applied to Non-Obese-Diabetic (NOD) mice, PNES resulted in increased lymphocytes accumulation in pLNs, reduced production of pro-inflammatory cytokines and inhibition of autoreactive T cell proliferation in pLNs in a β_2ARs dependent manner. Most importantly, PNES inhibited disease progression when applied to recently diabetic NOD mice by inhibiting pathogenic effector T lymphocytes. Our data provide a rationale for the use of bio-electronic medicine as a potential treatment modality for T1D.

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DOI: 10.1002/eji.201970400

P0282

Resveratrol ameliorates lupus through differential generation of Tregs and Th17 cells by epigenetic modifications of FoxP3 and IL-17 genes

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Resveratrol (RES), a polyphenolic compound found in plant products including red grapes, red wine, blue berries etc., possesses anti-inflammatory, antioxidant, and anticancer properties. In this study, we investigated the use of RES in the treatment of lupus using a mouse model. Lupus was first developed in SJL/J mice using pristane and then treated with vehicle or RES. RES treatment significantly suppressed pristane-induced lupus in SJL/J mice. Upon examination of generation of regulatory T cells (Tregs) and Th17 cells, we observed significant upregulation of FoxP3 expression but downregulation of IL-17 expression, demonstrating generation of anti-inflammatory Tregs but decrease in pro-inflammatory Th17 cells following RES treatment. There was a significant difference in the miR expression profiles in T cells of RES-treated mice, when compared to vehicle-treated mice. We observed dysregulation of 112 miRs including downregulation of miRs that regulate FoxP3 and upregulation of miRs that regulate IL-17. We observed significant demethylation of Foxp3 promoter but methylation of IL-17 promoter. Culture of activated T cells in the presence of RES showed significant increase in Tregs but decrease in Th17 cells in vitro. Together, these data suggest that RES regulates epigenetic modulation involving miRs and DNA methylation of target genes that leads to T cell differentiation into Tregs/Th17 cells causing suppression of lupus.

The present study was supported in part by NIH grants (R01AI123947, R01AI129788, P01AT003961, P20 GM103641, R01AT006888).

P0283

1,25-dihydroxyvitamin D3 but not 20S-hydroxyvitamin D3 exhibits immunomodulatory roles on T cell subsets in collagen-induced arthritis

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Objectives: To explore whether a non-calcemic analog of vitamin D, 20S-hydroxyvitamin D3 has comparative immunomodulatory effects with 1,25-dihydroxyvitamin D3 on collagen-induced arthritis (CIA).
Methods: DBA1/J mice induced for CIA were intraperitoneally treated with Vitamin D. CIA clinical symptoms and inflammatory responses including Th1/Th17/Tregs percentages were determined and compared. Mouse naïve $CD4^+$ T cells were polarized to Th17 cells or iTregs with or without Vitamin D, and then differentiated cells percentages, IL-6R, IL-23R, (p)-STAT3 and MAPK (ERK/JNK/P38) were analyzed by flow cytometry, qPCR and/or western blots.
Results: 1,25-dihydroxyvitamin D3 but not 20S-hydroxyvitamin D3 significantly delayed CIA onset, decreased incidence and clinical scores of arthritis, downregulated serum IgG levels and ameliorated bone erosion. 1,25-dihydroxyvitamin D3 but not 20S-hydroxyvitamin D3 downregulated IL-17A production in $CD4^+$ T cells while increased $CD4^+Foxp3^+Nrp\text{-}1^+$ cells both in draining lymph nodes and

DOI: 10.1002/eji.201970400

synovial fluid in arthritic mice. 1,25-dihydroxyvitamin D3 but not 20S-hydroxyvitamin D3 inhibited Th17 cells and induced iTregs differentiation *in vitro*. 1,25-dihydroxyvitamin D3 increased the expression and activation of STAT3, inhibited IL-23R expression and the phosphorylation of P38 during Th17 cells differentiation while 20S-hydroxyvitamin D3 did not show such effects on these molecules. **Conclusions:** 20S-hydroxyvitamin D3 exhibits no immunomodulatory roles on T cell subsets in collagen-induced arthritis and the *in vitro* differentiation of T cells, suggesting that more studies are needed to confirm the anti-inflammatory effect of 20S-hydroxyvitamin D3 before substituting 1,25-dihydroxyvitamin D3 for therapeutic use in autoimmune and inflammatory diseases.

P0284

Roles of lipid mediators in psoriatic skin inflammation

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Psoriasis is a chronic inflammatory skin disease thought to arise as a result of infiltration of inflammatory cells and activation of keratinocytes. Psoriasis has been regarded as a classical Th1 disease; however, Th17 cells are attracting much interest. Previous knowledge on the pathology of psoriasis has been obtained mostly through the study of human data and samples. In addition, recently developed animal models for psoriatic skin inflammation recapitulates the hallmarks of human psoriasis and provide us new insights on psoriasis. Lipid mediators and their receptors regulate a variety of physiological processes and involved in multiple pathologies including immune-related diseases. Here, we explored novel lipid mediators involved in psoriatic skin inflammation using clinical samples and imiquimod-induced psoriatic skin inflammation model. In particular, mass spectrometry analysis for multiplex quantitation of eicosanoids using imiquimod-treated murine skin samples revealed unique kinetics of various lipid mediators in the time-series from early induction phase to chronic resolution phase. These data motivated us to focus on some lipid mediators including leukotriene B4 (LTB4). As an example, BLT1 (known as LTB4 receptor) and CXCR2 have been revealed to coordinately promote neutrophil infiltration into the skin during the early phase of imiquimod-induced inflammation. Further analysis on other lipid mediators is in progress and some preliminary data indicates the novel functions of lipid mediators in the pathogenesis in psoriasis. These insights may aid in the rational design of novel treatments for broad spectrum of inflammatory and immune disorders beyond psoriasis.

P0285

Increased expression of the proprotein convertase enzyme FURIN in primary Sjögren´s syndrome

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Objectives: Proprotein convertase enzyme FURIN is critical in maintaining peripheral immune tolerance by regulating maturation of anti-inflammatory cytokine TGFβ-1. It is profoundly expressed in

DOI: 10.1002/eji.201970400

T helper type 1 (Th1) cells and co-regulated with IFN-γ cytokine via IL-12/Stat4 pathway. Previous studies have established that FURIN is upregulated in inflammatory conditions including atherosclerosis, rheumatoid arthritis and systemic lupus erythematosus. Here, we investigated the levels of FURIN in plasma and peripheral blood mononuclear cells (PBMCs) in patients with primary Sjögren's syndrome (pSS) and healthy controls. Additionally, we studied how FURIN plasma levels correlate with demographic and clinical characteristics in pSS patients.
Methods: FURIN plasma levels were determined using ELISA and mRNA expressions in PBMCs were quantitated using qPCR assay. Statistical analyses were performed with SPSS Statistics (IBM, version 20).
Results: FURIN was found to be significantly upregulated at both protein and mRNA level in pSS patients compared to healthy controls. In pSS patients, high FURIN protein level was especially associated with elevated IFN-γ levels and duration of sicca symptoms in the eyes. Results also displayed a trend showing lower levels of serum beta-2 microglobulin, ESR and a lower systemic disease activity index ESSDAI among patients with high FURIN level in plasma.
Conclusions: The expression of proprotein convertase enzyme FURIN is significantly increased in pSS. Elevated FURIN levels associate with high levels of Th1 type cytokine and long duration of dry eye symptoms. Patients with high FURIN levels also tend to show milder clinical findings indicating lower diseases activity.

P0286

Bach2 deficiency promotes autoreactive B cells to produce IgG class-switched autoantibodies pathogenic for lupus through T-dependent extrafollicular pathway

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IgG class-switched, but not IgM unswitched, autoantibodies play a crucial role in the development of systemic lupus erythematosus, but the mechanism on their production remains unclear. Despite defects in Ig class switch recombination and germinal center formation upon deliberate immunization, Bach2-deficient mice spontaneously accumulated IgG autoantibody-producing cells outside of germinal centers, accompanied with manifestations akin to lupus nephritis in a regulatory T cell-independent manner. B cell-specific deletion of Bach2 was not sufficient for this extrafollicular event, but required Bach2-deficient $CD4^+$ T cells. Bach2 deficiency promoted $CD4^+$ T cells to differentiate into extrafollicular and follicular helper T cells and this effect was associated with the repressor activity of Bach2 on *Icos* gene. These results altogether suggest that Bach2-deficient autoreactive B cells predominantly react in extrafollicular sites instead of germinal centers to give rise to IgG class-switched pathogenic plasma cells under the help of $Bach2^-Icos^{hi}$ helper T cells. Thus, our study reveals a novel mechanism by which genetic deficiency of Bach2 predisposes to lupus.

DOI: 10.1002/eji.201970400

P0287

POLM, a novel candidate gene in an Iranian family with autosomal recessive pattern inheritance of multiple sclerosis

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) mediated mainly by CD4+ T cells. Approximately 15-20% of MS patients reported to have a family history. Recently, whole exome sequencing (WES) and RNA-sequencing have been applied to unravel the genetic etiology of MS. In the present study, we aimed to examine rare genetic mutations in a multi-incidence MS Iranian family (more than 3 affected) and the possible effect of the identified mutations on the expression level of those genes in CD4+ T cells.
Whole exome sequencing was used to identify novel rare variants in a family with an autosomal recessive inheritance of relapsing-remitting MS (RRMS). Additionally, the functional impact of the variants was characterized by performing RNA-seq analysis of CD4+ T cells in RRMS patients at relapse phase (n=4) and healthy controls (n=2).
We determined a novel homozygous rare variant in POLM gene (chr7: 44118394 G>C) which results in a missense change (G176A) and predicted damaging on protein function (CADD=25.4). The variant segregated with the disease. Furthermore, 3930 upregulated and 2810 downregulated genes in CD4+ T cells (for FC, a cut-off at ±2 was chosen) were extracted. Interestingly, POLM gene differentially expressed in MS patients which led to upregulation of DNA repair.
Our results pinpoint different variant of POLM gene, belongs to a DNA polymerases gene family. Several evidences suggest a role for DNA repair genes in the etiology of MS. Combination of DNA and RNA sequencing will improve our understanding of the disease pathogenesis.

P0288

Quantification of mitochondrial DNA damage and copy number in circulating blood of patients with systemic sclerosis by a qPCR-based assay

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Background: Although not fully understood, oxidative stress has been implicated in the pathogenesis of different autoimmune diseases such as systemic sclerosis(SSc). Accumulating evidence indicates that oxidative stress can induce mitochondrial DNA (mtDNA) damage and variations in mtDNA copy number(mtDNAcn). The aim of this study was to explore mtDNAcn and oxidative DNA damage byproducts in peripheral blood of patients with systemic sclerosis and healthy controls (HCs).
Methods: Forty six patients with systemic sclerosis and forty nine healthy subjects were studied. Quantitative real-time PCR used to measure the relative mtDNAcn and the oxidative damage (oxidized purines) of each sample.
Results: The mean mtDNAcn was lower in patients with SSc than in HCs whereas the degree of mtDNA damage was significantly higher in cases as compared to controls. Moreover, there was a negative correlation between mtDNAcn and oxidative DNA damage.
Conclusion: These data suggest that alteration in mtDNAcn and increased oxidative DNA damage may be involved in the pathogenesis of SSc.

DOI: 10.1002/eji.201970400

P0289

Alterations in modulation of B cell activation by microparticles of patients with rheumatoid arthritis

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The fundamental role of B cells in the immunopathogenesis of rheumatoid arthritis (RA) is already known. Furthermore, an increased number of circulating microparticles (MPs) with presence of autoantigens, alarmins and immune complexes (ICs) has been described in patients with RA (PRA). The effect of MPs in B cell responses in PRA and healthy controls (HC) is unknown. We propose that MPs interact with B cells and modulate their response.
Enriched B cells of PRA and HC were cultured for 24 and 72 hours in absence and presence of MPs from HC (HC-MPs) and PRA (PRA-MPs). Both sources of MPs alone did not induce any change in the expression of activation markers. In anti-BCR stimulated B cells from HC and PRA the presence of HC-MPs and PRA-MPs reduced the frequency of CD69+/CD80+/CD86+ cells. With anti-BCR plus CpG stimulus, both sources of MPs reduced the frequency of CD69+/CD80+/CD86+ in B cells from HC but only a partial reduction was observed in cells from PRA. Also, HC-MPs and PRA-MPs decreased the frequency of proliferating B cells from HC, but only PRA-MPs forming ICs regulated the proliferation of B cells from PRA. Moreover, both sources of MPs reduced calcium mobilization in anti-BCR stimulated B cells from HC and PRA.
These results suggest that MPs from HC and PRA can regulate B cells activation even since an early time, but this modulation seems to be less effective in B cell from PRA; this phenomenon could contribute to the immunopathogenesis of RA.

P0808

The correlation between serum vitamin D concentration and Graves´ disease and ocular complication

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Background: Vitamin D deficiency is a global problem. Low level of vitamin D influences the onset of GD. The study was to investigate the relation of vitamin D level to GD and ocular complications (GO), and explore association of vitamin D with development and grade of GO in GD patients.
Methods: 100 GD patient, aged 16-70 years and 100 sex- and age-matched euthyroid healthy controls were enrolled. Serum levels of 25-hydroxyvitamin D [25(OH)D], thyroid hormones, thyroid stimulating hormone (TSH), thyroid autoantibodies were measured in all subjects.
Results: Vitamin D deficiency was widely found in all subjects, especially in GD patients. In GD patients compared with healthy controls, levels of thyroid hormones, thyroid autoantibodies were elevated, but the serum 25(OH) D3 level was decreased. The serum vitamin D level was significantly decreased among GD patients with GO. Vitamin D was the independent factor associated with GO development on multivariate analysis. When using EUGOGO criteria to classify the severity and activity of GO in GD patients, vitamin D in moderate to severe group was lower than that in inactive

DOI: 10.1002/eji.201970400

group compared with mild group and active group.
Conclusions: The level of serum 25(OH) D3 in GD patients was lower than that in the control group, and it was significant decreased in the presence of GO compared with the absence of GO. Vitamin D deficiency and insufficantly were significantly associated with the development and grade of GO in GD patients.

P0809

Inhibitory effects of TeAb-Anti-CCP-ScFv-p53 on peripheral T/B cell activation in patients with rheumatoic arthritis

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Objective: To investigate how TeAb-Anti-CCP-ScFv-p53 acts on the activation and cytokines/autoantibodies secretion of in vitro cultured T/B cells from RA patients.
Methods: Peripheral mononuclear cells were isolated from RA patients and cultured in vitro upon CCP antigen stimulation. The efficacy of TeAb-Anti-CCP-ScFv-p53 on the number and function of CCP antibody-secreting B cells was examined.
Results: Treatment of TeAb (100ug/mL) for 7 days decreased the concentration of CCP antibody, IL-6, TNFa and IL17a in the supernatant, the number of CCP antibody-secreting B cells, and the ratio of Th17 cells (CD3+CD8-IL17+/CD3+CD8-), but did not affect the secretion of Treg associated cytokines, TGFβ and IL-23.
Conclusion: TeAb-Anti-CCP-ScFv-p53 suppresses the number and function of CCP antibody-secreting B cells, as well as T cell activation under CCP antibody stimulation.

P0810

C5a/C5aR pathway is critical for the initiation of inflammation in psoriasis by potentiating plasmacytoid dendritic cells functions

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Psoriasis is one of the most common chronic inflammatory skin diseases, affecting approximately 2% of the population. The lack of characterization of the pathogenesis of psoriasis has hindered efficient clinical treatment of the disease. In our study, we observed that expression of complement component 5a receptor (C5aR) was significantly increased in skin lesions of both imiquimod (IMQ) and IL23-induced psoriatic mice and patients with psoriasis. C5aR deficiency or treatment with C5aR antagonists in mice significantly attenuated psoriasis-like skin lesions and expression of inflammatory cytokines and chemokines. Moreover, C5aR deficiency significantly downregulated IMQ-induced infiltration in psoriatic skin lesions and functions of plasmacytoid dendritic cells (pDCs), evidenced by the remarkable reduction in the IMQ-induced production of interferon-α (IFN-α) and tumor necrosis factor α (TNF-α), and FMS-like tyrosine kinase 3 ligand (FLT3L)-dependent pDCs differentiation. Accordingly, in vitro treatment with recombinant C5a accelerated pDCs migration and the differentiation of bone marrow cells into pDCs. Furthermore, biopsies of psoriatic patients showed a dramatic increase of $C5aR^+$ pDCs infiltration in psoriatic skin lesions, compared to healthy subjects. Our results provide direct evidence that C5a/C5aR signaling plays a critical role in the pathogenesis of psoriasis by potentiating pDCs differentiation and functions. Inhibition of C5a/C5aR pathway is

DOI: 10.1002/eji.201970400

expected to be beneficial in the treatment of patients with psoriasis.
Keywords: complement system, C5a/C5aR, psoriasis, pDCs, inflammation, mouse models

P0811

Neutrophil extracellular trap formation is associated with autophagy-related signalling in ANCA-associated vasculitis

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Increasing evidence indicates that aberrant neutrophil extracellular trap (NET) formation could contribute to the pathogenesis of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Recent research has provided evidence that a novel type of ANCA autoantibody, anti-lysosomal membrane protein-2 (LAMP-2) antibody, may have a pathogenic role in AAV. The current study sought to determine whether LAMP-2, as a novel antigen of ANCA, was present on NETs in AAV patients, the influence of the anti-LAMP-2 antibody on the neutrophil apoptosis rate and the role of autophagy in anti-LAMP-2 antibodyinduced NET formation. NET formation was assessed using immunofluorescence microscopy, scanning electron microscopy or live cell imaging. The neutrophil apoptosis rate was analysed using fluorescence activated cell sorting (FACS). Autophagy was detected using LC3B accumulation and transmission electron microscopy. The results showed that enhanced NET formation, which contains LAMP-2, was observed in kidney biopsies and neutrophils from AAV patients. The apoptosis rate decreased significantly in human neutrophils stimulated with anti-LAMP-2 antibody, and this effect was attenuated by the inhibitors of autophagy 3-methyladenine (3MA) and 2-morpholin-4-yl-8- phenylchromen-4-one (LY294002). The anti-LAMP-2 antibody-stimulated NET formation was unaffected by benzyloxycarbonyl-Val- Ala-Asp(OMe)-fluoromethylketone (zVAD-fmk) and necrostatin-1 (Nec-1), which are inhibitors of apoptosis and necrosis, respectively, but was inhibited by 3MA and LY294002. Moreover, the proportion of LC3BI that was converted to LC3BII increased significantly, and massive vacuolizations that exhibited characteristics typical of autophagy were detected in neutrophils stimulated with anti-LAMP-2 antibody. Our results provide further evidence that autophagy is involved in ANCAinduced NET formation in human neutrophils.

P0812

S1P promotes the secretion of IL-17 by CD4SP thymocytes in EAMG rat

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Sphingosine-1-phosphate (S1P) is a potent lipid mediator which is produced by the metabolism of sphingolipids in cells.S1P exerts various biological activities by binding to the corresponding receptors.It has been reported that S1P can not only mediate the output of mature thymocytes,but also affect the differentiation and maturation of thymocytes through G protein-mediated signaling pathways.Our previous study found an increased expression of S1P receptor 1(S1P1) in thymus of patients with myasthenia gravis(MG), which is an autoimmune disease often accompanied with abnormal proliferation and differentiation of thymocytes. The relationship of high expression of S1P1 with the aberrant proliferation and differentiation of thymocytes in MG thymus was evaluated in EAMG rat model immunized with AchR polypeptides. Our results showed enhanced expression of Foxo1,KLF2 and S1P1 in both CD4 single positive cells and CD4CD8 double negative thymocytes in EAMG rat. The amount of $CD4^{+}CD8^{-}IL\text{-}17^{+}$thymocytes was significantly increased in EAMG thymus.

DOI: 10.1002/eji.201970400

CD4 single positive cells were co-cultured with AChR pulsed bone marrow derived-dendritic cells, supplied with IL-2. S1P or FTY720 was added at day 7 of co-culture. S1P could promote $CD4^+$ thymocytes secrete IL-5,IL-6,IL-17A and IL-22,and reduce the production of IFN-ɣ.The results suggested that S1P can promote the differentiation of $CD4^+$ single positive thymocytes into Th17 cells and the expression of IL-17 and other inflammatory cytokines under AChR antigen stimulation.

P0813

Analysis of the results of 2967 sera autoantibodies by indirect immunofluorescence assay and line immunoblot assay

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Objective: A total of 2967 serum samples from July 1, 2014 to January 31, 2016 were retrospectively analyzed in this paper. Meanwhile, ANA was detected by indirect immunofluorescence method (IIF) and immune imprinting method (LIA) was used to detect the ANA ANAs.This paper discusses and analyzes the correlation between the detection results of the two methods.In addition, the clinical significance of combined detection of autoantibodies by the two methods in the diagnosis of autoimmune diseases was analyzed.
Methods: Serum samples from 2967 suspected or confirmed autoimmune diseases were detected by IIF and LIA methods.Collect test results for comparative analysis.Furthermore, the correlation between the results of the two detection methods was studied.
Results: This study showed that the positive rate of IIF method and LIA method was 16.35% and 11.53% respectively;LIA method was the most positive method for detecting anti-SSA antibodies, accounting for 36.48% of the positive rate.LIA detection rate was relatively high when the fluorescence titer was 1:320.
Conclusion: In ANA detection, the detection rate of IIF method was higher than LIA method, and the specificity of LIA method was higher than IIF method.When screened by IIF method alone, it is easy to lead to patients who with the anti-SSA and anti-AMA-M2 antibodies as the main antibodies, the ANA results are false negative.There was no significant correlation between the detection rate of LIA method and the fluorescence titer.Combined detection of the two methods can improve the accuracy of autoimmune disease diagnosis and reduce the rate of missed detection and misdiagnosis.

P0814

Environmental exposure to cadmium accelerates rheumatoid arthritis development by co-opting the ion channel ZIP8

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Rheumatoid arthritis is a chronic autoimmune disease that results in the destruction of joints and restriction of motion. While the root causes of disease are not fully understood, a number of lifestyle-based risk factors have been previously reported, including obesity, silica exposure, and smoking. The mechanisms underlying these risk factors are also incompletely understood, although exposure to heavy metals through smoking has been previously suggested to contribute. In this report, we observed increased accumulation of cadmium in the synovial fluid of smokers, and accelerated development of RA in DBA mice exposed to cadmium. This exposure could also be observed to drive increased activation of immune cells *in vitro*, as activated immune cells displayed increased expression of the ion channel ZIP8 compared to resting counterparts. Presence of ZIP8 significantly

DOI: 10.1002/eji.201970400

increased import of cadmium into immune cells, while knockdown of ZIP8 was sufficient to reverse the accumulation and reduce the strength of macrophage activation responses. Collectively, these results demonstrate that patients with RA may more easily retain cadmium due to the presence of activated immune cells in their synovial fluid, and that this exposure may accelerate disease development.

P0815

Anti-glomerular basement membrane disease with special epitope recognizing

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Anti-glomerular basement membrane (anti-GBM) disease is an autoimmune disorder that presents as the production of autoantibodies against the GBM, rapidly progressive glomerulonephritis and pulmonary hemorrhage.
Here we reported an unusual case of anti-GBM disease that the patient, with an indolent course and normal kidney function, possessed high titers of autoantibodies that could only be detected by a commercial ELISA kit.
A 47-year-old woman was referred to our Hospital with a 2-year history of persistent microscopic hematuria and without proteinuria.
GBM antibodies were tested positive 130 RU/ml (< 20 RU/ml), by enzyme-linked immunosorbent assay using purified bovine a(IV)NC1 (Euroimmun, Lu¨beck, Germany) as solid-phase ligands.
A renal biopsy showed primarily minimal changes with one fibrouscellular and two fibrous crescent formation out of forty glomeruli.
Series of sera from the patient recognized none of the recombinant human a chains, though all of them tested positive by the commercial ELISA kit. A western blot analysis confirmed the negative recognition of all five a chains of this patient. The serum did recognize purified hexamers from bovine GBM, detected by both ELISA and western blot analysis. Thus, the patient serum showed high titers of anti-GBM antibodies recognizing only bovine purified a chains, but not the classic antigens or epitopes we have known up to now.
Conclusion: This case reported a patient possessing higher titer of anti-GBM antibodies that recognized distinct epitopes on bovine a(IV)NC1 and native hexamers, but not presented on either of the five human a(IV)NC1.

P0816

The role of protein disulfide-isomerase A3 as autoantigen in the pathogenesis of autoimmune thyroiditis (AIT) and AIT-related brain damage in adult mice

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Objective: The aim of this study was to investigate whether protein disulfide-isomerase A3 (PDIA3) could serve as an autoantigen shared by thyroid and central nervous system (CNS) and the effect of PDIA3Ab on inducing autoimmune injury in thyroid and brain.
Methods: PDIA3-immunised CBA/J mice model was established by subcutaneous injection with PDIA3 recombinant protein twice with 2 weeks interval. Serum levels of PDIA3-specific IgG, Tg-Ab, TSH and TT4 were measured by ELISA in 4, 6, 10, 14 and 18 weeks after the last immunization. PDIA3, CD45, cleaved Caspase-3, IgG, C3 and MAC were detected by immunofluorescence staining. Morris water maze (MWM), LTP, TUNEL assays and scanning electron microscopy were used to evaluate the functional and morphological change in brain.

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DOI: 10.1002/eji.201970400

Results: Expression of PDIA3 was localized in thyroid follicular epithelial cells, thyroid vascular endothelial cells, cerebral vascular endothelial cells, neurons and astrocytes in normal CBA/J mice. Evident PDIA3Ab deposition was co-localized with C3 and MAC in thyroid and brain tissues of PDIA3 groups compared with control groups after 10 weeks after the last immunization. Elevated serum TSH levels began after 14 weeks of the last immunization. The decline in learning and memory was confirmed by MWM and LTP after 10 weeks of the last immunization. Moreover, apoptosis, perivascular edema and astrocytes activation could be observed in brain tissues of PDIA3 group.
Conclusions: PDIA3 can serve as an autoantigen shared by thyroid and CNS. PDIA3Ab-induced complement system activation may involve in the pathogenesis of AIT and AIT-related brain damage.

P0817

The clinical significance of anti-mitochondrial antibody (AMA) in patients with positive AMA at the first visit

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Objective: It is still under controversy whether there is any correlation between AMA and other biochemical indexes in primary biliary cholangitis (PBC), or whether AMA could be used to indicate the disease progress. We sought to explore the measuring level of AMA and its clinical implication in different groups of patients with positive AMA at the first visit.
Methods: We collected the demographic data, blood indexes, biochemical indexes and immune indexes of 1323 newly-detected AMA and/or AMA-M2 positive patients from January 2013 to December 2016 through the Information System of Peking University People´s Hospital. For those who had visited more than once, we adopted the information when AMA and/or AMA-M2 was detected positive for the first time.
Results: Patients were categorized as either newly diagnosed with PBC (new-PBC group, n=350) or non-diagnosed with PBC (non-PBC group, n=973). The median serum levels of AMA and AMA-M2 in the latter group were lower with statistical significance than in the former (AMA: 1:80 vs 1:320; $p < 0.001$; AMA-M2: 47 RU/ml vs 180 RU/ml; $p < 0.001$). In non-PBC group, serum AMA-M2 levels were correlated with age, IgG, IgM, AST, GGT, ALP, total protein and TC, and intra-group patients with higher AMA titers had significantly higher IgM compared to those with lower AMA titers ($p < 0.05$); In new-PBC group, serum AMA-M2 levels were correlated with age, PLT, IgM, total protein and TC, and intra-group patients with higher AMA titers had significantly higher IgM, GGT and ALP ($p < 0.05$).
Conclusion: AMA level is correlated with some clinical parameters, indicating its correlation to disease severity.

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DOI: 10.1002/eji.201970400

P0818

Expression and clinical significance of OX40 and OX40L in peripheral blood of patients with type 1 diabetes

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Type 1 diabetes (T1D) as a chronic and organ-specific autoimmune diseasewas influenced by inherited or environmental factors, leading to the loss of insulin production in beta cells when immune cells invade the pancreatic islets. OX40(CD134) and OX40L(CD252), members of the TNF receptor superfamily(TNFRSF) and TNF superfamily (TNFSF), playing important roles in T cells expansion, proliferation and differentiation. This study analyzed the expression of membrane OX40 and OX40L(mOX40 and mOX40L) and levels of soluble OX40 and OX40L (sOX40 and sOX40L) in T1Dpatients to analyze the clinical significance. The expression of mOX40 and mOX40L on immune cells was detected by flow cytometry. Levels of sOX40,sOX40L and cytokines in sera were measured by ELISA. We demonstrated for the first time that increased sOX40 and sOX40L levels and reduced mOX40 and mOX40L expression in T1Dpatients.We also found a positive correlation between sOX40 expression and UA (r=0.3376, *p*=0.0189) and Hemoglobin A1c (HbA1c) (r=0.3045, *p*=0.0616), as well as between sOX40L and Hemoglobin A1c(r=0.3131, *p*< 0.0001). Meanwhile, sOX40L expression of T1D patients was positively correlated with sera levels of IL-2(r=0.3676, *p*=0.0147), IL-6(r=0.3139, *p*=0.0485), IL-10(r=0.3455, *p*=0.0362) and IFN-γ (r=0.5201, *p*=0.0056). These results suggest that sOX40/OX40L signal may be promising biomarkers and OX40/OX40L pathway was involved in the pathogenesis of T1D.

P0819

B cell subsets associated with clinical characteristics in systemic lupus erythematosus

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Objective: To investigate the relationship between B cell subsets and systemic lupus erythematosus (SLE).
Methods: From February 2016 to March 2017, sixty patients with active systemic lupus erythematosus (SLE) were enrolled in the study. All SLE patients were diagnosed according to the 1997 revised classification criteria of the American College of Rheumatology. Flow cytometry analysis for total B cell, memory B cell ,naïve B cell, double negative B cell, non-switched memory B cell, switched memory B cells, B10 cell and plasmablast was performed in all patients.
Results: The percentage of plasmablast was positively correlated with SLEDAI score (r=0.38, p =0.004), ESR (r=0.489, p=0.00), lymphocytes (r=0.343, p=0.011). A negative correlation was observed between plasmablast and C3 (r=0.427, p=0001), C4 (r=0.374, p=0.011). A significant increase in the percentage of plasmalast was found in patients with moderate or high disease activity compared to the patients with low disease activity (p=0.012). B10 cell showed a negative correlation with ESR (r=0.345, p=0.011), CRP (r=0.317, p=0.022) and 24-h urine protein excretion (r=0.442, p =0.002). A negative correlation between double negative B cell and C3 (r=0.427, p=0.001) was found to exist. The percentage of double negative B cells was higher in the patients with decreased C3 compared to the patients with normal C3.
Conclusion: The results showed that plasmablast and double negative B cells could be actively involved in the pathogenesis of SLE. B10 cell, as a regulatory B cell is involved in regulating autoimmunity in SLE.
Keywords: SLE; B cell subsets; SLEDAI

DOI: 10.1002/eji.201970400

P0820

Effect and mechanism of dental pulp mesenchymal stem cell in rat knee osteoarthritis

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Objective: To investigate the effect and mechanism of the dental pulp mesenchymal stem cells (DPST) in rat osteoarthritis model.
Methods: (1) DPST were extracted from the teeth of children and wisdom teeth of teenagers less than 20 years of age. (2) 40 SD rats were randomly divided into four groups: A, B, C, and D. Group B, C, and D were all injected with papain in the articular cavity for modeling. Group A: healthy control group; Group B: model group treated with normal saline; group C treated with sodium hyaluronate; Group D treated with DPST. The treatment was given once a week for continuous 4 weeks. Level of IL - 1βand TNF-α were examined with ELISA in knee joint synovia and HE staining of cartilage was carried out.
Results: (1) The cells was shown with high expression of CD73, CD90, CD105 on the cell surface. It meets the identification criteria of stem cells and could differentiate from chondrocytes and adipocytes in the induction medium. (2) MANKIN grading analysis showed HE staining score in group B,C,D is significantly higher in comparison with group A ($P < 0.05$). (3) The levels of TNF-α and IL-1β were significantly increased in group B, C and D compared to group A and decreased in group CD compared to group B ($P < 0.05$).
Conclusion: Pulp mesenchymal stem cells can effectively reduce the level of inflammatory factors in knee joint of OA rats and improve the score of HE staining MANKIN in cartilage.

P0821

Osteoporosis risk in connective tissue disease related interstitial pulmonary disease

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Connective tissue diseases are often lead to thoracic changes, including due to interstia lung disease. Osteoporosis is also a common complication of connective tissue disease, but the occurrence and characteristics of osteoporosis in CTD-ILD are still unclear.
In this study, 109 patients diagnosed as CTD-ILD in the frist affiliated hospital of soochow university between January 2018 and February 2019 were reviewed and analyzed. In these patients,23 with osteoporosis (21.1%, including 13 with osteoporosis fracture, 11.9%). All the 23 patients with osteoporosis were female, with an average age of 62.07+10.25 years old. Among the 13 patients with osteoporosis related fractures, there were 5 with RA-ILD, 3 with pSS-ILD and 3 with UCTD, and 2 patients with PM/DM.The onset age of patients with osteoporosis was 52.34+13.70 years old. Interstitial lung lesions were the first symptom in 5 patients, and the duration of other patients with ILD ranged from 1 to 34 years, 15 patients (65.2%) had osteoporosis or even fractures within 2 years of ILD diagnosed.Among the patients with osteoporosis, 6 (26.1%) were treated with pirfenidone, statistically different with patients without osteoporosis (19.7%, 17/86).Bone mineral density index and lung ventilation index were significantly reduced in patients with osteoporosis.
In brief, there was an increased risk of osteoporosis in CTD-ILD patients. Women, late onset, rheumatoid arthritis and Primary sjogren´s syndrome may be the risk predicators .

DOI: 10.1002/eji.201970400

P0822

Regulation of the inflammasome adaptor ASC mediated singling and arthritis diseases

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Interlenkin-1 (IL-1 β) is a key factor of cartilage destruction in rheumatoid arthritis (RA). The understanding regulation of IL-1β mediated arthritis is limited due to the absence of suitable mouse models. Previously, we found that apoptosiss-associated speck-like protein containing a caspase-recruitment domain (ASC) promoted IL-1β secretion and synovial inflammation in the K/BxN serum transfer induced arthritis models. Arthritis disease crucially relies on the ASC inflammasome. Presently, we found that the transcriptional factor promyelocytic leukaemia zinc finger protein (PLZF) deficient mice exhibited decreased clinical and histological scores in arthritis models, which was similar to the ASC-deficient mice, suggesting the essential role of PLZF in arthritis disease. Loss of PLZF reduced Caspase-1 activation and IL-1β secretion in response to multi-NLRP3 stimuli in macrophages without affecting transcription and translation of inflammasome constitutes. Since PLZF serves as a transcription factor and mostly locates in nuclear, our data showed that PLZF was definitely required for the distribution of ASC from nucleus to cytosol. Furthermore the frequencies of ASC speck-containing cells and the formation of ASC dimer and oligomer were significantly decreased in PLZF-deficient macrophages. Moreover, we found the direct interaction between PLZF and ASC protein through immune-precipitation. Taken together, our findings indicated that PLZF may works as a novel regulator of ASC inflammasome by facilitating its translocation from nuclear to cytosol through direct binding to ASC protein, mediating IL-1β release and subsequently promoting rheumatoid arthritis associated inflammasome disease and cartilage destruction.

P0823

Ion channel expression aberration in rheumatoid arthritis

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Rheumatoid arthritis is a multifaceted autoimmune disease in which persistent inflammation in the synovium leads to the creation of a complex microenvironment. While much of the research focus in RA has been on the cell-cell interactions that occur between different infiltrating immune and stromal cells present, other abiotic factors in the microenvironment may also play significant roles. Through detailed meta-analysis using new dimension-reduction and clustering methods, we identify significant aberrations in the expression profiles of ion channels between RA patients and healthy controls. This aberration alone is sufficient to distinguish the two groups, and can also be observed in the peripheral blood of RA patients. This profile is linked to the cellular composition of the samples. Treatment with DMARDs partly reverses these aberrations, although ion expression profile itself appears insufficient to predict drug response. Since these differences in ion channel expression include changes linked to calcium transport, we used flow cytometry to verify that the calcium content of synovial immune cells is indeed varied, and that activated immune cells accumulate increased amounts of the ion. Collectively, these analyses uncover a previously underappreciated importance of ion channels in RA.

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DOI: 10.1002/eji.201970400

P0824

Determination of plasma antiglycan autoantibody in patients with IgA nephropathy and its correlation with clinical characteristics

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Objective: To establish the measurement of IgA1 O-glycan-specific antiglycan antibodies in patients with IgA nephropathy(IgAN), and evaluate their role in the development of IgAN.
Methods: A cohort of 170 IgAN patients that were followed regularly at the Peking University Institute of Nephrology and 30 healthy controls enrolled in this study. Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of galactose-deficient IgA1(Gd-IgA1) and plasma antiglycan autoantibody (IgG and IgA1). The correlation between antiglycan autoantibody, IgAN and clinicopathological parameters was analyzed by linear correlation and multiple linear regression analysis.
Results: IgG and IgA1 antiglycan autoantibodies that specifically recognized Fab-HR antigens can be detected in both IgAN and healthy control group. There was no significant difference in the absolute levels of plasma IgG antiglycan autoantibodies between IgAN and healthy controls（p=0.963). After adjustment of the plasma levels of IgG, the normalized antiglycan autoantibodies (ln IgG autoantibody/IgG) in patients with IgAN were significantly higher than that in healthy controls[（0.58±0.31）vs（0.37±0.11）, $P < 0.001$]. Moreover they were significantly associated with 24 h proteinuria excretion after adjusting for baseline clinical and pathological factors (β =0.713, 95%CI 0.323-1.102, P < 0.01). The area under ROC curve (AUC) of normalized IgG antiglycan autoantibody in the diagnosis of IgAN was 0.764. Using the cut-off value of 0.3957, the sensitivity and specificity of normalized IgG antiglycan autoantibody for IgAN were 0.729 and 0.700 respectively.
Conclusions: Gd-IgA1-specific antiglycan autoantibodies can be detected both in IgAN patients and healthy controls. They were elevated in some patients with IgAN and possibly involved in the development in these patients.

P0825

Community alteration of the cutaneous microbiota in SLE

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Background：In recent years, more and more studies have shown that skin microbiome has altered in a variety of skin diseases including psoriasis, atopic dermatitis and vitiligo, suggesting that disorders of skin microbiome are associated with a variety of inflammatory and autoimmune diseases.Although a few studies have investigated the association between systemic lupus erythematosus (SLE) and gut commensal,the alteration of cutaneous microbiome in SLE is still unknown.Our study is the first attempt to explored the cutaneous microbiome differences between SLE, healthy controls,and dermatomyositis (DM) which is an another autoimmune diseases sometimes may be difficult to differentiate from SLE.
Result: Using high-throughput 16S rRNA gene sequencing for assaying cutaneous bacterial communities of 69 SLE patients,49 healthy controls and 20 DM patients, we observed decreasing diversity in community richness and evenness and greater heterogeneity in SLE and DM from control to unaffected to lesion specimens.Staphylococcus taxa,specifically, Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus hominis were enriched in SLE lesional skin, while Rhodococcus ,Cutibacterium were decreased in SLE.Based on the results of KW test、ROC curve

DOI: 10.1002/eji.201970400

and Lefse analysis, it was identified that Staphylococcus taxa might serve as potential markers for disease statue. Picrust predicted glycerophospholipid metabolism and pentose phosphate KEGG pathways were significantly enriched in SLE patients.
Conclusion:The composition and function shifts of cutaneous microbiota in SLE indicate the microbial dysbiosis might be associated with the pathogenesis of SLE, which may provide potentially reliable biomarkers or therapeutic targets for the future translational research.

P0826

The expression of NOD2, NLRP3 and NLRC5 and renal injury in anti-neutrophil cytoplasmic antibody-associated vasculitis

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Background: Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are intracellular sensors of pathogens and molecules from damaged cells to regulate the inflammatory response in the innate immune system. This study aimed to investigate the expression of nucleotide-binding oligomerization domain containing protein 2 (NOD2), NOD-like receptor family pyrin domain containing 3 (NLRP3) and NOD-like receptor family CARD domain containing 5 (NLRC5) in kidneys of AAV patients, and further explored their associations with clinical and pathological parameters.
Methods: Thirty-four AAV patients in active stage were recruited. Their renal specimens were processed by immunohistochemistry to detect the expression of three NLRs, and by double immunofluorescence to detect NLRs on intrinsic and infiltrating cells. The associations between expression of NLRs and clinicopathological parameters were analyzed.
Results: The expression of NOD2, NLRP3 and NLRC5 was significantly higher in kidneys of AAV patients than normal controls, minimal change disease or class IV lupus nephritis, and they co-localized with podocytes and infiltrating inflammatory cells. The mean optical density of NOD2 in glomeruli was significantly higher in crescentic class than non-crescentic class, and correlated with proteinuria level and serum creatinine at renal biopsy. The mean optical density of NLRC5 in glomeruli was significantly higher in crescentic class than non-crescentic class, and correlated with proteinuria level, Birmingham Vasculitis Activity Score and the proportion of crescents in the renal specimen.
Conclusions: The expression of three NLRs was upregulated in kidneys of AAV patients. The expression of NOD2 and NLRC5 was associated with the severity of renal lesions in AAV.

P0827

Necrostatin-1 ameliorates Collagen-induced arthritis mice by inhibiting MLKL to reduce NETs formation

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Rheumatoid arthritis (RA) is a chronic inflammatory disorder in which neutrophil extracellular traps (NETs) have been considered involved in the development and progression. Here, we showed that Nec-1, a necroptosis inhibitor, significantly reduced the incidence of arthritis in Collagen-induced arthritis mouse model.Furthemore,Nec-1 not only effectively relieved the degree of joint swelling, but also decreased the expression of proinflammatory cytokines in serum and joints. We found that Nec-1

DOI: 10.1002/eji.201970400

reduced the formation of NETs in CIA model mice as well as in human neutrophils triggered by PMA in vitro, while had no effects on reactive oxygen species(ROS) levels .Of note, live cell imaging video taken by a fully automated live cell imaging assay showed that Nec-1 treated neutrophils that have been stimulated with PMA retained intact cell membrane. Collectively, these data demonstrated that Nec-1 may interfere with the pore-forming pathways on the host neutrophil cell membrane, and reduce Nets release, which may contribute to the ameliorating effect of Nec-1 in CIA model mice.

P0828

Normal serum matrix metalloproteinase-3 predicts radiographic remission in rheumatoid arthritis: one-year result from a real-world prospective cohort

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Background: Treat-to-target is a guiding principle for treatment of rheumatoid arthritis (RA), but how to select right target at right time remains unresolved. Our study aimed to explore whether serum matrix metalloproteinase-3 (sMMP-3) is a candidate for therapeutic target.
Methods: Active RA patients (DAS28-ESR>3.2) from a real-world prospective cohort were followed up at 0, 1st, 3rd, 6th and 12th months. Dynamic sMMP-3 were detected by ELISA. X-ray assessment of hands/wrists was repeated at baseline and the 12th month. Radiographic remission was defined as $\triangle$mTSS < 0.5 units.
Results: Among 306 RA patients recruited, there were 52.9% patients achieved LDA (DAS28-ESR< 3.2), 34.0% achieved clinical remission (DAS28-ESR< 2.6) and 69.3% showed radiographic remission at one year. RA patients with normal sMMP-3 at the 1st, 3rd and 6th months showed significantly higher cumulative probability of achieving one-year radiographic remission than those with normal CRP or ESR (1st month: 91.9% vs. 75.0% vs. 70.1%, 3rd month: 93.2% vs. 74.6% vs. 75.0%, 6th month: 90.9% vs. 76.9% vs. 75.0%, all P< 0.05). After adjustment for confounding factors (gender, age, disease duration, baseline bony erosion and previous treatment), multivariate logistic regression analysis showed normal sMMP-3 at the 1st, 3rd and 6th months predicted one-year radiographic remission (*OR*: 6.945, 8.333, 6.342, respectively, all P< 0.05). RA patients with more frequencies of normal sMMP-3 during follow-up were more probable to achieve one-year radiographic remission (*OR*: 1.734, 95%*CI*:1.326-2.267, P< 0.001).
Conclusion: Normal sMMP-3 after treatment predicts one-year radiographic remission in RA, which implied normal sMMP-3 might be an additional therapeutic target.

P0829

Analysis of myositis auto-antibodies in Chinese patients with cancer-associated myositis

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Objective: Cancer-associated myositis (CAM) has a poor prognosis and causes higher mortality. Generally, myositis-specific auto-antibodies (MSAs) and myositis-associated auto-antibodies (MAAs) have been shown to be useful biomarkers for its diagnosis.
Methods: In the present study, the focus was given in assessing the presence, prevalence and

DOI: 10.1002/eji.201970400

diagnostic values of myositis auto-antibodies in Chinese patients displaying CAM diagnosis. The sera collected from 49 CAM patients, 108 dermatomyositis/polymyositis (DM/PM) patients without cancer, 105 disease controls and 60 healthy controls were detected for the presence of 16 autoantigens (Jo-1, OJ, EJ, PL-7, PL-12, MDA5, TIF1γ, Mi-2α, Mi-2β, SAE1, NXP2, SRP, Ku, PM-Scl75, PM-Scl100, Ro-52) using a commercial Euroline assay.
Results: The frequency of anti-TIF1γ was significantly higher in CAM patients than in DM/PM patients without cancer (46.94% *vs.* 14.81%, $P < 0.001$). Importantly, the sensitivity and specificity for this MSA was 46.94% and 85.19%, respectively, with a positive predictive value (PPV) and negative predictive value (NPV) of 58.97% and 77.97%, respectively. These helped to differentiate CAM patients from DM/PM patients without cancer. However, there was no difference in other MSAs and MAAs between CAM and DM/PM patients without cancer.
Conclusions: The present study indicated that anti-TIF1γ levels could serve as important biomarkers for CAM diagnosis, and help in distinguishing between CAM and DM/PM patients without cancer.

P0830

Genetic and functional analysis of two missense mutations in CD46 predispose to postpartum atypical hemolytic uremic syndrome

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Postpartum atypical hemolytic uremic syndrome (aHUS) is a disease with a triad of hemolytic anemia, thrombocytopenia and acute renal failure after the pregnancy, which might be attributed to the uncontrolled complement activation. Membrane cofactor protein (*MCP*; *CD46*), a widely expressed transmembrane complement regulator, could inhibit complement activation by regulating C3b deposition on targets. Herein, we sequenced four generations of a postpartum-aHUS patient and found two missense mutations in *CD46*, a novel mutation (c.403G>C, p.G135R) from her father and a once reported mutation (c.293C>T, p.T98I) without functional tests from her mother. The G135R mutation caused a significantly reduced membrane expression of CD46 in peripheral blood lymphocyte and renal. Moreover, the expressed G135R protein was in precursor form, indicating an insufficient efficiency compared with wild type. The C3b binding ability of T98I mutant was significantly decreased while the C4b binding ability of the two mutants was slightly declined. The cofactor ability of the two mutants for factor I in the degradation of C3b was demonstrated to be impaired, and the cofactor ability of G135R in the degradation of C4b was also inhibited. This study reported the first case of a four-generation postpartum-aHUS pedigree and for the first time confirmed the pathogenic effect of G135R/T98I mutant, and then elucidated that deficiency in complement activity was predisposed to the development of postpartum-aHUS

P0831

Anti-melanoma differentiation-associated gene 5 in Chinese patients with dermatomyositis

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DOI: 10.1002/eji.201970400

Objective: The goal of our study was to determine anti-MDA5 autoantibody in Chinese patients with dermatomyositis (DM).
Methods: Patients, disease controls and healthy controls were identified in Peking Union Medical College Hospital. Anti-MDA5 was measured by serum enzyme-linked immunosorbent assay.
Results: We identified 191 DM patients (64.9% women, mean age 48.3 years), 92 disease controls (64.3% women, mean age 49.2 years) and 129 healthy controls (63.8% women, mean age 44.2 years). The frequencies of anti-MDA5-positivity in the 3 cohorts (MDA-5 positive: DM 13.1%, disease control 4.3% and healthy control 0%, respectively). Anti-MDA-5-positivity was significantly associated with ILD, since 50% of MDA-5-positive subjects had ILD versus 25.5% of MDA-5-negative subjects ($P< 0.05$).
Conclusion: Anti-MDA-5 antibody is significantly associated with ILD in Chinese DM patients.

P0832

Identification of novel autoantibodies for idiopathic inflammatory myopathies using human proteome microarray

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Objectives: Idiopathic inflammatory myopathies (IIMs) are a group of clinically heterogeneous, inflammatory muscle disorders. Autoantibodies are important biomarkers for IIMs, aiding in diagnosis. We aimed to employed human proteome microarrays, composed of about 20,000 unique human proteins, to identify IIMs-specific autoantibodies.
Methods: A Three-Phase strategy was used. To screen candidate autoantigens, in Phase I, 90 serum samples collected from 40 IIMs patients, 30 autoimmune disease controls and 20 healthy subjects were probed to human proteome microarrays. To verify these candidates, in Phase II, a focused array with candidate IIMs-associated autoantigens was constructed, and this was used to profile a much larger cohort, comprised of serum samples collected from 397 IIMs patients, 197 disease controls and 98 healthy controls. In Phase III, sera with high signal values (value > 3 standard deviation of the mean of the healthy group) for verified proteins were validated using western blot analysis.
Results: Ninety-one candidate autoantigens that were significantly associated with IIMs were identified in Phase I. After verification in Phase II, GNMT was considered as a new IIMs-specific autoantigen. Polymyositis and dermatomyositis serums had high signal values for GNMT, while no healthy subject showed high signal values. In Phase III, 13 of the 25 polymyositis, four of the ten dermatomyositis, five of the six cancer-associated myositis, two of the two juvenile dermatomyositis, and one of the two disease controls were positive for anti-GNMT autoantibodies using western blot.
Conclusion: Anti-GNMT autoantibodies serve as a novel biomarker for IIMs and is expected to help clinical diagnosis.

P0833

Myositis-specific autoantibodies in dermatomyositis/polymyositis with interstitial lung disease

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Aim: The prevalence and diagnostic values of myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) in dermatomyositis/polymyositis (DM/PM) were studied.
Method: A commercial immunoblot assay with 16 autoantigens was used to detect MSAs and MAAs in serum samples from 130 DM/PM patients, 100 disease controls, and 50 healthy subjects.
Results: The prevalence of anti-Jo-1, anti-MDA5, anti-TIF1γ, anti-Mi-2α, and anti-Mi-2β was

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DOI: 10.1002/eji.201970400

significantly higher in DM/PM than in other connective-tissue diseases (CTDs). Moreover, anti-MDA5 and anti-Ro-52 were significantly higher in DM/PM with interstitial lung disease (ILD) than in DM/PM without ILD, while that of anti-TIF1γ and anti-NXP2 were significantly lower in DM/PM with ILD than in DM/PM without ILD. For distinguishing DM/PM from other CTDs, the sensitivity, specificity, and positive predictive value (PPV) for anti-MDA5 were 28.46, 99.00, and 97.37%, respectively, with a positive likelihood ratio (LR+) of 28.46; they were 46.15, 58.00, and 58.82%, respectively, for anti-Ro-52 with an LR+ of 1.10. For distinguishing DM/PM with ILD from DM/PM without ILD, the sensitivity, specificity, and PPV for anti-MDA5 were 45.57, 100.00, and 100.00%, respectively, and for anti-Ro-52 were 60.76, 73.91, and 80.00%, respectively.
Conclusion: MSAs and MAAs serve as biomarkers for differentiating DM/PM from other CTDs as well as distinguishing DM/PM with ILD from DM/PM without ILD.

P0834

Estrogen receptor β activation stimulates the development of experimental autoimmune thyroiditis through up-regulation of Th17-type responses

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Objective: Estrogens play important roles in autoimmune thyroiditis. The aim of this work was to investigate which estrogen receptor (ER) subtype (α or β) mediates the stimulatory effects.
Methods: To investigate the individual role of ERα and ERβ activation, we treated bilaterally ovariectomized CBA/J mice with propyl-pyrazole-triol (PPT, ERα selective agonist), diarylpropionitrile (DPN, ERβ selective agonist) or control vehicle followed by thyroglobulin (Tg)-immunization to induce experimental autoimmune thyroiditis (EAT), and observed the aggravation of EAT after diarylpropionitrile (DPN, ERβ selective agonist) administration.
Results: The mRNA levels of interleukin(IL)-17A, IL-21 and RORγt and percentages of T helper (Th) 17 cells were up-regulated in the splenocytes of DPN-treated mice. Activated ERβ protein was found not only directly binding to the DNA promoters of IL-17A and IL-21 by ChIP-PCR, but also indirectly promoting IL-21 and RORγt gene transcription through interaction with NF-κB by Co-IP analysis. Moreover, the expressions of co-stimulatory molecules CD86 and CD80 were significantly increased on antigen-presenting cells (APCs), including splenic dendritic and thyroid follicular cells, in DPN-treated mice as compared with that of the control animals.
Conclusions: It suggests that ERβ is the predominant ER subtype responsible for EAT development, and its activation may enhance Th17-type responses through genomic pathways and alteration of APCs´ activities.
Keywords: Autoimmunity; Estrogen receptor; Subtype-selective agonist; Thyroiditis

P0835

NLRP3/IL-1β pathway is critical in skin damage in a lupus-like mouse model induced by galectin3 immunization

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DOI: 10.1002/eji.201970400

Systematic lupus erythematosus (SLE) is an autoimmune disease that may involve all organs and tissues along with various serological immune disorders. Skin damage is one of the earliest signs and related to 4 of 11 diagnostic criteria. Our previous study showed that anti-galectin3 antibody is closely associated with skin damages in SLE. However, the molecular mechanisms remain elusive. We here report that mice immunized by galectin3 protein developed lupus-like disorders including skin alopecia, nephritis and lung inflammation along with the induction of lupus-related antibodies including anti-nuclear(ANA), anti-double-strand DNA(dsDNA)antibodies and anti-galectin3 antibody as well. The overexpression of IL-1β and NLRP3 was observed at the site of skin biopsy both from mice and human diseases. Skin inflammation was greatly reduced by subcutaneous injection of anti- IL-1β antibodies into lesional skin, indicative a pathogenic role of IL-1β in lupus skin damage. The production of IL-1β was induce by anti-galectin3 antibody through NLRP3 pathway. Collectively, our results suggest that the NLRP3/IL-1β pathway is critical for the development of lupus skin lesions and imply anti- IL-1β antibody as a potential therapy for the disease.

P0836

Pathogenesis and clinical characteristics of specific pathological manifestations of adult-onset Still's disease atypical persistent skin eruptions

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Adult onset still's disease (AOSD) is one kind of rare multisystemic autoinflammatory disease with unknown etiology, and in a long time the mainstream opinion about its skin appearance is evanescent skin eruptions (ESEs) accompanied with fever. Atypical persistent skin eruptions (APSEs) as a new skin type of AOSD have been noticed in recent years. Necrotic keratinocytes in the upper 1/3 of the epidermis is a specific pathological feature of APSEs, but the mechanism of this distinguished pathological phenomenon is unclear yet. In this research we summarized clinical and pathological data of 29 APSEs and 8 ESEs patients and found that necrotic keratinocytes in the upper 1/3 of the epidermis could be found in all APSEs patients but hardly in ESEs patients. Lymphadenopathy could be found in 75% APSEs patients, and during the follow- up, 3 APSEs patients could not exclude hematological malignancy, and 2 APSEs patients be diagnosed as malignancy. Therefore, we believe that AOSD with APSEs is likely to develop as hematological malignancy, also AOSD with APSEs and lymphoma may belong to the same lineage. We also observed that CD4+ T cells, CD11b+ cells and CD68+cells are increasingly infiltrated in APSEs rather than ESEs. IL-1β and IFN-γ are specifically expressed in the upper 1/3 layer of the epidermis, as same as necrotic keratinocytes, which may related to the pathogenesis of APSEs-specific pathological phenomena.

P0837

Heparanase mediated disorder of coagulation mechanism in membranous nephropathy

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Objective: Heparanase plays an important role in coagulation disorders. The purpose of this study was to investigate the significance of heparanase-mediated disorder of blood coagulation in membranous nephropathy.
Methods: The experiment was divided into two groups: MN and healthy control group. According to the serum albumin content, simultaneously, the MN group was divided into two groups: A (Alb< 20g/L)

and B (Alb>20g/L). The levels of HPA,TF and TFPI in serum were detected.
Result: The HPA concentration in the MN group was 72.66 U / ml, in the healthy group was 10.53 U / ml, P < 0.05; The TFPI in the MN group was 20800.00 pg / ml, in the healthy group was 17198.00pg / ml, P = 0.18; The TF concentration in the MN group was 60.58 pg/ml, in the healthy group was 15.29 pg/ml, P< 0.05; The TF/TFPI ratio was 0.00334 in the MN group and 0.00098 in the healthy group, P< 0.05.The HPA levels in the MN A and B groups were 79.16 U / ml, 70.07 U / ml, P < 0.05; The TFPI in the MN A and B groups were 21819.75pg / ml, 16647.00pg / ml, P = 0.13 ;The levels of TF in MN A and B groups were 50.35pg/ml and 68.46pg/ml, P=0.05; The TF/TFPI in the MN A and B groups were 0.00289 and 0.00393, P=0.06.
Conclusion: HPA, TF, TFPIand TF/TFPI may be involved in the formation of coagulopathy in the MN; HPA may be an indicator of early signs of coagulopathy.

P0838

Anti-LAMP-2 antibodies induced NETs transfer autoantigens to dentritic cells and result in CD4+ T cells differentiation into Th17 cells

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Objective: Anti-lysosomal membrane protein-2 (LAMP-2) antibodies are a new subtype of anti-neutrophil cytoplasmic antibody (ANCA). Interactions between ANCA and neutrophils have been proved to be critical in the pathogenesis of ANCA associated vasculitis (AAV). This study was designed to investigate the effects of anti-LAMP-2-antibodies-induced neutrophils extracelluar traps (NETs) formation on dendritic cells (DCs) and its possibility to stimulate CD4+ T cells differentiation.
Methods: Neutrophils were isolated from health individuals by density centrifugation, and then stimulated by anti-LAMP-2 antibodies for 3 hours. NETs were detected by immunofluorescence staining and laser confocal microscopy. DCs were generated from human peripheral blood mononuclear cells and allowed to interact with neutrophils for 3 hours in the presence or absence of anti-LAMP-2 antibodies. Effects of anti-LAMP-2-antibodies-induced NETs on DC phenotypes and its ability to stimulate CD4+T cells differentiation were therefore measured by flow cytometry.
Results: Anti-LAMP-2 antibodies-induced NETs up-regulated maturation markers of DCs, including CD80/CD83/CD86/HLA-DR. In addition, it increased the production of IL-1β, IL-6, IL-8 and IL-23 by DCs, which have been proved to be imperative factors for Th17 cells differentiation. Then, we confirmed that CD4+ T cells had differentiated into Th17 cells in this cytokine microenvironment. Furthermore, DNase I, which has been regarded as degradation regulator of NETs, inhibited the influences made by anti-LAMP-2 antibodies-induced NETs.
Conclusion: Our findings suggest that autoantigens which were exposed along with anti-LAMP-2 antibodies-induced NETs acts as danger signals leading to autoimmunity. This study provides new evidence that anti-LAMP-2 antibodies-induced NETs could play a critical role in pathogenesis of AAV.

P0839

Dendritic cell-associated B7-H3 suppresses the production of autoantibodies and renal inflammation in a mouse model of systemic lupus erythematosus

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DOI: 10.1002/eji.201970400

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B7-H3 immune modulatory molecule has been implicated in the generation and pathogenesis of autoimmune diseases, the mechanism of action is less known. We explored the role of B7-H3 in the induction of autoantibodies and organ-directed inflammation in a murine systemic lupus erythematosus (SLE) model in which the immunization with DNA extracted from activated T cells induced the production of anti-DNA autoantibodies and subsequent glomerulonephritis, two hallmarks of human SLE. Mice deficient of B7-H3 or treated with a B7-H3 specific antibody produced significantly higher levels of anti-DNA autoantibodies and more severe glomerulonephritis than wild type mice, indicating an inhibitory function of B7-H3 in this model. Interestingly, immunization of mice with DNA-pulsed dendritic cells induced severe SLE symptoms while B7-H3 on dendritic cells is required in this process. Importantly, treatment of mice with recombinant B7-H3Ig fusion protein effectively ameliorated progression of murine SLE, accompanied with decreased level of anti-DNA autoantibodies and alleviated glomerulonephritis, decreased autoantibody deposition and complement deposition in kidney. Our findings implicate a potential role of B7-H3 on dendritic cells in the induction of SLE and as a potential target for the treatment of autoimmune diseases.

P0840

The reciprocal regulation between C-Maf and bBlimp-1 in IL-27-dependent IL-10 expression and its impact on intestinal homeostasis

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IL-27 promotes IL-10-producing T cell differentiation through induction of AhR and c-Maf, and enhances IL-10 production in various T cell subsets resulting in the conversion of effector T cells into T regulatory type 1 (Tr1)-like cells. Recently, B lymphocyte-induced maturation protein-1 (Blimp-1) has been demonstrated to play an important role in IL-10 production from Tr1 cells. However, how the reciprocal regulation between c-Maf and Blimp-1 mediates IL-27-induced IL-10 expression and modulates the colitogenicity of T cells is still unclear. To pursue this critical issue, we knocked down IL-27 by introducing a shRNA transgene to target the p28 subunit in T cell-specific Blimp-1 knockout (CKO) mice and examined the severity of disease in these CKO/IL-27p28 knockdown (KD) mice. The onset of weight loss and diarrhea in CKO/IL-27p28 KD mice was observed at earlier age than CKO mice. Although IFN-g and IL-17 levels of $CD4^+$ T cells were downregulated, IL-10 expression in Th1 cells was significantly lower in the lymphoid organs of CKO/IL-27p28 KD mice. To further investigate the effect of c-Maf on the pathogenesis of Blimp-1 deficiency-mediated colitis, we crossed T cell-specific c-Maf transgenic mice with CKO mice. The onset of colitis in these mice was delayed. The Th1 and Th17 populations were increased whereas the population of IL-10-producing $CD4^+$ T cells from lymphoid organs of CKO/c-Maf mice were higher than those of CKO mice. In conclusion, the reciprocal regulation between c-Maf and Blimp-1 in IL-27-dependent IL-10 expression is essential for modulating the colitogenicity of T cells to maintain intestinal homeostasis.

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DOI: 10.1002/eji.201970400

P0841

Reg3β from cardiomyocytes regulated macrophage migration, proliferation and functional skewing in experimental autoimmune myocarditis

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Macrophages play critical roles in inflammatory initiation, development, resolution and cardiac regeneration of myocarditis. However, Reg3β, as a member of regenerating family of proteins, contributes to dedifferentiation of injury cardiomyocytes as well as cardiac function remodeling. It remains unclear whether Reg3β was associated with macrophages reprogramming during autoimmune myocarditis. Our results showed that Reg3β could effectively recruit macrophages, promoted their proliferation and phagocytosis, and facilitated their polarized into M2 macrophages. Macrophage, especially M1 phenotype contributed to Reg3β production by cardiomyocytes. Our data also indicated that Reg3β was involved in self-protection mechanism following cardiac injury or stress. This suggests that Reg3β might be a critically protective factor of myocardium.

P0842

TL1A/TNFR2-mediated mitochondrial dysfunction of FLS increased inflammatory response in rheumatoid arthritis via ROS generation

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Rheumatoid arthritis (RA) is the major autoimmune destructive disease of joints with complicated pathogenesis. Pro-inflammatory cytokines play major roles in the regulation of synovial inflammation. The contribution of tumor necrosis factor-like ligand 1A (TL1A) in RA pathogenesis especially on fibroblast-like synoviocytes (FLS) has been strongly suggested in basic and clinical research. This study investigated the role of TL1A for mitochondrial dysfunction, oxidative stress induced in mitochondria and inflammatory response in RA-FLS. During the experiments, RA-FLS were incubated with TL1A and TNF receptor 2 (TNFR2) antagonist. Mitochondrion membrane potential and respiration-associated genes of mitochondrion were measured in both TL1A stimulated and non-stimulated RA-FLS. Additionally, the effects of TL1A on ROS production in mitochondria and inflammatory response in RA-FLS were also assessed. In the results, we found that TL1A induced mitochondrial dysfunction by weakening mitochondrial respiration and membrane potential, which was blocked by TNFR2 antagonist. Increased ROS synthesis in impaired mitochondria was observed with mito sox immunofluorescent staining, and increased expression of IL-1β, IL-6, IL-8 and MMP9 was detected in TL1A-treated RA-FLS. Inhibition of Mitochondria-derived ROS compromised the production of inflammatory factors in TL1A-stimulated RA-FLS, suggesting that mitochondrial dysfunction mediated by TL1A/TNFR2 axis might amplify the inflammatory response through Mitochondria-derived ROS regulation. Collectively, our results revealed that TL1A might be involved in making FLS more aggressive in RA pathogenesis by cell respiration interruption. Targeting TL1A/TNFR2 axis would hold promise in the management of RA.

DOI: 10.1002/eji.201970400

P0843

The expression and biological effects of OX40 molecule on peripheral Tfh cells in Graves' Disease

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OX40/OX40L signaling and Tfh cells both play important roles in the development of Graves´ disease. However, the relationship between OX40 and Tfh cells in this disease is still unknown. Here we show that compared with thyroid adenoma patients and healthy volunteers, the expression of OX40 on peripheral Tfh cells was significantly raised in patients with Graves´ disease, so did the expression of OX40L on peripheral B cells and monocytes. In addition, the proportion of Tfh17 cells in $OX40^+$ Tfh cells was also higher than that in $OX40^-$ Tfh cells, indicating $OX40^+$ Tfh cells are the main cells to promote antibody production. After adding sOX40L protein to $CD4^+$ T cells, the expression of CXCR5, PD-1, ICOS, Bcl6 and IL21 were all up-regulated, suggesting that OX40 signal can promote Tfh cell differentiation. Further, sOX40L protein was also added to sorted Tfh cells from tonsil and the results showed that OX40/OX40L signal can maintain the proportion of Tfh cells. Also, Bcl6 was upregulated and Blimp1 was downregulated in these OX40-stimulating Tfh cells. In addition, the expression of CD45RO was elevated and the proportion of live cells was significantly higher than that of the control group after stimulation with sOX40L protein. These findings indicate that OX40L expressed on antigen-presenting cells may bind to OX40 on Tfh cells to promote the differentiation of Tfh cells and memory Tfh cells, maintain survival of Tfh cells, which may help B cells secrete autoantibodies and participate in the occurrence and development of Graves´ disease.

P0844

Positionally cloned Ym1 regulates alternative activation of macrophages and contributes to arthritis development

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Chitinase-like proteins have been found to cause unbalance in immune regulation and tissue remodeling, leading to pathology. We have used genetically segregating mouse strain crosses, and identified the *Chil3* (Ym1) gene as a likely candidate gene for arthritis. Ym1 expression is virtually absent in RIIIS/J derived congenic mice due to the polymorphisms in its promoter. Ym1 congenic fragment showed protective effect on collagen-induced arthritis, and collagen antibody-induced arthritis which was triggered by mannan. Mannan intraperitoneal injection to mice could induce psoriatic arthritis which was ameliorated when Ym1 was deficiency. Depletion of macrophages, or adoptive transfer macrophages derived from congenic mice both could reduce arthritis severity. Bone marrow derived macrophages from Ym1 congenic mice tended to be M2 polarization, but were impaired in M1 polarization. Consistently, the M2 phenotype of peritoneal macrophages in mannan treated congenic mice was enhanced compared to wildtype control. In addition, supplement of Ym1 protein to congenic mice reversed the alleviated disease and impaired the M2 polarization. We concluded that Ym1 deficiency manipulated the alternative activation of macrophages, alleviating arthritis development. Our study identified Ym1 as a novel immune regulator of experimental arthritis in mice, thereby indicating a previously unrecognized immunomodulatory mechanism of pathogen derived pattern recognition molecules altering susceptibility to chronic inflammatory joint disease.

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DOI: 10.1002/eji.201970400

P0845

Haploinsufficiency of A20(HA20): updates on the genetics, phenotype, pathogenesis and treatment

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Background: A20, a protein encoded by TNFAIP3, plays a vital role in the negative regulation of inflammation and immunity. Loss-of-function mutation in TNFAIP3 leads to a new described autoinflammatory disease haploinsufficiency of A20 (HA20). It is still a poorly known disease for the limited patients. Therefore, we reviewed aiming to improve clinical recognition of this disorder and to make an early diagnosis, as a result, patients can get targeted therapies earlier and improved outcomes.
Methods: We reviewed the cases reported, summarized the mutations as well as clinical manifestations of a total of 61 patients from 26 families with a genetic diagnosis of germ-line mutations in TNFAIP3/A20 that have been reported in the literature. We also discussed the pathogenesis and treatment of HA20.
Results: A total of 24 pathogenic variants of A20 had been reported. The clinical phenotypes varied considerably even within families harbouring the same mutation, prior to establish the genetic cause, the diagnoses in these cases ranged from Behcet's disease (BD), rheumatoid arthritis (RA), rheumatic fever, juvenile idiopathic arthritis (JIA) to systemic lupus erythematosus (SLE), even adult-onset Stills' disease (AOSD). The HA20 patients presented with inflammatory signatures in NF-κB signaling were mostly responsive to treatment.
Conclusions: HA20 is a monogenic autoinflammatory disease with varies clinical manifestations. It´s hard to develop a set of diagnostic criteria for the extensive heterogeneity, and genetic sequencing is required for a definitive diagnosis. Except for symptomatic treatments, early use of biotherapies was effective for patients.
Keywords: autoinflammatory disease, HA20, TNFAIP3, NF-κB signaling, treatment

P0846

The application value of serum SAA in rheumatoid arthritis disease

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Objective: To investigate the application value of serum amyloid A (SAA) in rheumatoid arthritis (RA) disease.
Methods: Collect 263 confirmed RA patients. According to the DAS28-ESR, RA patients were divided into four groups: 25 cases in RA remission group, 24 cases in low activity group, 124 cases in moderate activity group, 90 cases in high activity group.Collect the clinical and laboratory data.Serum SAA levels were determined by ELISA.SPSS17.0 statistical software was used to analyze the experimental data and clinical data.
Results: SAA level in remission group [2.20 (0.99, 5.63) ug/ml], low activity group [2.06 (1.43, 5.37) ug/ml], moderate activity group [6.51 (6.01, 7.82) ug/ml], high activity group [13.79 (7.31, 29.93) ug/ml], there was a statistical difference between the four groups (c^2 = 66.89, P < 0.01).SAA was positively correlated with synovitis (r= 0.28, *P* < 0.01) in remission RA patients under MSUS

DOI: 10.1002/eji.201970400

examination.The optimal cutoff value of serum SAA for a assessment of RA activity was 5.33ug/ml, and the sensitivity and specificity were88.2%and 82.0%, respectively. The AUC was 0.84.When SAA combined with CRP was used to evaluate RA disease activity, the area under ROC curve was 0.87.
Conclusion: SAA is an important serological indicator for evaluating RA disease activity. When combined with CRP can improve the evaluation value of RA disease activity. The elevated SAA level in RA patients may indicate the existence of subclinical synovitis.
Keywords: Arthritis;rheumatoid;Serum amyloid A;Disease activity ;Combination

P0847

Serum phospholipase A2 receptor antibodies and immunoglobulin G subtypes in adult idiopathic membranous nephropathy: clinical value assessment

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Background: Idiopathic membranous nephropathy (IMN) is widely considered as an organ-specific autoimmune disorder. Implicated in its pathogenesis are the phospholipase A2 receptors (PLA_2R) expressed on glomerular podocytes against which serum antibodies are formed. In this study we quantified and assessed the clinical value of total serum PLA_2R antibodies and the subtype antibodies in IMN.
Methods: We measured serum levels of total PLA_2R antibodies and IgG subtype antibodies by Enzyme Linked Immunosorbent Assay (ELISA) in 146 biopsy-proven IMN patients, 51 non-IMN patients and 62 healthy controls. We went ahead and determined the diagnostic potential of total serum PLA2R antibodies and assessed if a relationship exists between the dominat subtype antibody and the clinical parameters.
Results: The diagnostic sensitivity and specificity of total serum PLA_2R antibody for IMN were found at 69.9% and 100% respectively. Significant differences in systolic blood pressure, serum Cystatin C, serum albumin and estimated glomerular filtration rate (eGFR) were found between the antibody-positive and antibody-negative groups of IMN patients. Subtype antibody 4 and 1 exhibited the highest positive rates of 94.4% and 91.6% respectively. The mean serum proportion of subtype antibodies was 65.4, 12.7, 7.6 and 4.6% for subtype 4, 1, 3 and 2 respectively. Serum levels of total protein and albumin were significantly decreased among patients with high serum titres of antibody subtype 4.
Conclusion: Our findings underscore the diagnostic potential of total serum PLA_2R antibodies and highlight the importance of antibody subtype 4 over other subtype antibodies in IMN.

DOI: 10.1002/eji.201970400

P0848

P2X7R promote the attack of acute gouty arthritis in rats from clinical to pathology

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Objectives: Activation of the P2X7R signaling pathway by changes in extracellular ATP concentrations leads to the development of acute gouty arthritis and the production of pro-inflammatory.
Methods: After establishment of acute gouty arthritis model, rats were given P2X7R agonist ATP, P2X7R inhibitor BBG and PBS, respectively. The clinical manifestations and histopathology of the ankle joints of each group rat were observed at 6h, 12h, 24h, 48h and 72h.
Results: 1. P2X7R regulates the development of acute gouty arthritis:At 12h, the clinical scores of ATP group were significantly higher than those of BBG group and control group, and the control group was higher than BBG group; At 12h and 24h, the swelling index and infiltration of mononuclear cell in ATP group was significantly higher than that in BBG group and control group; The neutrophils infiltration in ATP group was the highest among the three groups at 24h; **2.** P2X7R regulates pro-inflammatory cytokines production:At 24h, the level of IL-1β in ATP group was significantly higher than BBG group and control group; At 6h and 24h, higher level of IL-6 and TNF-α in ATP group compared with BBG group and control group.
Conclusion: Activation of P2X7R can significantly promote the attack of acute gouty arthritis and the production of IL-1β, IL-6 and TNF-α, suggesting that P2X7R affects the development of acute gouty arthritis and regulates the secretion of pro-inflammatory cytokines.

P0849

The correlation between VCAM-1 and renal injury of systemic lupus erythematosus

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Objective: To investigate the correlation between serum vascular cell adhesion molecule-1 (VCAM-1) level and disease activity and renal injury in patients with systemic lupus erythematosus (SLE) .
Methods: 107 SLE patients were divided into stable disease group (SLEDAI＜5) and active disease group (SLEDAI≥5)according to the systemic lupus erythematosus disease activity index (SLEDAI).Serum levels of VCAM-1 and other laboratory indexes were measured while pathological types of kidneys were noted.All subjects were analyzed by SPSS17.0 with $P < 0.05$ as the statistical significance.
Results: The active disease group had statistically higher levels of ESR,24-hour urinary protein quantification,ds-DNA,anti-complement C1q antibody,VCAM-1 and lower levels of complement C3 and C4 than stable disease group (P＜0.05).At the same time ,a positive correlation was found between the VCAM-1 and 24-hour urinary protein quantification (r_s＝0.335,P＝0.012) while a negative correlation was found between the VCAM-1 and complement C1q(r_s＝-0.317,P＝0.032)in the active disease group. According to the Pathological classification of lupus nephritis,proliferative sclerosis LN(type III, type IV and type VI) had a statistically higher level of 24-hour urinary protein quantification and VCAM-1 than mesenchymal proliferative LN (type I and type II) (P＜0.05).
Conclusion: VCAM-1 positively correlate with the disease activity of SLE and it may suggest kidney injury,especially proliferative sclerosis type.

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DOI: 10.1002/eji.201970400

Keywords: Systemic lupus erythematosus , Lupus nephritis , Vascular cell adhesion molecule-1 , Pathology

P0850

APLNR is involved in ATRA-induced growth inhibition of nasopharyngeal carcinoma and could suppress EMT process through PI3K/Akt/mTOR signaling pathway

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The apelin receptor (APLNR) is a G-protein coupled receptor (GPCR) involved in many pathophysiological processes; however, the correlation between APLNR expression and nasopharyngeal carcinoma (NPC) has not been reported. In this study, we used cDNA microarray and tissue microarray to determine APLNR expression levels in NPC tissues. We found that APLNR expression was reduced in NPC tissues compared to noncancerous nasopharyngeal epithelial (NPE) tissues. Furthermore, positive expression of APLNR in NPC predicted a better prognosis (disease free survival (DFS): P=0.001, overall survival (OS): $P<0.001$). Moreover, ingenuity pathway analysis (IPA) revealed that an indirect interaction existed between APLNR and retinoic acid (RA) in the cancer regulatory network. Consistently, after treatment with all-trans-retinoic acid (ATRA), we found that APLNR was significantly upregulated in NPC cell lines (5-8F and HNE1), while proliferation of NPC cells was inhibited. Cell cycle arrest occurred in the G0/G1 phase. In contrast, knockdown of APLNR diminished ATRA-induced growth inhibition of NPC cells. In addition, we surprisingly found that APLNR also played an important role in migration and invasion of NPC by wound healing and transwell assays. Western blot results revealed that hallmarks of epithelial-mesenchymal transition (EMT) were altered as well, suggesting that APLNR was capable of inhibiting EMT in NPC cells. Our study further demonstrated that low expression of APLNR promoted EMT in NPC cells by activating the PI3K/Akt/mTOR signaling pathway. Taken together, our data suggested that APLNR could potentially predict prognosis for NPC patients and inhibit proliferation, migration, invasion and EMT in nasopharyngeal cancer cells.

P0851

Changes and significance of BAFF and IL-10 in the adjuvant treatment of SLE in children with Huaiqihuang granules

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Objective: To investigate the efficacy and safety of Huangqi granules (HQH) in the treatment of children with SLE.
Methods: Children with SLE were divided into groups A, 15 cases (glucocorticoids and (or) immunosuppressive agents combined with Huaiqihuang,) and Group B ,15 cases(treated by only glucocorticoids and (or) immunosuppressive agents) ; group C ,10 cases(,healthy physical examination in the same period) .Serum BAFF and IL-10 levels were measured at the time before treatment and 3 months after treatment, and SLEDAI scores, laboratory indicators, and adverse drug reactions were also compared.
Results: (1) Compare to group C, the serum levels of BAFF and IL-10 in group A and B were higher

DOI: 10.1002/eji.201970400

(p< 0.05). Immunoglobulin was higher than normal range, complement decreased, CD3+, the proportions of CD4+, CD4+/CD8+ and NK cells were low, the proportion of CD8+ and CD19+ were high, the ANA titers were elevated, and the ds-DNA antibodies were positive. All cases had proteinuria in varying degrees. (2) After 3 months of treatment, SLEDAI score, serum BAFF, IL-10, IgG, and CD19+ in group A and B were all decreased (p< 0.05), and the proportion of complement and CD3+ was increased (p< 0.05). The amplitude of immune indicators changes in Group A was greater than that in group B (p< 0.05);
Conclusion: Huaiqihuang granules can be used to adjuvant therapy of children with SLE,which may be through the reduction of serum BAFF levels, reduce B cell activation, reduce IL-10 secretion, regulate T, B cell imbalance, reduce autoantibody production, and promote disease recovery.

P0852

Clinical analysis of neuroblastoma associated with opsoclonus-myoclonus syndrome

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Objective: To analyze the clinical features of neuroblastoma (NB) associated with opsoclonus - myoclonus syndrome (OMS) and improve the understanding of this disease.
Methods: One case of NB associated with OMS was introduced and its clinical features were analyzed in combination with previous literatures.
Results: NB can occur with OMS as the first symptom. The main symptoms are opsoclonus (involuntary, rapid, irregular eye movement), myoclonic, ataxia, motor and language development regression, and irritability. There was no abnormal in the EEG and MRI of the head. This case was initially misdiagnosed as viral cerebellitis.The image examination of chest revealed a mediastinal tumor and the pathological diagnosis was neuroblastoma (differentiated type). The symptoms of OMS were not significantly improved after tumor resection, but corticosteroids therapy showed effectiveness presenting as the remission of opsoclonus, then myoclonus ,ataxia, and at last the motor and language ability.
Conclusion: NB associated with OMS is a rare autoimmune disorder characterized by neurological damage as paraneoplastic syndrome, which is common in childhood. It clinically manifests opsoclonus, myoclonus, ataxia, behavioral changes. Children confirmed as OMS should have a routine trunk radiographic examination to find NB. The OMS symptoms can not be improved by tumor resection. In the case of NB with OMS, in terms of tumors, the majority of children can survive for a long time, but they are often left with long-term motor, behavioral, and cognitive sequelae.

P0853

Establishment of a humanized lupus nephritis mouse model

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Objective: NCG mice were used to establish a humanized Lupus Nephritis (LN) model. The immune reconstruction and CD4+ T cells subsets in donor and mice were detected. Survival time, renal pathology of mice and their correlation with donors' performance were observed.
Methods: NCG mice were intravenous and intraperitoneal injected with peripheral blood mononuclear cells (PBMCs) from healthy individuals (HC mice) or LN patients (LN mice) and euthanized 8 weeks

DOI: 10.1002/eji.201970400

after engraftment. The proportion of hCD45+ leukocytes cells, hCD3+ T cells, hCD19+ B cells, hCD4+hCD25^{high}hCD127^{low} Treg cells, hCD4+hCXCR5+hPD-1+ Tfh cells, hCD4+hIFN-γ+ Th1 and hCD4+IL-17A+ Th17 cells in donors' PBMCs and mice spleens were detected by flow cytometry. Renal lesions of mice were observed by Periodic Acid-Schiff (PAS) staining.
Results: hCD45+ leukocytes were successfully transfered. The survival time of LN mice is negatively correlated with the 24-hour urinary protein level of LN patients and shorter than HC mice. The reconstructed immune cells were mostly T cells. B cells exist in some LN mice. The percentage of Treg cells is relatively higher in LN patients but lower in LN mice. The hCD4+hCD161+ cells have more IL-17A secretion than that of hCD4+hCD161- cells both in human and mice. The glomerular lesions of LN mice whose donors have high 24-hour urinary protein levels were relatively severe while that were mild in HC mice.
Conclusion: The humanized LN mouse model has good correspondence with donors, which may be useful for studing the pathogenesis and treatment of severe LN.
National Natural Science Foundation of China (81471601)

P0854

Mechanism of hydrogen on alleviating inflammatory bowel disease by promoting the production of short-chain fatty acids in the intestine

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Hydrogen as a new medical gas exerts organ-protective effects through regulating oxidative stress, inflammation and apoptosis. Some evidence suggests the protective effects of hydrogen in various models of inflammatory bowel disease (IBD). However, the exact mechanism underlying this protective effect of hydrogen against IBD is not fully understood. The present study was designed to investigate whether hydrogen-rich saline (HS) attenuates IBD in mice model and the underlying mechanisms. The results showed that HS administration significantly attenuated inflammatory injury, including increase of weight and colon length, improvement of colonic histology, decreased level Of IL-6, TNF-α in serum, as well as inhibition of TLR4-NF-κB signaling pathway in colonic tissues. Furthermore, HS significantly promoted the production of short-chain fatty acids (SCFAs), including acetate and butyrate, by upregulating of specific SCFAs-producing bacterial abundance. Increased SCFAs facilitated the development of Treg cells , and enhanced intestinal epithelial barrier function by inducing goblet cell differentiation and mucous production, as well as decreasing the abundance of pathogenic E.coli, thus this is beneficial to the maintenance of intestinal immune homeostasis. Additionally, HS increased the abundance of intestinal Clostridium, thus the resulting butyrate further promoted the activation of PPAR-γ signal and inhibition of iNOS expression. Therefore, we believed Hydrogen is involved in the metabolic reprogramming of colon epithelial cells through regulating PPAR-γ signal, which promotes the recovery of the intestinal anaerobic environment. Taken together, HS exerts protection against IBD in mice, mediated in part by promoting the production of SCFAs.
This work was supported by fund(No.81771711, J16LK02)

P0855

Value of serum matrix metalloproteinase-3 in patient with Rheumatoid Arthritis

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DOI: 10.1002/eji.201970400

Objective: To investigate the value of serum matrix metalloproteinase-3（sMMP-3）in the assessment of active disease and bone erosion in patient with Rheumatoid Arthritis (RA).
Methods: Two hundred RA patients from The People´s Hospital of the Xinjiang Uygur Autonomous Region.The level of disease activity by DAS28 score. 78 healthy subjects were recruited as controls.
Results: （1） sMMP-3 was significantly highers in RA patients38.47ng/mLthan healthy subjects5.91ng/mL（P＜0.001）,sMMP-3 was highers in RA patients with High disease activity 47.29ng/mL than Medium disease activity 2.19ng/mL（P＜0.05）， the Medium disease activity than remission and Low disease activity18.39ng/mL（P＜0.001）；（2）The level of sMMP-3 was significantly correlated with DAS28（r=0.516, P＜0.001）、ESR（r=0.352, P＜0.001）、CRP（r=0.357, P＜0.001）；（3）The level of MMP-3 with bone erosion in RA patients was 41.88 ng/mLwhich was higher than that without bone erosion 37.18 ng/mL (P < 0.05)；Logistic single-factor regression analysis showed that ESR, DAS28 and CRP were risk factors for bone erosion (P < 0.05), while Logistic multiple-factor regression analysis showed that only sMMP-3 was risk factor for bone erosion (P < 0.05).
Conclusions: sMMP-3 can be used to evaluate the disease activity in RA patients and may be a risk factor for predicting bone erosion.

P0856

Mannan induced psoriasis is more severe in female mice

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Psoriasis is an autoimmune, chronic skin disease with a prevalence of 1-3% in the world population affecting males and females equally. However, in females an earlier disease onset and higher risk for psoriasis development at different age groups were reported. Estrogens have both immunosuppressive and immunostimulatory properties. Hence, the role of sex in psoriasis is considered to be still controversial. Animal models are useful tools in dissecting pathogenic pathways but the commonly used imiquimod induced psoriasis model shows no differences between male and female mice. Hence, we developed a new inflammatory psoriasis model using mannan from *Saccharomyces cerevisiae*. 5 mg of mannan mixed with incomplete Freund's adjuvant or vaseline applied on the back of several inbred mouse strains induced psoriasis with a female preponderance. Histological evaluation of skin samples showed thickening of epidermis and increased cell proliferation. The number of inflammatory cells including neutrophils, DCs, macrophages and gδ T cells and, pro-inflammatory cytokines were increased in the dermis, inguinal lymph nodes and spleen of psoriasis mice. Absence of endogenous estrogen decreased psoriasis symptoms by affecting the number of gδ T cells and DCs and down regulated the expression of pro-inflammatory cytokines. Further studies are underway to understand the role of exogenous estrogen and phytoestrogens as well as estrogen independent mechanisms operating in female mice having psoriasis symptoms.

P0857

The correlation between serum visfatin level and atherosclerosis in patients with rheumatoid arthritis

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DOI: 10.1002/eji.201970400

Objective: Our aim was to investigate the Correlation between serum visfatin levels and atherosclerosis in rheumatoid arthritis（RA）patients.
Methods: A total of 113 RA patients were included. Exclude other rheumatic immune diseases, hypertension, diabetes, history of coronary heart disease, smoking history.According to carotid ultrasound examination,patients were divided into RA with atherosclerosis group and RA without atherosclerosis group. The general data of age, sex, course of disease, height, weight, time of morning stiffness, number of joint tenderness, swelling and (VAS) pain score of visual analogue scale were collected.serumvisfatin, ESR,CRP,anti-CCP antibody,RF.The data were processed by SPSS17.0 statistical software.
Results: Serum visfatin levels in patients with carotid atherosclerosis was higher than that without arteriosclerosis in RA patient（14.1（8.6-17.8）ng/ml vs 10.4（10.3-19.7）ng/ml, P=0.038）. The disease activity score was no significant difference between the two groups(P>0.05).The serum visfatin level was positively correlated with the RF (r_s=0.38,P=0.00), anti CCP antibody(r_s=0.25,P=0.018), CRP(r_s=0.22, P=0.041）,TC（r_s=0.228, P=0.021）、LDL（r_s=0.196, P=0.049）.Univariate logistic regression analysis showed that age (OR=1.127,95%CI 1.071~1.186, P = 0.000), LDL (OR=2.328,95%CI 1.225 ~4.423, P = 0.01) and serum visfatin level (OR=1.123,95%CI 1.011~1.248, P =0. 031) is associated with carotid arteriosclerosis in patients with RA. multivariate logistic regression analysis showed that age (OR=1.132,95%CI 1.059~1.211, P =0.000) was a risk factor for atherosclerosis in patients with RA.
Conclusions: The increase of serum visfatin levels may promote the development of atherosclerosis in patients with RA.
Key words: Rheumatoid Arthritis;Visfatin;Atherosclerosis;Intima-Media Thickness

P0858

Short-term outcomes in patients with systemic juvenile idiopathic arthritis associated refractory macrophage activation syndrome treated with Tocilizumab, single-centre experience

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Objective: To assess the short-term outcomes among patients with Systemic juvenile idiopathic arthritis (sJIA) associated refractory Macrophage activation syndrome (MAS) who were treated with Tocilizumab (TCZ).
Methods: We collected clinical and laboratory outcomes retrospectively for 6 patients diagnosed as sJIA-associated refractory MAS who were hospitalized at our hospital from Jan 2016 to Jan 2019. The following laboratory findings were assessed before and after TCZ therapy: white blood cell count (WBC), hemoglobin (HB), platelet (PLT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, alanine aminotransferase (ALT) and serum ferritin (SF).
Results: Six patients (2 boys and 4 girls) with sJIA-associated refractory MAS were identified. Mean age at time of disease onset was 4.5 years and the youngest was 1y5m. Before the initiation of treatment, clinical manifestations included fever (6/6), rash (4/6), arthrophlogosis (4/6), abdominal pain and hematochezia (2/6), diarrhea (1/6). For treatment before intravenous TCZ, we used high-dose methylprednisolone pulse, immunoglobulin and cyclosporin A. We found the outcome within 7 days was favorable after TCZ therapy: clinical manifestations were all improved, 5 patients were afebrile in 24 hours and WBC count, HB, CRP, ESR, albumin, ALT returned to normal obviously within a week. The improvement of SF is not obvious during first week. PLT in one patient with refractory thrombocytopenia didn't return to normal until the second TCZ intravenous infusion.
Conclusions: TCZ can provide effective protection against sJIA-associated MAS, especially refractory MAS.
Keywords: Tocilizumab, Systemic juvenile idiopathic arthritis, Macrophage activation syndrome, Short-term outcome

DOI: 10.1002/eji.201970400

P0859

Value of KL-6 in diagnosis of connective tissue disease with interstitial lung disease

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Objective: To evaluate the application of the serum Krebs von den Lungen-6 (KL-6）for the interstitial lung disease(ILDs) associated with connective tissue diseases(CTDs).
Methods: Patients with CTDs who visited our Hospital between January, 2016 and December, 2017, and whose serum KL-6 level was measured were included. We analyzed 175 patients with CTDs ,84 CTDs associated ILD,91 CTDs patients without ILD. Record age、gender、diagnosis、serum KL-6 levels、pulmonary function tests and performed in parallel were reviewed. Statistical analysis was performed using SPSS (version21.0) statistical package.
Results: The significantly higher levels of KL-6 were determined in the CTD-ILD group than in the CTDs without pulmonary involvement group(P＜0.05）.By the ROC curves of serum KL-6 levels in 175 patients. The optimal cutoff value of serum KL-6 for a diagnosis of CTD-ILD was 409U/ml, and the sensitivity and specificity were82.1% and 86.8%, respectively. The AUC was 0.905.Serum KL-6 correlated negatively with vital capacity(VC) (%predicted)、forced vital capacity(FVC%)(%predicted)、forced expiratory volume in one second（FEV1%)(%predicted) and diffusing capacity of the lung for carbon monoxide (DLcoSB) (% predicted) .Serum KL-6 correlated Positive correlation with Gross glass score. The optimal cutoff value of serum KL-6 for a diagnosis active of CTD-ILD was 1018U/ml, and the sensitivity and specificity were88.2% and 64.0%, respectively.
Conclusion: The serum KL-6 is a valuable biomarker for CTD-ILD diagnosis， and it is an important serum marker for detection of CTD-ILD activity.

P0860

Value of anti-C1q antibody C3 and C4 in diagnosis of neuropsychiatric systemic lupus erythematosus

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Objective: To evaluate the application of anti-C1q antibody and C3 and C4 in the diagnosis of neuropsychiatric sys- temic lupus erythematosus(NPSLE) .
Methods: We conducted a cross-sectional study of 22 patients with NPSLE and 66 patients SLE. Anti-C1q antibody was assayed by ELISA， C3 and C4 was assayed by Immunoturbidimetry. The correlation between the clinical manifestations and complement was analyzed. Logistic regression analysis was performed to find the association of anti-C1q antibodies with NPSLE.
Results: The anti C1q antibody level of NPSLE was significantly higher than non-NPSLE patients, complement C4 signifi- cantly lower than non-NPSLE， using ＲOC curve analysis of anti-C1q antibody sensitivity and specificity in the diagnosis of NPSLE pa- tients were 63. 6%， 66. 7%， after using single factor analysis found that resistance against C1q antibody， anti-ribosomal P protein anti-bodies and anti-nucleosome antibodies were the predictive factors of NPSLE， but the multi-factor Logistic regression analysis was no as- sociated with lupus encephalopathy. But complement C4 reduce had associated with SLE patients with cerebrovascular accident.
Conclusion: The anti-C1q antibody has certain diagnostic value in the diagnosis of NPSLE, complement C4 may participate the occurrence of cerebrovascular accident of NPSLE.

DOI: 10.1002/eji.201970400

Keywords: Complement; Anti-C1q antibody; Neuropsychiatric systemic lupus erythematosus; Systemic lupus erythematosus

P0861

Mechanisms of an autoimmunity syndrome in mice caused by a novel dominant mutation in *Aire*

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Expression of the autoimmune regulator (AIRE) gene in the thymus is required for negative selection of autoreactive thymocytes and the establishment of central immune tolerance. Autosomal recessive loss-of-function mutations in AIRE cause autoimmune polyglandular syndrome type 1 (APS1), which manifests as a classic triad of hypoparathyroidism, adrenal insufficiency, and candidiasis. Recently, a kindred was identified with a C311Y mutation in the first plant homeodomain (PHD1) zinc finger of AIRE that presented with an autosomal dominant autoimmune phenotype distinct from APS-1. To determine the mechanisms underlying auto-immunity in this family, we generated a novel C311Y knockin (*Aire*$^{CY/+}$) mouse model using CRISPR targeting. As expected, *Aire*$^{CY/+}$ mice developed spontaneous organ-specific autoimmunity. As compared to *Aire*$^{-/-}$ mice and *Aire*$^{GW/+}$ mice, which carry a previously described autosomal dominant mutation in the SAND domain, *Aire*$^{CY/+}$ mice had: altered TSA expression in medullary thymic epithelial cells (mTECs), a unique pattern of affected peripheral organs, decreased frequency of antigen-specific T cells after immunization with an Aire-dependent TSA, and a less severe block in terminal mTEC differentiation. In sum, our findings further elucidate the role of the PHD1 domain in AIRE function and describe a novel mouse model that will facilitate its further characterization.

P0862

Extrapituitary prolactin promotes the generation of Eomes-positive helper T cells mediating chronic neuroinflammation

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A novel eomesodermin-positive CD4^{+}T cells (Eomes^{+} Th cells) been identified as a major pathogenic component in late/chronic stage of experimental autoimmune encephalomyelitis (EAE) and secondary progressive multiple sclerosis (SP-MS). However, the mode of their development under smoldering neuroinflammation remains elusive. We now demonstrate that antigen-presenting cells (APCs) such as B cells and non-B MHC class II^{+} myeloid cells accumulated in the central nervous system (CNS) under chronic inflammation and attained prolactin (PRL)-producing capacity. Further analysis indicated that such APCs producing PRL induced potentially cytotoxic Eomes-expressing Th cells which could be blocked in the presence of anti-MHC class II antibody. Furthermore,PRL levels in the cerebrospinal fluid were significantly increased in the late phase of EAE. PRL blockade by bromocriptine or Zbtb20-specific siRNA significantly reduced the numbers of Eomes^{+} Th cells in the CNS and ameliorated clinical signs of chronic EAE. The PRL dependency of Eomes^{+} Th cells was confirmed in a series of *in vitro* and *ex vivo* experiments. Accordingly, several cytokines produced in the CNS under chronic inflammatory condition induce PRL expression in APCs. Collectively, our current data clearly describe that prolonged neuroinflammation shapes the profiles of CNS APCs to facilitate the development of Eomes-expressing Th cells and promote the progression of chronic disease through the ectopic expression of prolactin.

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DOI: 10.1002/eji.201970400

P0863

Characterizing molecular mimicry in peripheral blood-derived autoreactive CD4$^+$ T cells in multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease in central nervous system and strongly associated with the HLA-DR allele DRA1*01:01/ DRB1*15:01(DR2b). Autoreactive CD4$^+$ T cells are considered critical for the pathogenesis of MS, and molecular mimicry between foreign- and self-antigens is considered as a main mechanism for their activation. However, the foreign agent/s, which may activate autoreactive T cells in MS, are not well defined, although EBV and *Akkermansia* have been suggested as important environmental pathogens. In this study, we successfully generated a DR2b-restricted CD4$^+$ T cell clone (TCC), 1175SA_TCC14, from peripheral blood of a MS patient by limiting dilution. Analysis of TCR Vβ sequences of 1175SA_TCC14 and comparison with TCR Vβ sequences found in brain lesions of the same MS patient showed that 1175SA_TCC14 was clonally expanded in MS brain lesions. Using an unbiased and systematic antigen search strategy, which combines testing the clone with combinatorial peptide libraries and subsequent bioinformatics analyses, we identified RAS guanyl-releasing protein-2 (RASGRP2) as target autoantigen of 1175SA_TCC14. Proteomics analysis demonstrated high abundance of RASGRP2 in brain. Next, the scoring matrix of 1175SA_TCC14 was used to score contiguous decapeptides of EBV and *Akkermansia*. Candidate peptides were selected and synthesized. Proliferative response testing showed that 1175SA_TCC14 responded to both EBV- and *Akkermansia*-derived peptides, but their stimulatory potency is lower than RASGRP2$_{(78\text{-}87)}$. Our study supports that molecular mimicry derived from suggested environmental triggers of MS, i.e. EBV and *Akkermansia*, can activate brain-infiltrating autoreactive CD4$^+$ T cells in MS.

P0864

Staphylococcus aureus-induced tissue-resident memory T helper 17 cells (T_{RM}17 cells) drive renal autoimmune disease

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Tissue-resident memory T (T_{RM}) cells represent a type of memory cells that reside in peripheral organs without recirculating. A tissue-resident memory phenotype has been described for both CD4$^+$ and CD8$^+$ T cells. T_{RM} cells provide rapid on-site immune protection against previous exposed pathogens. Here, we used flow cytometry and single cell RNA-sequencing to examine T cells in human and murine kidneys. We found a substantial population of CD4$^+$ CD69$^+$ T_{RM} cells of the T_H17 phenotype in human, and operationally named them T_{RM}17 cells. CD4$^+$ T_H17 cells are involved in the response to pathogens such as S. *aureus* and play a role in autoimmunity like crescentic glomerulonephritis (cGN).

DOI: 10.1002/eji.201970400

To analyze the role of $T_{RM}17$ cells, we established a mouse model of S. *aureus* infection that displays accumulation of long-term persisting T_H17 cells in the kidney. Induction of cGN in mice which had recovered from bacterial infection resulted in an enhanced renal T_H17 response and development of a severe cGN. In contrast, prior infection with *Listeria monocytogenes*, which causes a T_H1 immune response, did not alter renal autoimmunity. By labelling T cells in the kidney using photoconvertible Kaede-transgenic mice, we were able to demonstrate that S. *aureus* induced $T_{RM}17$ cells contributed to the enhanced local T_H17 response in cGN. In addition, depletion of T_H17 cells after S. *aureus* infection or neutralization of IL-17A ameliorated renal autoimmune disease, showing the impact of resident T_H17 cells in the kidney. Our data suggest that $T_{RM}17$ cells have a previously unknown role in amplifying organ-specific autoimmunity.

P0865

Altered signalling in patients with primary Sjögren's syndrome: a single-cell network analysis by mass cytometry

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Primary Sjögren's syndrome (pSS) is a chronic, inflammatory, systemic autoimmune disorder characterized by exocrinopathies, mainly in the salivary and lacrimal glands, along with other extraglandular manifestations (EGM). Enhanced activation potential of signalling pathways may play a crucial role in the aetiology and progression of the disease. In our previous work using phospho-flow cytometry, we had analysed MAPK/ERK and JAK/STAT signalling networks in peripheral blood mononuclear cells (PBMCs) and observed altered signalling profiles in pSS patients upon Interferon (IFN) and Toll-like receptor (TLR) stimulation.
We will validate and refine our previous results using mass cytometry with PBMCs from 16 female pSS patients and 8 healthy donors. Our panel consists of 20 surface markers enabling us to differentiate various immune cell populations and includes 9 phospho-proteins to study the intracellular signalling patterns.
We will investigate the proportion of the various cell populations within PBMCs. We will also look for any significant differences in the phosphorylation profiles between patients and healthy donors upon stimulation. We will try to stratify the patients depending on their phosphorylation status and see if there is any correlation between the phospho-epitopes and clinical parameters like EGM, presence or absence of autoantibodies and IFN score.
This will contribute to a better stratification of patients in order to improve diagnostics and treatment modalities for this heterogeneous patient group

P0866

Evaluation of IgM autoantibody in autoimmune hemolytic anemia

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Autoimmune hemolytic anemia (AIHA) is defined as the increased destruction of red blood cells (RBCs) by the production of anti-RBC autoantibodies. AIHA is commonly caused by IgG classes of autoantibodies. Although IgM autoantibodies are induced in AIHA, the role of IgM autoantibody

DOI: 10.1002/eji.201970400

against RBCs is not well known. In this study, we evaluated the role of IgM autoantibodies in AIHA by using CD40 knock-out (CD40KO) mice.
We used a well-established mouse model of AIHA induced by repeated alloimmunization with rat RBCs to develop autoantibodies against mouse RBCs. To evaluate the presence of anti-RBC autoantibodies and hemolysis in AIHA model mice, levels of RBC-bound IgG or IgM antibodies in the periphery, count of reticulocytes, and transfusion assay were assessed. Rosette formation was also assessed to evaluate the presence of RBC-bound B cells.
The amount of RBC-bound IgG was increased in WT mice, whereas either RBC-bound IgG or IgM were not detected in CD40KO mice. However, the number of reticulocytes and the rosette-forming B cells were increased in CD40KO mice. Furthermore, increased destruction of RBCs was observed in CD40KO mice. These results suggested that IgM autoantibody might have equivalent efficacy in developing AIHA compared to IgG autoantibody.

P0867

Mono-allelic breakdown of central tolerance

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Tolerance to self-antigens is a complex process beginning with the education of developing thymocytes by rare medullary thymic epithelial cells (mTEC) in the thymus. During this process, mTECs express and display ~90% of the body's coding proteome in order to eliminate autoreactive thymocytes that could react with self-antigens and cause autoimmunity. Crucial to this process is the Autoimmune Regulator (AIRE) protein, as it is responsible for expression of hundreds of tissue-restricted antigens in mature mTECs. Indeed, dysfunction of the AIRE gene in humans results in a multi-organ autoimmune syndrome, Autoimmune Polyendocrine Syndrome type 1 (APS-1). Historically, APS-1 has been shown to follow an autosomal recessive mode of inheritance, however, our recent data demonstrate that even mono-allelic mutations in *AIRE* can underlie organ-specific autoimmunity, characterized with later onsets and milder phenotypes than classical APS-1, yet with significantly higher prevalence. To better characterize the breakdown of self-tolerance by dominant-negative AIRE mutations, we have created several mouse models carrying missense or nonsense mutations within Aire's PHD1 domain. Interestingly, $Aire^{+/C313Y}$ heterozygous mice phenocopied traits of $Aire^{-/-}$ mice, such as wasting disease coupled with development of organ-specific autoimmunity, increased frequencies of mature mTECs, decreased frequencies of thymic Tregs and profound reduction in the expression of Aire-target genes in mTECs. These perturbations were not found in $Aire^{+/V303M}$ or $Aire^{+/C313X}$ heterozygous mice, highlighting the importance of the mutation context in autoimmunity development. These findings clearly validate that mono-allelic mutations in Aire can give rise to devastating autoimmunity, and prompt further reevaluation of non-classical APS patients.

DOI: 10.1002/eji.201970400

P0868

Autoantibody single B cell sorting from patient with membranous nephropathy and associated clinical applications

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Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults and one-third of patients develop end-stage renal disease (ESRD). The resent report assumes circulating autoantibodies against the podocyte surface antigens phospholipase A2 receptor (PLA2R) may play an important role in the development of membranous nephropathy (MN). But the role of monoclonal PLA2R antibody causes MN was still unclear. In this study, we quantify PLA2R antibody titer by ELISA and analyze genotyped DQ alleles in our patients. Then we produce PLA2R protein as bait for monoclonal PLA2R antibody from MN patients. Then we use the method of single B cell expression cloning, to separate the specific B lymphocyte which producing these autoantibodies. Therefore, we will study about antibody-antigen interaction, immune cell-typing, complement dysregulation, gene predominance and polymorphism. Connecting to clinical response, complication and prognosis, the study will clarify the mechanism of membranous nephropathy.

P0869

CD5L is a modulator of Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is one of the most prevalent arthritic diseases in the EU, accounting for over 2.9 million diagnosed cases. Due to its prevalence and treatment costs, RA represents a significant economic burden worldwide. This chronic autoimmune and inflammatory disease is characterized by synovial fibroblast hyperplasia and bone and cartilage erosion. A variety of determinants contribute for its pathogenesis, including T-cell and B-cell dependent pathways. However, how this inflammation is initiated, propagated and maintained remains controversial. Unbiased proteomic studies identified the circulating scavenger receptor CD5L as a highly enriched component of serum and synovial tissues of arthritic patients (1). We analyzed the presence of CD5L in samples from RA patients covering different stages of the disease, and observed increased CD5L levels concomitant with the severity of the disease and a direct correlation with the Sharp RTG Score or IL-6 serum levels. Conversely, COMP serum levels, described to decrease in the advanced stages of the disease, are inversely proportional to CD5L concentrations. These correlations, however, did not clarify whether CD5L is an immune component that helps resolving the condition or a factor that actually aggravates the disease. To clarify this aspect, we experimentally induced arthritis in *Cd5l* knockout mice. These animals showed a higher incidence of RA, higher severity and a much lower recovery rate when compared with WT mice, implying that CD5L is not a promoter of the disease, but rather a fundamental protective molecule against inflammation.

References:

1 - Balakrishnan L, et al. (2014) *Clin. Proteom.* 11:1

DOI: 10.1002/eji.201970400

P0870

Characterization of anti-interferon-γ autoantibodies from patients with mycobacterial disease in the monoclonal level

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Neutralizing anti-interferon-γ (IFN-γ) autoantibodies are an emerging etiology to patients suffered from the severe mycobacterial infection and other intracellular infections. Thus far, the molecular properties of these pathogenic autoantibodies are not well-elucidated. Therefore, we aimed to clone these autoantibodies through single human B cell capture method from patients and characterize them in the monoclonal level. We had successfully generated 19 mAbs; surprisingly, they exert extreme binding affinity with nanomolar range of KD from 10^{-9} to 10^{-11} M. Interestingly, twelve of them have the preference of V_H/V_L paired usage with diverse degree of somatic mutation. Further, the variant mAbs of unmutated common ancestor (UCA) still had higher binding ability to IFN-γ unexpectedly. Furthermore, anti-IFN-γ mAbs identified three non-overlapping regions.Particularly, we found out that neutralization is attributed to binding affinity regardless of binding sites. In advance, Site I mAb interfered with IFN-γand its receptor. Site II mAbs provide neutralization through higher binding affinity rather than interfering IFN-γ-receptor interaction. Beyond the neutralization, site III mAbs even lead to antibody-dependent cell-mediated cytotoxicity.These findings provide insights into complex mechanism lead to dysfunction of IFN-γ and acquired immunodeficiency underneath mycobacterial infection.

P0871

Characterization of anti-cytokine autoantibodies in human diseases

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The impact of anti-cytokine autoantibodies on human immunity is emerging in recent years, especially in susceptibility to infection. However, the function of these autoantibodies in human diseases and their mode of action in modulating our immune system remain unclear. Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are characterized by dysregulation of the immune system and production of autoantibodies against self-antigens, including cytokines. Taking advantage of the relatively high prevalence of anti-cytokine autoantibodies in these autoimmune diseases and in patients with severe infection without immunocompromised comorbidity, we aim to decipher the role of anti-cytokine autoantibody in human diseases. Here, we established a multiplex beads-based platform which can quickly and simultaneously detect the presence of multiple anti-cytokine autoantibodies in a small amount of patient's plasma. To rule out the non-specific binding signals, we further confirmed the results by ELISA and Western blot analysis. Our current results show two patients with anti-IL-1β autoantibody, a patient with anti-TNF-α autoantibody, a patient with anti-IL-6 autoantibody, a patient with anti-IFN-γ autoantibody and other positive cases. After that, we want to understand the biological function of these anti-cytokine autoantibodies in patients' plasma. We will evaluate the binding and neutralization ability of antibodies by in vitro and ex vivo functional assays.

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DOI: 10.1002/eji.201970400

Furthermore, by comparing the clinical manifestation with biological properties of these anti-cytokine autoantibodies, it will help us to better understand the mechanism of anti-cytokine autoantibodies in human diseases.

P0872

Diminished levels of soluble receptors of interleukin IL-23 and IL-17A (sIL-23R/sIL-17RA) in Patients with rheumatoid arthritis who present periodontitis

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Introduction: Rheumatoid arthritis (RA) and periodontitis (P) are chronic inflammatory diseases characterized by joint and alveolar erosion respectively. IL-23 and IL-17A have an important role in immunopathogenesis and the soluble receptors sIL-23R and sIL-17RA can inhibit this cytokine. In this regard there are reports with a discrepancy in IL-23 and IL-17 levels in RA and P. However, this IL-23/IL-17A axis has not been evaluated in patients with rheumatoid arthritis who present periodontitis (RAP). The aim of this study was to evaluate this axis in plasma of patients with RAP.
Material and methods: We included healthy subjects (HS), patients with P, RA and RAP. Of all the study subjects, peripheral blood was obtained by venipuncture; the plasma was isolated and stored at -80 ° C until quantification by ELISA of IL-23, IL-17A, sIL-23R and sIL-17RA. The results were analyzed by non-parametric test with SPSSv.25 software.
Results: The IL-23 in plasma did not present a significant difference between the study groups. IL-17A are higher in HS compared with P and RAP, likewise, we found significant differences between RA and RAP. On the other hand, only we observed a significant difference in sIL-23R between HS and P. Finally, sIL-17RA is significantly elevated in HS compared to the other study groups.
Conclusion: The sIL-23R and sIL-17RA are diminished in P, RA and RAP, it is possible that these receptors are not enough to capture the IL-23 and IL-17A and these cytokines can activate the Th17 cells or fibroblasts and indirectly stimulate the bone erosion.

P0873

Accumulation of autoreactive antibody-producing cells in the thymus of lupus-prone BWF1 mice

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the activation of autoreactive T and B cells, autoantibody production and immune complex deposition in several organs. Previous evidence showed an abnormal accumulation of B cells in the thymus of murine BWF1 diseased mice, a murine model of SLE. The role of this population in the progression of the autoimmune disease remains largely undefined. Using the BWF1 mice we observed that not only

DOI: 10.1002/eji.201970400

there is an increase of B cells but also plasma cells in diseased mice compared to healthy age-matched control mice. These thymic B cells are proliferating in situ and cluster in structures resembling ectopic germinal centers. Besides, a fraction of antibody-secreting cells detected in the thymus of diseased-BWF1 mice secretes anti-dsDNA autoantibodies.
On the other hand, thymic B cells from diseased-BWF1 mice can induce the differentiation of thymocytes to follicular helper T cells (T_{FH}). These data suggest that the accumulation of B cells in the thymus of BWF1 mice results in the expansion of follicular helper T cells in this organ. T_{FH} cells may, in turn, activate and differentiate B cells into autoreactive plasma cells. Thus, our data suggest that the thymus constitutes a niche that supports the maintenance of the humoral response in the development of murine SLE.

P0874

New immunoregulatory role for type-III interferon (IFN-III) in inflammatory bowel diseases (IBD)

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Inflammatory bowel disease (IBD) is chronic inflammation of the digestive tract. Currently, IBD affects 10 million people worldwide, and there is no known definitive cause or cure for IBD. Long term usage of conventional treatments such as anti-inflammatory and immunosuppressants could result in an increased chance of infection as the patient's immune system is weakened, further affecting the patient's quality of life. Recently, there have been an increasing number of studies that illustrated the importance of intestinal epithelial barrier in the pathogenesis of IBD. It is of our interest to identify a new immunotherapy candidate that can both suppress the expression of pro-inflammatory cytokine and induce repair of the intestinal epithelial barrier. Type III interferons (IFN-III) was first recognized as epithelium-specific cytokines and they are typically known for their anti-viral properties. Here, we present our recent work which elucidated a new immunoregulatory role of IFN-III using an in vitro IBD model. Using multiplex assay and ELISA to analyze the supernatant of the in vitro IBD model, expression of pro-inflammatory cytokines was markedly reduced with IFN-III treatment. Furthermore, IFN-III has shown significant activity in improving epithelial permeability compared to the untreated model. Taken together, IFN-III is a promising immunotherapy candidate for the treatment of IBD.

P0875

Vitamin D-associated abnormalities of peripheral immune cells in multiple sclerosis.

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Immune cells and Vitamin D play important roles in the aetiology of Multiple Sclerosis (MS). Two genes of the metabolic Vitamin D pathway, CYP27B1 and CYP24A1, which can be expressed by activated T helper cells have been identified as MS risk genes by genome-wide association studies (GWASs). We hypothesized that MS impacted the inflammatory profile of PBMCs and Vitamin D could modify all or some of PBMC populations. We tested the subpopulation and cytokine profile of peripheral immune cells both ex vivo and in vitro. PBMCs from MS patients (41 RRMS and 4 SPMS) and 12 healthy controls were collected, PBMCs subpopulation of T helper cells/monocytes and cytokine profile were tested by Flow cytometry and Cytometric Bead Array (CBA) kit, respectively. The MS risk SNPs around Vitamin D metabolism genes were genotyped by allele-specific PCR analysis (3'-mismatch method). Data were compared using nonparametric tests (i.e., Mann-Whitney, Kruskal-

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DOI: 10.1002/eji.201970400

Wallis and Friedman test) and linear regression for adjusting multiple factors. Ex vivo we found peripheral immune cell abnormalities associated with MS. We also found MS-specific dysregulation of cytokine profiles in PBMCs after 96 hours of tissue culture. Interestingly, the Th2 proportion both ex vivo and in vitro is correlated with the expanded disability scale score of MS patients. Furthermore, we found a consistent effect of the risk allele rs2248359-C on the proportions of various T cell subsets (Th1 and Th17.1). The presence of the double risk allele rs703842-AA reduced the IL-2 production. These observations demonstrate MS and Vitamin D-associated peripheral immune cell abnormalities.

P0876

Vitamin D supplementation reduces TLR7 and TLR9 expression in B cells from systemic lupus erythematosus patients

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Background/Purpose: Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease that is characterized by the production of autoantibodies directed against nuclear antigens, leading to multiple organ pathologies and organ destruction. Studies in murine SLE models have revealed a critical role for Toll-like receptors (TLRs) in the production of autoantibodies and development of other clinical disease manifestations. Vitamin D exerts important regulatory functions on cells from the innate/adaptive immune response and it has been suggested that vitamin D deficiency has an association with SLE disease activity and some clinical manifestations.
Methods: In this study, SLE patients with hypovitaminosis D (n=20, serum levels below 30 ng/ml) were supplemented with vitamin D (50.000 IU/week) or placebo during twelve weeks. TLR7 and TLR9 expression in B cells was evaluated by flow cytometry and mRNA expression by PCR, before and after vitamin D supplementation.
Results: We observed that when SLE patients are supplemented with vitamin D reaching normal values, TLR7 and TLR9 expression in B cells significantly decreases, by flow cytometry and PCR ($p = 0.026$ and $p = 0.04$, t test), compared to placebo. In these patients after a 3 month follow up period, vitamin D levels decrease and concomitantly, TLR7 and TLR9 expression in B cells increases.
Conclusion: Chilean SLE patients supplemented with vitamin D have lower TLR7 and TLR9 expression in B lymphocytes after treatment, when compared to placebo. Further studies are required to establish the effect of vitamin D in TLR7 and TLR9 expression.

DOI: 10.1002/eji.201970400

P0877

Novel cause of monogenic lupus illuminates disease pathogenesis

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease of uncertain aetiology and a well-established genetic basis. Females are nine times more likely to be affected than males. SLE is thought to be polygenic, although an increasing number of monogenic causes are being described. To date, there is no cure for this disease and current treatments often fail to treat the most serious manifestations. Better understanding of disease pathogenesis is critical for identification of more effective therapies. We have performed whole genome sequencing of probands with severe childhood-onset disease and have identified a novel cause of monogenic SLE. After identifying a *de novo* mutation in a proband, we subsequently identified additional SLE patients with damaging variants in the same gene.
Using CRISPR/cas9 we have generated a mouse model carrying the orthologous *de novo* variant. These mice develop lupus-like disease with an increased female to male bias. Disease and cellular manifestations include ANAs, thrombocytopenia, splenomegaly, increased Tfh cells, spontaneous germinal centres, activated CD4 and CD8 T cells, reduced marginal zone B cells and B1a cells, and accumulation of plasmablasts.
We have also identified an epistatic interaction between the *de novo* variant and a second rare variant present in the proband and validated this in mice: Mice carrying both human variants develop a more severe phenotype. Insights into the mechanism underpinning lupus development will be presented. These discoveries and bespoke mouse models of human lupus provide an opportunity to trial targeted therapies that may be effective in the patients.

P1413

Association of lymphopenia and infection in systemic lupus erythematosus

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Objective: To investigate the correlation between lymphopenia and infection in patients with systemic lupus erythematosus。
Method: This study enrolled 47 patients with SLE, the level of peripheral blood lymphocytes was detected by Flow cytometry ,and clinical data and laboratory examination were collected of all patients at the same time.Divided all patients into two groups by the level of peripheral blood lymphocyte count,compare the incidence of infection between the two groups.
Result: Of the 47 patients, 26 with lymphopenia,21 with normal lymphocyte level.In lymphopenia group,there were 12 patients with infection, including 6 cases of viral infection, 1 case of pulmonary infection, 1 case of urinary tract infection, 1 case of tuberculosis, and 2 cases of Brucellosis.In normal lymphocyte group,there were only 2 patients with infection.The incidence of infection was higher in lymphopenia group($P< 0.05$).Three patients in the lymphopenia group were infected with chronic B virus infection, one of which had virus replication.The rate of T-SPOT test positive was 33.3%(8/24)in

DOI: 10.1002/eji.201970400

the lymphopenia group, which was tend to higher than that in normal lymphocyte group(13%, 2/15) with no significant statistical difference(P = 0.263）.
Conclusion: Lymphopenia can lead to increased infection risk in SLE patients, especially virus infection. For SLE patients with hepatitis B virus infection, if with lymphopenia, HBV DNA replication should be detected regularly. In addition, the rate of T-SPOT test positive in SLE patients with lymphopenia increased, suggesting that there may be latent tuberculosis infection in these patients.

P1414

A case of rheumatoid arthritis complicated with finger gangrene and peripheral neuropathy

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A 43-year-old， male， "multiple joint swelling and pain for 7 years, fingertip numbness with blue and purple for 1 month". Since 2011, multiple joint swelling and pain occurred one after another. Other hospitals considered RA for diagnosis, hormone therapy was used intermittently. Since then, deformity of both hands and fingers joints was gradually found. One month ago, cyanosis, cold and pain occurred at the finger (toe) ends of the extremities, and numbness, weakness and difficulty in walking gradually appeared in both lower limbs. The skin at the end of the fingers of both hands is purple, skin temperature is low and tenderness is obvious. Methylprednisolone 80 mg QD intravenous drip, CTX0.2 mg QOD intravenous drip, low molecular weight heparin sodium 40 mg Q12h subcutaneous injection. Three days after the above treatment, the pain of the finger tip continued to worsen and began to blacken. Methylprednisolone was adjusted to 80 mg/bid and CTX 200 mg QOD was continued. After that, the static point of 80 mg QD was gradually decreased for 9 days, 60 mg QD for 7 days. After the above treatment, the patient´s fingertip pain was significantly improved and gradually separated from the analgesic drugs. The gangrene area was not enlarged, and the blue and purple around the gangrene disappeared. The level of sensory decline of both lower limbs moved down to the upper part of the ankle joint, and the sense of position was improved. There was still foot drop.

P1415

The role of erythroblast-like Ter cells in the pathogenesis of rheumatoid arthritis

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Objective: To explore the role of Ter cells in the pathogenesis of rheumatoid arthritis (RA).
Methods: DBA/1 mice were used to prepare collage-induced arthritis (CIA) model, the most commonly used animal model for RA research. According to the time of CIA onset and joint score, CIA mice were divided into three stages: early, peak and late stage, and CIA mice at peak stage were subdivided into high score and low score group. The frequencies of Ter cells and T and B cell subsets were detected by flow cytometry accordingly.
Results: Ter cells were expanded in CIA mice, and the frequencies in the high score group were significantly lower than those in the low score group. Th1 and germinal center B cells (GCB) in the high score group mice were significantly higher than those in the low score group. Correlation analysis showed that Ter cells in peak stage CIA mice were strongly negatively correlated with $CD4^+$T, Th1, Th17, and GCB cells, while strongly positively correlated with B10 cells. In late stage CIA mice, Ter cells were decreased, with frequencies lower than the peak stage.
Conclusion: Ter cells were significantly increased in CIA mice at peak stage, and were negatively

DOI: 10.1002/eji.201970400

correlated with joint scores and pathogenic immune cells. Moreover, Ter cells were decreased in CIA mice at late stage. Ter cells might be involved in RA pathogenesis as an immunomodulatory cell, which needs to be further studied.

Key Words: CIA；RA; Ter cells; inflammation

P1416

Milk fat globule-EGF factor 8 suppresses the aberrant immune response of systemic lupus erythematosus-derived neutrophils and associated tissue damage

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Abnormal features of the SLE-derived neutrophils, promoted aberrant immune response, have inspired new studies of the induction of autoimmunity and the development of organ damage in SLE. In this study, we explore the effect of MFG-E8 on the aberrant neutrophil features in pristane-induced lupus. SLE patients and mice with pristane-induced lupus develop autoantibodies associated with MFG-E8 overproduction. However, the deletion of MFG-E8 leads to uncontrolled early pulmonary and peritoneal inflammation and tissue damage in mice with pristane-induced lupus. Consistent with these findings, MFG-E8-deficient mice that are exposed to pristane show enhanced neutrophil accumulation and increased neutrophil death, including apoptosis, necrosis and NETosis, as well as impaired phagocytosis of macrophages. The consequences are the expansion of diffuse pulmonary hemorrhage, increased anti-nuclear antibody, anti-dsDNA antibody and anti-neutrophil cytoplasmic antibody levels, and enhanced immune complexes deposition and neutrophil extracellular traps (NETs) formation in the lung and kidney tissues of MFG-E8-deficient mice exposed to pristane. In patients with SLE and mice with pristane-induced lupus, neutrophil accumulation is elevated, which depends on higher expression of the surface receptor CXCR2. After pretreatment with recombinant MFG-E8, the surface expression of CXCR2 on neutrophil is downregulated, and the MFG-E8 deletion increase CXCR2 expression by ~40%. These studies indicate that MFG-E8 reduces neutrophil migration and NETosis via downregulating surface CXCR2 expression in parallel with its role in the phagocytosis of apoptotic neutrophils, suggesting that MFG-E8 may serve as a therapeutic agent for attenuating the early inflammatory responses of SLE and protect patients from lupus-related damage.

P1417

Chromatin accessibility landscape in healthy and scleroderma skin nominate dendritic cells in disease pathogenesis

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DOI: 10.1002/eji.201970400

Here we surveyed the genome-wide active regulatory elements in fresh human skin cells, using Assay of Transposase Accessible Chromatin with sequencing (ATAC-seq) in longitudinal skin from healthy volunteers and affected/unaffected skin from patients with systemic sclerosis (SSc). This study represents the first chromatin mapping of small and rare skin cell types harvested directly from human skin without clonal expansion. We created high-resolution epigenetic regulomes of 8 cell types in normal and SSc skins. In addition, we performed differential analysis on cell type specific peaks and SSc associated SNP enrichment between healthy individual and SSc patients. We also systematically surveyed the ligand-receptor cross-talks between 4 major cell types in skin and predicted several novel ligand-receptor partners highly interacted at lesion site. Integration of all results indicated that dendritic cells are the most differential in terms of chromatin accessibility compared with other cell types, suggesting that malfunction of dendritic cells could also be a source of pathogenesis of SSc.

P1418

Dihydroartemisinin prevents the senescence of myeloid-derived suppressor cells from lupus mice via Nrf2/HO-1 pathway

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Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease characterized by multi-organ injury. However, whether myeloid-derived suppressor cells (MDSCs) senescence exists and participates in SLE pathogenesis remains unclear. And whether dihydroartemisinin (DHA) attenuates the symptoms of SLE via relieving MDSCs senescence remains elusive. In the present study, we measured the senescence of MDSCs in SLE using SA-β-gal staining and western blot analysis of aging-related protein P21 and P53. We identified that the MDSCs senescence promoted the SLE progress by adaptive transfer MDSCs assays. Meanwhile, we further showed DHA ameliorated the symptoms of pristane-induced lupus by histopathological detection, western blot analysis, immunofluorescence, QPCR and flow cytometry analysis. DHA reversed oxidative stress-induced MDSCs senescence by detecting SA-β-gal staining and the aging-related proteins expression of P21 and P53. Furthermore, mechanistic analysis indicated that the inhibitory effect of DHA on MDSCs senescence was blocked by ML385, the specific antagonist of Nrf2, which revealed that the effect of DHA on MDSCs senescence was dependent on the induction of Nrf2/HO-1 pathway. Of note, we revealed that DHA inhibited MDSCs senescence to ameliorate the SLE development by adaptive transfer DHA-treated MDSCs assays. In conclusion, MDSCs senescence played a vital role in the pathogenesis of SLE, and DHA attenuated the symptoms of SLE via relieving MDSCs aging involved in the induction of Nrf2/HO-1 pathway.

P1419

The level of soluble tim3/galectin9 in serum with primary immune thrombocytopenia patients and its clinical significance

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Objective: To investigate the role and significance of serum soluble Tim3 (sTim3)、Galectin9 between Th1 (IFN-γ)、Th2 (IL-4)、Th17 (IL-17)、and Treg (TGF-β) cytokines in patients with primary immune thrombocytopenia(ITP).
Method: Peripheral blood was collected from 35 newly diagnosed ITP patients and 20 healthy

DOI: 10.1002/eji.201970400

controls. The serum levels of sTim3, Galectin9 and IFN-γ, IL-4, IL-17 and TGF-β were detected by ELISA.
Result: Compared with the 20 healthy controls,the levels of serum sTim3 and Galectin9 in 35 patients with ITP elevated (*P*< 0.001); The platelet counts decreased in 35 ITP patients when compared to healthy controls(*P*< 0.001); Th1 and Th17 related cytokines expression levels were increased (*P*< 0.05), but Th2 and Treg related cytokines expression levels were decreased (*P*< 0.05); The sTim3 levels were positively correlated with both IFN-γand PLT (all *P*< 0.001), but the sTim3 levels had no correlation with IL-4, IL-17, TGF-β(all *P*>0.05); The Galectin9 levels were negatively correlated with sTim3, IFN-γ, IL-4, IL-17, TGF-β, PLT (all *P*>0.05).
Conclusion: The increased expression of sTim3 and Galectin9 in the serum of ITP patients may have a role in immune imbalance of ITP.

P1420

MiR-150 deletion accelerates STZ-induced TIDM by disordering cellular immunity

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Type 1 diabetes mellitus (T1DM) occurs as a result of a chronic and progressive autoimmune destruction of insulin-secreting β cells. The disease aetiology is not understood completely. Both antibody mediated humoral immunity and CD4+T cell mediated cellular immunity involved in the development of TIDM. MiR-150 is one of the critical microRNAs that regulate the development and functions of both B cells and T cells. However, how miR-150 is involved in the development of TIDM is still unclear now. In this study, miR-150 knock-out (150KO) and wild type normal control (WT) mice were used to induce TIDM by streptozocin (STZ). Alterations of circulating diabetes-associated autoantibodies were detected by ELISA. CD4+,CD8+T cells, B cells, CD4+Foxp3+regulatory T cells (Tregs), IFN-γ and IL-17 in CD4+ T cells were detected by flowcytometry. The present results indicated that miR-150 deletion accelerated STZ-induced TIDM without increasing the serum level of circulating diabetes-associated autoantibodies. However, miR-150 deletion resulted in the decreased ratio of Tregs as well as the increased ratio of IFN-r and IL-17 expressing CD4+T cells in the spleen of STZ-treated mice. The current findings may provide new clues for the pathogenesis of TIDM.

P1421

Mitochondrial complex I inhibition by rotenone attenuates experimental autoimmune disease in murine lupus

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Systemic lupus erythematosus (SLE) is systemic autoimmune disease involved in multiple organs illness. Recent researches suggest that mitochondrial dysfunction plays an important role in the pathogenesis of SLE. Mitochondrial dysfunction could cause excessive oxidative stress and inflammation in the pathological conditions, while a suitable suppression of injured mitochondrial activity could reverse the oxidative stress and thus attenuated the tissue damage. In the present study, we utilized rotenone, an inhibitor of mitochondrial complex I, to investigate the effect of mitochondrial complex I inhibition on lupus symptoms in the MRL/lpr mice. Rotenone with 100 ppm was added in the jelly normal diet when the MRL/lpr mice were 23 weeks, and all the experimental mice were collected when they were 33 weeks. Data showed that treatment with rotenone significantly suppressed the

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DOI: 10.1002/eji.201970400

serum anti-DNA antibody level in MRL/lpr mice. Consistently, the percentage of B220+CD69+ cells in spleen and the size of spleen were decreased in rotenone-treat group. Besides, the myocardial function of the MRL/lpr mice was detected using the serum markers lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Results showed that rotenone obviously improved the myocardial function in MRL/lpr mice. Above all, our study provided the first evidence for the protective role of mitochondrial complex I inhibitor in lupus and suggested a new potential therapeutic avenue for the lupus clinical treatment through suitable regulation of damaged mitochondrion.

P1422

Analysis of single nucleotide polymorphisms in susceptibility genes for type 1 diabetes in Zhejiang Han population

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Objective: To study the association between single nucleotide polymorphisms for three genes *PD-1*, *CTLA4* and *TCF7L2* and the susceptibility to type 1 diabetes mellitus in Zhejiang Han population.
Methods: In this case-control study, healthy individuals and type 1 diabetes patients were randomly recruited in Zhejiang Han population. SNPs rs36084323, rs2227981 and rs2227982 in *PD-1* gene were genotyped by using the standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analytical method and verified by PCR-Sequencing Based Typing (PCR-SBT). The genotypes of rs11568821, rs41386349 and rs10204525 in *PD-1*; rs5742909, rs231775 and rs3087243 in *CTLA4* and rs7903146, rs12255372 and rs290487 in *TCF7L2* were determined by PCR-SBT. By using an SPSS 21.0 software package, χ^2 test was conducted to analyze the distribution of SNPs allele and genotype frequency in case and control groups. Binary Logistic regression was used to assess the association between SNPs alleles and genotypes frequency for T1DM. The haplotype and linkage disequilibrium analyses of each gene were performed using the SHEsis.
Conclusions: 1. rs36084323, rs41386349 and rs10204525 loci in *PD-1* gene were associated with susceptibility of T1DM in Zhejiang Han population.
2. In Zhejiang Han population, rs5742909, rs231775 and rs3087243 loci in *CTLA4* gene were associated with T1DM. Haplotype CGG is a risk factor for T1DM, although haplotype TAG is a protective factor for T1DM.
3. rs290487 in *TCF7L2* gene was associated with T1DM susceptibility. Allele T and genotype TT were T1DM risk factors. Haplotype CGT may be a risk factor for T1DM.

P1423

The expression level of tristetraprolin and its gene single-nucleotide polymorphisms in rheumatoid arthritis patients

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Objective: The aim of our study was to evaluate tristetraprolin (TTP) expression and gene single-nucleotide polymorphisms (SNPs) in rheumatoid arthritis (RA) patients.
Methods: The mRNA expression levels of TTP in peripheral blood mononuclear cells (PBMCs) were examined by real-time quantitative polymerase chain reaction (RT-qPCR) from 36 RA patients and 41

DOI: 10.1002/eji.201970400

controls. Simultaneously, two SNPs (rs251864 and rs3746083) of TTP were genotyped using TaqMan SNP genotyping assays in 580 RA patients and 742 controls. Data were analyzed by SPSS 19.0 software.
Results: The mRNA expression level of TTP was decreased in PBMCs of patients with RA when compared to healthy controls ($P< 0.001$). There were significant associations of TTP mRNA expression level with anti-CCP and RF in RA patients (both $P< 0.05$). Significant associations between rs3746083 polymorphism and RA were discovered in the allele and genotype distributions, as well as the dominant model (all $P< 0.05$). Moreover, we found the allele frequency of rs3746083 was significantly associated with RF phenotype in the RA patients ($\chi2=4.690$, $P=0.030$). However, we did not detect any significant associations between rs251864 and RA susceptibility and autoantibody profiles (all $P>0.05$). There were no associations between TTP mRNA expression level and the genotypes in RA patients (all $P>0.05$).

P1424

The autoantibody to estrogen receptor GPR30 in system lupus erythematosus, a potential serological biomarker associated with disease acitivity

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of large numbers of autoantibodies. The incidence of SLE in females is much higher than that in males, indicating the important role of estrogen/estrogen receptors in SLE. Autoantibodies specific to estrogen receptor-a (ERa) have been found in SLE patients and proved to associate with disease activity. GPR30 was a newly described estrogen receptor and was proved to participate in immune regulation. Whether autoantibodies to GRP30 were also presented in SLE patients is still unknown. Anti-GPR30 antibodies and the concentration of secreted GPR30 in SLE patients and healthy volunteers were detected by ELISA. The correlations between the anti-GPR30 antibody titer and clinical parameters were analyzed. Peripheral blood mononuclear cells were stimulated with an anti-GPR30 antibody to evaluate the function of anti-GPR30 antibodies. Anti-GPR30 antibodies were present in 40.3% of the SLE patients but in only 5.7% of healthy controls. There were significant associations between the anti-GPR30 antibody titer and clinical parameters of SLE patients, such as the SLE Disease Activity Index score. The inflammatory cytokines produced by lymphocytes were significantly increased after the anti-GPR30 antibody stimulation, as well as the phosphorylation of ERK. The secreted GPR30 in SLE patients also higher than healthy control and positively correlated with anti-GPR30 antibody titer. Our data suggest the anti-GPR30 autoantibody might be a potential biomarker for SLE diagnosis and disease activity evaluation and might contribute to part of the mechanism underlying the gender differences of SLE.

P1425

GMSC-Derived exosomes alleviate collagen-induced arthritis in mice

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Rheumatoid arthritis (RA) is a chronic, progressive and inflammatory autoimmune disease which primarily affects the small arthrodial joints. The aim of this study was to test whether transplantation of gingival mesenchymal stem cells (GMSC)-derived exosomes could alleviate collagen-induced arthritis (CIA) in mice, and to explore the underlying mechanism. GMSC were isolated from human gingival connective tissue, and the cell supernatant was collected to isolate the exosomes. The exosomes

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DOI: 10.1002/eji.201970400

were characterized by transmission electron microscopy, Western blot and size distribution analysis. CIA model was induced in DBA/1J mice by immunization with bovine type II collagen. GMSC, GMSC-Derived exosomes and PBS were injected intravenously into the mice on day 21 after immunization, and the swelling degree of the hind limb joints was scored every two days. Histopathological changes, the levels of cytokines (IFN-γ, TNF-α, IL-17A and IL-10) as well as $CD4^+$ Th cell subsets, the expression of IκBα and COX-2 were detected. The results showed that in vivo GMSC and GMSC-Derived exosomes attenuated the degree of joint swelling of mice and the development of joint histopathology. Moreover, it downregulated the levels of pro-inflammatory cytokines and upregulated anti-inflammatory factors. Importantly, GMSC and GMSC-Derived exosomes significantly reduced the percentages of Th1 and Th17, increased the percentages of Tregs. In conclusion, we demonstrated that GMSC-Derived exosomes has the same effect with GMSC on alleviating CIA and its mechanism may be related to the inhibition of NF-κB signaling pathway and the promotion of Treg. This study provides a new cell-free approach to the treatment of RA.

P1426

PADI4 promotes the polarization of M1 and hypoxia is associated with M2 macrophage activation in rheumatoid arthritis

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Objective: Regions of low oxygen tension (hypoxia) is a common feature of Rheumatoid arthritis (RA), which induce peptidyl arginine deiminase 4 (PADI4) overexpression in fibroblast-like synoviocytes (FLSs) and macrophages of RA. The imbalance between classically activated macrophage(M1) and alternative activated macrophages(M2) is proposed to participate in pathology of RA. The present study aimed to investigate the effect of hypoxia and PADI4 on the polarization of macrophages.
Methods: The distribution of M1- and M2-type macrophages in synovial tissues from RA and osteoarthritis (OA) patients was examined by immunohistochemical and double immunofluorescence analysis. THP-1 were induced into macrophages under normoxic (21% oxygen) or hypoxic (3% oxygen) concentrations. M1(marked by CD197) and M2(marked by CD206) detected by Immunofluorescence and quantitative real-time PCR, transfection of AdPADI4 and PADI4 inhibitor was conducted to manipulate the expression of PADI4.Protein expression of HIF-1α and HIF-2α was detected by Western blot.
Results: M1 and M2 were both abundant in RA synovial tissues whose M1/M2 ratio was 1.633 ± 0.1443. Hypoxia enhance the gene and protein expression of M1 and M2 markers. HIF-1α was expressed in both phenotypes and HIF-2α mainly expressed in M2 phenotype. M1-type genes expression were enhanced when PADI4 overexpression and decreased after using PADI4 inhibitor, while M2-type genes expression has no change
Conclusion: PADI4 and hypoxia are responsible for the imbalance of M1 and M2 and can be the target to improve the inflammatory microenvironment

DOI: 10.1002/eji.201970400

P1427

Headache of patients with systemic lupus erythematosus caused by intracisternal deposited lupus IgG through meningitis

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by high levels of autoantibodies and multi-organ tissue damage. Although neuropsychiatric symptoms are frequent in SLE patients (NPSLE), its pathogenesis remains unclear. In this study, we investigated pathogenesis of neuropsychiatric symptoms in SLE patients.
Methods: We analyzed the medical records of 1131 patients with SLE and investigated pathogenesis of NPSLE in animal experiments.
Findings: We found that headache is the most common clinical manifestation in patients with NPSLE. Meningitis is spontaneously developed and IgG deposits in meningeal membrane in lupus-prone mice. Meningitis was established in normal mice by intracisternal injection of lupus serum. This meningitis depends on the dose of IgG, but is not associated with the kind of autoantibody and disease activity. IgG deposition, monocytes/macrophages and TNF, complement and FcgRI are required for development of meningitis induced by lupus serum. Blockade of IgG signaling inhibits meningitis induced by lupus serum.
Conclusion: The deposited IgG in meningeal membrane exerts an important role in the pathogenesis of headache in patient with NPSLE. This finding will promote the development of an effective therapeutic strategy for NPSLE patients.

P1428

Monocytes/macrophages not keratinocytes and their products play an important role in skin inflammation induced by skin deposited lupus IgG

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Background: Skin injury is second common in patients with systemic lupus erythematosus (SLE), but pathogenesis of skin injury remains unclear. Based on skin deposition of IgG in SLE, the role of skin deposited IgG in the pathogenesis of skin injury in SLE was studied.
Methods: We established skin deposited lupus IgG by intradermal injection of lupus IgG in mice and investigated the role of skin deposited lupus IgG in pathogenesis of skin injury in SLE.
Findings: We found that skin deposited lupus IgG made by intradermal injection of lupus serum IgG induced skin inflammation, which appeared at 3 h and peaked at 3 d after intradermal injection. This skin inflammation was related to the dose of injected IgG but not to systemic disease activity. This skin inflammation was significantly decreased in mice depleted of monocytes but not lymphocytes. This skin inflammation was markedly decreased in mice with TNF-a deficiency. Lupus IgG promoted monocyte differentiation into dendritic cells (DCs) and enhanced the expression of TNF-α. TNF-α played a major role in the proliferation of keratinocytes.
Conclusions: The results indicate that the deposition of IgG in skin and monocytes/macrophages exert an important role in the pathogenesis of lupus skin injury. IgG signaling pathway is a therapeutic target in skin injury of SLE.

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DOI: 10.1002/eji.201970400

P1429

Association of serum KL-6 with systemic sclerosis associated interstitial lung disease: a meta-analysis

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by multiple-organ involvement among which Scleroderma-associated interstitial lung disease (SSc-ILD) occurs up to 70-90%. Studies have shown that serum KL-6 may be a serological indicator of SSc-ILD.
Methods: To explore the association of KL-6 in SSc-ILD, the related literatures were searched in Pubmed and Embase. The relationship between KL-6 expression and SSc-ILD was analyzed by using the standard mean difference of 95% confidence interval (CI) by random effects model.
Results: A total of nine studies, 429 cases and 198 controls were included in the meta-analysis. The analysis shows that KL-6 was elevated in patients with systemic sclerosis and was significantly associated with interstitial lung disease as following: KL-6 for total SSc and HC (MD = 1.17, 95% CI [0.75, 1.58], *P* = 0.01), KL-6 for SSc-ILD and HC (MD = 2.03, 95% CI [1.33, 2.73], *P* = 0.01), KL-6 for SSc without ILD and HC(MD = 0.79, 95% CI [0.48, 1.10], *P* = 0.01) , KL-6 for SSc-ILD and SSc without ILD(MD = 1.94, 95% CI [1.08, 2.79], *P* = 0.01).
Conclusion: KL-6 has significantly elevated levels of expression in total SSc, especially in SSc-ILD. A cut-off of KL-6 can be set up for distinguish patients with ILD and those without ILD.
Keywords: KL-6; Systemic sclerosis; interstitial lung disease; Meta-analysis.

P1430

Regulatory T cells levels in systemic lupus erythematosus patients: a meta-analysis

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Objective: The contribution of regulatory T cells (Tregs) to systemic lupus erythematosus (SLE) pathogenesis remains a matter of debate. The objective of this study is to quantify the association between peripheral blood Tregs and disease status in SLE patients.
Method: EMBASE and PubMed databases were searched using “systemic lupus erythematosus” and “regulatory T cells” as relevant key terms. A meta-analysis of studies that examined the proportion of Tregs among peripheral blood mononuclear cells (PBMCs) and $CD4^+$T cells was performed using Stata software. Subgroup analysis was performed based on ethnic groups and Treg definition markers.
Results: The Treg/PBMC and Treg/$CD4^+$T cell ratios were significantly lower in SLE patients than in healthy controls (HCs), whereas patients with active and inactive SLE showed no difference in these indicators. A subgroup analysis indicated that Asian SLE patients had a substantially lower proportion of Tregs/PBMCs than HCs, but this difference was not seen for Caucasian and Latin American SLE patients. Patients defined by $CD4^+CD25^+Foxp3^+$, $CD4^+CD25^+$ and $CD4^+Foxp3^+$ had a much lower Treg/PBMC ratio compared to HCs. Ethnic groups and choice of Treg definition markers had no influence on the proportion of Tregs/$CD4^+$T cells.
Conclusion: The proportion of Tregs among both PBMCs and $CD4^+$T cells was significantly decreased in SLE patients. Ethnic group and Treg definition markers may influence the proportion of Tregs among PBMCs. Further study of the correlation between SLE disease activity and the

DOI: 10.1002/eji.201970400

proportion of Tregs in peripheral blood is needed to determine the physiological role of this association.

P1431

ACPAs activate NLRP3 inflammasome to facilitate IL-1β production in rheumatoid arthritis

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Objective: Anti-citrullinated protein antibodies (ACPAs), informative rheumatoid arthritis (RA) biomarkers, play crucial roles in RA pathogenesis whose underlying mechanism merits investigation.
Methods: PBMC-derived macrophages and THP-1 cells were used in the study. Immunofluorescence staining, Co-IP and SPR were used to identify the co-location and interaction of CD147, ACPA and integrin β1. Western blot was used to investigate underlying signaling pathway in the inflammation.
Results: CD147 and ACPAs co-localized on the cell surface whose interaction were further verified by Co-IP and SPR experiments. Co-IP experiments demonstrated that the ACPA enhanced interactions between CD147 and integrin β1, promoted Akt signaling pathway, and led to the downstream NF-κB signaling pathway activation. Such reaction give rise to the nuclear translocation of transcription factor p65, promoting the transcription of NLRP3 molecule and of pro-IL-1β (the prime process), and launching ATP-mediated NLRP3 inflammasome activation. The interaction between CD147 and integrin β1 could be blocked by GRGDS which would cause a decrease in Akt signaling pathway, and reduce NLRP3 inflammasome activation and IL-1β production.
Conclusion: These results indicated that ACPA promotes the interaction of CD147 with integrin β1 to enhance the Akt/NF-κB signaling pathway in prime and activation of NLRP3 inflamasome.

P1432

Patients with Takayasu´s arteritis showed inactive by NIH standards but ^{18}F-FDG-PET/CT still active after 6 months with glucocorticoid plus cyclophosphamide

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Objective: The commonly adopted method of defning active disease in Takayasu's arteritis (TAK) is the defnition used by the US National Institutes of Health (NIH). ^{18}FDG-PET/CT appears to be a promising technique for the diagnosis and assessment of disease activity in patients of TAK. **To assess disease activity of TAK patients before and after treatment by ^{18}F-FDG-PET/CT and NIH criteria.**
Methods: All 15 patients fulflled the clinical criteria according to the American College of Rheumatology criteria and treat with glucocorticoid and cyclophosphamide . Two nuclear physicians visually assessed the degree of ^{18}F-FDG uptake for 6months. ^{18}F-FDG-PET/CT and the inflammatory vascular lesion were evaluated by standardized uptake value (SUV) of ^{18}F-FDG accumulation were interpreted as active vasculitic lesions.
Results: 15 patients were in the inactive stage according to the level of disease activity as clinically assessed by the NIH criteria after treatment with glucocorticoid plus cyclophosphamide for six months. But no patients was observed significant ^{18}F-FDG reduction with after treatment (SUV≤1.2). ^{18}F-FDG-PET/CT localised 18F-FDG accumulation in the inflammatory lesion in the patients with TAK who had inactive disease (n=15) assessed by the NIH criteria. ^{18}F-FDG PET/CT revealed intense ^{18}F-FDG

DOI: 10.1002/eji.201970400

accumulation (SUV max 2.84) in the vasculature of 15 patients in the inactive stage of TAK.
Conclusion: Although TAK patients were treated with glucocorticoid and cyclophosphamide for six months and met NIH standards, they were still in the state of disease activity after ^{18}F-FDG PET/CT detection. TAK patients still need more long-term effective treatment and observation.

P1433

Anti-inflammatory and immunomodulatory drug CP-25 alleviates experimental arthritis in mice by inhibiting B cells functions through regulating BAFF signaling and comparative efficacy with biological agents

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Aim: This study was to explore the regulatory effect of CP-25 on TRAF2-NF-κB signaling mediated by BAFF/ BAFF receptor in B cell of mice with collagen induced arthritis (CIA). To compare the efficacy of CP-25 with rituximab and etanercept.
Methods: CIA model was established in DBA/1 mice. CIA mice were given CP-25 for 40 days. Arthritis index (AI) and swollen joint count (SJC), spleen and joints histopathology, serum BAFF levels were analized. The percentages of B cell subsets ($CD19^+$ B cells, $CD19^+CD27^+$ B cells), BAFF receptor (BAFFR and TACI) expressions were analyzed by flow cytometry. The expressions of TRAF2, MKK3, MKK6, p-P38 and p-NF-κB65 in NF-κB signaling were analyzed by western blot.
Results: $CD19^+$ B cells, $CD19^+CD27^+$ B cells, $CD19^+BAFFR^+$, and $CD19^+TACI^+$ cells were increased in CIA mice. CP-25 suppressed AI and SJC, alleviated histopathology of spleen and joints, reduced serum BAFF levels, decreased percentages of B cell subsets, inhibited BAFFR receptor expressions, prevented the expressions of TRAF2, MKK3, MKK6, p-P38 and p-NF-κB65. Compared with Etanercept and Rituximab, CP-25 restored abnormal T cells proliferation and B cells percentages to normal level, and recovered the high expressions of moleculars in NF-κB signaling to normal level. The action intensity of Rituximab and Etanercept was stronger than CP-25. Rituximab and Etanercept inhibited above index to below normal levels.
Conclusion: In summary, our findings provided evidence that CP-25 alleviated CIA and regulated the functions of B cells through BAFF/BAFF receptor-NF-κB signaling. CP-25 would be a promising anti-inflammatory immune and soft regulatory drug.

P1434

The $CD4^+$ memory T cells' hyper-activiation and proliferation function by CD147 signal pathway in RA patients

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Rheumatoid arthritis (RA) is a disabling and common autoimmune disease, and the cause of RA is unknown. Recently, $CD4^+$ memory T cells have been placed at the center of early RA pathogenesis. **Our previous research found CD147-CD98 complex was indicated to mediate cell activation on human $CD4^+$T cells. The most important, CD147 is predominantly up-regulated on $CD4^+$ Tm cells from PBMCs of active RA patients. Anti-CD147 mAb 5A12 specifically inhibits Tm cells activation and proliferation by decreasing ZAP70, LAT and ERK phosphorylation. Knocking down CD147 or CD147 mutation on Tm cells exhibits similar inhibitory effects to 5A12,**

DOI: 10.1002/eji.201970400

compared with wild type CD147. On the basis of these researches, we propose to identify the role of CD147 signal pathway on the $CD4^+$ Tm cells, from RA patients and health control. The followed experiments we planned to do include CD147 on $CD4^+$ Tm cells the activation; the effects of down-regulation of CD147 complex to these functions using RNAi method; the effect of monoclonal antibody against CD147 on $CD4^+$ Tm cells function and mechanism of the antagonistic effect by using Laser scanning confocal microscope, Luminex, western and FCM, and the *in vivo* effect by $CD147^{T\text{-}KO}$ conditionally knocked out mice etc. **These studies go deep into previous research that CD147 affects functions of RA $CD4^+$ Tm cells, and further research will help elucidate the pathogenesis of RA which is expected to find potential targets on T cells to explore new treatment strategy in RA.**

P1435

Circulating angiogenic T cells in patients with systemic lupus erythematosus and rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), especially with lupus nephritis (LN), are chronic inflammatory diseases that associated with accelerated atherosclerosis. Since the recently identified angiogenic T cells (Tang) are involved in endothelial repair coupled with endothelial progenitor cells (EPC), **this study investigated the circulating Tang cells in SLE and RA patients, and their potential correlations with disease features**.
Methods: Samples were obtained from patients with SLE, RA and health controls (HC). Circulating Tang cells (CD3+CD31+CXCR4+ T cells) and EPC were assessed by flow cytometry. The plasma cytokine levels were assessed by enzyme-linked immunosorbent assay.
Results: The percentage of circulating Tang cells in RA and LN patients was significantly increased as compared to the non-LN patients and HC, and they were positively correlated with the level of EPC and proangiogenic cytokine levels. Additionally, the percentages of Tang cells in autoantibody positivity SLE and RA patients were significantly increased as compared to their negative counterparts and HC. And circulating Tang cell percentages were positively correlated with the extent of proteinuria in LN patients.
Conclusions: The increased levels of circulating Tang cells in LN and RA patients might play a role in the balance of endothelium dysfunction in these patients. Autoantibody positivity may identify a group of SLE and RA patients with increased Tang cells and **circulating Tang cells may be viewed as a potentially useful biomarker of endothelial damage and cardiovascular risk in SLE and RA**.

P1436

Frequencies circulating CD4+CD161+ and CD161+ Th17 cells correlate with disease activity in rheumatoid arthritis

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DOI: 10.1002/eji.201970400

Objectives: Rheumatoid arthritis (RA) is a common autoimmune disease that is primarily driven byeffector T cells, particularly Th17 cells, which are mainly contained within CD4+CD161+ T cells.**This study aimed to investigate the profiles of CD4+CD161+ T cells and their subsets[T-helper (Th)17, Th1, andTh17/Th1] in RA patients**.
Methods: Peripheral blood (PB)from RA patients and healthy controls (HC)was collected.The percentages of CD4+CD161+ T cells and theirsubsets, and the plasma levels of related cytokines were assessed by flow cytometry andcytometric bead array (CBA) analysis, respectively.
Results: The percentage of circulating CD4+CD161+ Tcellsand CD161+ Th17 in RA patients increasedsignificantly and was higher in patients with active disease status (DAS28 > 3.2)compared with those with low diseasestatus(DAS28≤3.2), and correlated positively with DAS28, CRP, ESR, IL-17, and IL-6 levels in RA patients. The percentage of circulating CD161+ Th1 decreased and correlated negatively with DAS28, CRP, and ESR levels in RA patients, while thepercentage of CD161+ Th17/Th1 was unchanged in RA patients and was notcorrelated with RA disease activity.
Conclusions: They support the hypothesis that Th17 cells are involved in the pathogenesis of RAand that **CD161+ Th17/CD161+ Th1-cell imbalance may contribute to the development of RA**, and suggest that **circulating CD4+CD161+ T cells, CD161+ Th17 and CD161+ Th1 cells in RA patientsreflect the degree of disease activity and maybe potential biomarkers of RA disease activity.**

P1437

Two new inflammatory markers associated with dsease activity score-28 in patients with rheumatoid arthritis: albumin to fibrinogen ratio and C-reactive protein to albumin ratio

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Objective: The albumin to fibrinogen ratio (AFR) and C-reactive protein to albumin ratio (CAR) have emerged as useful biomarkers to predict systemic inflammation. The aim here is to investigate the relation between AFR/CAR and Disease Activity Score of 28 joints (DAS-28) in rheumatoid arthritis (RA).
Methods: This retrospective study included 160 patients with RA and 159 healthy controls. We divided the RA patients into two groups according to the DAS 28-ESR score. Group 1 included 40 patients with a score of lower than 2.6 (patients in remission) and Group 2 included 120 patients with a score of 2.6 or higher (patients with active disease). The correlations between AFR, CAR and the disease activity were analyzed.
Results: For RA patients, the AFR was lower than those in the control group ($P < 0.001$). Patients in group 2 had higher CAR than those in group 1 ($P < 0.001$). The AFR was lower in group 2 than that in group 1. A positively correlation was observed between DAS 28-ESR score and CAR ($r = 0.645$, $P < 0.001$), while the correlation between DAS 28-ESR and AFR ($r = -0.836$, $P < 0.001$) was negative. AFR was related with decreased risk of RA disease activity (EXP (B) = 0.33, 95% CI (0.21-0.53), $P < 0.001$).
Conclusions: AFR and CAR are two novel inflammatory markers for monitoring disease activity in patients with RA.

DOI: 10.1002/eji.201970400

P1438

The study of immunological mechanism of the mice models of IB induced by TNBS

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Abstract: IBD, an important autoimmune disease, has currently an unclear pathogenesis. And the cytokines always play the key role in autoimmune diseases. To study the pathogenesis of IBD. Firstly, The IBD model was successfully established in the BALB/c female mice induced by TNBS/50% ethanol into intestine in according to pathological Changes observed in histological slices in the colon of mice. By Real-time PCR, we found, in the model groups established for 3 day, the expression of cytokines related to Th17(IL-21, IL-23） significantly increased, but other cytokines unsignificantly changed; In the model groups established for 6 day, the expression of Th1 cytokine (IL-2, IFN gam a, IL-12), Treg cytokine IL-10,Th17 transcription factor ROR γt increased with statistical significance in mesenteric lymph nodes; Th17 related cytokines (IL-21, IL-23, IL-17) were still higher, but had varying degrees of decline compared with model groups established for 3 days. These conclusion showed Th17 related cytokines played an important part by analyzing the change of cytokines; In the model groups established for 6 days(colonic inflammation is in the peak), the model was based on Th1 and Th17 mediated inflammatory bowel disease at the same time and suggested that Th1, Th17 play their roles respectively in the occurrence and development of IBD, and that Th17 cell played an significant role in early inflammatory.

P1439

Construction of a novel MuSK cell-based assay as a diagnostic tool for myasthenia gravis

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Background and purpose: To compare an in-house live muscle-specific kinase (MuSK) antibody cell-based assay (CBA) with a commercially available MuSK-Ab radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and fixed CBA (indirect immunofluorescence assay, IFA) using serum from patients with myasthenia gravis (MG) and other autoimmune diseases.
Methods: Sera from 624 and 251 AChR-Ab positive and negative patients,previously found to be seronegative was tested by ELISA for AChR and MuSK antibodies. and ganglioside (n = 30), autoimmune encephalitis [GABAB-positive (n = 5) and NMDAR (n = 8)], anti-aquaporin-4 (n = 23), muscle disease myositis spectrum (n = 30), and systemic lupus erythematosus (n = 23) antibodies, compared with sera from healthy control individuals (n = 20)were tested for binding to MuSK with different assays.
Results: 46 (18.3%) MuSK-Ab positive in 251 AChR-Ab negative MG serum and 4 (0.6%) MuSK-CBA Ab positive in 624 AChR-Ab positive MG serum.only 42 MuSK-Ab positive in 251 AChR-Ab negative MG with ELISA,44 with IFA.
Conclusion: Most MuSK-positive patients with generalized MG had severe bulbar dysfunction and respiratory insufficiency.CBA was a more specific and sensitive method than RIA and ELISA. Interestingly, the constructed live MuSK-CBA was more sensitive than the commercial fixed CBA (MuSK-IFA); however, CBA may exhibit interference from anti-nuclear antibodies.

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DOI: 10.1002/eji.201970400

P1440

CCL5 and related genes might be the potential diagnostic biomarkers for the therapeutic strategies of rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) is a rheumatic disease. The aim of this study was to identify gene signatures in RA and uncover their potential mechanisms.
Method: Gene expression profiles of GSE1919, GSE55235, GSE55457, and GSE77928 were downloaded from GEO database. The above four series contained 76 samples, including 44 RA patients and 32 normal controls. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed, and protein-protein interaction (PPI) network of the differentially expressed genes (DEGs) was constructed by Cytoscape software.
Results: Up-regulated DEGs were significantly enriched in biological processes, including immune response, positive regulation of immune system process and regulation of immune system process, while down-regulated DEGs were significantly enriched in biological processes, including response to oxygen-containing compound, cellular lipid metabolic process, and lipid metabolic process. KEGG pathway analysis showed the up-regulated DEGs were enriched in cytokine-cytokine receptor interaction, chemokine signaling pathway, and primary immunodeficiency. The 104 hub genes, which were significantly differently expressed between patients and normal controls in at least two datasets, were identified from the PPI network, and subnetworks revealed that these genes were involved in significant pathways, including cytokine-cytokine receptor interaction, chemokine signaling pathway, and primary immunodeficiency.
Conclusion: The study indicated that identified DEGs and hub genes promote our understanding of molecular mechanisms underlying the development of RA, such as C-C motif chemokine 5 (CCL5), might have a negative impact in the development of RA. CCL5 and its related genes might be the potential diagnostic biomarkers for the therapeutic strategies of RA.

P1441

Monocytic myeloid-derived suppressor cells are novel osteoclast precursors enhancing osteolysis in rheumatoid arthritis

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Objective: Our previous work has established a pro-inflammatory role of myeloid-derived suppressor cells (MDSCs) in autoimmune rheumatoid arthritis (RA), which is attributed to their capability in augmenting a pathogenic T helper (Th) 17 response. However, the potential involvement of these functionally plastic myeloid cells in RA-associated bone destruction is poorly understood. Here, we investigate the association of MDSCs with bone erosion in mouse models of RA as well as RA patients.

DOI: 10.1002/eji.201970400

Methods: MDSCs from arthritic mice or RA patients were cultured in the presence of M-CSF and RANK-L for osteoclasts (OCs) differenciation. The contribution of MDSCs to bone destruction in arthritic mice was examined by depletion or adoptive transfer of MDSCs. Correlations between MDSC frequency and bone erosion was analyzed in patients with RA to validate the bone destructive potential of MDSCs in clinic.
Results: MDSCs from arthritic mice and RA patients, particularly $CD11b^{+}Ly6C^{high}$ monocytic-MDSCs (M-MDSCs), could differentiate efficiently into functional multinucleated $TRAP^{+}$ OCs in the presence of M-CSF and RANK-L. Intriguingly, Th17 cells can facilitate differentiation of OCs from MDSCs by providing RANK-L. Selective depletion of M-MDSCs in arthritic mice with 5-fluorouracil not only attenuated a Th17 response, but also ameliorated bone destruction that coincided with marked reduction of OCs in the inflamed joints. Additionally, bone erosion was aggravated upon adoptive transfer of M-MDSCs in arthritic mice. Furthermore, MDSCs from RA patients also displayed pro-osteoclastogenic activity.
Conclusion: Our results reveal a unique pathogenic role of M-MDSCs in driving RA progression through their differentiation into OCs for bone destruction.

P1442

Expression and clinical significance of microRNA-155 in the plasma of patients with primary Sjögren´s syndrome

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Objective: To explore the role and clinical significance of microRNA-155(miRNA-155) in plasma from patients with primary Sjögren´s syndrome(pSS) by observing its expression level in different groups and the change of clinical indexes and experimental test indexes.
Methods: We collected 75 pSS patients（pSS group）, including 41 cases without glucocorticoid treatment (untreatment group) and 34 cases with glucocorticoid treatment (treatment group), and 40 healthy controls (health control group). We recorded disease-related clinical indicators of all pSS patients, and measured the expression of miRNA-155 in plasma. And then we performed statistical analysis by different groups.
Results: 1.The expression level of plasma miRNA-155 in pSS group were lower than that in health control group ($P< 0.01$), while that in the treatment group was higher than that in untreated group, but no statistical differences ($P>0.05$). 2. In different subgroups: The miRNA-155 level in patients with pathological grade III of labial gland subgroup was higher than that in pathological IV subgroup（$P< 0.05$). And the similar result were find in anti-SSA/SSB antibody positive subgroup and high-level ESR subgroup ($P< 0.01$ or $P< 0.05$). But plasma miRNA-155 level in fibrous pneumonia subgroup was lower than that in without fibrous pneumonia subgroup ($P< 0.05$). 3. There was no significant correlation between IgG and miRNA-155. The levels of IgA and ESR were positively correlated with miRNA-155.
Conclusion: The miRNA-155 level was lower in pSS patients, which reminds us miRNA-155 might involved in the pathogenesis of pSS, glucocorticoid immune response and the co-occurrence of fibrous pneumonia in pSS patients.

DOI: 10.1002/eji.201970400

P1443

The association between rs2476601 polymorphism in PTPN22 gene and risk of alopecia areata: a meta-analysis of case-control studies

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The single nucleotide polymorphism (SNP) rs2476601 of the protein tyrosine phosphatase, nonreceptor type 22 (PTPN22) gene has been presented to implicate in the pathogenesis of alopecia areata (AA) in a few association investigations with limited sample size and inconsistent conclusions. The aim of the current meta-analysis was to assess and synthesize the presently available data on the connection between rs2476601 and AA vulnerability. Six electronic databases including EMBASE, PubMed, Web of Science, the Cochrane Library, Wanfang data and the China National Knowledge Infrastructure database (CNKI) were systematically retrieved for relevant observational studies published previous to November 2018. Total odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were analysed to evaluate the correlation between PTPN22 polymorphism and AA. Risk of bias was estimated according to the Newcastle-Ottawa Scale (NOS). Sensitivity analyses were carried out using the RevMan 5.3 software. In general, 5 case-control studies including 1129 AA patients and 1702 healthy control individuals were obtained for this meta-analysis. The pooled results suggested that rs2476601 SNP was significantly associated with AA susceptibility under allelic model (C vs. T, OR=0.77, 95%CI 0.64-0.92, P=0.003) and recessive model (CC vs. CT+TT, OR=0.73, 95%CI 0.60-0.88, P=0.001). On the basis of the results of the current research, the rs2476601 polymorphism of PTPN22 gene is significantly correlated with AA susceptibility. The C-allele and CC-genotype carriers at this locus have a lower risk of AA.

P1444

MiR-497-5p, miR-195-5p and miR-455-3p function as tumor suppressors by targeting hTERT in melanoma A375 cells

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Objective: The aim of this study was to screen and identify the miRNAs involved in the regulation of hTERT in melanoma.
Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry were performed to detect hTERT mRNA and protein expression in 36 formalin-fixed paraffin-embedded melanoma tissue samples and 36 pigmented nevi cases. Bioinformatics analysis and custom miRNA polymerase chain reaction array were performed for predicting, screening and verifying miRNAs involved in the regulation of the hTERT gene. miRNA mimics and inhibitors were transfected into melanoma A375 cells. The relative expression of miR-497-5p, miR-195-5p, miR-455-3p and hTERT mRNA, the protein expression of hTERT were determined. Cell biological function experiments were performed. A direct target gene of the miRNAs investigated was analyzed.
Results: MiR-497-5p, miR-195-5p, miR-455-3p were significantly downregulated, while hTERT was upregulated in melanoma tissues. hTERT expression level was inversely correlated with miR-497-5p, miR-195-5p and miR-455-3p. Overexpression of miR-497-5p, miR-195-5p and miR-455-3p inhibited A375 cell proliferation, migration and invasion. Arrested the cell cycle, induced cell apoptosis and decreased hTERT expression at both mRNA and protein levels. Suppression of miR-497-5p, miR-195-5p and miR-455-3p partially reversed the inhibitory effects. hTERT was also identified as a direct target of miR-497-5p, miR-195-5p and miR-455-3p via dual luciferase reporter activity assay.
Conclusions: MiR-497-5p, miR-195-5p and miR-455-3p act as tumor suppressors by targeting

DOI: 10.1002/eji.201970400

hTERT in melanoma A375 cells. Therefore, miR-497-5p, miR-195-5p and miR-455-3p could be potential therapeutic targets for melanoma.

P1445

Aberrant hTERT promoter methylation predicts prognosis in Chinese patients with acral and mucosal melanoma

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Objective: To evaluate the methylation level of human telomerase reverse transcriptase (hTERT) promoter region CpG island (CGIs) and its prognostic impact in Chinese patients with acral and mucosal melanoma.
Methods: Fresh frozen tissues were taken from 14 patients with melanoma and 14 pigmented nevus control subjects. Bisulfite sequencing PCR (BSP) combined with TA clone sequencing was used to assess the methylation of hTERT promoter CGIs. The relative expression level of hTERT mRNA was determined by quantitative real-time polymerase chain reaction (qRT-PCR).
Results: CGIs-1 (-1392--1098 bp), CGIs-2 (-945--669 bp), and CGIs-3 (-445--48 bp) were selected. The results indicated that the methylation level of hTERT promotor CGIs in melanoma was greater than pigmented nevus (CGIs-1: 69.2±18.7% vs 46.8±20.4%, CGIs-2: 73.8±14.7% vs 55.6±16.0%, CGIs-3: 5.8±2.2% vs 2.2±1.3%, $P< 0.05$). The relative expression level of hTERT in melanoma was greater than in pigmented nevus (50.39±9.16 vs 26.10±7.25, t=7.778, $P< 0.001$). Linear regression analysis showed that the methylation level of the hTERT gene promoter of melanoma was positively correlated with the relative expression level of hTERT mRNA (R^2=0.490, F=13.478, P=0.003). Combined with the analysis of clinicopathological features, the methylation level of CGIs-2 in melanoma with lymph node metastasis was greater than in melanoma without lymph node metastasis, and the methylation level of CGIs-2 increased with TNM staging.
Conclusion: The methylation level of CGIs-2 was associated with hTERT mRNA expression, lymph node metastasis and TNM staging, suggesting that CGIs-2 hypermethylation may be used to evaluate the prognosis in Chinese patients with acral and mucosal melanoma.

P1446

Beneficial effects of gut microbiome mediated ellagic acid treatment on CNS autoimmunity

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Multiple sclerosis (MS) is a CNS autoimmunity characterized by demyelination and inflammatory infiltration with high disability rate. Gut microbiome contribute to several CNS disorders including MS, and diet is a critical determinant of the gut microbial composition. However, the interaction between the diet, gut microbiota, and immune system of MS remains unexplored. Here, we found that oral ellagic acid (EA, 25mg/kg/day), a natural polyphenolic compound rich in fruits and nuts with low bioavailability, effectively ameliorated clinical course and pathology of the MS model, experimental autoimmune encephalomyelitis (EAE). EA treatment suppressed CNS inflammatory infiltration and myelin loss, altered T cell differentiation with a reduction of IL-17 producing T cells and an increase in regulatory T cells. Further, we found that EA remodeled gut microbiome composition and metabolic pathways of EAE mice, led to an increase in Lactobacillaceae, Verrucomicrobiaceae, Planococcaceae families and a decrease in Erysipelotrichaceae, Acidaminococcaceae families. Fecal microbiome transplantation from EA-treated EAE mice to recipient EAE mice significantly alleviated the severity of

DOI: 10.1002/eji.201970400

the disease. In conclusion, EA exerts potent therapeutic effects on EAE that are at least partially mediated by the gut microbiome.

P1447

Blocking AIM2 inflammasome dependent DNA sensing pathway abrogates Pristane-induced lupus

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Accumulated evidence has shown that double-strand DNA(dsDNA) is closely involved in the pathogenesis of lupus. Absence in melanoma 2 (AIM2) inflammasome has been described to be positively correlated with lupus disease activity. In this study, we aimed to investigate the effect of AIM2-dependent DNA sensing pathway in pristane-induced lupus(PIL) mice model. PIL mice model were induced in both wild type(WT) and AIM2$^{-/-}$ mice.Twenty four weeks later cytokines secretion, kidney histopathology and AIM2 expression in kidney were tested. Our data showed that AIM2 expression was increased in glomerulus of PIL WT mice compared with healthy WT mice. And compared with WT PIL mice, AIM2$^{-/-}$ PIL mice displayed less proliferation of mesangial cells and downregulation of IgG and C3, decreased GSDMD in glomerulus, while increased endothelial cells Then we tested the cytokines in ascites in PIL AIM2$^{-/-}$ mice and PIL WT mice, and found that IL-1β expression of ascites was markedly lower in PIL AIM2$^{-/-}$ mice than that in PIL WT mice. Then human umbilical vein endothelial cells (HUVECs) were cultured, transfected with calf-thymus DNA(CT-DNA) in vitro. We found dsDNA efficiently induced the expressions of AIM2, caspase-1 and GSDMD, then pyroptosis. These results provide a new insight into the mechanism of lupus and indicate AIM2 inflammasome dependent DNA sensing pathway may serve as a new target against lupus.

P1448

Hepatocyte-derived Igκ exerts protective effect against autoimmune hepatitis in mice

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Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized by hypergammaglobulinemia, the presence of autoantibodies and inflammation within the liver. We recently reported that Ig heavy chains and light chains were expressed in sorted liver epithelial cells of μMT mice, and the sequence characteristics of hepatocyte-derived Ig is different from classical B-cell-derived Ig. And growing evidence indicates that free light chain is closely related to autoimmune diseases. However, the potential function and molecular mechanism of hepatocyte-derived Igκ in AIH is still unclear. Here, we demonstrate that AIH displayed elevated levels of Igκ on hepatocyte, and then there was the unique localization of Igκ in hepatocyte, which showed a cytoplasmic filamentous network and point shape around nucleus. Furthermore, Igκ knockdown in normal liver cell lines induced apoptosis, and decreased expression of Fas apoptotic inhibitory molecule and BCL2-like 1. More importantly, the function of Igκ were investigated in two mouse models of AIH, including Con A-induced acute hepatitis and S-100 liver antigen-induced chronic hepatitis. Compared to wild type mice (WT), hepatocyte-specific Igκ knockout mice (KO) had higher Ishak score, increased aspartate aminotransferase (AST) and alanine transaminase (ALT) levels in peripheral blood, and the

DOI: 10.1002/eji.201970400

expression of inflammatory factor MCP-1 was elevated. TUNEL staining results also showed that Igκ deficiency had increased hepatocytes apoptosis. Interestingly, loss of Igκ had no effect on leukocytes infiltration into liver tissue and expression of TNF-α in leukocytes. Together, hepatocyte-derived Igκ might inhibit hepatocyte apoptosis and exert a protective effect in autoimmune hepatitis.

P1449

Exogenous IL-9 ameliorates experimental autoimmune myasthenia gravis symptoms in rats

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Objective: To investigate the impact and mechanism of IL-9 activity of Experimental Autoimmune Myasthenia Gravis (EAMG) through in vivo and in vitro experiments.
Methods: EAMG model were induced by using the $AChR_{97-116}$ peptide of Lewis rats. Rats in the rrIL-9 treatment group were injected i.p. with 15 ng of rrIL-9 dissolved in 0.3 ml of PBS every 2 days beginning on the day of EAMG induction. Immunofluorescence method was used to detect the pathological change of neuromuscular junction (NMJ). ELISA was used to measure the levels of anti-AChR Ab in serum. Real-Time PCR was used to determine the expression level of transcription factors T - bet, Foxp3, STAT3, STAT5 and FACS was used to determine the effects of rrIL-9 on Th subsets both in vivo and in vitro.
Results: Compared with EAMG group, rrIL-9 treatment groups showed decreased clinical scores and increased body weigh and attenuated NMJ destruction; reduced the levels of anti-AChR Ab in serum; decreased the level of Th1 cells and increased the level of Treg cells and the transcription factor T-bet and Foxp3 showed the similar change; In vitro rrIL-9 treatment inhibited the differentiation of AChR-specific Th1 cells and promoted the development of Treg cells, and T-bet and Foxp3 showed the similar change; reduced STAT3 mRNA and increased STAT5 mRNA expression levels both in vivo and in vitro experiments.
Conclusion: RrIL-9 Abs influenced the balance between STAT3/STAT5 and Th1/Treg cells thereby altering EAMG disease presentation.

P1450

Mesenchymal cells secrete small molecules that regulate immune cell functions

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Intercellular communications among different cell types through soluble mediators or cognate interactions are an integral means of maintaining homeostasis in multicellular organisms. Here, we identified two novel small molecules secreted by fibroblasts that regulate the specific functions of different immune cells. The first molecule (JND1) enhances TNFα-induced gene transcription in human monocytes while the second molecule (LNY1) suppresses proliferation of mouse and human lymphocytes. JND1 specifically elevates TNFα-induced expression of cytokines and chemokines, but not other genes such as IκBα and CD44. Interestingly, it does not affect TNFα-induced gene expression in epithelial cells, indicating its cellular target is specifically expressed in monocytes. The second molecule LNY1 inhibits proliferation of T and B cells by generating reactive oxygen species (ROS). It also suppresses the metabolic switch in activated T cells. Furthermore it inhibits TCR-activated NFAT and calcium influx. Consequently, activated lymphocytes in the presence of LNY1 undergo apoptosis. Our data suggest that the interplay between immune cells and non-immune cells

DOI: 10.1002/eji.201970400

may provide therapeutic opportunities in the form of new drug targets and novel drug candidates for inflammatory diseases such as rheumatoid arthritis and IBD.

P1451

lincRNA-Cox2 regulates NLRP3 inflammasome and Th17 cells mediated neuroinflammation

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Inflammasome activation plays key roles in host defense, but also contributes to the pathogenesis of auto-inflammatory, and neurodegenerative diseases. As autophagy is connected with both the innate and adaptive immune systems, autophagic dysfunction is also closely related to inflammation, infection, and neurodegeneration. Here we identify that lincRNA-Cox2, previously known as a mediator of both the activation and repression of immune genes expression in innate immune cells, could bind NF-κB p65 and promote its nuclear translocation and transcription, modulating the expression of inflammasome sensor NLRP3 and adaptor ASC. Knockdown of lincRNA-Cox2 inhibited the inflammasome activation and prevented the lincRNA-Cox2-triggered caspase-1 activation, leading to decreased IL-1β secretion and weakened TIR-domain-containing adapter-inducing interferon-β (TRIF) cleavage, thereby enhancing TRIF-mediated autophagy. Elucidation of the link between lincRNA-Cox2 and the inflammasome-autophagy crosstalk in macrophage and microglia reveals a role for lncRNAs in activation of NLRP3 inflammasome and autophagy, and provides new opportunities for therapeutic intervention in neuroinflammation-dependent diseases. Furthermore, our study found that knockdown of lincRNA-Cox2 inhibited the differentiation of Th17 cells, which may alleviate the development of EAE.

P1452

MHC class II risk alleles and amino acid residues in primary membranous nephropathy

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DOI: 10.1002/eji.201970400

Epitopes of phospholipase A2 receptor (PLA2R), the target antigen in primary membranous nephropathy (pMN), must be presented by the HLA-encoded MHC class II molecules to stimulate autoantibody production. A genome-wide association study identified risk alleles at HLA and PLA2R loci, with the top variant rs2187668 within HLA-DQA1 showing a risk effect greater than that of the top variant rs4664308 within PLA2R1. How the HLA risk alleles affect epitope presentation by MHC class II molecules in pMN is unknown. Here, we genotyped 261 patients with pMN and 599 healthy controls at the HLA-DRB1, HLA-DQA1, HLA-DQB1, and HLA-DPB1 loci with four-digit resolution and extracted the encoded amino acid sequences from the IMGT/HLA database. We predicted T cell epitopes of PLA2R and constructed MHC- DR molecule-PLA2R peptide-T cell receptor structures using Modeler. We identified DRB1*1501 (OR, 4.65; 95% CI, 3.39 to 6.41; P,0.001) and DRB1*0301 (OR, 3.96; 95% CI, 2.61 to 6.05; P,0.001) as independent risk alleles for pMN and associated with circulating anti-PLA2R antibodies. Strong gene-gene interaction was noted between rs4664308(AA) and HLA-DRB1*1501/ DRB1*0301. Amino acid positions 13 (P,0.001) and 71 (P,0.001) in the MHC-DRb1 chain independently associated with pMN. Structural models showed arginine13 and alanine71, encoded by DRB1*1501, and lysine71, encoded by DRB1*0301, facilitate interactions with T cell epitopes of PLA2R. In conclusion, we identified two risk alleles of HLA class II genes and three amino acid residues on positions 13 and 71 of the MHC-DRb1 chain that may confer susceptibility to pMN by presenting T cell epitopes on PLA2R.

P1453

Targeting HMGB1 by ethyl pyruvate ameliorates systemic lupus erythematosus and reverses the senescent phenotype of bone marrow-mesenchymal stem cells

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease involving multiple organs and systems. Mesenchymal stem cells (MSCs) from SLE patients have demonstrated defects such as impaired growth, senescence phenotype and immunomodulatory functions. Some studies have suggested the close connection between inflammation microenvironment and cellular senescence. In the current study, we detected cytokines levels in bone marrow supernatant by the quantitative proteomics analysis, and found HMGB1 was remarkably increased in bone marrow from SLE patients. Senescence associated-β-galactosidase (SA-β-gal) staining, F-actin staining and flow cytometry were used to detect the senescence of cells. After stimulation of HMGB1 in normal MSCs, the ratio of SA-β-gal positive in BM-MSCs was increased, the organization of cytoskeleton was disordered, and TLR4-NF-κB signaling was activated. Finally, Ethyl pyruvate (EP) (40 mg/kg and 100 mg/kg, three times a week), a high security HMGB1 inhibitor, was injected intraperitoneally to treat MRL/lpr mice for 8 weeks. We demonstrated that EP alleviated the clinical aspects of lupus nephritis and prolonged survival of MRL/lpr mice. In the meantime, EP reversed the senescent phenotype of BM-MSCs from MRL/lpr mice. HMGB1 could be a promising target in SLE patients, and might be one of the reasons of recurrence after MSCs transplantation.

DOI: 10.1002/eji.201970400

P1454

G-MDSC-derived exosomes attenuate collagen-induced arthritis by impairing Th1 and Th17 cell responses

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The therapeutic effect of myeloid-derived suppressor cells (MDSCs) in mice with collagen-induced arthritis (CIA) remains controversial. We analyzed the role of exosomes derived from granulocytic MDSCs (G-MDSCs) in CIA and explored the potential mechanism underlying the immunosuppressive effect. In the CIA mice, G-MDSC-derived exosomes (G-exo) efficiently reduced the mean arthritis index, leukocyte infiltration and joint destruction. G-exo decreased the percentages of Th1 and Th17 cells both in vivo and in vitro. MiR-29a-3p and miR-93-5p contained in G-exo were verified to inhibit Th1 and Th17 cell differentiation by targeting T-bet and STAT3, respectively. Notably, the delivery of exogenous miR-29a-3p and miR-93-5p enhanced the effect of bone marrow-derived G-exo on attenuating arthritis progression in CIA mice. Exosomes derived from human MDSCs, which overexpressed miR-29a-3p and miR-93-5p, suppressed Th1 and Th17 cell differentiation in vitro. These data shown that G-exo alleviated CIA by suppressing Th1 and Th17 cell responses. Mechanistically, miR-29a-3p and miR-93-5p were verified to inhibit the differentiation of Th1 and Th17 cells, respectively. Our findings demonstrated the therapeutic potential of G-MDSC-derived exosomal miRNAs in autoimmune arthritis.

P1455

Loss of ubiquitin conjugating enzyme E2 (UBC9) in macrophages exacerbates multiple low-dose streptozotocin-induced diabetes by attenuating M2 macrophage polarization

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Type 1 diabetes (T1D) is characterized by the selective autoimmune destruction of islet β cells, and macrophages play a significant role in this process. Small ubiquitin-like modification (SUMOylation) is an important posttranslational modification involved in the development and pathogenesis of T1D, but its function in macrophages remains unexplored. We presently developed and used macrophage-specific ubiquitin conjugating enzyme E2 (*Ubc9*) knockout (KO) mice ($Ubc9^{-/-}$ mice) to address the impact of SUMOylation on macrophage function in a T1D model. We observed that blocking UBC9 in macrophages exacerbates multiple-low dose streptozotocin (MLD-STZ)-induced diabetes. Specifically, after STZ treatment, blood glucose levels were consistently elevated in the $Ubc9^{-/-}$ mice. The $Ubc9^{-/-}$ mice exhibited a higher diabetes onset rate than $Ubc9^{fl/fl}$ control mice (85% vs. 55%, respectively). Cellular studies revealed that the loss of *Ubc9* impaired bone marrow-derived macrophages (BMDMs) energy metabolism and attenuated M2 macrophage polarization, subsequently enhancing T cells activation capability. Pancreas-resident macrophages, rather than migrant macrophages, played a predominant role in MLD-STZ-induced diabetes. Mechanistically, UBC9-mediated SUMOylation of interferon regulator factor 4 (IRF4) enhanced the nuclear translocation, transcriptional activity and protein stability of IRF4, thereby promoting macrophage alternative activation. The present findings indicate that SUMOylation is a key epigenetic regulator of macrophages, affecting the function of key transcription factors such as IRF4, and modulating macrophage contribution to inflammatory and autoimmune diseases.

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DOI: 10.1002/eji.201970400

P1456

The activation of B cell NLRP3 inflammasome correlated with the disease activity in systemic lupus erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease with significant morbidity and mortality. B cell abnormality is an important characteristic of the disease. Evidences revealed that NLRP3 inflammasome play important roles in the development of SLE. Studies found NLRP3 inflammasome can be assembled in B cells, however, it is unclear that whether B cell intrinsic NLRP3 inflammasome involved in the pathogenesis of SLE.
Methods: The frequency of circulating B cells with activated NLRP3 inflammasome were examined by flow cytometry in SLE patients and healthy controls. The correlation between the level of NLRP3 inflammasome activation in B cells with the disease activity and B cells function were evaluated in SLE patients.
Results: We found the level of activated NLRP3 inflammasome was higher in B cells of SLE patients than control group and positively correlated with SLEDAI score and serum anti-dsDNA antibody level. Inhibited activation of NLRP3 inflammasome were noticed in B cells of SLE patient with successful treatments. B cells with activated NLRP3 inflammasome exhibited with increased activation and proliferation level. In addition, the titer of plasma IL-6 and IFN-α positively correlated with B cell intrinsic NLRP3 inflammasome activation in SLE patients.
Conclusion: The activation of NLRP3 inflammasome in B cells may be involved in the pathogenesis of SLE.

P1457

The efficacy and safety of antithrombotic therapy in patients with positive antiphospholipid antibodies receiving invasive procedures: experience from a single tertiary center

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Objectives: To evaluate the efficacy and safety of antithrombotic prophylaxis and to explore potential risk factors for thrombotic/bleeding events in patients with positive antiphospholipid (aPL) antibodies receiving invasive procedures.
Methods: All aPL-positive patients who underwent invasive procedures in Peking Union Medical College Hospital, from January 2002 to April 2018, were retrospectively enrolled. Demographic features, clinical features, antiphospholipid antibody profiles, types of invasive procedures, and antithrombotic management, as well as complications and outcomes, were systematically reviewed and recorded.
Results: A total of 111 aPL-positive patients with 130 invasive procedures were enrolled. 109 (83.8%) cases were on regular antithrombotic therapy which started at least one month prior to the invasive procedures, with 58 (44.6%) receiving anticoagulation therapy, 27 (20.8%) receiving antiplatelet therapy, and 24 (18.5%) receiving both. During the periprocedural period, the median time free of antithrombotic therapy was 2.5 d (interquartile range 1.5-6.0 d). Two (1.5%) periprocedural thrombotic events and 18 (13.8%) bleeding events were identified. Large open/laparoscopic surgeries of the thorax and abdomen were associated with a higher risk of bleeding (OR 3.46, 95% CI 1.24-9.67,

DOI: 10.1002/eji.201970400

p=0.014). All bleeding events were manageable and not life-threatening.
Conclusions: Aggressive antithrombotic therapy was associated with fewer thrombotic events in aPL-positive patients receiving invasive procedures, but might contribute to an increased bleeding rate, especially in large open surgeries. This study justifies more caution in prophylactic antithrombotic therapy in periprocedural aPL-positive patients.

P1458

Different clinical characteristics between elderly rheumatoid arthritis patients with elderly onset and younger onset

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Background: To investigate clinical characteristics of elderly rheumatoid arthritis (RA).
Methods: Clinical data of consecutive RA patients including disease activity, function and radiographic assessment were collected. Elderly RA was defined as age ≥ 60 years.
Results: ① There were 516 RA patients recruited with median age 51(42,60) years, median disease duration 53(24,109) months and 82.4% women. There were 15.3% elderly RA patients with younger onset and 10.7% elderly onset RA patients. ② Compared with younger RA patients, elderly onset RA patients had higher proportion of male (36.4% vs. 14.4%), shorter disease duration (24 months vs. 48 months), higher ESR (42 mm/h vs. 26 mm/h), higher rate of active smoking (36.4% vs. 15.1%) and physical dysfunction (HAQ-DI >1, 30.9% vs. 16.0%). Compared with younger RA patients, elderly RA patients with younger onset had longer disease duration (120 months vs. 48 months), higher ESR (40 mm/h vs. 26 mm/h), higher rate of radiographic joint damage (mTSS >10, 82.3% vs. 51.0%, all $P<0.05$). Elderly RA patients had more comorbidities than younger RA patients. ③ Compared with younger RA patients with matched gender and disease duration, elderly onset RA patients had higher ESR (42mm/h vs. 26mm/h), higher rate of active smoking (36.4% vs. 26.4%) and physical dysfunction (30.9% vs. 15.5%). After adjustment for potential confounders, multivariate logistic regression analysis showed elderly onset RA was positively associated with physical dysfunction (*OR*: 3.328*, 95%CI*: 1.403-7.473)
Conclusion: Elderly onset RA patients have clinical characteristics of active smoking, higher ESR, physical dysfunction and more comorbidities.

P1459

Activation of latent tuberculosis infection in rheumatic disease patients: a risk analysis

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Aim: To evaluate the risk of activation of latent tuberculosis infection (LTBI) in Chinese patients with rheumatic diseases who have received glucocorticoid treatment.
Methods: We conducted a 2-year study, enrolling 1788 patients with rheumatic diseases who were treated with glucocorticoid for at least 4 weeks at the Department of Immunology and Rheumatology, The First Affiliated Hospital of Nanchang University. Interferon- release assays (IGRA) were performed with patient blood samples obtained at baseline. Patient data, including age, gender, body mass index (BMI), duration and dosage of glucocorticoid and disease-modifying antirheumatic drug (DMARDs)

DOI: 10.1002/eji.201970400

treatment and comorbidities (malignancies, diabetes, chronic renal failure, silicosis) were collected. Patients were followed for 2 years to detect the emergence of active tuberculosis (TB).
Results: 21.8% (349/1600) of the patients tested positive in IGRA, indicating LTBI. 2-year follow-up showed that 18 (5.16%) patients with positive IGRA but only 4 (0.35%) patients with negative IGRA developed active TB. Univariate analysis showed that low BMI, administration of high dose glucocorticoids, and comorbidities that included interstitial lung disease and malignant cancers were significantly associated with LTBI activation. A subgroup analysis revealed that DMARDs therapy and chronic renal failure increased the risk of activation of LTBI in patients having received high but not low dose glucocorticoids.
Conclusion: Our results suggest that screening and preventive therapy of LTBI may be advisable for Chinese rheumatic disease patients undergoing prolonged high dose glucocorticoid therapy.

P1460

Role of combination examination of cytokines and microcirculation in the diagnosis of neuropsychiatric lupus

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Objective: To explore role of cytokines combined microcirculation of nail fold and bulbar conjunctiva in diagnosis of central nervous system-neuropsychiatric systemic lupus erythematosus(CNS-NPSLE).
Methods: 50 patients with CNS-NPSLE, 50 SLE patients without neuropsychiatric disorders and 30 patients with CNS infection were enrolled from December 2016 to December 2018. Microcirculation in nail fold and bulbar conjunctival were performed in all patients. The levels of cytokines (IL-2R, IL-1β, IL-6, IL-8, IL-10 and TNF-α)in serum and CSF were determined by chemiluminescence method.
Results: Twenty-six patients developed two or more neuropsychiatric subtypes. The most common neuropsychiatric disorder is seizures, followed by headache. The levels of IL-6 and IL-8 of cerebrospinal fluid in CNS-NPSLE group were significantly higher than those in non-NPSLE group ($P< 0.001$), but with no significant difference compared to CNS infection group ($P>0.05$). The levels of IL-2R, IL-1β, IL-10 and TNF-α in cerebrospinal fluid had no significant differences in each group ($P>0.05$). No significant difference was found for serum cytokine levels among the three groups ($P>0.05$).The cumulative score of nail fold microcirculation in CNS-NPSLE group was significantly higher than that in CNS infection group ($P< 0.001$), but no significant difference with non-NPSLE group ($P>0.05$).The cumulative score of bulbar conjunctival microcirculation in CNS-NPSLE group was significantly higher than that in the group of non-NPSLE and intracranial infection ($P< 0.001$).
Conclusion: Increased level of IL-6 and IL-8 in the CSF and cumulative scores of bulbar conjunctiva microcirculation may predict CNS-NPSLE and could act as diagnostic tool to differentiate CNS-NPSLE from non-NPSLE and CNS infection.

P1461

Identifying disease associated T cell receptors as the potential biomarker for immune-related diseases

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DOI: 10.1002/eji.201970400

The human adaptive immune system provides protection against internal and external antigens which probably affect the body's health. The protection function is directly triggered by the receptors on the surfaces of T cells (TCRs) and B cells (BCRs) to specifically recognize antigens. However, up to now, it is frustrating to exactly discriminate some immune related diseases, especially for autoimmune diseases that are fairly complicated for pathogenesis and have not exactly diagnostic method for early stage patients. Here, we proposed a pan-immunome project, building an integrated T cell receptor repertoire map for immune related disease, and aimed to explore and mine the disease's immune characteristics. We have collected more than ten thousand of samples comprising sorts of autoimmune diseases and healthy samples. All samples have been amplified TCR β repertoire and sequenced using high throughput sequencer. To store the data of this project, we have established a database (PIPD, https://db.cngb.org/pird/) in China National Genebank. Additionally, we found that the diversity and clonality of TCR repertoire are significantly different in diseases and control, as some clones undergo expansion to response the antigens of disease. Using bioinformatic method, we identified the clones that associate with disease from TCR b repertoire of large-scale of patients. Then, using machine learning, a statistical model was generated to classify diseases using those disease-associated TCRs, and performed well with high specificity and sensitivity. Thus, immune repertoire could be the biomarker for molecular classification of diseases. It is worthwhile to build an enormous immune database for the potential applications.

P1462

Effects of Toll-like receptor 4 on β2-glycoprotein I-induced splenic T cell subsets differentiation

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β2GPI is the main autoantigen in anti-phospholipid syndrome (APS), which has been demonstrated to promote the maturation of bone marrow derived dendritic cells (BMDCs) in a Toll-like receptor 4 (TLR4) dependent manner. However, β2GPI-induced T cells differentiation and the role of TLR4 in this process remain controversial. In this study, we focused on the differentiation of splenic T cells in β2GPI immunized Balb/c, C3H/HeN (TLR4 intact) and C3H/HeJ (TLR4 functional mutant) mice. According to our results, Th2 dominated differentiation has been observed in β2GPI immunized Balb/c and C3H/HeN mice, which was characterized by the up-regulated Th2 markers (GATA3 and IL-4). In addition, Treg marker (Foxp3 and TGF-β1) and Th1 markers (T-bet and IFN-γ) were reduced in these mice, implicated a suppressed Th1 and Treg differentiation after β2GPI immunization. However, the Th2-biased differentiation has not been observed in β2GPI immunized C3H/HeJ mice or in physiological saline treated C3H/HeN or Balb/c mice, indicating the importance of TLR4 in this β2GPI-induced Th2 differentiation. In addition, the observed Th2 differentiation could be strengthened by both dendritic cells (DCs) secreted cytokines and DCs-T cells contact, while the DCs-T cells contact showed a unique and indispensable role in suppressing Th1 function. Furthermore, the Th2 biased differentiation was more noticeable in mice received 4 times β2GPI immunization than those received 2 times, suggesting the amplifying effects of anti-β2GPI Ab on β2GPI induced Th2 response. These findings may partly explain the immune imbalance in APS patient through the view angle of T cell differentiation and anti-β2GPI antibody production.

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DOI: 10.1002/eji.201970400

P1463

Hippocampal atrophy in systemic lupus erythematosus patients without major neuropsychiatric manifestations

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This study was conducted to explore hippocampal structural changes and their possible associations with clinical characteristics, emotional status and treatment methods in patients with systemic lupus erythematosus (SLE) without major neuropsychiatric manifestations (non-NPSLE). Eighty-five non-NPSLE patients with normal conventional magnetic resonance imaging (MRI) and seventy-seven matched healthy control (HC) subjects were recruited. All participants underwent the standard high-resolution volumetric MRI. Bilateral hippocampal volume (HIPV) and hippocampal density (HIPD) were calculated respectively for each participant. We found that the bilateral HIPV and HIPD of the SLE patient group was significantly less than that of HC group. The bilateral HIPV of female patients were significantly less than male patients. SLE disease activity index (SLEDAI) was negatively correlated with the bilateral HIPV and the right HIPD. Urine protein was negatively correlated with the bilateral HIPV and HIPD. Hydroxychloroquine (HCQ) showed a protective effect on right HIPV. In conclusion, we found that the early hippocampal atrophy could occur before obvious neuropsychiatric manifestations and might be associated with SLE disease activity and organ damages. Early detection and intervention of hippocampal damage might prevent the progression to NPSLE. More studies are needed to fully understand the underlying mechanisms of hippocampal atrophy in SLE.

P1464

IgG immunocomplexes sensitize human monocytes for inflammatory hyperactivity via transcriptomic and epigenetic reprogramming in rheumatoid arthritis

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Prevalence of circulating immunocomplexes (ICs) strongly correlates with rheumatoid arthritis (RA) in humans. Deposits of IgG-ICs are abundant in affected joints of patients, yet molecular mechanisms for the pathogenic roles of such ICs are not fully understood. In this study, we present evidence that IgG-ICs precipitated from RA sera sensitized human monocytes for a long- lasting inflammatory functional state, characterized by a strong TNF-a response to cellular proteins representing damage- associated molecular patterns and microbe-derived pathogen-associated molecular patterns. Importantly, plate-coated human IgG (a mimic of deposited IC without Ag restriction) exhibited a similarly robust ability of monocyte sensitization in vitro. The plate-coated human IgG-induced functional programming is accompanied by transcriptomic and epigenetic modification of various inflammatory cytokines and negative regulator genes. Moreover, macrophages freshly isolated from synovia of patients with RA, but not sera-negative arthropathy, displayed a signature gene expression profile highly similar to that of IC-sensitized human monocytes, indicative of historical priming events by IgG-ICs in vivo. Thus, the

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DOI: 10.1002/eji.201970400

ability of IgG-ICs to drive sustainable functional sensitization/reprogramming of monocytes and macrophages toward inflammation may render them key players in the development of RA.

P1465

Artemisinin analog SM934 ameliorates dextran sulfate sodium-induced chronic colitis through regulating CD4+ T cell proliferation and differentiation via mTORC1 pathway

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SM934, a water soluble artemisinin derivative possesses immunoregulatory properties. The purpose of this study was to investigate the protective effects of SM934 on dextran sulfate sodium (DSS)-induced chronic experimental colitis and clarified the possible mechanisms.
Mice were treated by 2.5% DSS for one week, subsequently drinking water for 11 days as the first cycle. In the second cycle, DSS was still given 7 days as before, and then drinkng water as normal. Meanwhile, the colitis mice were orally administered SM934(10, 3, 1 mg/kg) from day 13 onward for 30 days. SM934 improved weight loss, alleviated disease activity index (DAI) colon length shortening, colonic pathological damage, reduced pro-inflammatory mediators and preserved tight junction (TJ) protein ZO-1, occludin expression challenged by DSS in mice. Furthermore, SM934 treatment reduced the CD4+ T cell numbers and inhibited CD4+ T cell proliferation of Mesenteric lymph nodes (MLN) cells on colitis mice. Notably, SM934 treatment inhibited the development of Th1 and Th17 cells and elevated the percentage of Treg cells in MLN cells, and pronouncedly downregulated the phosphorylation of STAT1 and STAT3 in colon tissues. Additionally, SM934 was capable of suppressing the mammalian target of rapamycin complex 1 (mTORC1) pathway in colon tissues as demonstrated by inhibiting phosphorylation of 4E-BP1, p70S6K and RPS6, mTOR.
Our documentation showed that SM934 ameliorates chronic colitis through regulating CD4+ T cell proliferation and differentiation via mTORC1 pathway.

P1466

The relationship between serum levels of growth differentiation factor 15 (GDF-15) and severity of rheumatoid arthritis

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Background: Rheumatoid arthritis is a chronic systemic inflammatory disease that primarily affects the joints with synovium. The serum levels of cytokine GDF-15 may increase under inflammatory conditions and tissue damage. The aim of this study was to determine the relationship between serum GDF-15 levels and severity of rheumatoid arthritis.
Methods: The present case-control study was done on 100 women with rheumatoid arthritis and 55 healthy women. Diagnosis of rheumatoid arthritis was made using clinical manifestations, physical examination and laboratory tests. Diagnostic confirmation made by rheumatologist based on ACR/EULAR criteria, and the disease activity score-28 (DAS28) was used to evaluate the disease severity. Blood samples were collected from all participants. GDF-15 serum level was quantitatively measured using ELISA method (e-Bioscience, USA).
Results: The mean and standard deviation of GDF-15 in the case group was significantly higher than those in the control group ($p < 0.001$). Also, serum GDF-15 levels were positively correlated with

DOI: 10.1002/eji.201970400

DAS28 score, ESR level, and CRP level (p< 0.001).
Conclusion: Serum levels of GDF-15 are correlated with the severity of rheumatoid arthritis. Such factor may be used as a prognostic marker of the disease.

P1467

Abnormalities in the gut mucosa of non-obese diabetic mice precede the onset of type 1 diabetes

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Introduction: The intestinal microbiota has been highly associated with the development of type 1 diabetes (T1D), however, the influence of immune and non-immune components of intestinal mucosa is poorly investigated. In this study, we investigated the alterations in the gut mucosa of NOD mice to understand the relationship between the gut-pancreas axis on the development of type 1 diabetes.
Material and methods: Non-obese diabetic (NOD) mice was compared with non-obese diabetic resistant (NOR) mice age matched at either 4-6 weeks or 16-20 weeks.
Results: NOD mice at 4-6 weeks already presented impaired oral tolerance induction and defective protective/regulatory mechanisms in the intestinal mucosa, including decreased of goblet cells, diminished mucus production (decrease of MUC2 and increase of MUC1 expression), lower levels of secretory IgA and anti-inflammatory cytokine IL-10. This context was associated to a process of dysbiosis and increased translocation of bacteria from the intestine to the pancreatic lymph nodes. These alterations were aggravated after onset of T1D. Pre-diabetic and diabetic NOD mice at 16-20 weeks of age presented several alterations in gut immune cells such as increased frequency of pro-inflammatory dendritic cells (DCs), reduction of tolerogenic DCs, imbalance in the inflammation/regulation axis (Th1, Th17, Treg cells), increase in type 3 innate lymphoid cells (ILC3) and decrease in ILC2 in the small intestine.
Conclusion: Disorders in the intestinal mucosa of NOD mice occur at early age and they can potentially interfere with the development of T1D due to the close relationship between the gut and the pancreas.

P1468

The association between rs1800629 and rs2234649 polymorphisms and risk for non-segmental vitiligo

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Objective: The present case-control study investigated the association of the polymorphisms rs1800629 (*TNF* -308 G>A) and rs2234649 (*TNFRSF1A* -383 A>C) with the risk for non-segmental vitiligo in Western Mexico population.
Materials and methods: Genotyping of the rs1800629 and rs2234649 polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism method in100 non-segmental

DOI: 10.1002/eji.201970400

vitiligo patients and 142 healthy subjects.
Results: According to a genetic model, we found a significant association ($p < 0.001$) of genotypes carrying -308*A and -383*C risk alleles with the susceptibility to non-segmental.
Conclusions: The rs1800629 and rs2234649 polymorphisms could represent new genetic markers of susceptibility for the development of non-segmental vitiligo in the population of western Mexico.
Key words: non-segmetal vitiligo, TNF-a, polymorphisms

P1469

The effects of germline STAT3-activating mutations from autoimmunity and lymphoid malignancy on mouse and human T cell development and function

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Signal transducer and activator of transcription 3 (STAT3) is a latent transcription factor with pleiotropic roles in hematopoietic and non-hematopoietic cells, that regulates gene expression downstream of cell surface cytokine and hormone receptors. Heterozygous germline loss-of-function mutations in *STAT3* lead to the primary immunodeficiency hyper-IgE syndrome (HIES) while somatic gain-of-function mutations in *STAT3* recur in human solid organ malignancies and non-Hodgkin lymphoma. Recently, germline heterozygous gain-of-function mutations in *STAT3* were shown to cause early-onset and multi-organ autoimmunity with aspects of immunodeficiency. Affected individuals share characteristics with autoimmune lymphoproliferative syndrome (ALPS) and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, including reduced T regulatory cell numbers and suppressive activity. They present with variable early-onset autoimmune symptoms including type 1 diabetes, juvenile-onset rheumatoid arthritis, gut enteropathies and autoimmune cytopenias. Whilst many effects of STAT3 loss-of-function on immune cells have been described, the mechanisms behind autoimmunity and immunodeficiency in patients with STAT3-activating mutations remain unclear.
Here, we present a detailed characterisation of T cell development and maturation in young and old mice on two different backgrounds with Crispr-engineered germline activating mutations in two domains of STAT3. We use mixed chimeras, flow cytometric analysis, T cell receptor deep-sequencing and high-throughput single-cell transcriptomics to reveal cell-extrinsic and -autonomous roles of STAT3 activation in T cells in autoimmunity versus immune malignancy. To our knowledge, this is the first report of mice with *Stat3* germline activating mutations identical to those in autoimmunity or malignancy. We validate our key findings in humans with gain-of-function germline *STAT3* mutations and childhood-onset autoimmunity.

P1470

HLA-B27 genotype in BIRDEM: correlation with clinical findings, inflammatory markers and auto-antibody

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Background: Human Leukocyte Antigen B27 (HLA-B27) is strongly associated with ankylosing spondylitis (AS) and other associated inflammatory diseases. We correlated referred patients

DOI: 10.1002/eji.201970400

symptoms with inflammatory markers, autoantibody and molecular tests in our study.
Methods: 100 referred patients to Dept. of Immunology of BIRDEM, Dhaka from January 28 to March 24, 2019 with the complaints of back pain, joint pain, knee pain, eye pain etc. has been enrolled. The HLA-B27 genotype was performed in LightCyecler 480 (Roche, Switzerland) platform using kits from TIB Molbiol (Germany).
Results: Among 100 referred patients, 28 were positive for HLA-B27 genotype (male 82.1% and female 17.9%) and gender distribution was found statistically significant (p=0.012). HLA-B27 positive genotype was found 42.9% among 21-30 years age group and it was statistically significant (p=0.050). Within 28 positive patients, 15 had low back pain (10 with also other joint pain), 3 with eye pain only, 3 with knee pain only, 2 with only spine pain, and 5 with knee pain accompanied by other joint pain. Among markers of inflammation, C-reactive protein (CRP) between HLA-B27 positive and negative patients found significant (p=0.044) whereas ESR was non-significant (p=0.234). The anti cyclic citrullinated peptide (anti-CCP) antibody was also found significant between HLA-B27 positive and negative genotype cases (p=0.010).
Conclusion: The HLA-B27 genotype positivity was found predominantly in male with 21-30 years age group and most of them were referred with low back pain. The value of markers of chronic inflammation (e.g. CRP) and anti-CCP antibody was found statistically significant with HLA-B27 genotype.

P1471

Glucosamine induces Th17 cell differentiation by inhibiting CD122 expression which results in the aggravation of collagen-induced arthritis

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D-glucosamine (GlcN) is known to have a therapeutic role in osteoarthritis. However, effect of GlcN on rheumatoid arthritis and molecular mechanisms how GlcN influences on immune cells are not fully understood. First, we investigated to understand the mechanism how GlcN influences on the T cell activation *in vitro*. We obtained the following results; 1) GlcN inhibited the T-cell proliferation and division. 2) Interleukin-2 (IL-2) production was significantly increased in GlcN-treated T cell culture medium. 3) IL-2 receptor beta chain (CD122) was significantly decreased by GlcN. 4) GlcN markedly reduced the IFN-γ and IL-17A expression. According to the inhibitory role of GlcN on T cells, we investigated whether GlcN has a therapeutic efficacy in collagen-induced arthritis. Surprisingly, in contrast to the results *in vitro*, mice received GlcN showed early onset and increased severity. Since IL-17 plays as a pathogenic cytokine in rheumatoid arthritis, we differentiated CD4+ T cells into Th17 cells. GlcN significantly increased Th17 differentiation and inhibited CD122 expression. Thus, GlcN induces Th17 cell differentiation by inhibiting IL-2 signaling, which may result in the aggravation of collagen-induced arthritis.

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DOI: 10.1002/eji.201970400

P1472

Expression of antimicrobial peptides during the development and progression of type II collagen-induced arthritis and its association with parameters of inflammation and joint damage

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Aims: To evaluate the expression of AMPs, CRAMP, mBD-3 and mBD-4 during the development and progression of type II collagen-induced arthritis and to analyze the association of these with pro and anti-inflammatory cytokines, as well as joint-damage.
Methods: Type II collagen-induced arthritis (CIA) was induced in DBA/1J mice. The joints were allocated at the onset, peak and decline phase of CIA. The expression of CRAMP, mBD-3, mBD-4, IL-10, IFN-g, IL-17, MMP-3 and RANK/RANKL were evaluated using immunohistochemistry and PCR assays. Cell-infiltration in the joint was evaluated using hematoxylin and eosin staining. Tartrate-resistant acid phosphatase (TRAP) staining was using to test osteoclasts activity. Safranin-O staining was used to cartilage detection.
Results: It was observed that the expression of mBD4 increases in the peak phase of CIA, and presented positive association with joint-infiltrating cells. CRAMP expression (LL-37 orthologous) increases as the development of the CIA reaching its higher expression at the peak-phase and decline-phase of the arthritis. In addition, CRAMP expression showed a positive association with joint infiltrating cells, the cartilage lost and the expression of MMP-3.
Conclusion: We speculated that CRAMP an anti-inflammatory role since its expression is augmented still in the decline-phase of arthritis, frequently associated with anti-inflammatory cytokines such as IL-10.

P1473

Clinical-immunological correlation of autoantibodies of systemic sclerosis: preliminary study on Moroccan patients

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Introduction: Systemic sclerosis is a multisystem connective tissue disorder characterized by excessive fibrosis of skin and internal organs and microvascular damage. It is believed that the SSc-specific autoantibodies are responsible both for amplifying immune response and targeting cell types that are relevant in the pathophysiology of SSc.
Objectives: To correlate the profile of the following specific autoantibodies of SSc with clinical manifestations observed in 23 patients from Morocco.
Methods: The occurrence of specific autoantibodies in 23 patients with SSc was investigated by indirect immunofluorescence and Immunodot, correlating the type of autoantibody with clinical manifestations.
Results: Among all patients evaluated, we found a predominance of females (90%), meanage 50

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DOI: 10.1002/eji.201970400

years old, Caucasian (100%), time of diagnosis between 1 and 10 years (50%), and disease duration of 9,5 years. According to the specific autoantibody profile, 4 patients were ACA-positive (17,4%), 6 were positive for anti-topoI (26,1%), 6 were positive for anti-RNP (26,1%), 3 were positive for anti PM-Scl (13%), 4 were positive for anti Ro-52 (17,4%) and 3 showed positive for anti SSA (13%). The anti-topo I autoantibody correlated with diffuse scleroderma, with greater disease severity and activity, with worse quality of life measured by the SHAQ index, with a higher prevalence of objective Raynaud's phenomenon and digital pitting scars of fingertips. The ACA correlated with limited scleroderma, with earlier onset of disease .The anti-RNP correlated with diffuse scleroderma.
Conclusions: The clinical subtype of the disease and some clinical manifestations in SSc may correlate positively with the presence of specific autoantibodies.

P1474

The connection of experimental data and clinical information of patients with auto-antibodies against IFN-γ

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With more and more patients found, there are several critical clinical problems were observed in patients with anti-IFN-γ autoantibodies. For example, even most, if not all, the patients presented severe mycobacterial infection as the major clinical illness. However, the outcomes are diverted: around 1/3 of cases respond well to anti-mycobacterial antibiotic treatment; it means that 2/3 of patients painfully suffered from persistent or recurrent mycobacterial infection despite the same anti-mycobacterial antibiotic regime. Moreover, recently we found that anti-IFN-γ autoantibodies were also found in a few patients with tuberculosis. TB and NTM are very different in term of pathogenicity; therefore, the mysterious question is why patients with anti-IFN-γ autoantibodies showed the different infection.
We developed several new experimental assays in lab to measure the absolute titer and neutralizing activity of anti-IFN-γ autoantibodies. We detail measure the property of anti-IFN-γ autoantibodies in patient plasma and try to provide the molecular explanations for these unsolved clinical tackles. We hypothesize that the patient outcome and clinical manifestation might associate with antibodies titer and/or neutralizing activity, and we did see some clues now. Moreover, different from genetic deficient, autoantibodies-mediated immune suppression might evolve in long term due to the titer change may help to reflect the outcome of patients and how to give the treatment or diagnosis of patients.

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DOI: 10.1002/eji.201970400

P1475

Ubiquitous transgenic expression of IL-23 to mediate disease pathogenesis in spondyloarthritis

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IL-23 cytokine has been implicated in the development of spondyloarthritis, including ankylosing spondylitis (AS), and enteropathic arthritis, such asinflammatory bowel disease. Here, we showed thatIL-23 overexpression in a mouse model induced multiorgan inflammation characterised by systemic inflammationand infiltrates of lymphocytes in the intestine and kidney. In addition, mice with transgenic overexpression of IL-23 demonstrated acceleration of disease progression, which was characterised by increased accumulation of various hematopoietic cells, including Th17 and Tfh cells, and production of pro-inflammatory cytokines, such as IL-17 and IL-22. This model can be used as a tool in variouspreclinical studies to investigate the molecular and cellular pathogenesis of spondyloarthritis and in the evaluation of potential therapies.

P1476

Identification of unique, shared, anti-IA-2 B cell clonotypes: a proteomic approach to understanding pathophysiological mechanisms underpinning humoral autoimmunity in Type 1 diabetes

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Immune destruction of beta (b) cells is a key feature of type 1 diabetes (T1D), yet the pathophysiological role of autoantibodies (Abs) to autoantigens remains poorly understood. Recently, proteomic interrogation of Abs indicates that restricted heavy (H) and light (L) chain gene usage underpins targeting of classic autoantigens in systemic autoimmune diseases such as Lupus. In the current study, we have taken a similar approach to characterise B cell clonotypes resulting in autoimmune targeting of the major T1D autoantigen, IA-2. Firstly, we developed a novel ELISA assay for the major IA-2 epitopes using an MBP-fusion protein (FP) incorporating the intracellular domain of IA-2. We next established an ELISA-based affinity purification (AP) method using the IA-2-MBP FP to isolate, for the first time, enriched IA-2 specific Abs from 2 patients with verified (by RSR commercial ELISA) anti-IA-2 autoimmunity. The AP IA-2 Abs were then analysed for Ig H and L chain clonality, variable region gene usage and AA sequence using high-res electrophoretic fractionation and de-novo/database-driven analysis of AA peptides (TripleTOF 5600+ MS). We report the presence of 2 unique Ig Kappa-restricted clonotypes giving rise to IA-2 autoimmunity; namely a IGHV3.23 H chain paired with either a IGKV1.9 or IGKV3.20 L chain. Remarkably, both clones were present in AP Ig from 2 unrelated T1D patients, with shared AA replacement mutations. These extraordinary findings suggest the pathogenic mechanisms leading to IA-2 autoimmunity may be virtually identical across individuals, leading to the intriguing possibility of intervention in the processes underpinning the disease.

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DOI: 10.1002/eji.201970400

P1477

Lymphoma driver mutations in the pathogenic evolution of an iconic human autoantibody

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Pathogenic autoantibodies arise in many autoimmune diseases but it is not understood how the cells making them evade the immune checkpoints that normally perform quality control on antibody proteins. The recent emergence of single-cell technologies offers a powerful way to profile rogue B cells within complex and heterogeneous immune populations.
Here single cell multi-omic analysis demonstrates a shared mechanism with lymphoid malignancy in the formation of a public rheumatoid factor autoantibody responsible for 60% of cases of mixed cryoglobulinemic purpura. By combining single cell DNA and RNA sequencing with serum antibody peptide sequencing and antibody synthesis, rare circulating B lymphocytes making the pathogenic autoantibody were found to comprise a clonal tree accumulating mutations in antibody and lymphoma driver genes. A mutation corresponding to the most frequent site of lymphoma mutations in the cullin ubiquitin ligase subunit KLHL6 drove the accumulation of antibody mutations, which in turn conferred pathogenicity by increasing the propensity to form insoluble protein aggregates.
These results reveal a cascade of somatic errors in antibody protein quality control leading to an iconic pathogenic autoantibody and provide direct evidence for a shared pathogenesis of autoimmunity and lymphoid malignancy.

P1478

First insights into the gut microbiota composition of rheumatoid arthritis patients from Mexico

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Background: Rheumatoid arthritis (RA) is a prevalent systemic autoimmune disease and increasing evidence suggests that the gut microbiota is involved on its susceptibility and pathogenesis.
Objectives: The aim of this study was to compare the gut microbiota composition of Mexican RA patients and control subjects, and to evaluate its association with inflammatory and clinical biomarkers.
Methods: Microbial taxa relative abundance was determined by 16S rRNA sequencing in 27 RA patients and 23 control subjects. In RA patients, clinical activity (DAS28), functional disability (HAQ-DI) and inflammatory biomarkers (ESR and CRP) were measured, whereas autoantibody levels (RF and anti-CCP) were quantified by ELISA.
Results: RA patients exhibited lower bacterial richness than the healthy control group (p=0.05), and this parameter was correlated with several clinical parameters including rheumatoid factor, disease activity, functional capacity and the use of the biologic antirheumatic drug Abatacept. Analysis of the microbiota structure and composition displayed significant differences between RA patients and

DOI: 10.1002/eji.201970400

controls (p=0.02, by Permanova). The linear discriminant analysis (LDA) effect size (LEfSe) method revealed that the genus *Ruminococcus* is higher in the fecal microbiota of RA patients, whereas a lower abundance of *Alistipes*, *Odoribacter, Barnesiellaceae* y *Phascolarctobacterium* was detected in comparison to controls.
Conclusions: We have characterized the gut microbiota of mexican RA patients for the first time, and confirmed the presence of modifications on the richness, structure and abundance of intestinal bacteria, which may impact the progression and severity of the disease.

P1479

Aire-dependent establishment of self-tolerance

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Role of Aire in the establishment of self-tolerance still remains fully elucidated. With the use of genetically modified strain of mice of Aire gene, we have been studying the expression dynamics of Aire during the differentiation program of mTECs. Those studies have elucidated the existence of post-Aire stage of mTECs, Aire's effect on the mTEC differentiation program and Aire-dependent production of Ly6C/G$^+$mTECs acting in trans. We have been also trying to understand the tolerogenic function of Aire together with its possible clinical application for the treatment of tissue-specific autoimmune diseases. Unexpectedly, we found that additive expression of human AIRE (huAIRE) both in mTECs and peripheral antigen-presenting cells (APCs)under the control of MHC-II promoter in NOD, a mouse model of type I diabetes, induced de novo polymyositis-like autoimmunity. In contrast, huAIRE expression only in peripheral APCs rendered the mice resistance to T1D dueto the loss of Xcr1$^+$dendritic cells (DCs) (equivalent to CD103$^+$DCs), an essential component for activating diabetogenic T-cells inthe periphery. Our results reveal that tissue-specific autoimmunity isdifferentially controlled by a combination of thymic function and peripheral tolerance,which can be manipulated by expression of huAIRE/Aire in each or both of thetolerance mechanisms. Thus, elucidation of the exact function of Aire may illuminate novel aspect for the pathogenesis of tissue-specific autoimmunity and its physiological role for the establishment of self-tolerance.

P1480

Auto-immune process in hematologic malignancies

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Introduction: Auto-immune process can occurs frequently in cancers, especially in hematologic malignancies. They must be diagnosed sooner because they can influence prognosis and the choice of the treatment. However, in our country we have not sustainable data about these diseases.
Method: We conducted a retrospective study from medical files of patients who was hospitalised or followed in external consultations in the department of Internal Medicine at University Hospital Yalgado Ouedraogo in Ouagadougou (Burkina Faso). The study period go from 1st January to 31th December 2016. Only patients over than 15 years old have been inclued.
Results: The study population collected was 122 patients. Hematologic malignancies represented 80,33% (98/122) of patients. The annual incidence of hematologic malignancies was 8,16 cases. Auto-immune cytopenias represented essential of auto-immune process with 32,65% (32/98). Among cytopenias, auto-immune hemolytic anemia (AHA) was the major cause with 62,5% (20/32), allowed by thrombopenia 25% (8/32) and neutropenia 12,5% (4/32). The direct coombs test was positive in 80% of cases of anemia (16/20).

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DOI: 10.1002/eji.201970400

The mean age was 53,51 years [15 - 84]. The sex-ratio (M/W) was de 1,51. The distribution of hematologic cancers was : Acute malignancies = 8,16%, Myeloproliferative Syndroms (MS)= 10,20%, Lymphoproliferative Syndroms (LS)= 76,53%, Myelodysplasic Syndroms (MDS)= 5,11%. We found that AHA was statistically linked to lymphoproliferative syndroms (p=0,0023). Infections was the most frequent etiology of death with 52,95%. Therapeutic most used was corticotherapy in 91,83% of patients. The global lethaly was 96,93%.
Conclusion: The major cause of auto-immune process in our study was hemolytic anemia.

P1481

HLA-G 3'UTR polymorphisms and its soluble protein levels in patients with rheumatoid arthritis from Indian population: a case-control study

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Objective: Human Leucocyte Antigen (HLA)-G antigens are inducible non-classical major histocompatibility complex class Ib molecules which play an important role in the regulation of inflammatory processes and immunomodulation in autoimmune diseases. There are controversial reports concerning the impact of *HLA-G* gene polymorphism on rheumatoid arthritis (RA). This study was aimed at examining the impact of 14 bp ins/del (rs66554220) and +3142G>C (rs1063320) polymorphism, correlating these with soluble (s)HLA-G levels to understand the susceptibility to RA in our sample cohort.
Methods: Genomic DNA from 100 RA patients and 100 healthy controls was isolated using the salting out method. Genotyping of HLA-G +3142G>C and 14-bp ins/del variants was done by polymerase chain reaction restriction fragment length polymorphism (PCR-RFP) and PCR method, respectively; levels of sHLA-G were estimated by ELISA.
Results: The *HLAG* +3142G>C polymorphism significantly decreased the risk of RA in codominant (OR =1.2, 95% CI =0.63-2.2, p =0.5911, GC versus GG; OR =0.2, 95% CI =0.05-0.7, p =0.0059**, CC versus GG), recessive (OR = 0.2, 95% CI =0.04-0.59, p = 0.0024**, GG + GC versus CC), and allele (OR =0.7, 95% CI =0.5-1.1, p =0.1215, G versus C) inheritance models tested. Our finding did not support an association between *HLA-G* 14 bp ins/del variant and risk/protection of RA. The sHLA-G levels were significantly lower in +3142GG, +3142GCC, 14bp del/del, 14bp ins/ins.
Conclusion: In summary, our results showed that *HLA-G* +3142G>C gene polymorphism might contribute to decrease the risk of RA in our sample cohort.

P1482

Novel function of IL-17E in the autoimmune pathogenesis of experimental Sjogren´s syndrome

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IL-17 family has been implicated in the autoimmune pathogenesis. Our previous study has demonstrated an essential role of IL-17A in the development of primary Sjogren´s syndrome (pSS), an autoimmune disorder characterized by tissue destruction in salivary glands. However, the role of IL-17E in the pSS pathogenesis remained unclear. Recently, we have established a mouse model of experimental Sjogren´s syndrome (ESS) that highly recapitulates human pSS. In this study, we first detected significantly enhanced IL-17E signature in the salivary tissues of ESS mice. IL-17RB, the

DOI: 10.1002/eji.201970400

receptor of IL-17E was also found increased in the glandular-infiltrating cells. Moreover, we found elevated serum levels of IL-5 and IL-13, increased type 2 innate lymphoid cells (ILC2) and IL-17RB+CD4+ T cells with enhanced expression of TRAF6, the downstream signaling upon IL-17RB activation during ESS development. Interestingly, serum levels of IL-25 was found elevated at the disease chronic stages of ESS mice. Consistently, marked increase of IL-25-producing cells was also detected in the salivary gland of ESS mice with severe lymphocytic infiltrations. To further determine the therapeutic potential of targeting IL-17E, we performed anti-IL-17E neutralization using ESS mice at disease chronic stages. Notably, anti-IL-17E treatment resulted in clinical improvement in diseased mice, including ameliorated salivary histopathology and significantly decreased glandular-infiltrating cells, including ILC2 and Th1 cells when compared with those mice treated with vehicles, suggesting that IL-17E may serve as promising candidate in the treatment of human pSS.

P2001

To deepen the understanding of anti-NOR90/hUBF antibody: followup in a patient with juvenile systemic sclerosis and review of the literature

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Objective: To broaden clinical sights and faciliate diagnosis of patients who had features of SSc.
Methods: We report a yong chinese female patient who was finally diagnosed as juvenile SSc with positive anti-NOR90/hUBF antibody, and review the literature associated.
Results: A Chinese female patient developed red rash in facial area and extremities at age 6, accompanied by intermittent arthralgia. The rash went exacerated with cold. At age12, cryoglobulinemia was suspected althoug cryoglobulin was not detected. She was treated with hydroxychloroquine(HCQ) and beraprost, and symptoms were relieved. At age 15, her left first toe developed ischemic lesion and the necrotic phalange had to be resected, although treated with CTX and thalidomide . She came to our department and further tests revealed positive anti-NOR90/hUBF and weakly positive anti-RP155. A diagnosis of juvenile SSc was made. Low-dose methylprednisolone were added for immune treatment. The foot ulcers healed within 2 months. Literature review: Anti-NOR90/hUBF is directed against human upstream binding factor (hUBF), which participates in RNA transcription. Although there is a lack of large-scale research reported, anti-NOR90/hUBF is considered associated with limited cutaneous SSc with mild internal organ involvement. It had a low sensitivity and was positive in less than 5% patients with SSc. It is not specific for SSc, and can also be found in other CTDs such as SLE, SS, UCTD and RA.
Conclusion: Anti-NOR90/hUBF is one of SSc-related autoantibodies and indicates limited cutaneous SSc with relatively mild organ involvement. It can serve as an important hint for diagnosis and prognosis.

DOI: 10.1002/eji.201970400

P2002

Leptin/OB-R pathway promotes IL-4 secretion from B lymphocytes and induces salivary gland epithelial cell apoptosis in Sjögren´s syndrome

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Sjögren´s syndrome (SjS) is a chronic autoimmune epithelitis in which cell apoptosis promotes the formation of inflammatory lesions. We used immunohistochemistry and TUNEL to assay B cell infiltration and apoptosis in salivary gland tissue from 16-week-old NOD/LtJ mice with SjS. In co-cultures of primary salivary glandepithelial cells (SGECs) and spleen B cells, we assessed SGEC viability and apoptosis using CCK8 assays and flow cytometry. ELISAs were employed to assess cytokine levels in culture medium. Leptin protein, leptin receptor (OB-R), pro-and anti-apoptotic proteins, and Jak2/Stat3/ERK signaling molecules were analyzed using western blotting. B cell infiltration and salivary gland apoptosis were increased in salivary tissue from mice with SjS. Leptin treatment had no effect on cell viability or apoptosis among B cells and primary SGECs. B cell and SGEC co-culture systems showed that leptin increased apoptosis induced by B lymphocytes, reduced SGEC cell viability, and promoted IL-4 secretion from B cells. This suggests Leptin/OB-R signaling stimulates B cells-induced SGEC apoptosis via IL-4 secretion and OB-R- Jak2-Stat3 activation.

P2003

Identification of new autoantibodies for rheumatoid arthritis using human proteome microarrays

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Objective: To screen autoantibodies of RA with high sensitivity and specificity using human proteome microarrays.
Methods and Results: The serum of five RA patients and five healthy controls were selected to detect the RA-related autoantibodies by microarray, and 25 candidates were screened. Then the IgG and IgM RA-focused microarrays composed of the 25 proteins were screened with additional cohorts of 72 RA patients and 106 healthy controls. The statistical methods used here include cluster analysis, logistic regression and ROC analysis.The results of IgG protein microarray showed: (1) Expression of these 25 autoantibodies in RA patients was significantly higher than healthy controls ($P< 0.05$).(2) ROC analysis showed the combination of anti-RBPJ, anti-SH3BGR and anti-PAFAH1B3 autoantibody can be highly RA-specific biomarkers, with 66.7% sensitivity and 74.2% specificity, and the AUC is 0.734; the sensitivity and specificity of anti-CCP and RF-negative RA patients diagnosis were 77.8% and 65.6%, and the AUC was 0.720.The results of IgM protein microarray showed:(1) Only 10 out of the 25 candidates´ expression was significantly higher in RA patients ($P< 0.05$). (2) ROC analysis showed anti-PAFAH1B3 antibody was identified to diagnose RA with 76.2% sensitivity and 72.9% specificity, and the AUC was 0.768; however, there was no significance for the diagnosis of anti-CCP and RF-negative RA patients ($P=0.160$).
Conclusion: The combination of IgG-type antibodies anti-RBPJ, anti-SH3BGR and anti-PAFAH1B3, the IgM-type antibody anti-PAFAH1B3 as well, has high sensitivity and specificity for diagnosis of RA; especially the IgG-type autoantibody combination has great value for diagnosis of anti-CCP and RF-negative RA patients.

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DOI: 10.1002/eji.201970400

P2004

TNFAIP3-complex dysfunction inhibits autolysosome degradation regulating inflammatory response in ankylosing spondylitis monocytes

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Autolysosome is a stage of autophagy, which includes the fusion of autophagosome and lysosome. The dysfunction of autolysosome will cause protein degradation disorder and metabolic abnormalities. Ankylosing spondylitis (AS) is a chronic autoimmune inflammatory disease with severe inflammatory symptoms in axial skeleton. The unknown cause of inflammation is key to understand pathogenesis of ankylosing spondylitis. TNFAIP3, also named A20, is known with its ubiquitin-related functions to regulate immune activation, of which deficiency is highly related to autoimmune disease. However, the role of TNFAIP3 in autolysosome function contributing to human ankylosing spondylitis has not been reported. Our objective was to study the role and mechanism of TNFAIP3 and its complex in ankylosing spondylitis. First, **TNFAIP3 expression in AS peripheral blood monocytes was decreased (especially non-classical monocytes)**, but unchanged in T and B lymphocytes. In vitro, TNFAIP3 reduced IL1 secretion, which was partially depended on NF-kappaB pathway. In early stage of LPS stimulation, TNFAIP3 induced autophagy which restricted inflammasome activation. After TNFAIP3-interacting protein screening, we found **TNFAIP3-complex could increase autophagy-flex and autolysosome function during early LPS stimulation, and then restricted inflammasome activation**. For the further mechanism study, different fragments of TNFAIP3 were established, and **the functional regions of TNFAIP3 in autolysosome related inflammasome degradation were proved**. Finally, autophagy, inflammasome and IL1 level were tested in AS peripheral blood monocytes. Our study indicates that **TNFAIP3-complex related autolysosome dysfunction in ankylosing spondylitis monocytes leads to IL1 secretion and constant inflammatory reaction**.

P2005

A risk SNP of type 1 diabetes, rs3802604, regulates immunological function via fine-tuning *GATA3* expression in CD8$^+$ T cells

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Type 1 diabetes (T1D) is autoimmune diabetes involved with multiple genes; hence, many genome-wide association studies (GWAS) sought to explore critical genetic risk factors in T1D. Previously, our GWAS data reported an intronic single nucleotide polymorphism (SNP) in *GATA3* (rs3802604G>A), as a risk factor for T1D. However, how this SNP functions and contributes to autoimmunity is still unknown. Here, we demonstrate that rs3802604 modulate *GATA3* expression in CD8$^+$ T cells and *GATA3* expression level is correlated with T1D pathogenesis in the rodent model of T1D. Bioinformatics tools predicted that rs3802604 was in a putative regulatory element. We found that a higher level of GATA3 mini-gene transcript in cells transfected with the rs3802604G-carrying vector *in vitro* because of higher splicing efficiency of the precursor mRNA. Rs3802604G>A altered protein binding motifs, which were predicted with bioinformatics tools and confirmed with an electrophoretic mobility shift assay. Comparing to the homozygotes for A allele, T1D patients homozygous for G allele of rs3802604 demonstrated a higher level of GATA3 expression and IL-2 secretion in CD8$^+$ T cells but

DOI: 10.1002/eji.201970400

not in $CD4^+$ T cells. Furthermore, we found a higher expression level of GATA3 in $CD8^+$ T cells from non-obese diabetic (NOD) mice than the control. The GATA3 expression level in $CD8^+$ T cells was significantly higher in diabetic than in pre-diabetic NOD mice. Our results reveal a newly-identified T1D risk SNP, rs3802604, can fine-tune GATA3 expression and influence the immunological function of $CD8^+$ T cells; thus, it may contribute to T1D pathogenesis.

P2006

Molecular characterization and stratification of idiopathic inflammatory myopathies: on the basis of skeletal muscle transcriptome study

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iIdiopathic inflammatory myopathies (IIM) are a group of heterogenous autoimmune diseases characterised by muscle weakness. Here, we performed skeletal muscle RNA-sequencing (RNA-seq) to discover the muscle transcriptional signature of IIM with different myositis-specific antibodies (MSA) profiles and investigate the potential molecular classification for IIM. Muscle specimen were taken from 60 patients with IIM, 6 patients with non-IIM myopathies and 9 normal controls. IIM patients was dissected into eight groups based on their MSA profiles. Total RNA from muscle specimen were extracted and sequenced using Illumina HiSeq2500. Quantitative RT-PCR and immunohistochemistry were performed to validate the results of RNA-seq. To define the global muscle signature of IIM, all IIM samples were compared to normal controls and total of 1637 transcripts were differentially expressed. Unsupervised hierarchical clustering of these differentially expressed transcripts revealed a prevalent interferon (IFN) signature in IIM. Then clustering of the interferon-stimulated genes (ISGs) showed distinct ISG expression between different MSA subgroups. An IFN signature scoring system was established to quantify the IFN activity and subsequently IIM could be classified into IFN-Dominant, IFN-Moderate and IFN-Weak respectively based on the IFN intensity and MSA status. Moreover, the results of quantitative RT-PCR and immunohistochemistry also confirmed that IFN-Dominant group had much higher ISG expression than the IFN-Moderate and IFN-Weak group. In conclusion, we revealed a prominent IFN signature and MSA-based ISG expression heterogeneity in IIM through muscle transcriptomics.

P2007

Increased interleukin -22 levels in lupus nephritis and its association with disease severity in both patients and lupus-like mice model

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Objective: Interleukin-22 (IL-22) has been considered as an inflammatory cytokine. In the present study, we investigated the potential role of IL-22 in lupus nephritis (LN).
Methods: We examined the IL-22 levels of serum and kidney tissue from LN patients and MRL/lpr mice. An intraperitoneal injection of saline, isotype control antibody (IgG), prednisone (3mg/kg/mouse),

DOI: 10.1002/eji.201970400

or anti-IL-22 mAb (5µg/kg/mouse or 25µg/kg/mouse) was administered twice a week from 6 to 18 weeks of age.
Results: IL-22 levels in both serum and kidney were significantly higher in LN patients than healthy controls. The serum and renal levels of IL-22 in MRL/lpr mice were significantly increased over time. **After MRL/lpr mice were treated with anti-IL-22 monoclonal antibody (mAb) for 12 weeks, significantly less urine protein and lower serum levels of creatinine and urea nitrogen were found.** In addition, less renal injury score and fewer number of inflammatory cells per glomerulus were observed in MRL/lpr mice treated with anti-IL-22 mAb as compared with control groups.
Conclusions: Our results suggest that **IL-22 as a pathogenic cytokine might be a potential target for treatment of lupus nephritis.**

P2008

Nasal insufflation of a T cell epitope on human α3(IV)NC1 could prevent the development of experimental anti-GBM disease

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Anti-glomerular basement membrane (GBM) disease is a classical autoimmune disease with a rapid development and a poor prognosis.In this study, we aim to prevent the development of experimental anti-GBM disease by nasal administration of P14, the nephrogenic T cell epitope on human α3(IV)NC1.Four group of Wistar Kyoto (WKY) rats were respectively given with P14 nasally at a dose of 80µg, 40µg, 20µg, 10µg at day -3, -2, -1 before immunization with P14 to induce experimental anti-GBM disease. The urinary protein, serum creatinine and blood urea nitrogen were detected to evaluate renal function. Detection of IgG deposition and periodic acid-Schiff (PAS) staining of kidney section were used to assess the kidney injury. Nasal insufflation of P14 at a total dose of 120µg or 60µg could significantly prevent the development of anti-GBM disease in WKY rats, with an improvement of renal function and a mild kidney injury. And rats given nasally with P14 at a total dose of 120µg or 60µg also had a less level of circulating anti-α3(IV)NC1 antibody. Therefore, nasally insufflation of P14, the T cell epitope on human α3(IV)NC1 could significantly prevent the development of experiment anti-GBM disease in WKY rats, indicating a protective role of mucosal tolerance in the prevention of anti-GBM disease and providing a new therapeutic strategy for human anti-GBM disease.

P2009

The co-stimulatory molecule B7-H1 involves in the function of human gingiva-derived mesenchymal stem cells depending on STAT-3 signaling on autoimmune arthritis

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Objective: Human gingival-derived mesenchymal stem cells (GMSCs) are heterogeneous and identification of specific cell population that holds the immune regulatory and therapeutic effect is important. We herein demonstrate that B7-H1 expression on GMSCs provides an ideally molecular marker to identify the cell subset that has a functional activity
Methods: GMSCs were sorted into B7-H1 high or low two subsets by flow cytometry sorter. GMSCs

DOI: 10.1002/eji.201970400

are pretreated with the blocking antibody, rh-IFN-γ or STAT-3 inhibitor for 12h and then were used to conduct the suppressive experiments in vivo or in vitro. GMSCs were transferred to mice through tail veil on collagen-induced arthritis (CIA) mice.
Results: B7-H1 showed high expression on GMSCs compared with control cells prepuce derived fibroblasts (PDFs). The B7-H1high GMSCs subset showed much greater immune-suppressive effect compared with B7-H1low GMSCs subset. The former subset cells significantly decreased the severity of arthritis and pathology scores, down-regulated inflammatory cytokine (IFN-γ, IL-17a) production, up-regulated Treg cells. Blocking B7-H1 pathway resulted in the impairment of the suppressive effect from the GMSCs. On the other hand, over-expressed the B7-H1 on GMSC strengthened the immune-suppression of GMSCs. Pretreatment of the GMSCs with STAT-3 inhibitor significantly eliminated the suppressive effect of GMSCs.
Conclusion: B7-H1 expressed on GMSCs plays a crucial role in the immunosuppression of the GMSCs on CIA pathology and the effect depends upon STAT3 pathway. Thus, we have identified B7-H1+ GMSCs as a much more efficient GMSCs subset that may provide a new therapeutic strategy for patients with rheumatoid arthritis.

P2010

The role of circulating IgA1 complexes in the pathogenesis of IgA nephropathy

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IgA nephropathy (IgAN) is characterized by predominant IgA deposition in glomerular mesangium. Previously studies proved renal deposited IgA in IgAN came from circulating IgA1 containing complexes (CIC). Here we explored the composition of the CIC in IgAN to identify key components in the complexes. In total, 35 IgAN patients and 17 healthy controls were recruited for CIC isolation by affinity chromatography (jacalin column) and gel filtration chromatography (S300 molecular-exclusion column). Next, the isolated CIC were labeled by iTRAQ and quantitatively analyzed by mass spectrometry. Meanwhile, the isolated CIC (100ug/ml) were used to treat cultured human mesangial cells, followed by mesangial cells proliferation, as well as supernatant MCP-1 levels detection to monitor mesangial cells activation after CIC challenge. In CIC, 62 proteins showed significant different levels between IgAN and controls. Among them, the content of α1 microglobulin (A1M) in CIC was associated with not only in vitro mesangial cells proliferation and MCP-1 secretion, but also in vivo eGFR levels and tubulointerstitial lesions in IgAN patients. Then we recruited an independent population (218 IgAN patients and 51 healthy controls), and detected plasma levels of A1M-IgA complex using in house developed ELISA for validation. Our results showed the levels of A1M-IgA complex in IgAN patients were significantly higher than in controls. Moreover, A1M-IgA complex levels was also correlated with eGFR and Oxford T score (tubulointerstitial lesion score) in IgAN patients. Our results suggested A1M-IgA complex as an important component in CIC, and elevated A1M-IgA complex in CIC contributed to renal injury in IgAN.

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DOI: 10.1002/eji.201970400

P2011

High levels of plasma galactose-deficient IgA1 are associated with kidney progression in patients with IgA vasculitis nephritis

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Background: IgA vasculitis nephritis (IgA-VN) is the most common secondary IgA nephropathy (IgAN). Many studies have demonstrated that galactose deficient in the IgA1 hinge region (Gd-IgA1) is associated with the development and also progression in primary IgA nephropathy. In this study we aim to evaluate the roles of Gd-IgA1 of kidney progression in IgA-VN in a large Chinese cohort.
Methods: This cohort study enrolled 112 patients with IgA-VN, 15 patients with IgA vasculitis and none kidney involvement, and 108 patients with IgAN. Plasma IgA1 and galactose deficient IgA1 levels at biopsy were measured by ELISA. The primary end point was a 30% decline in eGFR or ESRD or death.
Results: The levels of Gd-IgA1 in IgA-VN patients and IgAN were higher than that of the healthy controls (302.86±54.93 U/ml vs. 303.16±59.43 U/ml vs. 281.30±43.74 U/ml; P=0.047) and also those with IgA vasculitis without kidney involvement (272.65±53.14 U/ml, P=0.036). After adjust clinical data, higher levels of Gd-IgA1 were independently associated with a greater risk of kidney failure (HR=1.703 per 1 standard deviation; 95% confidence interval (CI), 1.233-2.352, P=0.001). Compared with the first Gd-IgA1 quartiles (as a reference), the fourth Gd-IgA1 quartiles group retained a predictive value for poor renal outcome (HR=3.740; 95% CI, 1.204 -11.619; P=0.023).
Conclusions: These data indicate that Gd-IgA1 levels were similarly elevated in adult patients with IgAN and IgA-VN. Moreover, increased Gd-IgA1 levels were both involved in the development and progression in IgA-VN as that observed in IgAN.

P2012

Identification of target genes at juvenile idiopathic arthritis GWAS loci in human CD4$^+$ T cells and neutrophils

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Juvenile idiopathic arthritis (JIA) is a complex autoimmune disorder characterized by inflammation of the joints in childhood. Genome wide association studies (GWAS) have identified numerous JIA risk loci mostly located in noncoding regions of human genome, suggesting that these loci may act as cis-regulatory elements affecting the expression of their target genes. **We took an integrated multi-omics approach to identify target genes in two types of immune cells (CD4$^+$ T cells and Neutrophils) important in JIA pathogenesis.** We first extracted all 127 genomic regions that have been reported to be associated with JIA from GWAS catalog and then found 238 genes correlates with SNP genotype in whole blood in GTEx database (P-value < 0.05). Next, we examined which of these genes showed differential expression between JIA cases and controls in CD4$^+$ T cells and Neutrophils respectively by analyzing microarray data from GEO database. Our *in silico* analysis identified 3 pairs of JIA SNPs and target genes shared by CD4$^+$ T cells and Neutrophils, and 15 pairs uniquely in CD4$^+$

DOI: 10.1002/eji.201970400

T cells and 10 pairs only in Neutrophils (|FC|>1.2, PFP < 0.05). Next, we tried to understand functional mechanism of these genes by constructing protein-protein interaction network and conducting pathway enrichment analysis. In addition, we explored SNP-gene regulatory mechanism using Hi-C data in public domain. **Functional studies are underway to examine target genes SUOX in Jurkat cells and TRIM58 in HL-60 cells, which may help to understand how these genes mediate the effect of their corresponding GWAS loci in JIA pathogenesis.**

P2013

Plasma galactose-deficient IgA1 and C3 and CKD Progression in IgA nephropathy

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Background and objectives: Increased circulating galactose-deficient IgA1 and subsequently complement activation both play important roles in the pathophysiology of IgA nephropathy. However, their relationship to disease severity and progression remains unclear.
Design, setting, participants, & measurements: In this study, we assessed 1300 IgA nephropathy patients with a median follow-up period of 43 months. Levels of plasma galactose-deficient IgA1 and complement component C3 were measured at the time of biopsy, and renal survival was modeled using restricted cubic splines.
Results: The association of galactose-deficient IgA1 levels and renal outcomes showed a nonlinear relationship. The risk of kidney failure increased with the increase of plasma galactose-deficient IgA1 levels but reached a plateau when galactose-deficient IgA1 >324.7 U/ml, while the risk of kidney failure stably increased with the increase of galactose-deficient IgA1/C3 ratio. After adjusted for traditional risk factors, higher levels of galactose-deficient IgA1/C3 ratio were independently associated with kidney progression events (per ln (galactose-deficient IgA1/C3), HR=2.03, 95% CI=1.25-3.29, P=0.004). In reference to the first quartile of the galactose-deficient IgA1 /C3 ratio, HRs for the second quartile was 1.71(95% CI=1.01-2.89, P=0.05), 1.55(95% CI=0.91-2.63, P=0.10) for the third, and 2.17(95% CI=1.33-3.56, P=0.002) for the fourth quartile.
Conclusion: Plasma galactose-deficient IgA1 levels showed a non-linear relationship with renal outcomes, while galactose-deficient IgA1/C3 ratio was an independent risk factor for kidney progression events and showed a linear relationship with renal outcomes.

P2014

Activation of the PGC-1β/NFATc-1 pathway in circulating osteoclast precursors is associated with bone destruction in rheumatoid arthritis

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Objectives: Activation of osteoclastogenesis at the bone site in rheumatoid arthritis (RA) is well established. The mechanisms by which circulating osteoclast precursors contribute are still unclear. Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1β is implicated in transcriptional

DOI: 10.1002/eji.201970400

regulation of osteoclastogenesis in mouse models. This study investigates the contribution of PGC-1β in circulating osteoclast precursors and its link to RA bone destruction.
Methods: PGC-1β expression in RA peripheral CD14+ monocytes was increased and correlated with X-rays joint destruction. Cells from RA patients or healthy controls were transfected with a lentivirus vector for PGC-1β gene silencing or over-expression and cultured with M-CSF and RANKL. Bone resorption activity, bone degrading enzymes and signaling molecules were measured in these mature osteoclasts.
Results: Increased nuclear accumulation of PGC-1β was observed in RA peripheral CD14+ monocytes and these cells had stronger osteoclastogenesis than in healthy controls. PGC-1β protein expression was positively correlated with radiographic joint destruction (r=0.396~0.413, all P< 0.05). PGC-1β knockdown suppressed the expression of cathepsin K, TRAP and MMP-9 (51%~82% reduction), osteoclast differentiation and bone resorption activity. Conversely, PGC-1β over-expression increased these markers (1.5~1.8 folds) and osteoclastogenesis. VIVIT, an inhibitor of NFATc-1 activation, inhibited the effect of over-expressed PGC-1β by reducing cathepsin K, TRAP and MMP-9 expression. Chromatin immunoprecipitation and dual-luciferase reporter gene assay showed PGC-1β bound to NFATc-1 promoter, leading to transcription activation.
Conclusion: Activation of the PGC-1β/NFATc-1 pathway in circulating osteoclast precursors was associated with bone destruction in RA. This may represent a new treatment target.

P2015

Inhibition of Kappa free light chains (KFLC) alleviated inflammation and epidermal hyperplasia in imiquimod-induced psoriasis-like skin disease

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The production of Kappa free light chains (KFLC) of immunoglobulins is abnormally high in several pathological autoimmune conditions and considered as an inflammation marker, but the physiological role and molecular mechanism remain unclear. Psoriasis is a chronic recurrent skin inflammatory disease, and inhibition of inflammation may be an effective mean of treating psoriasis. In this study, by using an experimental mouse model of psoriasis induced by imiquimod (IMQ), we investigated the effect of KFLC inhibitor named F991 on psoriasis inflammation and epidermal hyperplasia. KFLC expression were located on sebaceous gland cells and epidermis and expression reduced by F991 treatment. In IMQ-inducing psoriasis skin lesion on BALB/c mice, F991 didn't affect psoriasis area and severity index (PASI) scoring including skin erythema, scaling, and papule formation. But Histologic analysis showed that epidermis thickening and excessive infiltration of inflammatory cells found in psoriasis-like skin were alleviated obviously by administration of F991. Moreover, F991 treatment reduced imiquimod-induced inflammatory cytokines significantly such as IL-6 and IL-17, while the IL-10 level increased a lot. No clear change of IFN-γ and TNF-α were found with F991 treatment. Taken together, targeting of KFLC could reduce psoriasis inflammation and epidermal hyperplasia. Our findings may open new perspectives for understanding the mechanisms and developing a therapeutic strategy for psoriasis.

DOI: 10.1002/eji.201970400

P2016

A novel autoimmune disease variant of IgG1 modulates the fate decision of autoreactive B cells

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Here we identify a novel variant of human membrane bound IgG1 which affects the fate decision of autoreactive IgG1+ B cells. The hIgG1-G396R positively correlates with the onset and progression of SLE in patients. Using knock-in mice harboring the corresponding G390R variant, we validate the pathogenic roles of this variant in vivo. In two types of inducible autoimmune murine models, G390R variant mice generate excessive amount of plasma cells with dominant autoreactive repertoires, leading to a burst of broad spectrum autoantibodies. This increase in autoreactive IgG1+ plasma cells is due to the biased differentiation from autoreactive IgG1+ GC B cells and memory B cells. We demonstrate that G390R variant revokes the anergic state of autoreactive IgG1+ memory B cells, which leads to their intensive proliferation and the collapse of immune tolerance.
Mechanistically, the G390R variant lowers the threshold for IgG1-BCR activation by potentiating the phosphorylation of IgG1 immunoglobulin tail tyrosine (ITT) motif by Lyn kinase, thereby altering the spatial organization and availability of phospho-ITT motif to enhance the recruitment of the Grb2 and Btk signaling molecules into the immunological synapses (IS). This triggers significantly longer dwell times of Grb2 in the IS, leading to hyper-activation of the phospho-ITT-Grb2-Btk signaling module upon antigen stimulation. The elevated BCR microclusters formation and excessive Grb2 recruitment were also confirmed in peripheral blood IgG1+ B cells from G396R homozygous SLE patients. Furthermore, we provided insights into the structural basis for the enhanced binding of G390R ITT motif to Lyn kinase by molecular dynamics simulations.

P2017

Normalization of PTEN expression, suppression of B cell hyperresponsiveness and inhibition of lupus development in MRL/lpr mice by miR-7 targeting

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Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by B cells hyperresponsiveness and multiple autoantibodies production. The development of SLE is regulated by numerous miRs, including mIR-7 that suppresses PTEN expression in SLE B cells, which leads to overactivation of the AKT signal pathway and increased B cell activation. Since PTEN can be regulated by numerous mIRs, it remains unclear whether mIR-7 plays an essential role in SLE pathogenesis. To address this issue, we treated $MRL^{lpr/lpr}$mice with an MiR-7 antagomir and analyzed the impact on B cell activation and lupus manifestations. We found that MiR-7 was up-regulated in $MRL^{lpr/lpr}$mice and directly targeted PTEN mRNA in B cells. miR-7 down-regulation of PTEN/AKT signaling promoted B cell differentiation into plasma cells and spontaneous germinal center (GC) formation. MiR-7 not only regulated B cell activation, but miR-7 antagomir intervention also down-regulated STAT3 phosphorylation and production of IL-21. As a result, blocking miR-7 in $MRL^{lpr/lpr}$mice with antagomir reduced follicular helper T (Tfh) expansion and IL-21 production by correcting the down-regulated PTEN expression. These findings collectively implied that miR-7 targeting normalized PTEN expression, inhibited the PI3K/AKT pathway in B cells and reduced Tfh-derived IL-21 stimulation of autoreactive GC B cell, and, thereby, prevented lupus development in $MRL^{lpr/lpr}$mice. Therefore, miR-7 intervention may serve as a potential treatment strategy in SLE.

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DOI: 10.1002/eji.201970400

P2018

Autoimmune liver disease-related autoantibodies in patients with biliary atresia

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Biliary atresia (BA) is a severe neonatal disease, characterized by progressive inflammatory fibrosis and obliteration of bile ducts. Accumulated evidence supports that autoimmune injury mechanism plays an important role in the pathogenesis of this disease. We investigated the prevalence and clinical significance of autoimmune liver disease (ALD)-related autoantibodies in sera of 124 BA patients and 140 age-matched controls by line-blot assay. The overall positive rate of autoantibodies in preoperative BA patients was 56.5%. ALD profile assay showed higher reaction to PBC-related autoantibodies in BA patients than that to AIH. Among these autoantibodies, anti-BPO was detected more frequently in the BA patients (14.8% vs 2.2%, P< 0.05). Accordingly, 25.8% (32/124) BA patients also showed a high positive reaction for anti-M2-3E compared to the controls (8.6%). The prevalence of ANAs in BA patients was 11.3%, which was higher than that in disease controls (3.3%, P < 0.05), but the reactivity to specific ANAs was only 8.2%. The prevalence of ANCAs in BA patients was also remarkably higher than that in the controls (37.9% vs 6.3%, P< 0.05), but showed no difference from that in patients with other cholestasis. ANCA positivity was closely associated with the occurrence of postoperative cholangitis (r=0.61, P< 0.05), whereas none of the autoantibodies showed a correlation to cytomegalovirus infection or the stages of liver fibrosis. It is concluded that high prevalence of autoantibodies in the BA developmental process strongly reveals the autoimmune-mediated pathogenesis. Serological ANCA positivity may be a useful predictive biomarker of postoperative cholangitis.

P2019

Annexin-A1 regulation of human peripheral blood regulatory T cells

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Annexin-A1(ANXA1) is a glucocorticoid-induced multifunctional protein involved in inflammatory response in rheumatoid arthritis（RA）. The level of ANXA1 in the plasma of RA patients and its potential for predicting and treating disease via its regulation of Treg cells needs to be defined in RA. First, we assessed the disease activity of RA patients by examining plasma samples from patients with RA versus healthy donors. We quantified Foxp3 expression in peripheral blood mononuclear cells (PBMCs) via real-time PCR as previously described and observed that Foxp3 mRNA levels were significantly lower in RA patients than in healthy subjects (P< 0.05). Administration of rhANXA1, either at 5 ng/mL or 10 ng/mL, significantly increased the levels of Foxp3 mRNA in PBMCs of RA patients and healthy controls (P< 0.05). Lastly, a significant decrease in the proportion of Tregs was observed in naïve RA patients versus healthy controls (*n*=13, P< 0.05). ANXA1 was correlated with an increased proportion of $CD4^{+}CD25^{+}CD127^{low}$ Tregs. ANXA1 may have a positive effect on Treg proportions via its upregulation of Foxp3 expression. Administration of ANXA1 has an anti-inflammation effect with relatively low doses of rhANXA1. The opposite effect of ANXA1 could be largely dosage-dependent. Therefore, this could be a promising therapeutic target for RA.

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DOI: 10.1002/eji.201970400

P2020

Defective EZH2 expression attenuates Treg differentiation in rheumatoid arthritis

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T cell subsets homeostasis is important in the pathogenesis of rheumatoid arthritis (RA). Here we show reduced expression of H3K27 methyltransferase EZH2 in CD4+ T cells from RA patients. EZH2 inhibition with GSK126 as well as siRNA could reduce naïve T cells differentiation into Treg. Moreover, EZH2 inhibition could downregulate TGF-β/SMAD signaling pathway and Foxp3 expression. Cocultivation with synovial fluid from RA patients resulted in EZH2 suppression in CD4+ T cells. Our data collectively indicate that defective EZH2 expression contributes to attenuated Treg differentiation in RA.

P2021

IL-17-triggered downregulation of miR-497 results in high HIF-1α expression and consequent IL-1β and IL-6 production by astrocytes in EAE mice

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Interleukin 17 (IL-17) is increasingly recognized as a key factor that contributes to the pathogenesis of multiple sclerosis (MS) and its experimental mouse autoimmune encephalomyelitis (EAE) model. However, the roles and regulatory mechanisms of IL-17-induced pro-inflammatory cytokine production in EAE mice remain largely unclear. In this study, the expression of IL-17, hypoxia inducible factor-1α (HIF-1α), IL-1β, IL-6 and microRNA-497 (miR-497), as well as their intrinsic associations, was investigated using EAE model mice and cultured astrocytes exposed to IL-17 in vitro. We observed markedly increased production of IL-17, HIF-1α, IL-1β and IL-6 in the brain tissues of EAEmice, while the expression and secretion of HIF-1α, IL-1β and IL-6 were also significantly increased when cultured primary astrocytes from mice were stimulated with IL-17. Meanwhile, the expression of miR-497 was downregulated both in vivo and in vitro. Subsequent in vitro experiments revealed that IL-17 induced the production of IL-1β and IL-6 in astrocytes through the upregulation of HIF-1α as a transcriptional factor, indicating that IL-17-mediated downregulation of miR-497 enhanced HIF-1α expression. Furthermore, astrocyte-specific knockdown of IL-17RA and HIF-1α or astrocyte-specific overexpression of miR-497 by infection with different lentiviral vectors containing an astrocyte-specific promotor markedly decreased IL-1β and IL-6 production in brain tissues and alleviated the pathological changes and score of EAEmice. Collectively, these findings indicate that decreased miR-497 expression is responsible for IL-17-triggered high HIF-1α expression and consequent IL-1β and IL-6 production by astrocytes in EAE mice.

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DOI: 10.1002/eji.201970400

P2022

Short-chain fatty acids ameliorate anti-glomerular basement membrane disease in WKY rats

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Short-chain fatty acids (SCFAs) had been reported to be protective in many diseases. The pathogenic role of autoreactive Th1 and Th17 cells in the pathogenesis of anti-GBM disease had been fully demonstrated. Thus, we speculated that SCFAs could ameliorate the development of anti-GBM disease.Experimental anti-GBM disease was constructed by immunizing Wistar Kyoto rats with a nephrogenic linear epitope $\alpha3_{127\text{-}148}$. Three major SCFAs, sodium acetate, sodium propionate or sodium butyrate, were respectively provided to the rats in the drinking water since immunization. The levels of urinary protein, serum creatinine and blood urea nitrogen were detected to evaluate the renal function. Kidney injury was accessed by immunofluorescence and PAS staining under light microscopy. Treatment with sodium acetate, sodium propionate, or sodium butyrate could amelioratethe severity of kidney injuries of anti-GBM disease in WKY rats. Particularly, rats treated with sodium butyrate had a significantly lower level of 24h urinary protein, serum creatinine and blood urea nitrogen than that of rats treated with control saline water on the day of sacrifice. They also showed a markedly reduction of the IgG deposition along the GBM and percentage of glomeruli affected by crescent formation. The glomerular $CD4^+$T cell and macrophage infiltration in rats treated with sodium butyrate were significantly reduced, as well as the level of circulating antibodies against anti-α3(IV)NC1.Therefore, the treatment with SCFAs showed a protective role in the development of anti-GBM disease in WKY rats, indicating a therapeutic implication for patients with anti-GBM disease.

P2023

The expression and clinical significance of different forms of LILRA3 in systemic lupus erythematosus

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Objective: Our previous study has shown that functional *Leukocyte immunoglobulin-like receptors A3 (LILRA3)* contributes to susceptibility and subphenotypes of systemic lupus erythematosus (SLE). However, the mechanism remains unclear. We aimed to evaluate the role of LILRA3 in SLE.
Methods: 126 SLE patients and 48 healthy controls were recruited in this study. Functional studies were performed using intracellular flow cytometry and ELISA.
Results: Both LILRA3 levels in serum and $CD14^+$ monocytes were significantly elevated in SLE patients compared with healthy controls. Elevated LILRA3 level was found positively correlated with SLEDAI. Furthermore, more elevated LILRA3 levels were found in patients with higher SLEDAI, presence of lupus nephritis and thrombocytopenia.
Conclusions: Both LILRA3 levels in serum and $CD14^+$ monocytes significantly increased in SLE and positively correlated with disease activity and severity. The up-regulation of LILRA3 expression may serve as a biomarker of disease activity and severity of SLE.

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DOI: 10.1002/eji.201970400

P2024

Myeloperoxidase autoantibodies induce platelet activation primed by low concentration thrombin

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Anti-neutrophil cytoplasmic autoantibodies (ANCAs) directed to myeloperoxidase autoantibodies (MPO) are in the circulation of most patients with microscopic polyangiitis and pauci-immune necrotizing crescentic glomerulonephritis. Although MPO-ANCA is capable of activating neutrophils to degranulate and produce oxygen radicals, the effect of it in platelet activation has not yet been unveiled. The current study demonstrates that MPO-ANCA causes normal human platelets to activate primed by low doses thrombin. Purified immunoglobulins, MPO-ANCA and its $F(ab')_2$ fragment significantly increase the CD62P expression of platelet in present of thrombin compared with controls. The existence of MPO is confirmed using mass spectrometry technology, after analyzing the $F(ab')_2$ fragment pull-downed proteins. After thrombin priming, platelet not only MPO expresses on its surface and has an activation-dependent manner, but also releases the protein to extracellular. These effects are markedly enhanced the activation of platelet. Additional, there is no significantly improved the CD62P-expression on platelets in MPO-ANCA group compared with controls for adding MPO inhibitor, indicating that primed platelets have ANCA antigen at their surfaces interact with the autoantibody. These observations suggest an *in vivo* pathogenic role of MPO-ANCA, thus potentially providing a novel avenue for treatment.

P2025

Distinct correlations between the CD163+ macrophages and the clinical manifestations in different progressive type of symptomatic knee osteoarthritis

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Background/Purpose: Symptomatic knee osteoarthritis (OA) is a heterogeneous disease. There's one subtype shows higher level of inflammation and higher risk of disease progression. In the current study, we analyzed the clinical characteristics of patients with different progressive type of OA, and the correlation between peripheral blood and synovial CD163 + macrophages and ERS, CRP, BMI, WOMAC scores in rapidly and slowly progressive OA patients.
Methods: We categorized 42 patients (32 female and 10 male) with OA for primary total knee arthroplasty (TKA) into rapidly progressive group（ROA,＜10 years, n=23,） and slowly progressive group (SOA, ≥10 years, n=19). The number of CD163+ macrophages in peripheral blood and synovium were detected by flow cytometry. The correlation with ERS, CRP, BMI, WOMAC pain, stiffness and function The correlation of CRP and BMI between the ROA and the SOA were analyzed.
Results: There was a significant positive correlation between the number of CD163+ macrophages (r=0.702, P=0.000). In ROA group, CD163+ macrophages were significantly increased (peripheral blood: 12.35±4.63 VS 2.26±1.08, t=9.88, p=0.00; synovial membrane: 37.55±13.08 VS 21.08±3.63 ; t=5.75, p=0.00). In ROA group, the WOMAC pain score was correlated with the number of CD163+ macrophages in peripheral blood (t=-0.228, p=0.037), but not with CRP, ESR and BMI. The WOMAC

DOI: 10.1002/eji.201970400

pain score was positively correlated with BMI (t = 0.565, P = 0.012) in SOA patients.
Conclusion: CD163+ macrophages and obesity may participate in different progressive type of OA.

P2026

Identification of novel serological biomarkers for systemic sclerosis diagnosis using protein array

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Objective: Systemic sclerosis (SSc) is a systemic autoimmune disorder which leads to vasculopathy, tissue fibrosis and autoantibodies. For SSc, biomarkers mean a lot to diagnosis in the clinics. To undertake this challenge, we employed the HuProt arrays, each comprised of 20,000 unique human proteins, to identify SSc-specific autoantibodies using a Two-Phase strategy established previously.
Methods: In Phase I, we profiled the autoimmunity on the HuProt arrays with 90 serum samples collected from 40 SSc patients, diagnosed autoimmune patients who suffer from rheumatoid arthritis (RA; n=6), systemic lupus erythematosus (SLE; n=7), Sjogren's syndrome (SS; n=7), dermatomyositis (DM; n=5), polymyositis (PM, n=5) and 20 healthy subjects, and identified 113 candidate autoantigens that were significantly associated with SSc. To validate these candidates, in Phase II we constructed a focused array with these 113 candidate SSc-associated antigens, and use it to profile a much larger cohort, comprised of serum samples collected from 400 SSc patients, 200 autoimmune patients (i.e. 42 RA, 44 SLE 44 SS, 30 DM and 30 PM), and 130 healthy controls.
Results: This allowed us to validate SNRPA as a SSc-specific autoantigen in SSc. The results showed that the positive rate of anti-SNRPA antibody in patients with SSc was 11.25%, significantly higher than 3.33% in all control groups and 0% in normal controls. The association of anti-SNRPA with SSc patients was further validated using the traditional Western blotting analysis.
Conclusion: In conclusion, anti-SNRPA antibody serves a novel autoantibody for SSc.

P2027

Induction of acute and chronic arthritis using monoclonal antibodies binding to cartilage proteins

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Objective: To develop and characterize a new arthritogenic monoclonal antibody cocktail binding to different cartilage proteins that can induce acute and chronic arthritis in mice.
Methods: Antibodies to unmodified and citrullinated collagen type II, type XI and cartilage oligomeric matrix protein were produced as culture supernatant and affinity purified using Gamma-bind plus column. Two antibody cocktails (Cab3 and Cab4) were injected intravenously into mice to induce arthritis. Well defined adjuvants, LPS or mannan were injected on either day 5 and/or 60 to enhance the incidence and severity of arthritis. Joint inflammation was followed up to 21 or 95 days. Antibody binding and complement activation on the cartilage surface were demonstrated by immunohistochemistry and immunofluorescence methods. Bone erosion and destruction were analyzed by histology and Micro-CT.
Results: The new antibody cocktails are more efficient than anti-CII antibody cocktail. Cab3 induced earlier and more severe disease. We have now established two new models of arthritis; Cab3-LPS induced arthritis (CLIA) and Cab3-mannan induced arthritis (CMIA). Cab4 was found to be efficient

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DOI: 10.1002/eji.201970400

cocktail to induce arthritis in moderately susceptible C57Bl/6 mice. We also found severe bone loss in both CLIA and CMIA models.
Conclusion: Two different antibody cocktails and methods for arthritis induction were established and characterized with monoclonal antibodies binding to different cartilage proteins. These models could be useful for screening potential drug candidates for treating arthritis.

P2028

The effect of autoimmune responses against α-enolase on the function of central nervous system

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Objective: Explore the effect of autoimmune responses against α-enolase (ENO1) on the function of central nervous system (CNS).
Methods: 5-week-old CBA/J female mice were randomly grouped and treated with 100 µg ENO1 recombinant protein (ENO1 group) or PBS (Control group) in Freund adjuvant twice with 2 weeks interval. The spatial cognitive learning ability and expression levels of related proteins were assessed and compared between two groups on week 10 (W10), week 14 (W14) and week 18 (W18) after the last immunization.
Results: MWM test showed that daily average escape latency times prolonged and the frequencies of passing through the objective zone and platform area also lowered in ENO1 group compared with control group on W14 and W18. Findings by LTP showed that f-EPSP slope in ENO1 group was markedly lower than that in control group (158.86 ± 11.80% vs 274.85 ± 15.56%). Evident edema and IgG deposition around microvessels were observed by electron microscope in cortex and hippocampus on W10 in ENO1-immunized mice. Compared with control group, the expression levels of endothelial tight junction proteins (Claudin-5 and Occludin) were significantly lower, and the expression levels of Iba-1, GFAP, IL-6, cyclin dependent -5 (CDK- 5) and phosphorylated Tau in ENO1 group were much higher in ENO1 group.
Conclusions: ENO1Ab can impair the function of vascular endothelial cells, which stimulates the hyperplasia and activation of glial cells and the overexpression of proinflammatory cytokines (e.g. IL-6), and eventually lead to the impairment of neuron function through abnormal hyperphosphorylation of tau, catalyzed by CDK-5.

P2029

PTX3 synegizes with C1q in promoting caspase-1-dependent pyroptosis and NLRP3 inflammasome over-activation in rheumatoid arthritis

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Inflammatory cytokine storm plays crucial roles in the pathogenesis of rheumatoid arthritis (RA), however, its underlying mechanisms remains unclear. In this study, we demonstrated increased caspase-1-dependent pyroptosis in RA monocytes. Serum from active RA could induce gasdermin D (GSDMD)-dependent pyroptosis in monocytes. RA neutrophils were the major cells producing increased level of pentraxin 3 (PTX3) that preferentially bound to $CD14^{+}$monocytes. And in RA patients, C1q promoted the binding of PTX3 to monocytes and helped switching apoptosis to pyroptosis by coordinately inducing GSDMD cleavage, cell swelling with large bubbles and

DOI: 10.1002/eji.201970400

inflammatory cytokine (TNF-a, IL-1b, IL-6 and IL-18) release. In addition, increased expression of activation marker CD40 as well as caspase-1-related late apoptotic cells (7-AAD$^+$annexin V$^+$) were also observed in RA monocytes after PTX3 plus C1q treatment.These findings collectively implicate an important role of PTX3 synegizing with C1q in promoting GSDMD-mediated, caspase-1-dependent pyroptosis in RA and shed lights on potential new treatment strategy targeting pyroptosis-mediated persistent inflammatory cytokine release.

P2030

A rare variant (rs933717) at FBXO31-MAP1LC3B in Chinese is associated with systemic lupus erythematosus

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Objectives: Recent evidence from genetic, cell biology and model animal studies have suggested a pivotal role of autophagy in mediating systemic lupus erythematosus (SLE). However, the genetic basis has not yet been thoroughly examined. The aim of the present study was therefore to identify additional susceptibility variants in autophagy-related genes, and their functional significance.
Methods: First, we performed a gene family-based genetic association analysis in patients with SLE using ImmunoChip, and selected the top-associated polymorphisms for replication in additional cohorts. Publically-available datasets including eQTL and ENCODE on transcription factor binding sites and cell-type specific differential expressions were analyzed to identify regulatory clues. The functional effects were tested by luciferase reporter assays, electrophoretic mobility gel shift assays (EMSA) and differential gene expression assays.
Results: In 14,474 samples, we observed that the rare Chinese variant rs933717T was associated with susceptibility to SLE (case 0.11% vs. control 0.87%, $p = 2.36 \times 10^{-10}$, OR = 0.13). The rs933717 risk allele C correlated with increased *MAP1LC3B* expression: increased *MAP1LC3B* mRNA was observed in patients with SLE and in lupus-prone mice. In reporter gene constructs, the risk allele increased luciferase activity up to 4-fold in both HEK293T and Jurkat cell lines, and the binding of HEK293T and Jurkat nuclear extracts to the risk allele was also increased.
Conclusion: We observed a likely genetic association between LC3B, a widely-used marker for autophagy, and susceptibility to SLE.

P2031

The possible effect of B-cell epitopes of epstein-barr virus early antigen, membrane antigen, latent membrane protein-1,and -2A on systemic lupus erythematosus

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This study was aimed to evaluate the role of B-cell epitopes of Epstein-Barr virus (EBV) Early antigen protein D (EA), membrane antigen (MA), latent membrane protein (LMP)-1, and LMP-2A in systemic lupus erythematosus (SLE). B-cell epitopes were predicted by bioinformatics technology. 60 female mice were ran-domized equally into 12 groups: 1-10 groups were immunized by epitope peptides (EPs) 1-10, respectively, while 11 and 12 groups were PBS and Keyhole limpet hemocyanin (KLH)

DOI: 10.1002/eji.201970400

control groups. Immunoglobulin G (IgG) and autoantibody to nuclear antigen (ANA) concentrations in mice serum were determined at week 8. Indirect levels of EP1-10 were further detected by enzyme-linked immuno sorbent assay (ELISA) in 119 SLE patients and 64 age- and gender-matched health controls (HCs). 10 probable EBV EA, MA, LMP-1, and LMP-2A B-cell epitopes related to SLE self-antigens were predicted and corresponding EP1-10 were synthesized. IgG concentrations at week 8 were increased in EP1-10 and KLH groups compared with PBS group in mice; while ANA levels were elevated in only EP1-4, EP6-7, and EP10 groups compared to KLH group by ELISA, and ANA-positive rates were increased in only EP1, EP2, EP4, EP6, and EP10 groups by indirect immunofluorescence assay. EP1-4, EP6, and EP10 indirect levels were increased in SLE patients than HCs, while EP1, EP3, EP6, and EP9 were correlated with SLE disease activity index score. In conclusion, EBV EA, MA, LMP-1, and LMP-2A B-cell EPs increased SLE-related autoantibodies in mice, and their indirect levels might be served as potential biomarkers for SLE diagnosis and disease severity.

P2032

The association of tea consumption with the disease activity of rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by synovitis and irreversible joint destruction. It has been reported that epigallocatechin gallate (EGCG), a polyphenol isolated from tea leave, mediated inhibition of G3BP1 provides a potential treatment for cGAS-related autoimmune diseases. But it has not been very clear that the relation between tea consumption and disease activity of RA. The present study is designed to investigate whether tea consumption has an influence on disease activity of RA.
Methods: This was a large scale, cross-sectional study composing of 733 patients recruited from Peking University People's Hospital from January to August, 2016. The tea consumption was assessed by a self-administered questionnaire. These patients were divided into two groups according to disease activity scores in 28 joints (DAS28)-ESR: DAS28-ESR < 3.2 and DAS28-ESR ≥ 3.2. The Mann-Whitney and chi-square test were used to determine any statistical differences between the means and proportions of the two groups. The stratified analysis was conducted to verify the stability of the results and multiple logistic regression analysis was applied to identify whether disease activity of RA could be influenced by tea independently.
Results: Multivariate analysis reveals that there is significant negative association between the disease activity of RA and tea consumption more than 750ml a day (OR = 0.28; 95%CI =0 .14-0.59, $P < 0.01$).
Conclusions: The tea consumption > 750ml/day is obviously negatively correlated with DAS28-ESR. Therefore, drinking more tea could be beneficial for the RA patients.
Key words: rheumatoid arthritis; DAS28-ESR; tea consumption

P2033

Researches on the change of TLR2, TLR4, TLR9 expression before and after treatment of microecologic preparation in patients with ulcerative colitis

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DOI: 10.1002/eji.201970400

To explore the action mechanism of microecologic preparation in the treatment of ulcerative colitis (UC) from the angle of TLR2, TLR4, TLR9. Thirty patients with mild or moderate UC (active phase) were randomly divided into group A,B and C(n=10). The patients in group A were treated with Medilac-S and SASP, those in group B were treated with bifidobacterium capsule and SASP, while those in group C, serving as the control, were only treated with SASP. The treatment lasted four weeks. Colonic biopsies were taken before and four weeks after treatment in order to immunohistochemically detect the expression of TLR2, TLR4, TLR9 protein. The mRNA levels of TLR2, TLR4, TLR9 were assessed by reverse transcriptase polymerase chain reaction. Immunohistochemistry and RT-PCR showed that TLR2, TLR4, TLR9 was intensely expressed in groups A, B, C before treatment and weakly or negative expressed in those groups after treatment. There was significant difference in the expression of TLR2, TLR4, TLR9 in those groups between before and after treatment ($P< 0.05$). Comparison among the three groups showed no significant difference in the TLR2, TLR4, TLR9 expression between groups A and B($P>0.05$)and significant difference existed between group A or B and group C ($P< 0.01$). Microecologic preparation can reduce the expression level of TLR2, TLR4, TLR9 in UC patients, the decreasing of TLR2, TLR4,TLR9 which may be the action mechanism of of microecologic preparation in the treatment of UC.
Funding: This work was financially supported by the Hainan Natural Science Foundation Project (No. 819MS063).

P2034

Joint deposited lupus IgG causes arthritis but inhibits bone destruction

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Background: Bone destruction is remarkable feature in inflammatory arthritis, however it remains unclear that the pathogenesis of arthritis lacking bone erosions in patients with systemic lupus erythematosus (SLE), which is a chronic autoimmune disease characterized by high levels of autoantibody and multi-organ tissue damage. We aimed to determine the role of autoantibody in the development of arthritis and bone destruction in SLE.
Methods: We analyzed medical record of SLE patients, investigated whether autoantibody induces arthritis lacking bone destruction in animal models, determined whether SLE autoantibody inhibits osteoclastogenesis induced by RANKL in vitro experiments.
Findings: We found that arthritis lacking bone erosions is common in SLE patients and lupus-prone mice. Intraarticular injection of lupus serum IgG induces arthritis. Severity of arthritis depends on dose of injected serum. Deposition of IgG, monocytes/macrophages and TNFa are all required for the development of arthritis. lupus serum IgG inhibits osteoclastogenesis. The deposited lupus IgG inhibits bone destruction through occupying signaling of FcgR/Syk. FcgRI/Syk signaling has regulatory effects in arthritis and bone destruction in animal models.
Conclusions: Our results demonstrate mechanism that joint deposited lupus IgG causes arthritis lacking bone erosions. We anticipate that our findings promote understanding of lupus arthritis and suggest that autoantibody IgG should also be of clinical values in inhibition of bone destruction although triggering organ tissue damage.

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DOI: 10.1002/eji.201970400

P2035

Investigating glandular fibrosis in patients with primary Sjögren´s syndrome and its potential mechanism

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Background: Primary Sjögren´s syndrome (pSS) is a complex autoimmune disease with exocrine gland inflammatory cell infiltration andirreversible fibrogenesis development at late stage of disease. The fibrosis mechanism of the salivary glands remains unclear. In present study, we evaluated the fibrosis of salivary gland in pSS patients, and explored the possible mechanism.
Methods: Histology was performed on pSS patients and normal controls for lymphocyte infiltration (Focal score, FS). Masson trichrome staining was utilized to measuretissue fibrosis conditions. Immunohistochemistry was also utilized to describetissue-infiltrating lymphocyte subtypes and collagen types. The correlationof degree of fibrosis in salivary gland was compared to clinical parameters of pSS patients and infiltrated lymphocyte subtypes.
Results: The histology results showed that T cell infiltraion are presented in anentire stage of pSS development, while the degree of B cell infiltration got worse compared tocollagen deposition in pSS salivary glands.,. Degree of salivary gland fibrosis was positively related to FS. The above results indicated that B lymphocytes infilitration might involved in glandular fibrogenesis
Conclusion: Lymphocyte infiltration was related to salivary gland fibrosis in pSS patients, and B cells might paly the major role in pSS glandular fibrogenesis . Therefore, B cell deletion might be a potential optional regimen to improve salivary gland fibrosis.

P2036

Immunomodulatory effect of Schisandrae oil in mouse model of autoimmune hepatitis induced by Concanavalin A

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Objective: To study the immunomodulatory effect of Schisandra oil (SCO) in mice model of autoimmune hepatitis (AIH) induced by Concanavalin A (ConA).
Methods: C57BL/6 mice were divided into control group, model group and SCO group. Mice in SCO group were given SCO at 5mg/kg by intragastric administration every day for 7 days, followed by ConA-induced model of AIH. 10 hours after ConA injection, the levels of serum transaminase ALT, AST and lactate dehydrogenase (LDH) were measured by the assay kits, the expression of a series of inflammatory cytokines in liver, spleen and mesenteric lymph nodes (MLN) were detected by qPCR, and the T cell activation and IFN-γ expression in spleen and MLN were examined by flow cytometry.
Results: Compared with control group, each indicator in model group were significantly higher. In SCO preventive treatment group, the levels of serum ALT, AST and LDH were significantly reduced, the expression levels of inflammatory cytokines in liver were downregulated to varying extents, the T cell activation in spleen and MLN was inhibited, the percentage of IFN-γ^+ $CD8^+$ and IFN-γ^+ $CD4^+$ T cells was decreased, and the frequency of Th2 and Th17 cells in spleen and MLN was also decreased at the same time.
Conclusion: SCO has a protective effect on immune liver injury by inhibiting the activation of T cells and reducing the expression of inflammatory cytokines, which reflects that SCO plays a role in the

DOI: 10.1002/eji.201970400

immunomodulation of AIH, indicating that SCO is of great significance for the maintenance of autoimmune homeostasis.

P2037

Complement activation products in the circulation and urine of primary membranous nephropathy

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Background: Complement activation plays a substantial role in the pathogenesis of primary membranous nephropathy (pMN). C5b-9, C3c, MBL, and factor B have been documented in the subepithelial immune deposits. However, the changing of complement activation products in circulation and urine is not clear.
Methods: We measured the circulating and urinary levels of C1q, MBL, C4d, Bb, properdin, C3a, C5a, and sC5b-9, in 134 patients with biopsy-proven pMN, by ELISA. All the plasma values were corrected by eGFR and all the urinary values were corrected by urinary creatinine and urinary protein excretion. Anti-PLA2R antibodies were measured in all patients.
Results: The plasma complement activation products were elevated both in the patients with and without anti-PLA2R antibodies. C3a levels were remarkably increased in the circulation and urine, much higher than the elevated levels of C5a. C5b-9 was in normal range in plasma, but significantly higher in urine. The urinary C5a had a positive correlation with anti-PLA2R antibody levels and urinary protein. Positive correlations were observed between plasma C4d/MBL and urinary protein, only in the patients with positive anti-PLA2R antibodies but not in those without. The plasma level of Bb was elevated and had positive correlation with urinary protein only in the patients without anti-PLA2R antibodies.
Conclusion: Complement activation products were remarkable increased in pMN and may serve as sensitive biomarkers of disease activity. The complement may be activated through lectin pathway with the existence of anti-PLA2R antibodies, while through alternative pathway in the absence of antibody.

P2038

Analysis of anti-RNA polymerase III antibodies in Chinese Han systemic sclerosis patients

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Aim: To assess the prevalence and clinical correlation of anti-RNA polymerase III antibodies (anti-RNAP III) in Chinese Han systemic sclerosis (SSc) patients.
Methods: Serum samples from 236 patients with SSc, 125 patients with connective tissue diseases (CTD), and 78 healthy controls (HCs), recruited from Peking Union Medical College Hospital and 21 other medical centers in China, were tested for antibodies to RNA polymerase III by means of a line immunoassay (LIA) or an enzyme-linked immunosorbent assay (ELISA) kit.
Results: Anti-RNAP III antibodies were found in 14/236 SSc patients (5.93%), 1/125 (0.80%) CTD patients, and 0/78 (0.00%) HCs. The prevalence of anti-RNAP III was higher in SSc patients than in the CTD and HC groups (p=0.002). The renal crisis was significantly more common in patients with anti-RNAP III than patients without anti-RNAP III (42.9% vs. 4.1%, $p < 0.0001$). Gastrointestinal

DOI: 10.1002/eji.201970400

involvement was significantly more common in patients without anti-RNAP III than patients with anti-RNAP III (53.6% vs. 21.4%, *p*=0.039). There was good agreement between the ELISA and line immunoassay (LIA) detection capabilities for anti-RNAP III.
Conclusions: The anti-RNAP III antibody, which was detected by ELISA, has diagnostic value for SSc and predictive value for SSc-related renal crisis. Both ELISA and LIA are very reliable methods for anti-RNAP III.

P2039

Evaluation of a commercial immunoassay for autoantibodies in Chinese Han systemic sclerosis population

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Objective: The autoantibody profile, which varies between races, has rarely been reported in Chinese Han systemic sclerosis (SSc) patients. The present study aims to investigate the autoantibody profile of Chinese Han SSc patients using a commercial line immunoassay.
Methods: The prevalence of autoantibodies to Ro-52, PDGFR, Ku, PM75, PM100, Th/To, NOR90, Fib, RP155, RP11, CENP-B, CENP-A and Scl-70 were analyzed in serum samples obtained from healthy controls (*n*=30), SSc patients (*n*=320) and non-SSc connective-tissue disease patients (*n*=100) using an Euroline Systemic Sclerosis Profile kit.
Results: SSc patients had increased prevalence of anti-RP11 (*P*=0.032), anti-CENPB (*P*< 0.001), anti-CENPA (*P*=0.001) and anti-Scl-70 (*P*< 0.001), but had decreased prevalence of anti-Ro-52 (P=0.004), when compared to non-SSc CTDs. Furthermore, SSc patients had increased prevalence of anti-Ro-52 (*P*=0.003), anti-CENPB (*P*=0.034), anti-CENPA (*P*=0.034) and anti-Scl-70 (*P*< 0.001), when compared to HCs. In addition, SSc patients with interstitial lung disease (ILD) had increased prevalence of anti-Ro-52 (*P*=0.046) and anti-Scl-70 (*P*=0.035. Moreover, SSc patients with pulmonary arterial hypertension (PAH) had increased prevalence of anti-Ro-52 (*P*=0.013) and decreased prevalence of anti-Scl-70 (*P*=0.001). Anti-Ro-52 and anti-Scl-70 had increased values for predicting ILD in SSc patients, while anti-Ro-52 had increased values for predicting PAH in SSc patients.
Conclusion: A commercial line immunoassay was used to evaluate the diagnostic value of autoantibodies in Chinese Han SSc patients. The results clearly indicate that the immunoblot assay has good diagnostic utility for SSc. Anti-Ro-52, anti-RP11, anti-CENPB, anti-CENPA and anti-Scl-70 are useful for diagnosing SSc in the Chinese Han population.

P2040

The immunological characteristics of serum anti-glomerular basement membrane antibodies in HIV/AIDS patients

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Anti-glomerular basement membrane disease (GBM) is an autoimmune disease caused by the deposition of circulating anti-GBM antibodies. The main target antigens are the non-collagen region of the α3(IV)NC1(α3 chain of type IV collagen). EA and EB are two classical antigen epitopes on α3. It reported that about 17% of AIDS patients can detect anti-GBM, but its immunological characteristics

DOI: 10.1002/eji.201970400

are still unclear. In this study, the immunological characteristics of the target antigens were clarified. Total 93 HIV/AIDS patients and 20 healthy volunteers were selected in Beijing Youan Hospital from 2017 to 2018. Recombinant human α1-α5(IV)NC1, chimeric protein EA, EB were used as solid phase antigens. ELISA was employed to measure concentrations and subtypes of serum IgG autoantibodies. Five patients (5/93, 5.38%) had detectable levels of autoantibodies against GBM, in which four (4/93, 4.30%) had anti-α3 and two (2/93, 2.15%) had anti-α5. IgG3 was dominant subtype of autoantibody against α3 (4/4, 100%). None of them recognized classical antigen epitopes EA and EB. Clinical data revealed that there was no proteinuria or hematuria in these positive patients. Furthermore, there were no significant differences in gender, age, peripheral blood CD4+ cell count and HIV viral load between anti-GBM positive or negative. Anti-GMB could be detected in a small population of HIV/AIDS patients, concentrations were quite low, and which did not recognize classical antigen epitopes. These data suggest that these anti-GMB positive patients might differ from those with classical anti-GBM diseases, so there´s no obvious pathological change.
Keywords: anti-GBM antibody, epitope, IgG subtype, AIDS

P2041

Family history of rheumatic diseases in patients with rheumatoid arthritis- a large-scale cross-sectional study

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Objectives: To determine the associations between family history of rheumatic diseases and clinical features in patients with rheumatoid arthritis (RA).
Methods: 890 RA patients were enrolled, and data were collected including the clinical and laboratory features of the patients. The complete data of 803 patients were finally acquired and compared between those who had family history of rheumatic diseases and those who had not.
Results: In this cohort, the male/female ratio was 1: 3.5, and the age of onset was 45.08±14.46 years. A total of 123 (15.32%) patients were accompanied with family history of rheumatic diseases, including rheumatoid arthritis, spondyloarthritis, Sjogren´s syndrome, systemic lupus erythematosus and systemic sclerosis. The clinical and laboratory characteristics were compared between the patients with and without family history. The onset-age of the subjects was significantly different between those with and without family history of rheumatic diseases (39.97 ±13.68 vs. 46.01 ±14.46; $P < 0.01$). Adjusted by gender, BMI, smoking and alcohol drinking, anti-CCP antibody and RF level, the age at disease onset in patients with family history was 4.54 years earlier than that in patients without family history ($P < 0.05$). Further hierarchical regression analysis showed that, the age at onset of RA patients with family history was 10.02 years earlier than that without family history among smoking patients ($P < 0.01$).
Conclusion: Family history of rheumatic diseases was a risk factor for early onset of RA, and may interact with smoking.
Keyword: Rheumatoid arthritis; Family history; Age at onset; Smoking

DOI: 10.1002/eji.201970400

P2042

Pathological cardiac hypertrophy: the synergistic effect of adenylyl cyclases inhibition in cardiac and immune cells during chronic catecholamine stress

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Chronic catecholamine stress mediated by β-adrenergic receptors (βARs) expressed in cardiac and immune cells has adverse effects on cardiac function. We hypothesis that pathological cardiac hypertrophy (PHC) occurring during chronic catecholamine stress may be resulting from a synergy of adenylyl cyclases (ACs) inhibition in cardiac and immune cells. Also, we hypothesis that ACs may have regulatory effects on the expressions and activities of G-protein-coupled receptor kinases (GRKs) in these cells based on the negative correlations they exhibit between healthy and failing hearts.
Therefore, the downregulation of ACs during chronic stress permits upregulation of GRK5/6 to initiate a GPCR-independent activation of ERK1/2 in a maladaptive manner. ERK1/2 then mediate excessive myocyte hypertrophy which causes arrhythmias, hypoxia and myocyte necrosis. Myocyte necrosis invokes a sterile inflammation (through NF-kB activation which gets sustained by ERK1/2) in an attempt to repress the necrotic myocytes death. However, downregulation of AC7 in immune cells permits hyperactive immune responses which exacerbates myocytes necrosis and pathologically remodels the heart with a marked fibrosis.
In preliminary experiments, ECG recordings for 21 days chronic stress modelled mice (n=7) showed that they had long QT syndrome. RT-qPCR results (n=3) also revealed gene levels of ACs (5, 6 and 7) and Interleukin (IL) -10 were downregulated, while, GRK2 and GRK5, ERK1/2, ANP, BNP, IL-1β, IL-6 and TNF-a gene levels were upregulated compared to their control groups. However, further experiments are necessary to validate our hypothesis and to elucidate the probable mechanism causing PHC during chronic catecholamine stress.

P2043

Identification of novel citrullinated autoantigens in the immune complexes of pateints with rheumatoid arthritis

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Background: A specific feature of the immune response in rheumatoid arthritis is the presence of anti-citrullinated protein antibodies (ACPAs) in sera. Accumulating evidence suggests that they play a role in the pathophysiology of RA. In this study, novel citrullinated autoantigens of RA patients were identified and clinical significance of the corresponding ACPAs was studied.
Methods: The immune complexes in the serum of 10 RA patients were isolated by Protein G chromatography, and then the citrullinated antigen spectrum in the immune complexes were identified by the Orbitrap high-resolution mass spectrometry. The levels of APCAs in serum of 120 RA, 32 OA, 30 SLE, 30 pSS and 51 HC were determined by ELISA (enzyme-linked immunosorbent assay). SPSS17.0 for windows and GraphPad Prism 5 were used to analyze the data.
Result: A total of 11 citrullinated proteins were identified in CICs from RA patients, which had not been reported in previous studies. The autoantibodies against these novel antigens were significantly increased in RA patients compared with that in healthy and disease controls. The sensitivity and specificity of antibodies in the diagnosis of RA in this study were 15.83-28.33% and 88.78-95.92% respectively. They showed supplementary diagnostic significance for CCP negative RA patients. Further correlation analysis showed that the above antibodies were correlated with IgG, IgA, IgM, ESR

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DOI: 10.1002/eji.201970400

and DAS28.
Conclusion: 11 novel citrullinated antigens derived from immune complexes were found and related to RA autoimmune response. The immune complexome analysis by proteomic CIC autoantigen screening is a feasible way of identifying novel citrullinated modifications.

P2044

Interleukin-2 deficiency associated with renal impairment in systemic lupus erythematosus

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Background: Impaired interleukin-2 (IL-2) production was reported in systemic lupus erythematosus (SLE). This study aimed to investigate the clinical relevance of serum IL-2 and therapeutic effects of recombinant IL-2 (rIL-2) in SLE, especially in lupus nephritis (LN).
Method: Serum samples were collected from 89 SLE patients and 66 age- and gender- matched healthy controls (HCs). All of them were treated with corticosteroids, antimalarials and/or immunosuppressants. Fifty out of 80 patients received low dose IL-2 therapy additionally.
Result: Decreased serum IL-2 was found in patients with active LN (P = 0.014) and correlated with 24-h urine protein excretion (r = -0.281, P = 0.026). Compared with LN patients with decreased levels of serum IL-2, patients with increased levels had better remission rate (P=0.041). Furthermore, patients with exogenous low-dose IL-2 supplement demonstrated better improved nephritis and higher remission rate (55.56%, P = 0.058) than those with conventional therapy. In addition, the percentages of regulator T (Treg) cells expanded in LN patients with low-dose recombinant human IL-2 treatment (P = 0.007), especially in LN patients achieving remission (P = 0.010).
Conclusion: IL-2 deficiency is associated with renal impairment that can be improved by exogenous IL-2 supplement.

P2045

Tissue specific autoantibodies improve diagnosis of primary Sjögren's syndrome in early stage and indicate localized salivary injury

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Objectives: Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by lymphocytic infiltration of exocrine glands. Due to the absence of specific clinical manifestations and biomarkers in the early stage, pSS is generally underrecognized. We aimed at elucidating the role of the tissue-specific autoantibodies (TSAs), i.e., anti-CA6, anti-SP1, and anti-PSP antibodies, in pSS.
Materials and methods: We enrolled 137 pSS patients, 32 secondary Sjögren's syndrome (sSS) patients and 127 healthy controls (HCs), whose serum and saliva samples were collected. TSA levels were detected by ELISA, and the medical records were reviewed.
Results: 1. The serum IgA levels of anti-CA6, anti-SP1 and anti-PSP were significantly higher in pSS and sSS patients. Anti-CA6 IgG was notably higher in pSS patients. 2. The positivity of anti-CA6, anti-PSP and all the three antibodies together were significantly increased in anti-SSA-negative pSS

DOI: 10.1002/eji.201970400

patients. 3. The average IgM levels of anti-CA6 and anti-SP1 decreased as the disease duration extended. 4. The anti-CA6-positive patients have significantly higher levels of serum IgA, while the anti-PSP-positive group has a notably higher serum IgM level. 5. Anti-α-fodrin antibody was elevated in TSA-positive patients, especially in the anti-CA6-positive group. 6. Preliminary detection of saliva TSAs showed all the IgG levels of these three antibodies increased significantly in pSS patients. **Conclusion:** TSAs improve diagnosis of pSS in the early stage, especially in anti-SSA-negative patients. Their tissue-specific nature indicates localized salivary injury, which deserves further studies to clarify the mechanism.

P2046

TGF-β isoforms as biomarkers in systemic sclerosis: association with inflammatory parameters and autoantibodies

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Introduction: Systemic sclerosis (SSc) is an autoimmune disease with high mortality, characterized by vascular abnormalities, chronic inflammation, and tissue fibrosis. Transforming growth factor β (TGF-β) has been identified as a regulator of pathological fibrogenesis and inflammation. Therefore, TGF-β isoforms could be a major role in the pathogenesis of SSc.
Aim: To measure the serum levels of TGF-β1, 2, and 3 in patients with SSc and to determine its association with the clinical variables of SSc patients.
Material and methods: A case-control study including 56 SSc patients classified according to the 2013 American College of Rheumatology/ European League Against Rheumatism SSc classification criteria and 115 unrelated control subjects (CS), both from southern Mexico, was performed. The three activated TGF-β isoforms were measured by a BioPlex MagPix system. Anti-centromere (ACAs), anti-topoisomerase (ATAs), anti-RNA Pol lll, and anti-fibrillarin antibodies were determinate by immunoenzymatic assays (ELISA).
Results: We observed significantly lower serum levels of the three activated TGF-β isoforms in SSc patients than in CS ($p< 0.0001$). All three isoforms were positively correlated with C-reactive protein, ACAs antibodies, and platelets ($p< 0.05$). Moreover, TGF-β3 was associated with anti-RNA Pol lll antibodies. No significant associations betweenTGF-β isoforms with skin fibrosis or mouth opening scores were observed in the SSc patients.
Conclusions: The association of TGF-β isoforms with clinical manifestations of SSc patients suggest a prominent role of this cytokine in inflammation and autoantibody production in SSc.

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DOI: 10.1002/eji.201970400

P2047

Increased risk of coronary heart disease among patients with primary Sjögren's syndrome: a nationwide population-based cohort study

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Aim: To investigate the association between primary Sjögren's syndrome (pSS) and coronary heart disease (CHD), and the influence of medications for pSS patients on risk of CHD.
Methods: The authors identified 4175 patients with a new diagnosis of pSS between 2002 and 2013 from the National Health Insurance Research database. The control-to-case ratio was 4:1. The risk and cumulative incidences of CHD were calculated.
Results: The adjusted hazard ratio (HR) of CHD for pSS patients was 1.17 (1.03-1.34) after adjusting for age, sex, comorbidities, and medications. The cumulative incidence for CHD in the pSS group was significantly higher than that in the control group (log-rank $p < 0.0001$). The risk of CHD in pSS patients was increased with age by 4% per year, and 45- to 59-year-olds were at the highest risk (HR = 1.464, 1.195-1.794). The application of corticosteroids (HR = 1.45, 1.07-1.97) as well as NSAIDs (HR = 1.31, 1.05-1.65) both increased the risk of CHD among pSS patients.
Conclusion: pSS is associated with an increased risk of subsequent CHD in Taiwan. Primary Sjögren's syndrome might be an independent risk factor for CHD. Use of corticosteroids and NSAIDs in the treatment of pSS patients increased the risk of developing CHD.

P2048

Investigating altered clozapine-mediated effects on myeloid cells during during multiple sclerosis

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Resident and infiltrating myeloid cells have arisen as viable therapeutic targets to reduce the damaging neuroinflammation associated with multiple sclerosis (MS). Previous work has revealed that atypical antipsychotic agents such as clozapine are effective at reducing neuroinflammation in experimental models by regulating innate immune responses; however, a recent clinical trial revealed an enhanced susceptibility to clozapine in people with progressive MS. We investigated how these medications affected the phenotype and function of macrophages, monocytes, and microglia *in vitro* and *in vivo* and found that while *in vitro* clozapine-treated macrophages could regulate Th cell cytokine production, *in vivo* treatment did not overtly alter the peripheral myeloid or T cell subsets. Instead, clozapine reduced innate cell activation and thus regulating neuroinflammation. To understand if immune cells from people with MS responded differently to clozapine than cells from healthy subjects, monocyte responses were evaluated and showed a distinct pattern of responsiveness supporting the idea that clozapine may have altered and unexpected effects during progressive MS.

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DOI: 10.1002/eji.201970400

P2049

Ethanol consumption inhibits the development of autoimmunity by blocking IL-21 secretion in T_{FH} cells

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Alcohol consumption has emerged as consistent protective factor for the development of autoimmune diseases such as rheumatoid arthritis (RA); however, the underlying mechanism for this tolerance-inducing effect of alcohol is unknown. We show that alcohol, as well as its metabolite acetate, specifically suppress interleukin-21 production from T follicular helper (T_{FH}) cells in vitro and in vivo, thereby exerting immune regulatory and tolerance-inducing properties. This effect of alcohol on T_{FH} cells was associated with reduced B cell numbers in the germinal centers, impaired autoantibody formation and the inhibition of development of experimental autoimmune arthritis and encephalomyelitis. In contrast, T cell independent immune responses and effector models of inflammation were not affected by alcohol exposure. In summary, our findings provide a new mechanistic explanation for the immune regulatory and tolerance-inducing effect of alcohol consumption.

P2050

Adiponectin exacerbates collagen-induced arthritis via enhancing B cell proliferation and differentiation

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Although B cells have been shown to contribute to rheumatoid arthritis (RA) pathogenesis by autoantibody production, antigen presentation and activation of CD4 T cells, as well as cytokine production, the precise roles of B cells in RA need to be further explored. Our previous studies have revealed that adiponectin (AD) is highly expressed in the inflamed synovial joint tissue and correlates closely with progressive bone erosion in RA patients. Therefore, the presentHere, we aimed studies were designed to investigate athe possible effects role of AD in regulatingon B cell proliferation and differentiation as well as the molecularrelative mechanisms. WeThe result showed found that AD could induce B cell proliferation and differentiation both in cell culture.collagen-induced arthritis (CIA, as a model of human RA) mice (in vivo) and in purified spleen B cells (in vitro). Notably, local intraarticular injection of AD promoted B cell expansion in joint tissues and exacerbates arthritic development in collagen-induced arthritis (CIA) mice. Mechanistically, AD induced the activation of PI3K/Akt1 and STAT3 was enhanced in B cells in response to AD stimulation, and PI3K/Akt1-activated STAT3

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DOI: 10.1002/eji.201970400

promoted AD-induced B cell proliferation and differentiation. Moreover, STAT3 activation up-regulated Blimp-1 expression at transcriptional level and promoted B cell differentiation. Taken together, our findings indicate that AD exacerbates CIA via enhancing B cell proliferation and differentiation mediated by PI3K/Akt1/STAT3 axis, which maythat might provide a new insight in understanding the pathogenesis of RA.

P2051

Characteristics of rheumatoid arthritis patients with autoimmune thyroid disease: a clinical analysis of 83 cases

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Objective: To investigate the clinical characteristics of patients with rheumatoid arthritis complicated with autoimmune thyroid disease (RA+AITD).
Methods: We performed a retrospective study to analyze the clinical data of 83 patients with RA+AITD from the second Xiangya Hospital during 2015-2017.
Results: Female rate was 92.8%. The average disease course of RA was 8.0 years. The average DAS28 score was 6.3. Positive autoantibody distribution: RF 92.4% (RF IgG25%, RF IgA 60.5%, RF IgM 90.8%), ACPA 82.7%, ANA 38.2%, SSA 14.5%, Ro-52 14.5%, SSB 4.8%, P-ANCA/MPO 19.6%. Positive thyroid-related autoantibody distribution: TPOAb 90.4%, TGAb 90.1%, TRAb 28.6%. About 50% patients had normal thyroid function, but 36.1% had hypothyroidism (including subclinical hypothyroidism) and 13.3% had hyperthyroidism. Thyroid ultrasound Doppler scanning showed 45.0% patients with RA+AITD had thyroid nodules and 37.5% had diffuse thyroid lesions. **Conclusion:** Compared with RA patients without AITD, patients with RA+AITD are more common in women with long course and high disease activity of RA. The patients with RA+AITD had no significant differences in positive rates of RF, ACPA, ANA, SSA, Ro-52 and SSB autoantibodies, but had significant higher rate of P-ANCA/MPO compared with RA patients without AITD. TPOAb and TGAb were the most common thyroid-related autoantibodies in patients with RA+AITD, followed by TRAb. Hypothyroidism was more common than hyperthyroidism. Thyroid nodules and diffuse thyroid lesions were often seen in thyroid ultrasound imaging. We recommend screening of thyroid function, thyroid-related autoantibodies, and thyroid ultrasound in RA patients, especially in females.

P2052

Steroid unresponsive Hashimoto´s encephalopathy treated with double filtration plasmapheresis

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To explore the treatment of unresponsive HE with corticosteroid therapy. Each patients were given steroid therapy after diagnosis and treated HE with double filtration plasmapheresis in patient presenting unresponsive to methylprednisolone therapy. Cognitive dysfunction is the most common clinical manifestation . HE is a rare encephalopathy associated with autoimmune thyroiditis. Although according to the previous studies, most HE cases have been highly responsive to corticosteroid treatment, still a few patients are resistant to the therapy. Overall, as for those who are resistant or

DOI: 10.1002/eji.201970400

contraindicative to steroids treatment, their prognosis could be quite poor, and DFPP may be an optional choice with no plasma limitation and could be used timely.

P2053

Anti-DFS antibodies in routine ANA testing in Tunisian patients

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Background: The presence of antinuclear antibodies (ANA) directed against intracellular antigens is a hallmark of connective tissue diseases. Anti-Dense Fine Speckled 70 (DFS70) antibodies were recently identified as associated to a specific ANA indirect immunofluorescence (IIF) pattern. They are a common finding in clinical laboratory testing. The aim of the study was to analyze the clinical value of anti-DFS70 antibodies in a cohort of patients undergoing routine ANA testing.
Methods: In this study we enrolled all sera submitted for ANA screening from January 2015 to February 2019 with a specific DFS IIF pattern on Hep-2 cells (Biorad ®) confirmed by immunodot (Euroimmun ®).
Results: Out of the 19072 sera tested for ANA in our lab, 7222 (37.86%) were positive. The DFS pattern was identified in 259 cases (3.58%). Patients within this subgroup had a mean age of 40 years (range 2 to 80) and a sex ratio of 1/5.
Associated autoimmune diseases (AID) were observed in 63 cases (24.32%). Patients revealing DFS pattern presented hematological (8.88%), neurological (8.1%), dermatological (6.94%) and renal signs (6.17%).
Monospecific DFS was detected in 23 patients (8.88%), among them only 5 patients had associated AID.
Anti DFS antibodies were associated to other ANA specificities mainly Ro52 (17.37%), SSA (14.28%) and histone (10.81%).
Conclusion: It has been reported that monospecific anti-DFS70 ANA enables reassurance of patients and doesn't characterize authentic AID. However, in our study, among the 23 patients with monospecific DFS pattern, 5 had AID. This profile requires further investigations and warrants close clinical follow-up.

P2054

Roles of GM-CSF in the pathogenesis of autoimmune diseases: an update

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) was first described as a growth factor that induces the differentiation and proliferation of myeloid progenitors in the bone marrow. GM-CSF also has an important cytokine effect in chronic inflammatory diseases by stimulating the activation and migration of myeloid cells to inflammation sites, promoting survival of target cells and stimulating the renewal of effector granulocytes and macrophages. Because of these pro-cellular effects, an imbalance in GM-CSF production/signaling may lead to harmful inflammatory conditions. In this context, GM-CSF has a pathogenic role in autoimmune diseases that are dependent on cellular immune responses such as multiple sclerosis (MS) and rheumatoid arthritis (RA). Conversely, a

DOI: 10.1002/eji.201970400

protective role has also been described in other autoimmune diseases where humoral responses are detrimental such as myasthenia graves (MG), Hashimoto's thyroiditis, inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE). In this review, we aimed for a comprehensive analysis of literature data on the multiple roles of GM-CSF in autoimmue diseases and possible therapeutic strategies that target GM-CSF production.

P2055

Conditional depletion of whole microRNA-183-96-182 cluster in lymphocytes suppresses autoantibody production in *Lpr* lupus mice

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The miR-183-96-182 (miR-183C) is a highly conserved miRNA cluster, which locates at human chromosome 7 and murine chromosome 6. miR-183C family expression is very low in the spleen cells of normal C57BL/6 mice, but can be highly inducd in activated T and B lymphocytes. Current studies suggest a functional compensation of miR-183c family members in immunity regulation. We have previously reported a significant upregulation of miR-183C in the splenic cells of different murine lupus models. In this study, we found that the upregulation of miR-183C in lupus mice is inversely related to the expression of Foxo1 and Foxo3a, which play important roles in the regulation of T lymphocyte homeostasis and function. Inhibition of miR-182 alone or whole miR-183C increases Foxo1 expression and significantly reduces lupus-related inflammatory cytokines such as IFNγ, IL-6, and IL-17 in *in vitro* activated splenocytes from both MRL and MRL-*lpr* mice. To further characterize the pathogenic contribution of miR-183C in lupus, we developed B6-*lpr* mice with conditional depletion of miR-183C or miR-182 alone in $CD2^+$ lymphocytes (miR-183C$^{-/-}$B6-lpr and miR-182$^{-/-}$B6-*lpr*). Surprisingly, depletion of miR-183C *in vivo* has no similar suppressive effect on the inflammatory response in splenocytes as that we observed in *in vitro* study. Impressively, we found that depletion of miR-183C, but not miR-182 alone significantly suppresses the development of anti-dsDNA autoantibodies in B6-*lpr* mice. There is also a trend of reduced splenomegaly in miR-183C$^{-/-}$ B6-*lpr* mice. Together, our results suggest that miR-183C might be a new potential target for lupus therapy.

P2056

CD36 regulates B-cell response in the systemic lupus erythematosus

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Scavenger receptor CD36 has been shown to mediate uptake of modified self-antigens such as apoptotic cells and oxidized LDL in macrophages and dendritic cells. Interestingly, CD36 was discovered to be expressed also specifically on an innate B cell subtype, marginal zone B cells (MZB). Here we investigate the role of CD36 in B cell activation in the context of apoptotic cell clearance and break of tolerance to self. We found that formation of germinal center B cells and autoantibody production in response to an increased load of apoptotic cells coincide with downregulation of CD36 on MZB cells. B cells lacking CD36 proved more easily activated in response to apoptotic cells, as was shown in bone marrow (BM) chimeras reconstituted with wild type and CD36-deficient BM. In steady-state, we found that CD36 co-localizes with the B cell receptor and the tyrosine kinase Lyn. Interestingly, upon cross-linking of the inhibitory Fc receptor, FcγRIIb, CD36 instead co-localizes with

DOI: 10.1002/eji.201970400

this receptor. This suggests that CD36 interacts with FcγRIIb to activate a negative signaling cascade in B cells. Our data implicate CD36 in the early regulation of B cell responses towards apoptotic cells and autoantibody production. In line with this we find that CD36-deficient B cells are more resistant to FcγRIIb-induced cell death. Thus, CD36 exerts its regulatory function by associating with a known negative signalling pathway in B cells involving Lyn and FcγRIIb. This finding could have implications for autoimmune diseases involving apoptotic cell-derived self-antigens.
Keywords: Marginal zone B cells, CD36, autoimmunity, immunology

P2057

A critical role of interleukin-17A in regulating autoreactive plasma cell response during lupus nephritis development

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Immune tolerance breakdown in systemic lupus erythematosus (SLE) leads to anti-nuclear autoantibody production and multiple organ damages, but the underlying mechanisms remain poorly understood. In this study, we revealed a pathogenic role of the proinflammatory cytokine interleukin-17A (IL-17A) in modulating autoantibody secretion by plasma cells during lupus development. Herein, we used chromatin-induced lupus mouse model and found that mice deficient for either IL-17A (*Il17a*$^{-/-}$) or IL17A receptor (*Il17rc*$^{-/-}$) showed significantly reduced serum anti-dsDNA IgG levels, reduced serum ANA levels, decreased glomerular immune complex deposition and ameliorated lupus progression compared with wild-type (WT) lupus mice. Flow cytometric analysis showed that CD19^{-}CD138^{+}Blimp-1^{high} plasma cells were markedly decreased in both *Il17a*$^{-/-}$ and *Il17rc*$^{-/-}$ lupus mice when compared with WT lupus mice. Moreover, within all B cell subsets, the surface IL17 receptors (IL-17ra and IL17-rc) showed significantly higher expression levels in CD19^{-}CD138^{+} plasma cells in WT lupus mice than in adjuvant-treated controls and other B cell subsets. In culture, IL-17A significantly increased anti-dsDNA IgG autoantibody production in purified plasma cells from lupus mice. Further mechanistic studies demonstrated that IL-17A could maintain the survival of lupus plasma cells *in vitro*. Our findings identify a novel function of IL-17A in triggering autoantibody production by plasma cells and provide potential therapeutic strategies for the treatment of SLE.

P2058

Functional activity of T-regulatory cells in healthy donors and patients with rheumatoid arthritis, in vitro assay

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In this study we assessed the influence of homeostatic factors IL-7 and IL-15 on the functional characteristics of T-regulatory cells (Treg) from healthy donors and patients with rheumatoid arthritis. The 20 donors and 10 patients with RA were enrolled. Treg phenotyping (CD3^{+}CD4^{+}CD25^{+}CD127^{lo}FoxP3^{+}) was performed by flow cytometry, also using CTLA-4 and PD-L1 as functional markers. IL-7, IL-15 and anti-CD3+IL-2 were chosen as activators during 7-days stimulation. Treg were isolated by magnetic separation and then labeled CFSE to assess proliferation. Treg suppressive activity was analyzed by inhibition of proliferation of CD4^{+} and CD8^{+}-cell.
We revealed an increasing of CD4^{+}CD25^{+}FoxP3^{+} cells under all stimulation condition in patients. That

DOI: 10.1002/eji.201970400

may indicate the induction of Treg from $CD4^+$. Herewith, patients Treg had a lower proliferative activity than Treg from donors under IL-7 and IL-15. But no significant differences were revealed regard suppressive activity of Treg between donors and RA-patients.
The PD-L1 and CTLA-4 surface expression on Treg changed similar in both groups. However peripheral blood Treg from patients had a lower density of CTLA-4.
The reduced proliferative activity of Treg in the group of RA-patients under IL-7 and IL-15 perhaps make a significant contribution to the development of the disease due to the delay of Treg pool reconstitution in the lymphopenia conditions on the initial stage. Furthermore, increased induction of $CD4^+CD25^+FoxP3^+$ cells in PBMC cultures in patients group associated with pathogenesis of RA, since induced Treg have unstable expression of FoxP3, and are capable to differentiate into pathogenic exFoxP3-Th17 lymphocytes under inflammatory condition.

P2059

Host-microbial crosstalk in the pathogenesis of inflammation and cancer in Primary Sclerosing Cholangitis (PSC)

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Primary sclerosing cholangitis (PSC) is a chronic inflammatory disorder of the hepatobiliary system associated with inflammatory bowel disease (IBD). PSC-IBD is linked to increased risk of colon cancer and hepatobiliary malignancies, with poor prognosis. Dysbiosis is present in PSC supporting a role for the microbiome in the pathogenesis of disease.
We aim to 1) assess host-microbial functions in PSC-IBD 2) evaluate whether PSC-IBD-associated pathways affect epithelial transformation.
Mucosal biopsies and brushings were collected from patients with PSC-IBD, patients with IBD without PSC and healthy controls. 16S rRNA sequencing was performed to characterise the adherent microbiome and 3' RNA sequencing to analyse intestinal transcriptomes. Patient-derived colonic organoids were stimulated with different cytokines for 24 hours and markers of cytokine downstream pathways, stemness and pluripotency were analysed by qPCR.
Higher microbial diversity was present in biopsies *versus* brushings with alterations in amplicon sequence variants (ASV) between patients and controls. A distinct transcriptomic profile was identified in the colon of patients with PSC-IBD with enrichment of pathways associated with IL22 and TGF-beta signalling. The IL22 receptor, *IL22RA1,* was induced by IFN-gamma stimulation in PSC-IBD-derived organoids that also over-expressed *OLFM4* and *POU5F1*.
Our findings indicate that microbes that are in closer proximity with the colonic crypts may play a role in the pathogenesis of inflammation and cancer in PSC-IBD. PSC-associated Th1 responses result in increased epithelial IL22 responsiveness. Higher expression of the stemness genes *OLFM4* and *POU5F1*, triggered by bacteria and IL22, suggest that microbial-driven IL22 responses may contribute to epithelial transformation in PSC-IBD.

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DOI: 10.1002/eji.201970400

P2060

Defective scotopic visual signaling promotes development of retinal inflammation

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Slow progression of photoreceptor neurodegeneration and a poor visual prognosis are often found in several forms of congenital stationary night blindness (CSNB). To date, 17 genes with more than 350 mutations affecting scotopic vision have been identified in association with CSNB. Although the role of these genes in neurotransmission through the retina in scotopic light condition is well described, much less is known whether and how these genes can modulate immune response in the retina. To address this, we have tested one of the genes (GPR179) associated with CSNB. We observed that GPR179 (GPR179 KO) knockout mice were more susceptible to develop experimental autoimmune uveoretinitis (EAU) compared to wild-type (WT) mice. GPR179 KO mice with EAU exhibited increased EAU clinical score characterized by retinal detachment and hemorrhages, upregulated expression of pro-inflammatory chemokines, higher activation status of microglia and astrocytes and increased infiltration of pathogenic CD4+ T cells in the retina compared to WT EAU mice. GPR179 KO EAU mice were also found to have worse visual acuity than WT EAU mice, even though they showed no impairment in visual acuity before the induction of EAU. However, we found that GPR179 KO mice exhibited an increased expression of IL-6 in the retina compared to WT mice, indicating a subclinical retinal inflammation state that may contribute to a severe EAU development. Our findings suggest that the retina with defective scotopic visual signaling associated with GPR179 deficiency is particularly vulnerable to autoimmune inflammation. *Supported by GACR18-11795Y, PRIMUS/17/MED/7 and ProgresQ35.*

P2061

IL-18 and TNF-α modulates the OPG/RANKL ratio and induces bone resorption in subject with rheumatoid arthritis- a correlative study

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Rheumatoid arthritis (RA) is the major health concern worldwide that is associated with inflamed synovium, bone erosion and abnormal metabolism. The autoimmunity in RA stimulates the principle immune cells to release pro-inflammatory cytokines from the activated T-cells which mediates the effect of oxidative stress thereby leading to multiple health complications. In the present study the association between inflammation and bone loss in RA were analyzed. 285 numbers of RA subjects and 50 healthy subjects were enrolled in this study. We have done Dual-energy X-ray absorptiometry (DEXA), estimated RF, ESR, CRP, IL-18, TNF-α, OPG and RANKL in all subjects. We did synovial tissue histology and immunohistochemistry for IL-18, TNF-α, OPG and RANKL in RA and non-RA trauma subjects. Our data showed increased expression of IL-18, TNF-α & RANKL and decreased expression of OPG in RA in comparison to healthy subjects. Statistically we found a negative correlation between OPG/RAKL and inflammatory cytokines IL-18 (r= -0.74) and TNF-α (r=-0.85) in RA subjects whereas a positive correlation was observed between OPG/RANKL and bone mineral density (BMD) of spine (r=0.71) and femoral neck. (r=0.79). The decreased OPG/RANKL ratio is the result of increased expression of RANKL in RA subjects where IL-18 and TNF-α play a potent role during RA. RANKL is the potent osteoclastogenic factor causing bone resorption in RA. The positive correlation between decreased OPG/RANKL ratio and BMD therefore relates its association with bone erosion process. Finally it can be concluded that IL-18 and TNF-α modulate the OPG/RANKL ratio thereby causing bone erosion.

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DOI: 10.1002/eji.201970400

P2062

A peep in to the prevalence and clinical significance of the uncommon ANA patterns in Central India

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Antinuclear antibodies have become an essential screening and diagnostic tool for autoimmune disorders. The clinical significance of the common ANA patterns and the corresponding antibody like nuclear homogenous and nuclear speckled has already been established. However the uncommon patterns remain elusive in terms of relative antibodies and their clinical association and are often considered negative. Thus a retrospective data analysis study was undertaken to know the prevalence of the uncommon ANA patterns in a tertiary care centre of Central India and to assess their clinical significance. ANA reports of 1232 patients were retrieved and analysed. ANA testing had been done by the gold standard Indirect Immunoflourescent Assay on Hep-2 cell slide. Among the 326 positive samples, 26% were uncommon nuclear, cytoplasmic and mitotic sub-patterns and mixed patterns, the nuclear sub patterns dominating the numbers. While in total the prevalence in women was higher, the male to female ratio was followed by cytoplasmic, nuclear and mixed patterns, but the distribution was almost equal in case of mitotic. Certain diseases were associated with some of the sub patterns. The high prevalence of these patterns points to their importance clinically and should encourage further evaluation of them and their corresponding antibodies and the clinical utility should be established to ascertain diagnostic gain in autoimmune and other disease conditions.
Key Words: Antinuclear Antibodies, Uncommon patterns, Immunoflourescent Assay, Central India

P2063

Oral glutamine intake suppresses collagen-induced arthritis through inducing MAPK phosphatase (MKP)-1

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L -Glutamine (Gln), a nonessential amino acid, is abundant in the plasma. Gln has been shown to have anti-inflammatory property. As a result, Gln protects animals from endotoxic shock, and attenuates many inflammatory diseases. Regarding the anti-inflammatory properties of Gln, we have shown that Gln can effectively deactivate both p38 MAPK and cytosolic phospholipase A_2 ($cPLA_2$) by rapid induction of MAPK phosphatase (MKP)-1. In this study, we explore the possibility that Gln can ameliorate collagen-induced arthritis (CIA). Oral Gln intake (0.5 g/kg/day) attenuated not only severity of arthritis based on clinical scores, hind paw thickness, and radiographic and pathologic findings, but also local and systemic levels of proinflammatory cytokines, such as TNF-a, IL-1b, IL-6, and leukotriene B4. Oral Gln inhibited p38 phosphorylation and NF-kB activation in the inflammed tissues. Gln administration resulted in early and enhanced MKP-1 induction. Importantly, MKP-1 small interfering RNA (siRNA), but not control siRNA, significantly abrogated the Gln-mediated 1) induction of MKP-1; 2) attenuation of arthritis; 3) inhibition of p38 phosphorylation, NF-kB activation and tissue levels of proinflammatory cytokines. These data indicated that Gln ameliorated CIA via inhibition of p38/NF-kB signaling in a MKP-1-dependent manner.

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DOI: 10.1002/eji.201970400

P2064

Role of TGF-β1 activation mediated by GARP in immune responses to protein immunization and autoimmune diseases

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Background: Garp is a transmembrane protein expressed on the surface of two types of immune cells,namely activated Tregs and B cells, where it mediates the release of active TGF-β1.
We investigated the role of Garp-dependent TGF-β1 activation by B cells and Tregs in response to protein immunization and in autoimmune diseases in mice.
Results: Mice carrying a B cell- or Treg- specific deletion of *Garp*(Mb1-Cre x Garp$^{fl/fl}$mice and Foxp3-Cre x Garp$^{fl/fl}$mice, respectively) were immunized with Cholera toxin B (CTB). Absence of Garp on B cells or Tregs did not influence the production of anti-CTB Ig.
Mice were also immunized withOVA to analyze CD4^{+}T cells responses in vitro. The absence of Garp on B cells or Tregs did not influence the secretion of IFNγ, IL-4 or IL-17 by CD4^{+}T cells.
Finally, we measured IgG deposition in the kidneys of aged mice by IF, as a read-out for autoimmune nephritis development. Mice carrying a B cell- specific Garp deletion appear to show IgG deposition in the kidneys, a phenotype we did not observe in mice carrying a Treg specific- Garp deletion.
Discussion: The absence of Garp on B cells or Tregs does not alter Ig and CD4^{+}T cell responses to protein immunization. In contrast, the absence of Garp on B cells, but not on Tregs, seems to be associated with IgG deposition in kidney. This suggests that Garp-dependent TGF-β1 activation by B cells, but not by Tregs, may be required to prevent auto-immunity.

P2065

In-vitro functional responses of dsg3-specific autoreactive Treg and Th17 cells in Pemphigus vulgaris

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Background: Pemphigus vulgaris (PV) is the consequence of a loss of natural tolerance against Dsg3. Treg and Th17 cells are critically involved in the maintenance of peripheral tolerance in autoimmune diseases. We aim to evaluate Th17 and Treg cell frequency and their *in-vitro* functional responses.
Methods: Total of 30 cases and 30 healthy control were enrolled for the study, clinically diagnosed as PV. Blood samples were collected for Peripheral blood mononuclear cells (PBMCs). PBMCs were cultured and stimulated with desmoglein antigen for 16 hours. Cultured cells were harvested and stained with CD3-PerCP, IL10-PE and IL17-AF647 antibodies and sorted using FACs AriaIII. These sorted TCC(T Cell Clone) were cultured with IL2, anti-CD3 and anti-CD28 antibodies. Proliferation index was determined by MTT assay. Culture supernatant were harvested for cytokines quantification. mRNA expression of Foxp3 and RORγt were analyzed using Real time PCR.
Results: The results showed decreased in the frequency of IL10 secreting T cell and proliferation of IL-17 secreting T cells, after stimulation with Dsg-3 *in-vitro*. Further, the cytokine level of IL10 and TGF-b were significantly($p < .05$) reduced in IL10+ TCC of cases. Similarly, for IL17+ TCC related cytokines, IL2, IL6, IL17($p < .05$) were significantly high, in cases. Foxp3 expression was downregulated whereas RORγt expression was significantly upreguated in TCC of cases.
Conclusion: Dsg-3 specifically proliferates IL-17 secreting T cells but has a counter effect on IL-10 secreting T cells promoting dysregulation of immunity. This may suggest that IL-17 secreting cells may have an essential role in triggering an inflammatory response in PV patients.

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DOI: 10.1002/eji.201970400

P2066

Decrease of disease score in in vivo model of multiple sclerosis targeting S100B

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S100B, a small-calcium binding protein, acts as an intracellular regulator and through extracellular signaling. It has been demonstrated a direct correlation between increased amount of astrocytic S100B and its role in demyelination and neuroinflammatory processes upon demyelination in *ex vivo* demyelinating model.
The aim of the study is to investigate the possible role of pentamidine isethionate, a small molecule with antiprotozoal activity, able to target S100B, in the modulation of clinical outcome of RR-EAE mouse model.
Experimental Autoimmune Encephalomyelitis (EAE) was induced in female SJL wild-type mice and four groups were formed: EAE with vehicle (saline), EAE treated with pentamidine, Control mice treated with pentamidine and untreated control group; the pentamidine dosage was, 4mg/kg, intraperithoneally. Weight and clinical score of the animals were evaluated each day post injection. Following sacrifice, brains were collected and analyzed for the expression of myelin and neuroinflammation by immunohistochemistry and qRealTime PCR. We observe that pentamidine-treated mice display a faster decrease in disease score during the remission.
Thus, these preliminary results indicate that S100B is involved in MS pathology and that its inhibition may be a new therapeutic strategy not only to reduce damage but, hopefully, to fasten the recovery.
Disclosure**:** Funded by FISM pilot Grant 2018.

P2067

A potential inflammatory role of IL-31 in psoriatic arthritis: a correlation with Th17 cytokine profile

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The psoriatic arthritis (PsA) is an autoimmune disease characterized by epidermal lesions and joint inflammation triggered by a strong deregulation of proinflammatory cytokines production. The PsA is considered a Th17 cytokine profile mediated disease, however the IL-31 has been implicated in inflammatory skin diseases.
Objective: the goal of this study was to determine the possible association of IL-31 and the Th17 cytokine profile in PsA.
This cross-sectional study was conducted in PsA patients and control subjects (CS). The serum levels of the cytokines was measured by magnetic bead-based assay using BioPlex/MagPix System.

DOI: 10.1002/eji.201970400

Results: As results significant differences were observed between study groups in IL-31 (PsA=77.23 pg/mL vs CS=64.4 pg/mL, p< 0.001), and Th17 cytokine profile serum levels, (IL-17A: PsA=6.36 pg/mL vs CS=2.97 pg/mL, p=0.02; IL-17F: PsA=44.15 pg/mL vs CS=23.36 pg/mL, p=0.01; IL-17E: PsA=3.03 pg/mL vs CS=0.82 pg/mL, p< 0.001; IL-21: PsA= 36.45 pg/mL vs CS=12.44 pg/mL, p=0.02). However, significant differences were not observed for IL-23 (PsA=31.2 pg/mL vs CS=53.26 pg/mL, p=0.58). Additionally, higher levels of IL-17A and IL-17E in the patients with psoriasis scalp were found. In addition, positive correlations between IL-31 with Th17 cytokine profile serum levels were found in PsA (IL-17A rs=0.64, p< 0.001; IL-17F rs=0.73, p< 0.001; IL-17E rs=0.70, p< 0.001; IL-21 rs=0.54, p=0.002; IL-23 rs=0.5, p< 0.01).
Conclusion: in PsA there are increased serum levels of IL-31, IL-17A, IL-17F, IL-17E, IL-21, but not IL-23. Moreover, there is a positive correlation between IL-31 with the Th17 cytokine profile, which could represent an important immunopathogenic mechanism in PsA

P2068

MBP85-99 specific TCR repertoire in DR2+ patients affected by multiple sclerosis

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T and B cells play a prominent role in the pathogenesis of Multiple Sclerosis and T cells specific for myelin antigens become able to cause damage and recruitment of other immune cells in the CNS. The T cell mediated self-reactivity against myelin is strongly linked to HLA; specifically in HLA-DR2+ subjects, an immunodominant T-cell epitope exists within MBP residues 85-99. TRBV-TRBJ spectratyping of the repertoire of T cells specific for MBP85-99 revealed the usage of a limited number TCRs. The analysis was performed on the blood and cerebrospinal fluids (CSF) samples of 30 DR2+ patients and 7 healthy DR2+ subjects, allowing to identify 2 TCR rearrangements shared by more than 50% of MS DR2+ patients during the active phase of the disease. The immunophenotyping analysis revealed that one of the two shared TCRs was exclusively used by IL-17 secreting cells and that it was present in CSF of patients in relapse but not during the remission and that it was down-regulated during remission after therapy. Twenty-five % of the investigated TCR rearrangements were used by IFN-g secreting cells.
To date there are no available biomarkers for response to therapy and therapy efficacy is measured on the absence of clinical and radiological activity during follow-up.
The identification of a limited number of TCRs involved in the recognition of MBP85-99 in complex with HLA-DR2 specifically associated with disease can lead to development of tools aimed at measuring their number in the blood of MS patients as potential biomarkers of disease.

P2069

Study of genetic variations B-cell scaffold protein with ankyin repeats 1(BANK-1) among Algerians patients with Systemic lupus erythematosus

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DOI: 10.1002/eji.201970400

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Background: GWAS studies have shown that genetic variants of BANK-1 are strongly associated with SLE and their products affect key functional domains of the protein and contribute to uncontrolled BCR signaling with B cell hyper-reactivity characteristic of SLE.
Objective: Find an association between BANK-1 rs10516487 and rs3733197 and susceptibility to SLE in Algerian patients.
Material and methods: This is a case-control study of 152 SLE patients (age: 37 ± 12 years, sex ratio: 1: 10) and 102 SSc patients (age: 44 ± 13 years, sex ratio: 1: 10) compared to 150 healthy controls (age: 28 ± 9 years, sex ratio: 1: 7). We performed BANK-1 gene SNPs genotyping with TaqMAN technology and we respected the Hardy-Weinberg equilibrium.
Results: The Analysis of results shows for BANK-1 rs10516487 that allele G (OR 1.07) and genotypes GG (OR 1.02) and AG (OR 1.07) are associated with SLE and allele A (OR 0.92) and genotype AA (OR 0.8) are protective whereas no difference exists between SLE and controls for BANK-1 rs3733197 for allelic frequencies despite the fact that AG genotype is more frequent in SLE patients (OR 1.30) and genotypes GG (OR 0.89) and AA (OR 0.52) more frequent in controls. As for the production of autoantibodies, our results show that BANK-1 rs3733197 genotype AG is associated with the production of anti-ds DNA (OR 1.51) and anti-SSA (OR 1.40) antibodies in SLE patients.
Conclusion: Our results show an association of SNPs BANK-1 rs101516487 and rs3733197 to the development of SLE in the Algerian population.

P2070

Evaluation of matrix metalloproteinase-3 (MMP-3) as a candidate biomarker in assessment of disease activity in Algerian rheumatoid arthritis patients

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Introduction/Objectives: Matrix metalloproteinase-3 (MMP-3) is a protease induced by inflammatory cytokines in rheumatoid arthritis (RA) synovium and degrades a number of extracellular matrix components of cartilage and bone. The aim of our study is to evaluate serum MMP-3 levels in RA patients compared to healthy subjects and control patients. Then we assess how this marker reflects disease activity in RA in correlation with inflammatory markers, erosion and autoantibodies status.
Material/Methods: Our study groups consisted of 116 RA patients, 66 healthy controls and 47 control patients [33 undifferentiated connective tissue disease (UCTD) and 14 Chronic Inflammatory diseases (CID)]. MMP-3 serum measurement was based on a quantitative ELISA assay (Aesku.Diagnostics, Germany). MMP-3 levels were correlated with: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity score-28 (DAS28), Rheumatoid factor (RF), anti-cyclic citrullinated antibodies (ACPA) and erosion status.
Results: Serum MMP-3 was significantly higher in sera of RA patients (49±46ng/ml) compared to healthy controls (18±14ng/ml) ($p< 0.001$) and UCTD patients (17±16ng/ml) ($p=0.004$). MMP-3 levels were increased in 56% of RA patients, particularly in female patients with high DAS28 (58±44ng/ml) compared to those with moderate DAS28 ($p=0.03$). This statistical increasing values were found also with positivity of CRP ($p=0.02$), RF ($p=0.03$), ACPA ($p=0.02$) and erosive status ($p=0.009$). Finally, MMP-3 levels correlated positively only with RF ($p< 0.0001$; $r=0.35$) and ACPA rates ($p=0.004$, $r=0.26$).
Conclusion: Measurement of serum MMP-3 provides a particularly useful marker of inflammatory

DOI: 10.1002/eji.201970400

activity in RA patients and may have a particular value in predicting the progression of erosive disease.

LB116

The role of TDO2-mediated kynurenine metabolic pathway in fibroblast-like synoviocyte from rats with adjuvant induced arthritis

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Objective: The kynurenine (Kyn) metabolic pathway is a key metabolic pathway regulating the immune responses, tryptophan-2, 3-dioxygenase (TDO2) is one of the rate-limiting enzymes in this pathway. Fibroblast-like synoviocyte (FLS) is an important and ultimately effective cells in the pathogenesis of rheumatoid arthritis (RA). We investigated the expression and function of TDO2 in FLS and immune cells in RA model.
Methods: Rats adjuvant-induced arthritis (AA) was induced by intradermal injection of complete Freund's adjuvant. The concentration of tryptophan (Trp) and Kyn was determined by high performance liquid chromatography to evaluate the enzyme activity of TDO2. Expression of TDO2 in synovial joints was detected by immunohistochemistry. The expression of TDO2 in FLS and macrophages was assayed by western blot and immunofluorescence staining. The effect of TDO2 inhibitors 680C91 and Kyn on the function of FLS was studied by CCK-8 and transwell.
Results: IDO1 was mainly involved in the development of AA rats at the initial stage, while TDO2 played a major role at the peak and remission of AA rats. Compared with the normal group, the expression of TDO2 in the FLS and macrophages of AA rats was significantly increased. Kyn promoted the proliferation, migration and invasion of normal and AA-FLS, and 680C91 had an inhibiting effect on the function of AA-FLS. Meanwhile, Kyn could reverse the proliferation of AA-FLS that has been inhibited by 680C91.
Conclusion: Our data suggested that TDO2 may be an important regulator in FLS function. TDO2 may be a potential target for treating RA.

P2736

A case of active and severe Graves' ophthalmopathy with onset of hypothyroidism

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Objective: We found a patient had severe GO with the onset of hypothyroidism, as well as high titer of serum thyrotropin receptor antibody (TRAb).
Methods: We retrospectively analyzed the clinical data of A 49-year-old, male patient with GO. She complained diplopia and eyelid edema 3 years ago. Physical examination showed eyelids edema, prominent eyes, ball conjunctiva, and redness of palpebral conjunctiva. Von Graefe sign and Mobius sign were positive. Left eye gazing up and outreach was limited. Diplopia were obvious when gazing up and gazing down. The score of clinical activity score (CAS) was 4/7. Thyroid hormone and antibody results were showed that TSH 22.34~145.80 mIU/ml (0.38~4.34 mIU/ml), FT_3 3.83~4.70 pmol/L (2.77~6.31 pmol/L), FT_4 10.64~15.55 pmol/L (10.45~24.38 pmol/L), TRAb 33.35 IU/L (0~1.75 IU/L), TPOAb 528 IU/ml (0~60 IU/ml), TgAb 670 IU/ml (0~60 IU/ml). ECT scan of thyroid showed bilateral thyroid nuclide uptake roughly enhanced (especially for left lobe). Orbit CT scan showed that up, lower and medial rectus muscles of both eyes were thicken. He was diagnosed with hypothyroidism and GO, and 25~50 ug levothyroixine given once daily. We chose intermediate-dose schedule of

DOI: 10.1002/eji.201970400

methylprednisolone, a starting dose of 0.5 g once weekly for 6 weeks, followed by 0.25 g once weekly for 6 weeks, combined with dose of 0.75 g Mycophenolate mofetil (MMF) daily for 24 weeks. Symptoms became better.
Results: It indicats that TRAb, thyrotropin stimulus-blocking antibody (TSBAb), may lead to hypofunction of thyroid and GO in this case.

P2737

Topical application of epigallocatechin-3-gallatereduces psoriasiform lesions in the flaky skin mouse model by inducing epidermal differentiation and inhibiting inflammation

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Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation and aberrant keratinocyte differentiation. Epigallocatechin-3-gallate, as anti-inflammatory agent, has been suggested for potential therapeutic use in this context, possibly by the inhibition of hyperproliferation as well as the enhancement of keratinocyte differentiation through directlyanti-inflammatory.
Objectives: To determine whether topical application of epigallocatechin-3-gallate can modulate pathological markers of psoriasiform lesions in flaky skin mice and to find out if the application of epigallocatechin-3-gallate would cause the increased epidermal differentiation and reduction in proliferation and inflammation.
Methods: Six-week-old female homozygous flaky skin mice (fsn/fsn) were treated topically with epigallocatechin-3-gallate(1 mg cm^2 skin areas), twice a day, up to 11 weeks of age.
Results: Treatment of flaky skin mice with epigallocatechin-3-gallate resulted in a reduction in (i) pathological markers of psoriasiform lesions; (ii) infiltration of inflammatory cells; and (iii) mRNA and protein expression of inflammatory cytokines. Epigallocatechin-3-gallate treatment also increased the expression and processing of caspase-14. Furthermore, there was a decrease in the expression of markers for cell proliferation (proliferating cell nuclear antigen) and modulation of tight junction proteins (occludin and claudin-1).
Conclusions: Epigallocatechin-3-gallate treatment may reduce psoriasiform lesions, and this effect may be the result of the enhancement of keratinocyte differentiation and the anti-inflammatory and immune adjustment.

P2738

The effect and mechanism of *Trichinella Spiralis* proteins from dfferent stages on collagen-induced arthritis in mice

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Background: Rheumatoid arthritis (RA) is a chronic joint inflammatory disease of unknown etiology. Pre-infection of *Trichinella spiralis* can reduce the clinical symptoms and joint pathological damage in mice with collagen-induced arthritis (CIA). This project aims to investigate the effects of soluble and excretory/secretory proteins from *T. spiralis* on CIA and its mechanism.
Methods: The soluble muscle larvae proteins (SMP), muscle larvae excretory/secretory proteins

DOI: 10.1002/eji.201970400

(MES), soluble adult protein (SAP) and adult excretory/secretory proteins (AES) were prepared from adult stage of *T. spiralis*. Thirty DBA/1 CIA model mice were randomly divided into 6 groups (CIA, SMP, SAP, MES, AES, and MTX, n=5), and received intraperitoneal injection of 100 µl solution respectively: normal saline for CIA group; 25 µg *T. spiralis* protein for treatment groups; methotrexate (3 mg/kg) for MTX group. CIA clinical parameters were continuously observed. X-ray imaging, feet pathology evaluation and cellular immune analysis were performed at end point.
Results: The clinical scores of the joints and the foot pads thickness in each treatment group decreased at different time. The SMP group and the MTX group were similar. The proportions of Th17 cells and IL-22$^+$ Th cells in each treatment group were significantly lower than that in CIA group ($p<$ 0.05), and the proportions of Th2 cells and Treg cells were significantly higher ($p<$ 0.05).
Conclusion: *T. spiralis* proteins had therapeutic effect on CIA, and SMP was better. The mechanisms might depend on the up-regulation of Th2 and Treg cells and the down-regulation of Th1, Th17 and IL-22$^+$ Th cells.

P2739

Upregulated IL-1 receptor-associated kinase 1 (IRAK1) in systemic lupus erythematosus: IRAK1 inhibition represses Th17 differentiation with therapeutic potential

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Systemic lupus erythematosus (SLE) is a typical autoimmune disease. Genome-wide analyses have revealed that interleukin-1 receptor-associated kinase 1 (IRAK1) is associated with susceptibility to SLE. Our previous study investigated the role of IRAK1 in nuclear factor-κB (NF-κB)-related pathways in a mouse model of lupus. In this study, we aimed to further explore the etiological role of IRAK1. The gene expression and phosphorylation of IRAK1 in CD4+ T cells from lupus patients and healthy controls were examined by quantitative reverse transcription-polymerase chain reaction and western blotting, respectively. The percentage of circulating Th17 cells and plasma IL-17A levels were evaluated by flow cytometry and enzyme-linked immunosorbent assay, respectively. The influence of IRAK1 suppression on Th17 development was assessed using an IRAK1 inhibitor and small interfering RNA. We found that IRAK1 transcript levels in CD4+ T cells were significantly upregulated in SLE patients in comparison to controls and were positively correlated with disease activity. In vitro experiments showed that lupus CD4+ T cells had more pronounced IRAK1 phosphorylation at threonine-209 upon IL-1β stimulation than did control cells. Moreover, IRAK1 expression was positively associated with Th17/IL-17A in patients. When naïve CD4+ T cells were polarized toward the Th17 subset, IRAK1 inhibition significantly repressed IL-17A production and the gene expression of Th17 markers, namely, retinoic acid receptor-related orphan receptor c, IL-23 receptor and IL-17A. In summary, IRAK1 is overexpressed and hyperactivated in CD4+ T cells from SLE patients. IRAK1 inhibition attenuates Th17 differentiation in the context of human SLE, suggesting a therapeutic opportunity.

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DOI: 10.1002/eji.201970400

P2740

Interleukin-1 receptor associated kinase 1 is a potential therapeutic target of anti-inflammatory therapy for systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease and currently has no effective therapy. The genome-wide analyses indicate that interleukin-1 receptor associated kinase 1 (IRAK1) is associated with the susceptibility of SLE in humans. In the present study, we identified that IRAK1 was overexpressed and hyper-activated in splenic mononuclear cells from B6.MRL-Faslpr/Nju (B6.lpr) mice and peripheral blood mononuclear cells (PBMCs) from SLE patients. Intraperitoneal treatment with a small molecular inhibitor of IRAK1 (IRAK1/4 inhibitor or IRAK-Inh) significantly mitigated inflammatory responses and renal injury in B6.lpr mice. IRAK-Inh treatment or knockdown of IRAK1 by specific siRNA decreased the relative levels of NF-κBp65 phosphorylation in human PBMCs from SLE patients. Therefore, IRAK1 may be a potential target for anti-inflammatory therapy for SLE and other inflammatory diseases.

P2741

NKG2D+CD4+ T cells kill regulatory T cells in a NKG2D-NKG2D ligand- dependent manner in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) features a decreased pool of CD4+CD25+Foxp3+ T regulatory (Treg) cells. We had previously observed NKG2D+CD4+ T cell expansion in contrast to a decreased pool of Treg cells in SLE patients, but whether NKG2D+CD4+ T cells contribute to the decreased Treg cells remains unclear. In the present study, we found that the NKG2D+CD4+ T cells efficiently killed NKG2D ligand (NKG2DL)+ Treg cells in vitro, whereby the surviving Treg cells in SLE patients showed no detectable expression of NKG2DLs. It was further found that MRL/lpr lupus mice have significantly increased percentage of NKG2D+CD4+ T cells and obvious decreased percentage of Treg cells, as compared with wild-type mice. Adoptively transferred NKG2DL+ Treg cells were found to be efficiently killed in MRL/lpr lupus mice, with NKG2D neutralization remarkably attenuating this killing. Anti-NKG2D or anti-interferon-alpha receptor (IFNAR) antibodies treatment in MRL/lpr mice restored Treg cells numbers and markedly ameliorated the lupus disease. These results suggest that NKG2D+CD4+ T cells are involved in the pathogenesis of SLE by killing Treg cells in a NKG2D-NKG2DL-dependent manner. Targeting the NKG2D-NKG2DL interaction might be a potential therapeutic strategy by which Treg cells can be protected from cytolysis in SLE patients.

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DOI: 10.1002/eji.201970400

P2742

Clinical analysis of Chinese anti-LRP4 antibodies in patients with myasthenia gravis

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Background and purpose: Low density lipoprotein receptor-associated protein 4 (LRP4) autoantibodies have recently been detected in myasthenia gravis (MG), but little is known about the clinical characteristics associated with this serological type. In this study, the clinical features of Chinese patients with anti-LRP4 antibody-positive MG were characterized.
Methods: A total of 2172 MG serum samples were collected from patients in various parts of China. ELISA was used to detect AChR-Ab and Titin-Ab, and cell-based assays were used to detect MuSK-Ab and LRP4-Ab. Clinical data for patients with MG were collected from different provinces in China.
Results: In total, 16 (0.8%) patients with LRP4-MG were found among 2172 total patients, including 3 patients with AChR/LRP4-MG. Additionally, 13 (2.9%) patients with LRP4-MG were found among 455 patients with double seronegative MG. The ratio of males to females for these 13 patients was 1:1.6 and 53.8% patients were children. A total of 91.7% of cases exhibited initial ocular involvement, and 58.3% of cases exhibited simple eye muscle involvement. Responses to acetylcholinesterase inhibitors and prednisone were observed.
Conclusion: The expanded sample confirmed that the positive rate of LRP4-Ab in China is lower than that in Western countries. Our results highlighted the differences between LRP4-MG and other antibody groups. Children and female patients in LRP4-MG have a higher prevalence, often involving the ocular muscles and limb muscles. The clinical symptoms are mild and satisfactory responses to treatment are often achieved.

P2743

GLS1-mediated glutaminolysis unbridled by MALT1 protease promotes psoriasis pathogenesis

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Psoriasis is a severe disease with the disturbance of the metabolism and inflammation, but the molecular mechanisms underlying this aspect of psoriasis pathology are poorly understood. Here, we reported that glutaminase 1 (GLS1), the first enzyme in glutaminolysis, was highly expressed, together with higher concentration of glutamate in individuals with psoriasis and psoriasis-like mouse model. More interestingly, the level of GLS1 was positively correlated with the disease activity and IL-17 levels in psoriasis. The aberrant GLS1-mediated glutaminolysis maintained high concentrations of acetyl-CoA to enhance histone acetylation of IL-17a promoter and transcriptional activity of RORC, which revealed an epigenetic mechanism of GLS1-mediated glutaminolysis in promoting helper T (Th) 17 cell differentiation and contributing to the immune imbalance in psoriasis. We further demonstrated that GLS1 expression was supported by MALT1 protease, which was constitutively active in psoriatic $CD4^+$ T cells and supported GLS1 expression by stabilizing c-Jun that directly bound to the GLS1 promoter region. Blockading either of GLS1 activity or MALT1 protease resolved Th17 differentiation and showed highly resistant to the psoriasis pathology upon IMQ-induced animal model. Finally, we found Th17-related cytokines enhanced GLS1 expression in keratinocytes, resulting in hyperproliferation and chemokine production of keratinocytes. Overall, our findings provided the role of MALT1-c-Jun-GLS1-Th17 axis in psoriasis pathogenesis, and revealed novel potential therapeutic targets for psoriasis.

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DOI: 10.1002/eji.201970400

P2744

Application of contrast-enhanced ultrasound result for monitoring the arterial inflammation in Takayasu´s arteritis in Chinese population

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Objective: To evaluate the utility of contrast-enhanced ultrasound (CEUS) in assessing vessel wall inflammation and disease activity in Takayasu´s arteritis (TA), and then compare them with those results of (18) F-fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET).
Method: We enrolled 71 patients with TA who had undergone carotid artery CEUS. 22 of 71 patients underwent ^{18}FDG-PET at the same time. The associations between the carotid CEUS vascularization grade and clinical data were assessed. The ability of evaluating disease activity in TA was further compared between the vascularization of carotid artery CEUS and vascular uptake of18FDG-PET.
Results: Scans for 45 of 71 patients who underwent CEUS showed active lesions (visual grade ≥2) in the left or right carotid vascular region. There was a statistically significant association between CEUS assessment and ITAS 2010 (P=0.01) or Kerr´s criteria (P=0.000). In 71 TA patients, there were 34 patients with clinically inactive disease according to ITAS 2010. In 34 TA patients with clinically inactive disease, 11 CEUS scans showed active lesion (visual grade≥2) in right or left carotid vascular region. In 22 TA patients of completed both CEUS and ^{18}F-FDG/PET, the right (or left) carotid CEUS vascularization grade positively correlated with vascular ^{18}F-FDG uptake (P=0.000). When visual grade≥2 in ^{18}F-FDG uptake was regarded as vascular inflammation, carotid CEUS showed a sensitivity of 100%, a specificity of 80%.
Conclusion: CEUS could find active lesion in carotid vascular region for TA patients with clinically inactive disease. Carotid CEUS may be a rapid and cost-effective substitute tool for angiography in the follow-up of TA.

P2745

Morin exerts anti-arthritic effects by attenuating synovial angiogenesis via activation of peroxisome proliferator activated receptor-γ

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Morin, a flavonoid occurring in many dietary plants, can reduce the number of synovial blood vessels and ameliorate collagen-induced arthritis (CIA) in rats. Herein, its underlying mechanisms in view of the peroxisome proliferator activated receptor-γ (PPARγ) pathway are addressed. In vitro, wound-healing and transwell assays are conducted to explore the effect of morin on HUVECs migration. Morin inhibits HUVECs migration and tube formation induced by VEGF, which is reversed by PPARγ antagonist GW9662 or siPPARγ. Molecular docking and competitive binding assays show that morin could bind to PPARγ. Morin increases the expression of PDK4 and CD36 in a PPARγ-dependent manner and increases the luciferase activity in cells transfected with PPARγ plasmid, which indicates that morin could activate PPARγ after binding. In addition, morin increases the expression of PTEN, a target gene of PPARγ that suppresses angiogenesis and inhibits PI3K/Akt signaling. The effects of morin on the PTEN-PI3K/Akt pathway are diminished by GW9662 and siPPARγ. In vivo studies show

DOI: 10.1002/eji.201970400

that morin ameliorates rat CIA, reduces synovial angiogenesis, and upregulates the expression of PTEN in the synovium, which is almost completely abolished by GW9662. **In conclusion, morin is a potential agonist of PPARγ, which attenuates synovial angiogenesis and arthritis via the PPARγ-PTEN-PI3K/Akt pathway.**

P2746

CD147: a target for lymphocyte lineage and rheumatoid arthritis therapy

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CD147, as a multifunctional transmembrane glycoprotein, is highly expressed on the surface of a variety of tumor and immune cells, promoting the development and invasion of tumors and rheumatoid arthritis.
Using T cell-specific deletion, we showed that in thymus CD147 is indispensable for thestable αβ T cell lineage commitment: loss of CD147 biases both multipotent DN and fully committedDP cells into innate NK-like lineages. Mechanistically, **CD147 deficiency results in impairedWnt signaling and expression of BCL11b, a master transcription factor in determining T cell identity**. Functional blocking of CD147 by antibody phenocopies genetic deletion to enrichNK-like cells in the periphery.
We also demonstrated that CD147 was predominantly upregulatedin Tm cells derived from RA patients. The anti-CD147 mAb 5A12 specifically inhibited Tm-cell activation, proliferation andfurther restrained osteoclastogenesis. **Using a structural-functional approach, we depicted the interface between 5A12 and CD147.This allowed us to identify two critical residues, Lys63 and Asp65, as potential targets for RA treatment,** as the double mutationK63A/D65A inhibited Tm-cell activation, mimicking the neutralization by 5A12.
We also showed for the first time that ACPAs could activate the NLRP3 inflammasome, which participates in RA pathogenesis. The interaction of CD147 and ITGB1 was enhanced in macrophages after ACPA treatment, followed by the activation of the downstream Akt/NF-κBsignalling pathway and successive induction of NLRP3 and pro-IL-1β.
Summary, besides CD147 can be a target for lymphocyte lineage, it is also an effective surface targets for immune modulation in tumor and RA therapy.

P2747

A novel DMARD, Iguratimod disrupts both IL-17 and TGF-β signaling in epithelial cells and attenuates bleomycin-induced interstitial lung disease in mice

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Subsets of patients with rheumatoid arthritis (RA) always develop interstitial lung disease (ILD). In addition to environmental and genetic factors, lots of traditional disease-modifying antirheumatic drugs (DMARDs) for RA treatment have been reported to induce the fibrotic lung abnormalities. Here we show that iguratimod, a novel DMARD, dose-dependently and potently diminished the severity of bleomycin-induced lung fibrosis by attenuating IL-17 and TGF-β signaling, which is distinct from methotrexate. In addition, iguratimod attenuated lung fibrosis in CIA mice. Furthermore, we found that,

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DOI: 10.1002/eji.201970400

Act1, the target protein of iguratimod, was induced in response to TGF-β and in turn promoted TGF-β induced epithelial-mesenchymal transition. Increased Act1 expression has been found in lungs of bleomycin-induced fibrosis and also in the lungs of patients with IPF. Our data demonstrate an important role of Act1 in TGF-β signaling and pulmonary fibrosis, providing a novel approach for targeting Act1 in treating clinically refractory fibrotic diseases, such as RA-ILD.

P2748

Anti-β_2GPI/β_2GPI induced neutrophil extracellular traps formation to promote thrombogenesis via the TLR4/MyD88/MAPKs axis activation

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Antiphospholipid antibodies (aPLs) are a large group of heterogeneous antibodies that bind to anionic phospholipids alone or in combination with phospholipid binding proteins. Increasing evidence has converged to indicate that aPLs especially anti-β_2 glycoprotein I antibody (anti-β_2GPI) correlate with stroke severity and outcome. Though studies have shown that aPLs promote thrombus formation in a neutrophil-dependent way, the underlying mechanisms remain largely unknown. In the present study, we investigated the effect of anti-β_2GPI in complex with β_2GPI (anti-β_2GPI/β_2GPI) on neutrophil extracellular traps (NETs) formation and thrombus generation *in vitro* and *in vivo*. We found that anti-β_2GPI/β_2GPI immune complex induced NETs formation in a time- and concentration-dependent manner. This effect was mediated by its interaction with TLR4 and the production of ROS. We demonstrated that MyD88-IRAKs-MAPKs, an intracellular signaling pathway, was involved in anti-β_2GPI/β_2GPI-induced NETs formation. We also presented evidence that tissue factor was expressed on anti-β_2GPI/β_2GPI-induced NETs, and NETs could promote platelet aggregation *in vitro*. In addition, we identified that anti-β_2GPI/β_2GPI-induced NETs enhanced thrombus formation *in vivo*, and this effect was counteracted by using DNase I. Our data suggest that anti-β_2GPI/β_2GPI induces NETs formation to promote thrombogenesis via the TLR4/MyD88/MAPKs axis activation, and could be a potentially novel target for aPLs related ischemic stroke.

P2749

Anti-beta$_2$ GPI/beta$_2$ GPI complexes induce platelet activation and promote thrombosis via p38MAPK: a pathway to targeted therapies

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Anti-beta$_2$ glycoprotein I (anti-beta$_2$ GPI) antibodies are important contributors to the development of thrombosis. Anti-beta$_2$ GPI antibody complexes with beta$_2$ GPI are well known to activate monocytes and endothelial cells via the intracellular NF-kB pathway with prothrombotic implications. By contrast, the interaction of anti-beta$_2$ GPI/beta$_2$ GPI complexes with platelets has not been extensively studied. The p38 mitogen-activated protein kinase (MAPK) pathway has been recognized to be an important intracellular signaling pathway in the coagulation cascade and an integral component of arterial and venous thrombosis. The present study reveals that levels of anti-beta$_2$ GPI/beta$_2$ GPI complexes in sera are positively associated with p38MAPK phosphorylation of platelets in thrombotic patients. Furthermore, SB203580 inhibits anti-beta$_2$ GPI/beta$_2$ GPI complex-induced platelet activation. Thrombus formation decreased in *p38MAPK*$^{-/-}$ mice after treatment with anti-beta$_2$ GPI/beta$_2$ GPI

DOI: 10.1002/eji.201970400

complexes. In conclusion, p38MAPK may be a treatment target for anti-beta$_2$ GPI antibody-associated thrombotic events.

P2750

Anti-phospholipid antibody-activated platelets lead to endothelial cell damage via MMP-1/PAR1 axis

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Antiphospholipid Syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity. It is classically associated with persistent positivity for anti-phospholipid antibodies (aPLs). APLs are autoantibodies directed primarily against phospholipid-bound proteins, with the most common target being β_2-glycoprotein I (β_2GPI). We and others have previously identified that anti-β_2GPI in complex with β_2GPI (anti-β_2GPI/β_2GPI) can activate platelets to promote thrombosis. However, the underlying mechanism remains to be elucidated. In the present study we investigated the effects of anti-β_2GPI/β_2GPI-activated platelets on endothelial cell damage which is a key factor in promoting thrombosis. Incubation platelets with anti-β_2GPI/β_2GPI induced platelets activation (glycoprotein IIb/IIIa and P-selectin expression) in a time- and concentration-dependent manner. Based on the expression of matrix metalloproteases (MMPs) on platelet's surface and their pro-inflammatory effects, we detected the level of several MMPs (MMP-1, MMP-2, MMP-3 and MMP-14) in platelets' culture supernatant, and found that anti-β_2GPI/β_2GPI promoted platelets MMP-1 release significantly. Co-culture human umbilical vein endothelial cells (HUVECs) with anti-β_2GPI/β_2GPI-activated platelets lead to increased lactate dehydrogenase (LDH), interleukin-1β (IL-1β) secretion, and endothelial monolayer permeability. Inhibition of protease-activated receptors (PARs) on HUVECs with RWJ-56110 reversed MMP-1 induced endothelial LDH, IL-1β secretion and permeability. Our data indicate that anti-β_2GPI/β_2GPI-activated platelets lead to endothelial damage via MMP-1/PAR1 axis, and could be a potentially novel target for aPLs induced thrombosis.

P2751

Clinical characteristics of immune thrombocytopenia associated with primary Sjogren´s syndrome: a retrospective analysis of 291 patients

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Background: Primary Sjogren´s syndrome (pSS) is one of the most common autoimmune diseases sometimes complicated with immune thrombocytopenia(ITP). The characterisistics of ITP associated with pSS patients and the potential risk factors were not very clear. This study aimed to explore the clinical characterisistics of ITP associated with pSS patients.
Method: A total of 291 SS patients hospitalized between 2015 and 2018 were retrospectively reviewed.
Results: Among the 291 patients included in the study, there were 35 patients in the PSS-ITP group, with a male to female ratio of 3:32, and an average age of onset of 46.8346±13.2869 years ; 256 patients in the non-ITP PSS , with a male to female ratio of 11:117, and an average age of onset of 40.0857±15.73563 years . Non-ITP in PSS group compared to patients with non-ITP PSS, the prevalence of interstitial lung disease, dry eye symptoms and joint involvement is less, difference is statistically significant. However, there were no statistically significant differences in the positive rates

DOI: 10.1002/eji.201970400

of C3, C4, antinuclear antibodies, anti-SSA antibodies and anti-SSB antibodies. The ESSDAI scores of the non-ITP pSS group and the ITP-pSS group were 4.1417±2.63819 and 5.3143±2.36110, respectively. The PSS with ITP group was significantly higher than the non-ITP pSS group, and the difference was statistically significant.
Conclusion: (1)The onset of age in ITP-PSS was younger than non-ITP pSS. (2)The non-ITP PSS group are more likely to develop interstitial lung disease and joint involvement.
Keywwords: primary Sjogren´s syndrome; immune thrombocytopenia;retrospective analysis.

P2752

Study on the mechanism of Guizhou Miao drug "Sidaxue" on inflammation and vascular hyperplasia of rheumatoid arthritis

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The main difficulty of rheumatoid arthritis treatment lies in the inability to effectively control arthritis and synovial vascular hyperplasia. It is known that IL-1β/NF-κB signaling pathway is the "switch" to activate many inflammatory factors, and VEGFR family is related to angiogenesis. "Sidaxue", a special prescription for treating RA in Miao area of Guizhou Province, has clinic application value due to its insignificant side effects and low price. This study explores the mechanism of "Sidaxue" on inflammation and vascular hyperplasia of rheumatoid arthritis by researching the effect of "Sidaxue" on the expression of IL-1β/NF-κB signaling pathway and VEGFR family factors in rats of type II Collagen Induced Arthritis model. The results of gene and protein expression showed that "Sidaxue" could down-regulate the expression of inflammatory factors such as IL-1β, TL-17, and TNF-α, inhibit the activation of NF-κBp65 and the expression of VEGF and VEGFR-2, and in turn alleviate arthritis and vascular hyperplasia. Pathological sections also showed that "Sidaxue" could control arthritis and vascular hyperplasia. Therefore, this study demonstrates that the "Sidaxue" can treat RA by controlling vascular hyperplasia and inflammation through inhibiting the expression of IL-1β/NF-κB signaling pathway and VEGFR family factors.
Keyword: Rheumatoid arthritis, Miao drug "Sidaxue", IL-1β/NF-κB signaling pathway, VEGFR family

P2753

A Study of Th17, Treg cell and the expression of relative cytokines in Antigen-induced arthritis in rabbits

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Objective: To investigate T helper cell 17(Th17), regulatory T (Treg) cell and the expression of relative cytokines in Antigen-induced arthritis in rabbits (AIA model).
Methods: 25 New Zealand white rabbits were randomized into 2 groups, 20 in experimental group and 5 in contrast group. High-resolution ultrasound was used to evaluate arthritis. Ultrasound guided biopsy was applied and the pathological sections with HE staining were observed by microscope and accessed. Flow cytometry was used to analyze the percentages of Th17 and Treg cells. The mRNA expressions of retinoid-related orphan receptor gamma t (RORγt) and fork-head box p3 (Foxp3) were detected by reverse transcription polymerase chain reaction (PCR). The concentrations of interleukin 17 (IL-17) was detected by enzyme-linked immunosorbent assay (ELISA).
Result: The percentages of Th17, RORγt mRNA and IL-17 in AIA model group increased significantly

DOI: 10.1002/eji.201970400

compared to healthy controls. The percentage of Treg cells, Foxp3 mRNA and TGF-β in AIA model group were significantly lower than that of in healthy controls (P< 0.05).
Conclusions: The changes for Th17 and Treg cells and their relative cytokines suggest that these factors can be predictions to the severity of arthritis in AIA model.

P2754

Application and preliminary validation of the Hip Inflammation MRI scoring system (HIMRISS) in spondyloarthritis

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Aims: To apply the MRI scoring system (HIMRISS, Hip Inflammation MRI Scoring System) on Ankylosing Spondylistis (AS) patients with hip joint involvement.
Methods: Four readers (including 2 radiologists and 2 rheumatologists) scored the MRI scans obtained from a total of 44 AS patients with hip lesions, and scored both hip joints of these patients at the same time. Inter-observer reliability among readers was assessed by variance analysis to obtain the intraclass correlation coefficient (ICC). The final patient HIMRISS score were sum value of two hip joints. The clinical significance were evaluated by pearson correlation analysis between other disease activity indexes, including Ankylosing Spondyliis Disease Activity Score (ASDAS), laboratoty features, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Harris hip scores.
Results: HIMRISS is a reliable MRI scoring method for bone merrow lesion (BML) and synovitis in SpA, and the ICC value could achieve to 0.9. The ICC values for detecting femoral BML, acetabular BML, and synovitis-effusion were 0.73, 0.84, and 0.88, respectively. HIMRISS values were significantly associated with ASDAS_CRP and ASDAS_ESR, and negatively associated with harris scores.
Conclusion: HIMRISS offers reliable MRI scoring method for hip joint in AS, and it is beneficial for early detection of inflammation before the occurrence of structural damage and fast quantification in disease activity assessment.

P2755

The role of humoral immunity at the early pathological stage of Sjogren´s syndrome-like autoimmune diseases

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Background: Humoral immunity play significant and essential roles at pathological stage of primary Sjogren´s syndrome. We reported that immune complex and complement deposition had been detected in not only Lip gland tissue from pSS patients but also salivary gland tissue from Gnaq-/- chimeras mouse model nine months post transplantation. However, whether humoral immunity occurs and is involved in initiating early pathological injuries towards salivary glands epithelial cell before lymphocytic infiltration has rarely been studied.
Objective: To investigate the roles of humoral immunity at the early pathological stage of Sjogren´s syndrome.
Methods: 1. Immuno-histochemistry and HE stain were performed using tissue specimens from clinic and mouse model. 2. Indirect immunofluorescence was applied to detect serum ANA level in mouse model. 3. Serum level of CIC and C3 were analyzed by ELISA kit. 4. Sjogren´s syndrome-like

DOI: 10.1002/eji.201970400

autoimmune diseases animal model were established through bone marrow transplantation from Gnaq-/- mouse to C57/BL6 wild-type.
Results: Through Immuno-histochemistry and HE stain, we found that immune complex combining with C3 and C1q deposited in Lip gland tissue and salivary gland tissue at early stage of Sjogren´s syndrome-like autoimmune diseases, while positive serum ANA had been detected but no lymphocytic infiltration had been observed. In vitro, we further demonstrated that humoral immunity actually contributed to the early direct injuries to salivary glands epithelial cells throuth complement dependent cytotoxicity pathway.
Conclusion: Humoral immunity occurs and initiates earlier pathological injuries before tissue infiltration of lymphocytic in pSS, thus this will possibly be a crucial and potential checkpoint.

P2756

The role of autoantibodies to DFS70/LEDGF/p75 in autoimmune disease

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In order to investigate the role of autoantibody to DFS70/LEDGF/p75 in AARD differential diagnosis and its positive distribution in the patients with ANA^+, autoantibodies to DFS70 were detected in 2356 of patients who were ordered ANA tests using IIF on the sections which has been covered with 9:1 of DFS70 knock out Hep-2 and non knock out Hep-2. ANA positive are 216 in 2356 patients (9.17%), whereas 17 patients were anti-DFS70 positive (0.72%). There were 88.23% of patients without AARDs and 11.76% of patients with AARDs in the anti-DFS70 positive patients. Therefore, the positive distribution discrepancy of anti-DFS70 was significant between the above two groups ($P< 0.05$). Overall, autoantibody to DFS70 presents in non-AARD patients while rarely in AARD patients. Thus, Anti-DFS70 positive is an important exclusion criterion for AARDs.

P2757

Comparative study on high resolution ultrasound and serology TNF-α to predict ectopic germinal center in antigen induced arthritis model (AIA) in rabbits

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Objective: To investigate and compare the value of high resolution ultrasound and serum TNF-αto predict ectopic germinal center on synovium tissue in antigen induced arthritis model (AIA)in rabbits.
Methods: Twenty-five New Zealand white rabbits were randomized into two groups, 20 in model group and 5 in control group. High resolution ultrasound was used to evaluate arthritis on both knees. Serum tumor necrosis factor-α(TNF-α) level were determined by ELISA. Ultrasound guided biopsy was applied and the pathological sections were observed by microscope and accessed in week 4, 8, 12, 15 after establish model. H&E and immunohistochemical staining was used to detect pathological changing and ectopic germinal center in synovium tissue. Analyzing the correlation between high resolution ultrasound, serum TNF-αand grades of chronic synovitis and ectopic germinal center.
Results: The thickness of membranes and TNF-αwere significantly higher in rabbits with ectopic germinal center than that of without ectopic germinal center under H&E and immunohistochemical staining($P< 0. 05$) .
Conclusion: Ultrasound scale and serum TNF-αlevel may be valuable to predict the presentation of ectopic germinal center in synovium tissue in AIA model.
Keywords: Rheumatoid arthritis, Animal model, Ultrasound, TNF-α, Ectopic germinal center

DOI: 10.1002/eji.201970400

P2758

Investigation on the changes and roles of peripheral blood lymphocyte subsets and platelet associated antibodies in children with primary immune thrombocytopenia

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To investigate the changes of peripheral blood lymphocyte subsets and PA-Ig in children with ITP, and to study their roles in mechanism and diagnosis of ITP. Counting and classification of peripheral blood cells in 67 cases of newly diagnosed children with ITP and 42 healthy children were completed by cell analyzer. Flow Cytometry was used to detect the expression of platelet associated antibodies and T lymphocyte subsets, NK and B cells in both ITP and controls. The statistical analysis was performed by SPSS 19.0 statistical software. The average counts of platelets, lymphocytes and percentage of lymphocytes in patients with ITP were less than those in controls. However, there were no statistical difference of the total WBC count between ITP children and controls. The level of PA-Ig in ITP patients were much higher than that in controls. Correlation analysis demonstrated that in ITP patients the platelets counts were negatively correlated with PA-Ig levels and not related with PA-Ig subsets. Multivariate linear regression analysis showed that platelet counts of ITP patients PLT=48.452-1.256*PA-IgG-1.033*PA-IgM-1.332*PA-IgA model had statistical significance; The average counts and percentages of $CD3^+$ and $CD4^+$ lymphocytes, NK cells, and the ratio of $CD4^+$ and $CD8^+$ lymphocytes in ITP patients were much less than controls. However, the average count and percentage of $CD8^+$ and $CD19^+$ lymphocytes were higher than controls. Both cellular and humoral immune systems were found dysfunction in ITP children. The detection of PA-Ig and lymphocyte subsets can provide laboratory basis for the diagnosis and treatment evaluation of ITP patients.

P2759

Association study of SIGIRR polymorphisms with rheumatoid arthritis in a Chinese population

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Objective: To investigate the association of two single-nucleotide polymorphisms (SNPs) in the single immunoglobulin IL-1-related receptor (SIGIRR) gene with rheumatoid arthritis (RA) in a Chinese population.
Methods: We examined 508 RA patients and 525 healthy control subjects. SIGIRR rs7396562 and rs3210908 polymorphisms were specified from genomic DNA using TaqMan genotyping assay on Fluidigm 192.24 system.
Results: The SIGIRR rs7396562 T allele was increased in RA patient group compared with control subjects (T versus G: OR =1.284, 95 % CI =1.078-1.529, *P*=0.005). Significant difference in genotype distribution was found in RA patients and controls ($\chi 2$ =9.203, *P*=0.010). We also discovered a statistical significance under the recessive model (TT versus TG + GG: OR =1.491, 95 % CI =1.149-1.934, *P*=0.003). However, we did not detect any significant associations between rs3210908 and RA susceptibility.
Conclusions: Our study represents the first report demonstrating an association of the SIGIRR rs7396562 polymorphism with RA susceptibility in a Chinese population.

DOI: 10.1002/eji.201970400

P2760

Gαq deficiency accelerates the onset of experimental lupus

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Systemic lupus erythematosus is diagnosed according to a spectrum of clinical manifestations and autoantibodies associated with abnormal expression of type I interferon signature genes. Tetramethylpentadecane (TMPD), also called as pristine, induced a murine model of systemic lupus erythematosus. Taking pristine to genetically intact mice results in the production of lupus-like autoantibodies and glomerulonephritis, similar to human lupus nephritis. Our previous studies have shown a crucial role of Gαq in immune system. We treated *Gnaq*$^{+/-}$ C57 mice with pristane using C57 mice as controls. *Gnaq*$^{+/-}$C57 mice developed antinuclear antibodies earlier than the control mice; also, their titers were higher than the controls. pDCs were increased in the peritoneal lavage fluid of *Gnaq*$^{+/-}$ C57 mice injected for 7 days compared to controls. Th1 and Th17 cells both were increased significantly in injected *Gnaq*$^{+/-}$ C57 mice after a month. *Gnaq*$^{+/-}$ C57 mice took place into more severe kidney damage than controls. Together all, our findings demonstrate that Gαq deficiency accelerated lupus occurrence.

P2761

CD226 attenuates treg proliferation via AKT/ERK signalling in an EAE model

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CD226 molecules play a vital role in the activation of effector CD4^{+}T cells during the process of immune response, but a cell-intrinsic function in CD4^{+}T subsets is not clear. Here, we show that *Cd226*$^{-/-}$ mice were resistant to MOG$_{35-55}$-induced experimental autoimmune encephalomyelitis with highly expressed IL-10^{+}CD4^{+}T cells and downregulated IL-17A^{+}CD4^{+}T cells when compared with wild type mice.Th17 cell infiltration into the CNS was largely decreased in the absence of CD226 during EAE. CD226 deficiency facilitated the proliferation of Treg cells with increased number in EAE mice and supported by the elevated iTreg proliferation *in vitro*. The Akt and Erk signalling pathways were involved in *Cd226*$^{-/-}$ Treg proliferation and function *in vivo* and *in vitro*. These findings collectively indicate that CD226 is a key molecule for Treg-mediated suppression because it inhibits Treg proliferation. Thus, we provide further mechanisms of CD226 molecules involved in Treg functions in EAE and the evidence that of the potential therapeutic effects of anti-CD226 on autoimmune diseases.

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DOI: 10.1002/eji.201970400

P2762

Mechanism research of 14-3-3 η protein on osteogenetic differentiation in bone marrow mesenchymal stem cells by regulating MAPK signal pathway

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Objective: Whether 14-3-3η protein could affects osteogenic differentiation of BMSCs in patients with ankylosing spondylitis by regulating MAPK signaling pathway.
Methods: Up-regulation and down-regulation of lentiviral vector transfecting 14-3-3η. QRT-PCR, Western-Blot, scratch test and co-immunoprecipitation. Constructing miRNA142-3P or miRNA142-3P overexpression or inhibition.
Results:
1. Knocked down 14-3-3η. Expression of hif-a, vegf, BMP-2, OPN, ost and Runx2 were detected at different time point (1d, 4d, 7d, 14d, 21d)by QRT-PCR and Western-Blot. The expressions of the above moleculars were significantly increased.
2. Knocked down 14-3-3η. The migration ability of BMSCs was obviously enhanced after 24h culture by scratch experimental .
3. Hypoxic environment, 14-3-3η and MAPK were down-regulated, Hif-α and BMP-2 were up-regulated, while 14-3-3η decreased with MAPK. Knocked down 14-3-3η, MAPK was down-regulated and expression of osteogenic protein BMP-2 were up-regulated. When adding caspase3 inhibitor, knocking down 14-3-3η again and MAPK was not cleaved by caspase3 and BMP-2 expression was not up-regulated.
4. 14-3-3η and MAPK were down-regulated while the level of BMP-2 was up-regulated compared with miR-142-3P was overexpressed. Inhibited miR-142-3P expression showed that 14-3-3η and MAPK were up-regulated, and osteogenic protein BMP-2 was down-regulated compared with control group. At the same time, mRNA of 14-3-3η was up-regulated compared with control group, while mRNA of miR-142-3P was down-regulated compared with control group.
Conclusion:
1. 14-3-3η could significantly inhibit osteogenic differentiation of BMSCs by activating MAPK signaling pathway;
2. miRNA142-3P was involved in the inhibition of osteogenic differentiation of BMSCs through the inhibition of 14-3-3η.
Keywords: 14-3-3η protein/Osteogenesis /MAPK/miRNA142-3P

P2763

Therapeutic effect and mechanism of low dose naltrexone(LDN) on the Collagen-induced-arthritis(CIA) rat models

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Objective: Our experiments study the of efficacy and mechanism of LDN in the treatment of RA to alleviate the joint inflammation through CIA rat models in vivo.
Methods: Rats were evaluated the severity of arthritis through pathological changes of ankle and synovial tissue by HE staining and semi-quantitative scoring. T-cell subsets were detected by flow-cytometry. Expression of cytokines were detected in peripheral serum by ELISA. Real time PCR, IH staining and WB methods were utilized to detect the expression of mRNA and protein of opioid receptors and TLR4/RANKL/NF-κB in synovial tissue and spleen.
Results: In the 10mg/kg-LDN group, severity of joint inflammation was the least, $CD4^+T/CD8^+T$,Th2/Th1 and Treg/Th17 were increased in the spleen lymphocytes. Contrary to IL-10, the expression

DOI: 10.1002/eji.201970400

of pro-inflammatory cytokines was decreased. MOR and DOR were strongly expressed in spleen, KOR and DOR were strongly expressed in synovial tissue. The expression of TLR4/NF-κB/RANKL was less in the spleen and synovium compared with the CIA group, but was more with the control group.
Conclusions: LDN can relieve the severity of arthritis and 10mg/kg is the optimal dose. LDN could combined with MOR and DOR in the spleen inhibiting the TLR4/NF-κB signaling pathway regulating the systemic immune response to alleviate the inflammation. Besides LDN could combined with KOR and DOR in the synovial tissue inhibiting TLR4/RANKL/NF-κB pathway as to decrease osteoclast differentiation decreasing the erosion of articular cartilage and bone tissue in the joint.
Key words： Low dose naltrexone(LDN); Collagen-induced- arthritis(CIA) rat models; immune regulation; T cell subsets; TLR4/RANKL/NF-κB

P2764

Gut microbiota modulator suppresses Th17 cell response in rheumatoid arthritis through induction of anti-inflammatory gut hormone

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Gut microbiota is well recognized to participate in the pathogenesis of many Th17 cell-drived autoimmune diseases. This study aimed to investigate the mechanism by which microbiota modulators repress Th17 cell responses using berberine as a tool drug. The results showed that oral berberine (200 mg/kg) markedly attenuated Th17 cell response and synovitis in collagen-induced arthritis (CIA) rats. The systematic analysis of gut microbiota and metabolites demonstrated that berberine ameliorated arthritis in rats mainly by promoting the expression and activity of butyryl-CoA:acetate-CoA transferase, a key enzyme responsible for butyrate generation, and by elevating the relative abundance of butyrate-producing communities in microbiota. Considering the extremely low oral bioavailability of butyrate, we screened endogenous immunosuppressive and anti-inflammatory mediators, and found that berberine selectively enhanced the secretion of cortistatin, a gut hormone. Co-administration of the receptor antagonists of cortistatin markedly weakened the Th17-suppressing and anti-CIA effects of berberine. Moreover, the elevation of cortistatin level correspond to the gut microbiota-modulation and butyrate-generation effect of berberine.
In conclusion, berberine ameliorated CIA in rat through a 'gut microbiota-cortistatin-Th17 cell' pathway. The gut microbiota modulators have potentials to treat Th17 cell-related diseases including rheumatoid arthritis with an indirect action mode.

P2765

Serum secretory phospholipase A2 group IB correlates with the severity of membranous nephropathy

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Background: Serum secretory phospholipase A2 group IB (sPLA2-IB) is involved in the pathological processes of membranous nephropathy (MN). To date, there is no large-scale study validating the usefulness of circulating sPLA2-IB in the follow-up of patients with MN.
Methods: A total of 158 patients with primary membranous nephropathy (pMN), 34 with secondary membranous nephropathy (sMN) and 53 healthy controls were enrolled. Histological staging was made for all MN patients. 36 of the pMN patients accepted immunosuppressive therapy and 11 sMN

DOI: 10.1002/eji.201970400

patients who received treatment of primary disease were followed up for 6 months. Serum sPLA2-IB, PLA2R-Ab, BUN, creatinine, total protein, albumin, cholesterol, triglyceride and 24-hour urine protein were measured at the time of diagnosis. SPLA2-IB and 24-hour urine protein were measured at the end of follow-up.
Results: Circulating sPLA2-IB levels were significantly higher in pMN and sMN patients compared to controls and negatively correlated with TP and albumin, whereas positively correlated with 24-hour urine protein. PLA2-IB was found increased with the severity of proteinuria. Through the 6-month follow-up, sPLA2-IB and 24 h-urine protein levels were found significantly decreased when patients with pMN or sMN reached remission. By ROC analysis, PLA2R-Ab was demonstrated to be most significant in the differential diagnosis of pMN and sMN compared with 24-hour urinary protein and serum sPLA2-IB.
Conclusion: sPLA2-IB was correlated with the level of proteinuria in MN patients suggesting to be a potential biomarker for monitoring disease severity and therapeutic effects of both pMN and sMN.

P2766

Hot spots in studies on international etiology for rheumatoid arthritis

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Objective: To analyze the published articles of etiology for rheumatoid arthritis and get the recent research hotspot.
Methods: Searching etiology for RA literatures through PubMed database of US Congress Library of Medicine in past five years, using BICOMB to bibliometric analysis and SPSS 19.0 to cluster analysis,plotting strategic coordinate figure.
Results: Four research hotspots were concluded by analyzing the key words: 1) therapeutic uses and adverse effects of antirheumatic agents; 2) genetics and complications of RA; 3) immunity of autoantigens and autoantibodies in RA;4)immune cells and microRNAs in RA.
Conclusion: Researches on RA etiology developed stably, with different current status and developing tendency,which provides reference for its related studies.
Keyword: Rheumatoid arthritis;Etiology;Hot spots in studies;Co-word clustering; Strategic coordinate

P2767

Associations of perceived social support and positive psychological resources with fatigue symptom in patients with rheumatoid arthritis

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Objective: This study aimed to assess the association between perceived social support (PSS) and fatigue and the roles of hope, optimism, general self-efficacy and resilience as mediators or moderators on PSS-fatigue association among RA patients in China.
Methods: A multi-center, cross-sectional study was conducted with RA inpatients in northeast China. The Multidimensional Fatigue Inventory, Multidimensional Scale of Perceived Social Support, Herth Hope Index, Life Orientation Test Revised, General Self-Efficacy Scale and Ego-Resiliency Scale were completed. The associations of PSS, hope, optimism, general self-efficacy and resilience with fatigue and the moderating roles of these positive psychological constructs were tested by hierarchical linear regression. Asymptotic and resampling strategies were utilized to assess the mediating roles of hope, optimism, general self-efficacy and resilience.

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Results: PSS, hope, optimism and resilience were negatively associated with RA-related fatigue, whereas DAS28-CRP was positively associated. Only resilience positively moderated the PSS-fatigue association (B = 0.03, β =0.13, P< 0.01). Hope, optimism and resilience may act as partial mediators in the association between PSS and fatigue symptoms (hope: a*b = -0.16, BCa 95%CI: -0.27, -0.03; optimism:a*b = -0.20, BCa 95%CI: -0.30, -0.10; resilience: a*b = -0.12, BCa 95%CI: -0.21±0.04).
Conclusions: Fatigue is severe among RA patients. Resilience may positively moderate the PSS-fatigue association. Hope, optimism and resilience may act as partial mediators in the association. PSS, hope, optimism and resilience may contribute as effective recourses to alleviate fatigue, upon which PSS probably has the greatest effect.
Key words: Rheumatoid Arthritis; Fatigue; Social Support; Hope; Optimism; General Selfefficacy; Resilience

P2768

Pentraxin 3(PTX3) inhibits osteoclast formation induced by fibroblast growth factor 2(FGF2) in rheumatoid arthritis(RA)

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Objective: The study confirmed that PTX3 has inhibitory effect on osteoclast formation induced by FGF2 in patients with RA.
Methods: The synovial membrane and fluid of 10 RA patients and 10 OA patients were collected. The levels of PTX3, FGF2 and RANKL in the synovial fluid were detected by ELISA,the levels of PTX3,FGF2 and RANKL in synovial membrane were detected by immunohistochemistry. The levels of PTX3, FGF2 and its receptor HSPG and FGFR-1 in RASF were detected by PCR. The effect of PTX3 on FGF2 and its receptor binding was observed by binding experiment of ^{125}i-FGF2 cells. RASF cells were treated with rhPTX3 at different concentrations, the expressions of ICAM-1 and RANKL were detected by PCR and Westernblot. PBMC was co-cultured with RASF, TRAP staining was used to identify osteoclasts.
Results: FGF2 was higher in the synovial tissue and synovial fluid of RA patients. FGF2 promoted the expression of ICAM-1 and RANKL. FGF2 can promote the growth of RASF, while PTX3 can inhibit the growth of RASF cells stimulated by FGF2. PTX3 can significantly inhibit the expression of FGF2-induced ICAM-1 and RANKL, and the inhibitory effect is greater with the increase of concentration. The number of TRAP positive cells in the FGF2 group was increased.FGF2 receptor HSPG in RA group was higher than OA group. rhPTX3 can inhibit the binding of FGF2 to its receptors.
Conclusion: PTX3 may inhibit the osteoclast induced by FGF2 by affecting the binding of FGF2 to the receptor HSPG.
Keywords: PTX3; FGF2; RA;RANKL;HSPG; osteoclast

P2769

Type I interferons are upregulated in the SAMHD1-deficient rat

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Loss of function mutations in human SAMHD1 gene cause Aicardi-Goutières syndrome (AGS), a rare autoimmune disease featured by high levels of type I interferons (IFN) and upregulation of IFN stimulated genes (ISGs). High type I interferons signature has been found in SAMHD1-knockout mice and SAMHD1 morphant zebrafishes. However, whether SAMHD1 deficiency leads to high expression

DOI: 10.1002/eji.201970400

of type I interferons in rats remains unknown. In this study, we established SAMHD1-null rat models using the CRISPR/Cas9 system and analyzed whether these rat models had the features of AGS. PCR identification results showed the fourth exon of rat SAMHD1 gene was deleted in the tails of these rat models by its specific sgRNAs using the CRISPR/Cas9 system. Western blot results showed that SAMHD1 proteins were not detectable in a variety of organs of these rat models. ELISA results indicated that serum IFN-α and IFN-β levels in SAMHD1-deficient rats were obviously higher than those of wild-type rats. And abnormal high levels of IFN-α and IFN-β were observed in various organs of SAMHD1-knockout rats using immunohistochemistry. Furthermore, qRT-PCR results demonstrated that rat IFN stimulated genes (ISGs) MX1 and ISG15 were upregulated in SAMHD1-knockout rats. Taken together, these results indicated that SAMHD1-deficient rat models had been successfully constructed and they had high type I interferons signature. Our findings suggested SAMHD1-deficient rats might be the ideal models for elucidating the cause of pathogenic type I IFN responses in autoimmune AGS.

P2770

MICB*002 and MICB*014 protect against rheumatoid arthritis, whereas MICA*009 and MICA*A6 are associated with rheumatoid arthritis in a Hainan Han Chinese population

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Aim: Rheumatoid arthritis (RA) as an inflammatory autoimmune disease affects the synovial joints as well as other organs and tissues. Since aberrant expression of MIC molecules has been observed in RA patient, MIC genotypes might play certain roles in the development of RA.
Method: To explore the association of MICA and MICB polymorphisms with RA in a Han Chinese population in Hainan Island, samples from 172 RA and 137 healthy controls were genotyped for MICA and MICB.
Results: Our results indicated that MICB*002 and MICB*014 were less frequent in RA patients than in controls (P = 0.000, 0.005) while there were higher percentages of RA patients carrying MICA*009 and MICA*A6 (P = 0.005).
Conclusion: Different MIC variants might modulate the autoimmune reaction differently in RA disease and therefore serve as protective or risk factors.

P2771

Iodide adjustment and/or 1,25(OH)$_2$D$_3$ supplementation partially reverse the imbalance of pro-inflammatory and anti-inflammatory cytokines in iodide-induced hypothyroidism and autoimmune thyroiditis in offspring rats

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We aim to investigate whether iodide adjustment from 100 high iodide intake (100 HI) to normal iodide intake (NI) and/or 1,25(OH)$_2$D$_3$ supplementation may improve high iodide induced hypothyroidism and autoimmune thyroiditis in the offspring Wistar rats. In the 100 HI group of maternal rats, high TPOAb and TgAb levels were detected, and in their offspring at postnatal (PN) 180 day, the FT3 and FT4 levels showed a significant decrease, while the levels of serum TSH, TPOAb and TgAb were significantly increased in 100 HI groups. The continued to 100 HI group displayed CD4$^+$ co-localized with thyroid hormone receptor (TR) or vitamin D receptor in infiltrated cells in the thyroid gland by double immunofluorescent staining. After the treatment with iodide adjustment and/or 1,25(OH)$_2$D$_3$

DOI: 10.1002/eji.201970400

supplementation, the serum level of FT3, the protein expression of TR in thyroid, % thyroid uptake were all significantly increased. The mRNA expression of IL-17A and IFN γ was significantly decreased, while the mRNA expression of IL-10 was significantly increased in both the thyroid gland and spleen. We conclude that in maternal rats, autoimmune thyroiditis can be induced following 100 HI on lactation days, and in their off spring at PN180, hypothyroidism complicated with autoimmune thyroiditis can occur. Iodide adjustment and/or 1,25$(OH)_2D_3$ supplementation may partially reverse high iodide intake induced imbalance of pro-inflammatory (IL-17A, IFN γ) and anti-inflammatory cytokines (IL-10). This may provide an insight in the improvement of iodide-induced thyroid disease in the offspring rats from immunological aspect. (Supported by NSFC: 81874257)

P2772

Mannan from the wall of Saccharomyces cerevisiae attenautes murine experimental colitis by regulating the balance of Th17 and Treg

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Background: Recent studies have shown that mannan from the cell wall of Saccharomyces cerevisiae can induce Treg cell differentiation in the co-culture of dendritic cells (DDR) and CD4+ T cells. However,whether mannan from the cell wall of Saccharomyces cerevisiae can inhibit inflammation in a mice model of IBD by up-regulationTreg cell has not been reported.
Methods: Colitis was induced by using DSS. Mannan from the cell wall of Saccharomyces cerevisiae or distilled water was given by gavage into mice with colitis every day and changes in body weight were evaluated. After 15 days, the treated mice were killed and evaluated for histological score, Foxp3 and RORγt expression in the colonic tissue, IL-17A and IL-10 expression in the peripheral blood.
Results: Wasting disease and histological damage were significantly attenuated in mannan-treated mice with colitis compared with those in the distilled water mice. In addition, the expression of RORγt and IL-17A were downregulated, whereas Foxp3 and IL-10 were upregulated in the colonic mucosa of mannan-treated mice.
Conclusions: Mannan from the cell wall of Saccharomyces cerevisiae can attenautes murine experimental colitis by inducing Treg cell differentiation and inhibiting Th17 cell differentiation.

P2773

Mesenchymal stem cells increase CD1c$^+$ tolerogenic dendritic cells by up-regulating FLT3 ligand in human systemic lupus erythematosus

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Allogeneic mesenchymal stem cells (MSCs) exhibit immunoregulatory function in human autoimmune diseases such as systemic lupus erythematosus (SLE), but the underlying mechanisms remain incompletely understood. We show here that the number of peripheral tolerogenic CD1c$^+$ dendritic cells (DCs) and the levels of serum Fms-related tyrosine kinase 3-ligand (FLT3L) were significantly decreased in SLE patients especially with lupus nephritis, compared to healthy controls. Transplantation of allogeneic umbilical cord-derived MSCs (UC-MSCs) significantly up-regulated peripheral blood CD1c$^+$DCs and serum FLT3L. Mechanistically, UC-MSCs expressed FLT3L that bound to Fms-related tyrosine kinase 3 (FLT3) on CD1c$^+$DCs to promote the proliferation and inhibit the apoptosis of tolerogenic CD1c$^+$DCs. Conversely, reduction of FLT3L with small interfering RNA

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DOI: 10.1002/eji.201970400

(siRNA) in MSCs abolished the up-regulation of tolerogenic CD1c$^+$DCs in lupus patients treated with MSCs. Interferon-γ (IFN-γ) induced FLT3L expression in UC-MSCs through Janus Kinase/Signal transducers and activators of transcription (JAK/STAT) signaling pathway. Thus, allogeneic MSCs might suppress inflammation in lupus through up-regulating tolerogenic DCs.

P2774

Autoantibody-mediated movement disorders

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The spectrum of autoantibody-mediated movement disorders includes a broad and clinically heterogeneous group of conditions. These autoantibodies could target the extracellular and intracellular domain of proteins of neuronal cells. Most of these movement disorders are the result of autoimmune encephalitis. Anti-N-methyl-d-aspartate-receptor encephalitis (NMDAR) encephalitis is the most common autoimmune encephalitis that is typically found in young women with teratoma. This kind of encephalitis is usually acute or subacute onset with significant psychiatric symptoms including agitation, mania, hallucination, aggression as well as cognitive dysfunction. Involuntary movements such as stereotype or oral facial dyskinesias are the clinical features of Anti-NMDAR encephalitis. Stiff person syndrome and stiff limb syndrome are related with GAD and amphiphysin autoantibodies. Other autoantibodies could also cause highly specific clinical phenotypes, such as faciobrachial dystonic seizures (FBDS) caused by LGI1 antbodies and neuromyotonia caused by CASPR2 antibodies. Opsoclonus myoclonus syndrome (OMS) could be the result of various autoantibodies including Ma2, Ri, Yo, DPPX antibodies. In Ruijin Hospital, we summarized 91 cases of autoimmune encephalitis. More than 30% of these patients developed movement disorders. In some patients, movement disorder could be the initial symptoms. The movement disorders always have good response to immune therapy. In this presentation, we will discuss the clinical features and treatment of autoantibody-mediated movement disorders in detail.

P2775

Toll-Like receptor 7 ameliorating the progression of the SLE on MRL/lpr mice via inhibiting T follicular helper cells differentiation

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It has been recently indicated TLR7 could induce CD4+T cell anergy. T follicular helper (Tfh) cells represent a CD4+T cell subset specialized for provision of help to B cells. Increased activity of Tfh cells plays a major pathogenic role in systemic lupus erythematosus (SLE). In this study, to identify the influence of TLR7 on Tfh cells in SLE mice mode, we analyzed the phenotypic and functional properties of Tfh cells in MRL/lpr mice after treatment with TLR7 ligands imiquimod. We showed that TLR7 downregulated Tfh cells development in vivo and ex vivo depend on many transcription factors besides Bcl-6. In addition, the frequency of Tfh cells correlated positively with the percentage of B cells, levels of serum anti-dsDNA antibodies and ANA. Our results demonstrate that TLR7 could inhibit Tfh cells immune response and TLR7 ligands may be used as a means of inducing 'tolerance' on Tfh cells in SLE.

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DOI: 10.1002/eji.201970400

P2776

The clinical diagnostic value of serum anti-C1q antibody level in SLE and LN patients

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Objective: To detect the associations between anti-C1q antibody and systemic lupus erythematosus（SLE）and that between anti-C1q antibody and lupus nephritis（LN).
Methods: The Anti-C1q antibody were measured using ELISA kit from 110 cases of SLE patients(SLE group) and 60 cases of healthy people(normal control group). The 110 SLE patients were divided into LN group with 41 patients and non LN group with 69 patients. The serum levels of Anti-C1q, anti-dsDNA antibody and biochemical markers of the two groups were detected, and the result were analyzed.
Results: The serum anti-C1q antibody level of SLE group was significantly higher than that of normal control group［（11.90±23.83）U/ml vs（2.73±4.18）U/ml，P＜0.05］. Serum level of anti-C1q antibody in LN group showed a strongly positive correlation with anti-dsDNA antibody and showed a negative correlation with C3 and C4.
Conclusion Anti-C1q antibody would help to characterize the disease activity of SLE，and are associated with SLE kindey damage. The serum anti-C1q antibody, AnuA and anti-dsDNA antibody combined can improve LN diagnostic specificity.

P2777

Unique features and clinical relevance of aberrantly differentiated atypical memory B cells in lupus patents

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A population of atypical memory B cells (AtMs) are greatly expanded in active lupus patients, but their generation and pathophysiological roles are poorly defined. The aim of this study was to comprehensively characterize lupus AtMs with a purpose to identify therapeutic clues to target this disease-relevant B cell population in lupus. We found that $CD24^{-}CD20^{hi}$ AtMs were strongly increased in two Chinese cohorts of treatment-naïve lupus patients. Gene expression profile indicated that B cell signaling and activation, lipid/saccharide metabolism and endocytosis pathways were abnormally up-regulated in lupus AtMs. Furthermore, lupus AtMs displayed a dysfunctional phenotype, underwent accelerated apoptosis, poorly co-stimulated T cells and produced less pro-inflammatory cytokines. Interestingly, lupus AtMs were in a paradoxically differentiated status with both B cell and plasma cell markers. In addition, we identified a key metabolic pathway which played a crucial role in their differentiation. Finally, AtMs were accumulated in the kidneys of lupus nephritis patients and associated with disease severity. These findings demonstrated that lupus AtMs are abnormally differentiated with metabolic and functional dysregulations. Specifically targeting AtMs by manipulating their metabolism might be an attractive option to improve therapeutic effectiveness in lupus patients.

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DOI: 10.1002/eji.201970400

P2778

Inhibition of microRNA-34a ameliorates murine collagen-induced arthritis

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Rheumatoid arthritis (RA) is one of the most frequently occurring autoimmne diseases, with symptoms including synovium hyperplasia, immune disorder, cartilage damage and bone resorption. It has previously been demonstrated that microRNA-34a (miR-34a) may participate in cell apoptosis, immune activation and bone metabolism, therefore the present study investigated the effects of miR-34a on RA. Collagen-induced arthritic (CIA) mice were employed as a murine model of experimental arthritis, and it was demonstrated that the level of miR-34a in the spleens, lymph nodes and synovium was increased in the CIA mice compared with normal DBA/1j mice. Administration of miR-34a antagomir, the chemically modified inhibitor, ameliorated CIA and delayed the onset of symptoms. Arthritis scores decreased and joint swelling was alleviated with the miR-34a antagomir treatment and the expression of inflammatory cytokines was decreased. miR-34a antagomir delivery significantly decreased the percentage of T cells present including T helper (Th) 1, Th2, Th17 and regulatory T cells. Furthermore miR-34a antagomir-treated CIA mice demonstrated decreased inflammatory-induced bone loss. Overall, **it was observed that inhibition of miR-34a ameliorated murine arthritis, downregulated T cell percentage and cytokine expression, and suppressed bone loss.** The experimental results suggest that inhibition of miR-34a may offer a novel alternative for the treatment of RA.

P2779

MiR-326 overexpression in T cells breaks Th17／Treg balance in MRL/lpr mice

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Objectives: We describe a role for miR-326 in driving deregulation of Th17／Treg balance in lupus mouse.
Methods: 3 groups of female MRL/lpr mice were injected with lentivirus-miR-326 (LV-326) or lentivirus-miR-326 specific inhibitor (LV-sponge) to increase or inhibit miR-326 expression, respectively, and lentivirus-no-encoding (LV-ctrl) as control. The percentage of Th17 cells and Tregs in spleen $CD4^{+}$T cells and kidney were detected by flow cytometry. Levels of serum cytokine (IL-17A, IFN-γ, TGF-β)were detected by CBA. Urinary collections (24 h) were collected and assessed for albumin using ELISA. Immunohistochemistry labeled kidney IL-17A and TGF-βexpression.
Results: Day 7 analysis of differentiationof Th17 cells and Tregs in spleen after injection of lentivirusshowed an increasedpercentage of Th17 in LV-326 mice, while reduction of percentage of Tregs in these mice. Testing the serum cytokines revealed that the significantly increased levels of IL-17A and IFN-γin LV-326 mice, and decreased in LV-sponge mice, no different has been found in TGF-βbetween the groups. Moreover, LV-326 mice showed higher levels of 24h urinary albumin than LV-sponge and LV-ctrl mice in day 7 after injection of lentivirus. The lentivirus-miR-326 injection promoted the infiltration of Th17 cells and simultaneously increased the expression of IL-17A in kidney, no different has been found of TGF-β.
Conclusions: Our data reveal that miR-326 overexpression increased the differentiationof Th17 cells and decreased thedifferentiationof Tregs, and accelerated the renal injury in MRL/lpr mice.These

DOI: 10.1002/eji.201970400

suggest that miR-326 may play a catalytic role in the development of lupus-like changes in lupus model mouse by breaks Th17/Treg balance.

P2780

Autoimmune encephalitis antibodies: comparative detection of serum and cerebrospinal fluid

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Autoimmune encephalitis（AIE）is a group of newly recognized inflammatory diseases of the central nervous system in association with different autoantibodies. In this study, we intended to study the distribution of AIE antibodies, including anti-NMDAR, Caspr2, LGI1 and $GABA_BR$, and provide support for clinical diagnosis of AIE, evaluation of disease progression and efficacy, and selection of clinical specimens. Serum and/or cerebrospinal fluid (CSF) were collected from the Department of Neurology, Huashan Hospital, Fudan University from December 1, 2015 to June 31, 2018. The antibody spectrum of AIE (anti-NMDA, anti-AMPAR1/2, anti-Caspr2, anti-LGI1 and anti-$GABA_BR$ antibody) was examined by cell-based assay (CBA) method. The results showed that among 183 cases of AIE patients with positive antibodies, the proportion of anti-NMDAR positive (67%) was the highest, followed by anti-LGI1 (16%), anti-$GABA_BR$ (8%), anti-Caspr2 (5%), and the anti-AMPAR was the lowest. In addition, for anti-NMDAR patients, the positive rate of CSF (7.13%) was significantly higher than serum (5.35%) (P< 0.05). And for those with anti-Caspr2 and/or LGI1, the positive rate of serum (0.83%; 1.78%) was significantly higher than in CSF (0.06%; 0.54%）(P< 0.01); while for anti-$GABA_BR$ patients, the difference of positive rate between serum (0.89%) and CSF (0.83%) was not enough to significant. We concluded that for anti-NMDAR encephalitis, the positive rate of CSF was higher than serum; for anti-Caspr2 and/or LGI1 encephalitis, the positive rate of serum was higher than in CSF; while for anti-$GABA_BR$ encephalitis, the positive rate between serum and CSF has no difference.

P2781

Soluble glucocorticoid-induced tumor necrosis factor receptor regulates Helios expression in Myasthenia gravis

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Background: Helios is important for functional and phenotype stability of regulatory T cells (Tregs). However, the role of Helios in autoimmune diseases and its regulation remains unclear. This study aimed to investigate the role of Helios+ Tregs in myasthenia gravis (MG) and glucocorticoid-induced tumor necrosis factor receptor (GITR) and its ligand (GITRL) in the modulation of Helios.
Method: Multicolor flow cytometry was performed to analyze Helios+ Tregs in peripheral blood from MG patients and healthy donors (HDs). Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of soluble GITRL/GITR in plasma. Tregs were isolated via magnetic separation and treated with recombinant GITRL and GITR-Fc. Membrane GITRL on Tregs and expression of

DOI: 10.1002/eji.201970400

Helios and other markers (FOXP3, CD25, CD39, CTLA-4, PD-L1 and IL-10) involved in immunosuppressive activity were determined by flow cytometry.
Result: Both Helios+ Tregs and soluble GITR were decreased in generalized MG (GMG) patients (n=14), compared with HDs (n=14) and ocular MG (OMG) patients (n=16). Helios+ Tregs express higher level of CD39 compared to Helios- Tregs. Further analysis indicates soluble GITR was negatively correlated with quantitative MG score and promoted Helios expression and enhanced function of Tregs independently of membrane GITRL.
Conclusion: This work demonstrates abnormal changes in Helios+ Tregs and soluble GITR in MG, as well as direct regulation of Helios by GITR in the context of Tregs. This work provides new insight into the role of GITR in the regulatory pathway of Helios and pathogenesis of MG.

P2782

Low-dose interleukin-2 treatment of active SLE with no increase in infection risk

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Interleukin 2 (IL-2) is critical for the development and maintenance of T cell subsets including regulatory T cells (Tregs) which is deficiencies in systemic lupus erythematosus (SLE). We described a randomized,double-blind, placebo-controlled clinical trial which enrolled 60 patients of active SLE who received low-dose IL-2 (n=30) or placebo (n=30) with standard care. At week 24, the SRI-4 response rate of IL-2 group [70.37% (19/27)] was significantly higher than the placebo group [44.0% (11/25), *P*=0.004]. The SELENA-SLEDAI scores reduced more rapidly in the IL-2 group (median from 12 to 4, *P*< 0.001) compared to placebo group (median from 11 to 6, P< 0.001). IL-2 treatment resulted incomplete remission of LN in 53.84%(7/13) patients compared to 16.67% (2/12) in the placebo group. At the end of 24 weeks, low dose IL-2 treatment was associated with significant reductions of proteinuria (*P*=0.001) and anti-dsDNA titres (*P*=0.059), and better improvement of clinical symptoms including rash (*P*=0.007), arthritis (*P*=0.003) and alopecia (*P*=0.039), as compared to those of the placebo group.Additionally, 44.82% (13/29) of IL-2-treated patients had prednisone tapered ≥ 50% at week 24, compared to 33.33% (9/27) in the placebo group. No serious infection was observed in IL-2 group, but 2 were infected in placebo group. Besides expansion of immune tolerance-enhancing Treg cells, low dose IL-2 also sustained homeostasis of cellular immunity, with enhanced NK cells. Low-dose IL-2 therapy was effective and induced remission in active SLE with no increased risk of infection, which could be due to enhanced NK cells.

P2783

The lymphocyte count and other parameters in the evaluation of SLE activity

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Background: There was some controversy on whether Neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and platelet to lymphocyte ratio (PLR) are associated with disease activity in Systemic Lupus Erythematosus patients.
Methods: The NLR, MLR, PLR, and lymphocyte count results were retrospectively analyzed in regard to their correlations with SLE disease activity index.
Results: The NLR, MLR, PLR and the lymphocyte count were all significantly different from the controls as expected. However, it was the lymphocyte count and the PLR, but not the NLR and MLR, that showed statistical difference between two subgroups. Further regression analyses shown that the

DOI: 10.1002/eji.201970400

PLR and the lymphocyte count correlations with the SLEDAI scores. The ROC curve analysis of these four parameters demonstrated that the lymphocyte count displayed the largest AUC.
Conclusion: The lymphocyte count correlates better than NLR, MLR and PLR with the SLEDAI, and thus it might be more suitable for the evaluation of SLE disease activity.

P2784

The role of AIM2 in regulation of losing self-tolerance in T and B cells in autoimmunity

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The whole pathogenesis of Systemic lupus erythematosus(SLE)is still unclear currently. However SLE is characterized by loss of self-tolerance with activation of autoreactive T and B cells, leading to pathogenic autoantibodies production and tissue injury. AIM2 is DNA sensor which can bind with external double strand DNA antigens and form AIM2 inflammasome. Our finding suggested a role of AIM2 in regulating T cell polarizing and higher AIM2 level in memory T cells. However, compared to health control, lower AIM2 level in B cells was found in lupus patients. In addition, AIM2 is mainly expressed in Naïve B cells, in all B cell subtypes. These results are also consistent with a standard vaccine mouse model by KLH and CFA immunization. In mouse which was immunized for 8 days, more memory cells were found in WT mouse, compared with AIM2 knockout mouse. AIM2 was considered to play an important role in innate immunity. Here we proved the role of AIM2 in regulation in T cell and B cell differentiation and activation in autoimmunity.

P2785

Correlation between insulin resistance and autoimmune thyroid disease and oxidative stress in patients with polycystic ovary syndrome

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Objective: To investigate the relationship between insulin resistance (IR) and autoimmune thyroid disease (AITD) and oxidative stress in patients with polycystic ovary syndrome (PCOS), and to provide a new basis for clinical research and treatment of PCOS patients with IR.
Methods: Serum thyroid parameters, oxidative stress (reactive oxygen ROS and total antioxidant capacity TAC), insulin and blood glucose levels were measured in 220 patients with PCOS. Patients with PCOS were divided into PCOS + AITD group and PCOS + non-AITD group according to whether AITD was combined, and those were divided into PCOS + IR group and PCOS + non-IR group according to whether or not to be combined IR. The relationship between IR, thyroid parameter levels and oxidative stress levels were compared in all PCOS patients.
Result: Serum thyroglobulin antibody (TG-Ab), thyroid peroxidase antibody (TPO-Ab) and thyroid stimulating hormone (TSH) levels and ROS levels in the PCOS+IR group were higher than those in the PCOS+ non-IR group ($P< 0.05$). The total TAC level was lower than that of the PCOS+ non-IR group ($P< 0.05$). The HOMA-IR, IR incidence, and ROS levels in the PCOS +AITD group were higher than those in the PCOS + non-AITD group ($P< 0.05$), while the total TAC level was lower than the PCOS+ non-AITD group ($P< 0.05$).
Conclusion: IR is closely related to AITD and oxidative stress in patients with PCOS. AITD and IR are

DOI: 10.1002/eji.201970400

also associated with oxidative stress. AITD and oxidative stress increase the risk of insulin resistance in PCOS patients.

P2786

E3 ligase FBXW7 aggravates TMPD-induced systemic lupus erythematosus by promoting cell apoptosis

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease, and the pathogenesis of SLE has not been fully elucidated. The E3 ubiquitin ligase FBXW7 has been well characterized in cancer as a tumor suppressor that can promote the ubiquitination and subsequent degradation of various oncoproteins. However, the potential role of FBXW7 in autoimmune diseases is unclear. In the present study, we identified that FBXW7 is a crucial exacerbating factor for SLE development and progression in a mouse model induced by 2, 6, 10, 14-tetramethylpentadecane (TMPD). Myeloid cell-specific FBXW7-deficient ($Lysm^{+}FBXW7^{f/f}$) C57BL/6 mice showed decreased immune complex accumulation, glomerulonephritis, glomerular mesangial cell proliferation, and base-membrane thickness in the kidney. $Lysm^{+}FBXW7^{f/f}$ mice produced fewer anti-Sm/RNP and anti-ANA autoantibodies and showed a decreased MHC II expression in B cells. In $Lysm^{+}FBXW7^{f/f}$ mice, we observed that cell apoptosis was reduced and that fewer $CD11b^{+}Ly6C^{hi}$ inflammatory monocytes were recruited to the peritoneal cavity. Consistently, diffuse pulmonary hemorrhage (DPH) was also decreased in $Lysm^{+}FBXW7^{f/f}$ mice. In terms of mechanism, we clarified that FBXW7 promoted TMPD-induced cell apoptosis by catalyzing MCL1 degradation through K48-linked ubiquitination. These results revealed that FBXW7 expression in myeloid cells played a crucial role in TMPD-induced SLE progression.

P2787

Detection and evaluation of islet autoantibodies related to type 1 diabetes by chemiluminescence and western blotting

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Objective: The study is to determine and compare the serum levels of GADA, ICA, IAA, IA2A and ZnT-8A in type 1 diabetic patients and healthy people by western blotting and chemiluminescence. To explore the diagnostic performance of the five antibodies in these two methods and to analyze which method is more efficient and more suitable for clinical type 1 diabetes diagnosis.
Methods: The subjects were divided into two group. The type 1 diabetes group and the healthy control group. The levels of GADA, ICA, IAA, IA2A and ZnT-8A in the two groups were detected by western blotting and chemiluminescence respectively. The detection rates of these markers were analyzed by chi-square test and ROC curves.
Results: The positive rates of IAA, GADA, IA2A, ICA and ZnT-8A in the type 1 diabetes group by chemiluminescence and by western blotting were both significantly higher than the healthy control group. There was a statistically significant difference between the positive rates of these markers in the type 1 diabetes group detected by chemiluminescence and western blotting. The chi-square test and ROC curve analysis both showed that the chemiluminescence method was more sensitive than the western blotting for detecting 5 antibodies in diabetes.
Conclusion: Chemiluminescence detection sensitivity of diabetic autoantibodies compared to western

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DOI: 10.1002/eji.201970400

blotting high more conducive to the diagnosis of type 1 diabetes
Keywords: Type 1 diabetes, islet autoantibodies, Western blot, Chemiluminescent method

P2788

High expression of MDR1 can aggravate resistance to methotrexate in RA patients

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Objective: To clarify the role of MDR1 gene in methotrexate (MTX) resistance in patients with rheumatoid arthritis (RA).
Methods: Fibroblast-like synoviocytes (FLS) from RA patients were infected with recombinant adenovirus Ad-EGFP-MDR1 in vitro to obtain MDR1 over-expressed RA FLS; the transcription level of MDR1 gene and the expression level of its coding product P-gp protein were detected by Real-time PCR and Western blot; the efflux function was verified by rhodamine 123 efflux experiment; the resistance to MTX was detected by MTT assay.
Results: RA FLS were infected with recombinant adenovirus Ad-EGFP-MDR1 72 hours later, the particles size in MDR1 over-expressed RA FLS increased, the cell volume became larger, and the growth rate became slower. Compared with the negative virus control RA FLS, the transcription level of MDR1, the expression level of P-gp protein and the ability of extracellular rhodamine 123 in MDR1 over-expressed RA FLS were significantly enhanced, and the survival rate of MDR1 over-expressed RA FLS was significantly increased at each concentration of MTX.
Conclusion: The high expression of MDR1 can affect the efflux ability to MTX by up-regulating the expression of P-gp, thus enhancing the drug resistance to MTX in RA FLS.

P2789

Associations of HLA class II alleles with clinical phenotype and outcome in patients with primary membranous nephropathy: a single amino acid makes the difference

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GWAS and HLA genotyping have revealed associations between HLA alleles and susceptibility to primary membranous nephropathy (pMN), but the associations with clinical phenotypes and kidney outcome are poorly defined. We previously identified DRB1*1501 and DRB1*0301 as independent risk alleles for pMN. Here, we investigated HLA associations with demographic characteristics, anti-phospholipase A2 receptor antibody (PLA2R-Ab), treatment response and kidney outcome after a median follow-up of 52.0 (37.1, 74.3) months in 258 patients. DRB1*0301, but not DRB1*1501, was associated with higher level of PLA2R-Ab (OR=1.58, 95%CI 1.13-2.22, *P*=0.008). Although DRB1*1502 which differs from DRB1*1501 by a single amino acid, was not a risk allele for pMN (OR=1.01, *P*=1.000), it was associated with lower eGFR both at baseline (OR=1.79, 95%CI 1.18-2.72, *P*=0.006) and at last follow-up (OR=1.72, 95%CI 1.17-2.53, *P*=0.006), a worse renal outcome by Kaplan-Meier analysis (*P*=0.013) and a higher risk of ESRD by Cox regression analysis (HR=4.52, 95%CI 1.22-16.74, *P*=0.024); nevertheless, the absence of remission remained the only independent risk factor for ESRD by multivariate analysis. DRB1*1502 was also associated with a higher PLA2R-Ab level [161.4 (28.1, 268.0) vs. 36.3 (2.8, 130.5) U/mL, P=0.013] and showed interaction with DRB1*0301 for this variable. Thus, our findings show that *HLA* genes control PLA2R antibody production and pMN severity and outcome. They additionally suggest that DRB1*1502 behaves like a modifier gene with a strong predictor value when associated with HLA risk alleles. Other modifier genes need further investigations in larger cohorts.

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DOI: 10.1002/eji.201970400

P2790

Risk HLA class II alleles and amino acid residues in MPO-ANCA associated vasculitis risk HLA class II alleles and amino acid residues in MPO-ANCA associated vasculitis

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A genome-wide associated study (GWAS) indicated that patients with MPO-ANCA associated vasculitis (AAV) were associated with *HLA-DQ*, while the susceptible alleles in these loci were under-investigated. This study genotyped 258 Chinese MPO-AAV patients and 597 healthy controls on HLA DRB1, DQA1, DQB1 and DPB1, and extracted the encoded amino acid sequences from the IMGT/HLA database. The replication cohort included 97 cases and 107 controls. T cell epitopes of MPO were predicted and docked to the HLA molecules. We found DQA1*0302 (OR=2.30, 95%CI 1.73-3.05, P=3.90×10^{-9}) and DQB1*0303 (OR=1.84, 95%CI 1.42-2.38, P=3.63×10^{-6}) were risk alleles for MPO-AAV. They were in overt linkage disequilibrium (r^2=0.69) and the haplotype DQA1*0302-DQB1*0303 was risk (haplotype score=6.39, P=1.61×10^{-10}) as well. Aspartate$_{160}$ on DQ α chain (OR=2.11, 95%CI 1.64-2.72, P=4.59×10^{-9}), encoded by DQA1*0302, and isoleucine$_{185}$ on DQ β chain (OR=1.72, 95%CI 1.37-2.16, P=2.20×10^{-6}), encoded by DQB1*0303, showed significantly risk effects to MPO-AAV. DQB1*0201 (OR=0.29, 95%CI 0.18-0.48, P=4.54×10^{-7}) and DQA1*0501 (OR=0.28, 95%CI 0.16-0.50, P=3.61×10^{-6}) showed negative associations with the disease. The risk alleles and amino acids were successfully validated in the replication cohort. Molecular docking revealed that the susceptible HLA molecule could present the predicted epitopes of MPO. This study identified two risk alleles of HLA II genes and their encoding amino acids, which confer susceptibility to MPO-AAV. It provides clues for MPO epitopes investigations, which is one of the key events in AAV pathogenesis.

P2791

C9 deficiency ameliorates DSS-induced colitis through decreasing macrophage and neutrophil infiltration and inflammatory cytokine release

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Using our novel C9 knockout mice(*C9*$^{-/-}$), we aim to evaluate the role and potential mechanisms of complement C9 deficiency in the development of DSS induced ulcerative colitis. Our data showed that, compared to WT mice, *C9*$^{-/-}$ mice showed lower level of stool traits index, disease activity index, body weight loss and mortality rate induced by DSS. However, *C9*$^{-/-}$ mice had longer colon(5.55±0.26cm) than in WT mice(4.8±0.26cm) when sacrificed on day 6. Spleen index, percent of spleen neutrophils (CD11b$^+$Ly6G$^+$ or CD11b$^+$Gr-1$^+$) and macrophages (CD11b$^+$Ly6C$^+$ or CD11b$^+$F4/80$^+$) declined in *C9*$^{-/-}$ mice. HE staining suggested that *C9* deficiency ameliorated colon inflammatory response in colon compared to that in WT mice. Decreased MAC deposition as well as neutrophil (Gr-1$^+$) and macrophage (CD68$^+$) infiltration in colon were also observed *C9*$^{-/-}$ mice. We further proved that colon tissue inflammatory cytokines including IL-1β, IL-6 and IL-8 decreased significantly in *C9*$^{-/-}$ mice, and a dramatic change between WT and *C9*$^{-/-}$ mice was observed on day 2 after DSS induction, cytokine levels peaked at day 4 and declined at day 6 in both groups. The change of cytokines was paralleled to that found in neutrophils and macrophages. These data together suggested that *C9* deficiency ameliorated DSS-induced colitis through decreasing macrophage and neutrophil infiltration and inflammatory cytokine release.
FUNDING: This project is supported by National Nature Science Foundation of China(#81873883),

DOI: 10.1002/eji.201970400

Project of Medical and Health Technology Development Program of Shandong Province (#2018WS065)

P2792

C9 knockout aggravates autoimmunity in lupus prone B6 mice

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We aim to explore the contribution of complement MAC in lupus nephritis. We generated B6*lpr* and B6.*C9*$^{-/-}$*lpr* mice, and analyzed severity of lupus associated injuries and phenocytes. Our data indicated that B6.*C9*$^{-/-}$*lpr* mice exhibited significantly larger pooled lymph nodes and spleen than that in B6.*lpr* mice, and higher level of urine protein and serum BUN were detected in B6.*C9*$^{-/-}$*lpr* mice. Anti-dsDNA and anti-ANA antibody level increased with time(5 to 10 month) in both mice strains, however B6.*C9*$^{-/-}$*lpr* mice produced 3 to 4 times of autoantibody compared to B6.*lpr* mice. HE and PAS staining suggested that B6.*C9*$^{-/-}$*lpr* had developed significantly more severe renal inflammation than in age and sex matched B6.*lpr* mice as B6.*C9*$^{-/-}$*lpr* mice displayed a markedly proliferative kidney pathology with glomerular cell proliferation and inflammatory cell infiltrates. We found that up to 25% B6.*C9*$^{-/-}$*lpr* mice died in a 12 month survival assay compared to that in B6.*lpr* mice(less than 5%). We further evaluated the autoimmune immune cell phenotypes in these mice. Total cell numbers of lymphocytes and absolute numbers of CD3^{+} T cells and CD19^{+} B cells in spleen and lymph node of B6.*C9*$^{-/-}$*lpr* mice significantly increased in comparison with B6.*lpr* mice. B6.*C9*$^{-/-}$*lpr* mice showed higher percentages of activated CD69^{+}CD4^{+} T cells, and lupus associated non-classical CD3^{+}B220^{+} T cells, CD3^{+}CD4^{-}CD8^{-} T cells. Our data indicated an novel role of *C9* deficiency in lupus autoimmunity.
Funding: This project is supported by National Nature Science Foundation of China (#81873883), Project of Medical and Health Technology Development Program of Shandong Province (#2018WS065)

P2793

RNA-seq of circular RNAs identified circPTPN22 as a potential new activity indicator in systemic lupus erythematosus

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Circular RNAs (circRNAs) are possible biomarkers for many diseases, but the knowledge of circRNAs in the peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE) remains limited. This study aimed to assess the expression of circRNAs in PBMCs from patients with SLE and healthy individuals by RNA sequencing (RNA-seq). In total, 128 circRNAs were significantly differentially expressed including 39 upregulated and 89 downregulated circRNAs in four new-onset SLE patients compared with 3 healthy controls. After verification of the four candidate circRNAs in 49 patients with SLE and 37 controls using quantitative real-time polymerase chain reaction (qRT-PCR) assays, a previously undescribed circRNA with potential translation activity, circPTPN22, was selected to confirm its clinical significance. Bioinformatics analysis demonstrated that the parent gene of circPTPN22 was protein tyrosine phosphatase nonreceptor type 22 (PTPN22), a potent regulator of T cell activation. The downregulation of circPTPN22 in patients with SLE was strongly negatively correlated with their SLEDAI scores. CircRNA-miRNA-mRNA co-expression network analysis indicated a correlation between circPTPN22 and the miRNAs and mRNAs related to

DOI: 10.1002/eji.201970400

immunological regulation including the development of SLE. Patients with higher SLEDAI scores had lower circPTPN22 expression levels, and long-term hormone treatment significantly increased circPTPN22 levels. Receiver operating characteristic (ROC) curve analysis indicated that circPTPN22 has good diagnostic value for SLE. Our data demonstrated the aberrant expression of circRNAs in patients with SLE compared with healthy controls; circPTPN22 might function as a diagnostic and disease severity indicator in SLE.

P2794

The regulation mechanism of G0S2 gene during the pothogenesy and treatment of myasthenia gravis (MG)

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Objective: This study aimed to explore the expression pattern and the methylation level of G0S2 in the PBMC from MG patients and try to reveal the relationship between G0S2 and MG. Explore the relationship of NFAT5 and G0S2 to reveal the regulation mechanism of G0S2 during the pothogenesy and treatment of MG.Meanwhile, try to demonstrate the potential therapeutic mechanism of tacrolimus on MG.
Methods: The relatively expression level of G0S2 in PBMC between healthy people and MG patients were compared with qRT-PCR. The methylation frequency of G0S2 promoter was detected by BSP and Pyrosequencing. The Dual-Luciferase Reporter System was used to reveal the relationship between G0S2 promoter and nuclear factor of activated T-cells 5 (NFAT5).
Results: The qRT-PCR results showed that the expression of G0S2 was significantly up-regulated in PBMC of MG patients, especially in B cells. And this expression difference was significantly associated with lower frequency of G0S2 promoter methylation. NFAT5, which combines to island 1 of G0S2 promoter, could suppress the expression of G0S2, but failed to affect the methylation of G0S2 promoter. Tacrolimus therapy could reduce the expression of GOS2 significantly in MG patients generally.
Conclusion: G0S2 was remarkably up-regulated in PBMC of MG patients. NFAT5 may affect the transcription initiation and down-regulate the expression of G0S2 by combining with the island 1 in promotor region, which could control the expression of G0S2 and thus to regulate the immune balance of lymphocytes. Therefore, G0S2 may be an immune regulatory factor in both MG occurrence and MG treatment.

P2795

An overlap of antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis and IgG4-related kidney disease

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Background: It has been reported that antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (ANCA-GN) and immunoglobulin G4 (IgG4)-related kidney disease (IgG4-RKD) could overlap. The current study aimed to investigate clinical and histopathological characteristics of patients with ANCA-GN and IgG4-RKD overlap syndrome.
Methods: Eleven patients with combined ANCA-GN and IgG4-RKD were included in this study. Their clinical and pathological data were analyzed.

DOI: 10.1002/eji.201970400

Results: Of the 11 patients, 8 were male and 3 were female. Seven, three and one patient were classified as microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiits (EGPA), respectively. Hematuria was absent or slight in 8 (72.7%) cases. Renal histology of these patients revealed concurrent ANCA-GN and IgG4-RKD. Regarding the histological classification of ANCA-GN, five (45.5%) and 6 (54.5%) patients were classified as focal and crescentic ANCA-GN, respectively. MPO-ANCA could be detected in 10/11 (91.0%) patients. IgG subclasses of MPO-ANCA were tested, and all were positive for IgG4-MPO-ANCA. In patients with combined ANCA-GN and IgG4-RKD, the percentage of positive IgG1-MPO-ANCA was significantly lower than the control group of 20 AAV patients without IgG4-RKD (P=0.002), and the percentage of positive IgG4-MPO-ANCA was marginally higher than the control group (P=0.14).
Conclusion: ANCA-GN and IgG4-RKD overlap syndrome concerned mainly MPO-ANCA positive patients, with a striking male predominance. The IgG subclass analysis of MPO-ANCA showed lower percentage of IgG1 subclass. The association between ANCA-GN and IgG4-RKD is possible and represents a special entity.

P2796

De novo fatty acid synthesis induced Sjögren's syndrome by promoting Th17 differentiation

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Primary Sjögren's syndrome (pSS) is a chronic inflammatory autoimmune characterized by inflammatory infiltration of immune cells within lacrimal and salivary glands (SGs), which leads to the dysfunction of these exocrine glands. Th17 cells and their secreted cytokines play an important role in the pathogenesis of Sjogren´s syndrome, but the mechanism is still unclear. Recent clinical studies have found that metabolic disorders may be closely related to Sjogren´s syndrome. Our studies have also found that FASN, the key enzyme of lipid synthesis and metabolism in peripheral blood of Sjogren´s syndrome patients, is significantly elevated, and mainly expressed in Th17 cells. Fatty acid synthase (FASN) is downstream of ACC, and previous studies have shown that FASN activity influences both cancer and inflammation. However, it remains to be determined whether FASN is a viable target for Sjögren's Syndrome. Here, we demonstrate that FASN is a critical metabolic induced Sjögren's syndrome by promoting the generation of inflammatory subsets of Th17 cells. In vivo, knocking out of FASN in Th17 cells, leads to reduction of Sjögren's syndrome. These study demonstrate that FASN-mediated metabolism disorder of fatty acid synthesis may be a new mechanism to regulate Th17 cell function and lead to Sjogren´s syndrome, which can provide new directions and ideas for the treatment of Sjogren´s syndrome.

P2797

Effect of NASP mutant on the autoimmunity of induced lupus model mice

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B6-WT mice and B6-NASP^{M} mice were immunized with DNA of ConA-activated mouse spleen lymphocytes. The serum anti-dsDNA antibody levels were determined by ELISA. The pathological changes of the kidneys were detected by HE staining. Deposition of immune complexes and complements were detected by immunofluorescence staining. Flow cytometry was used to detect changes of spleen lymphocyte subsets in mice, which further explored the mechanism by which NASP mutant genes affect immune response in mice.Compared with B6-WT mice, B6-NASP^{M} mice had no significant effect on body weight, kidney index and spleen index (P>0.05).The results of ELISA

DOI: 10.1002/eji.201970400

showed that the anti-ds-DNA antibody level of B6-NASPM mice was significantly increased.HE staining and immunofluorescence staining of mouse kidney showed that the B6-NASPM group had more obvious glomerular cell proliferation, IgG and complement C3 deposition than the B6-WT group. The results of flow cytometry showed that the proportion of CD3+ cells decreased (P< 0.01), the proportion of NK cells decreased significantly (P< 0.001), and the proportion of Breg cells increased (P< 0.05)in the B6-NASP^{M} mice.Mutations in the NASP gene can aggravate autoimmunity in a mouse model of lupus.
This project was supported by the National Natural Science Foundation of China (#81373185) and Natural Science Foundation of Shandong, China (ZR2018MH014)

P2798

Effect of mouse NASP gene mutation on spleen lymphocyte subsets

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The NASP gene mutant mouse was spotted by ES targeting technique. Six months old B6-WT mice and B6-NASP^{M} were used. The proportion of the immune cell subsets and cell cycle in the spleen were detected by flow cytometry. PCR and DNA sequencing showed that B6-NASP^{M} mice were successfully prepared. The mutation of NASP gene did not affect the expression of NASP protein in skin, spleen and liver and spleen index (P>0.05). Flow cytometry results showed that the proportion of CD3^{+} T cells in spleen decreased (P< 0.01)and the proportion of CD19^{+}B cells increased (P< 0.01) in B6-NASP^{M} mice; The proportion of CD3^{+}CD4^{+} T cells decreased (P< 0.05), the proportion of CD3^{+}CD8^{+} T cells increased (P< 0.01), the ratio of CD4/CD8 decreased (P< 0.01), and the proportion of NK cells decreased (P< 0.05). The S phase cells of spleen lymphocytes in B6-NASP^{M} mice were slightly higher than those in B6-WT mice (P< 0.05). Mutation of NASP gene does not affect the expression of NASP protein, but it can change the proportion of immune cells in mouse spleen, resulting in immune dysfunction in mice.
This project was supported by the National Natural Science Foundation of China (#81373185) and Natural Science Foundation of Shandong, China (ZR2018MH014)

P2799

Increased T Follicular helper-like Cells and tertiary lymphoid structures in the skin lesions of discoid lupus erythematosus

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Clinically, lupus erythematosus (LE) patients with skin lesions can be divided into two subsets based on the organs involved: cutaneous LE (CLE), such as discoid LE (DLE), and systemic LE (SLE), e.g., acute LE (ACLE). Interestingly, the excessive production of autoantibodies can be found in serum and skin lesions of SLE, however, increased autoantibodies appear in the inflammatory skin lesions rather than serum of DLE. By now the pathomechanism of immune deposition in DLE remains unclear. Herein, with Multiplex immunofluorescence microscopy, we demonstrate that in over half of 112 DLE biopsies, lymphoid aggregates ranged from tight clusters of B cells and T cells to highly organized structures that comprise functional germinal centres (In 58% of cases (66/112), there were well-circumscribed aggregates of CD19^{+} B cells with CD4^{+} T cells. In 8% of biopsies (10/112), structures similar to GCs of human tonsil were observed.). Moreover, compared with SLE, the percentage of T cells in DLE showed no statistical difference (n=20, *P*=0.158), but the percentage of B cells was significantly higher in DLE (n = 20, *P*=0.0022). 5 samples were selected respectively from two group of

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DOI: 10.1002/eji.201970400

LE to detect the proportions of Tfh cells (CD4/IL21/BCL6/PD-1/CXCR5). Then we found a significantly increased population of T follicular helper (Tfh)-like cells expressing IL21, PD-1 and Bcl-6 but lacking CXCR5 in the dermis of DLE as compared to the SLE (n=5, *P*=0.0060). Abnormal increased Tfh-like cells in dermis may contribute to the formation of ectopic GCs structures and immune deposition in DLE.

P2800

Antimicrobial peptide LL-37 forms complex with bacterial DNA to aggravate ulcerative colitis

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a major public health problem. In the intestinal milieu with abundant presence of microbes, and this enteric bacteria not only play a key role in intestinal homeostasis and function, but also in the onset and perpetuation of chronic intestinal inflammation.
Bacterial DNA (bacDNA) is frequently found in serum of patient with UC and CD, even blood bacterial culture is negative. How bacDNA evades immune elimination and is translocated into blood remain unclear. The human antimicrobial peptide cathelicidin (known as LL-37, Cramp in mice), for example, is found increased in inflamed and non-inflamed mucosa in patients with UC but not in patients with CD. We found that bacDNA avoids elimination and disables bacteria-killing function of LL-37 by forming complex with it, which is inducible after culture with bacteria or bacterial products. Elevated LL-37-bacDNA complex was found in plasma and lesions of patients with UC. LL-37-bacDNA promoted inflammation by inducing Th1, Th2 and Th17 differentiation and activating toll-like receptor-9 (TLR9). The complex also increased paracellular permeability, which possibly combines its inflammatory effects to promote local damage and bacDNA translocation into blood. Cramp-bacDNA aggravated mouse colitis severity while interference with the complex ameliorated the disease. The study identifies that inflammatogenic bacDNA utilizes LL-37 as a vehicle for blood translocation and to evade immune elimination.

P2801

IL-27 promotes hPMSCs ability to induce the generation of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells via the JAK/STAT pathway in the treatment of experimental GVHD

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Human mesenchymal stromal cells (MSCs) harbor immunomodulatory properties to induce the generation of suppressive T cells. MSCs have been successfully used in treating graft-versus-host disease (GVHD) accompanied by abundant inflammatory cytokines such as IL-27. This study investigated the effects of IL-27 on the human placenta-derived MSCs (hPMSCs) to induce generation of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells in vitro and in the humanized xenogenic GVHD NOD/SCID model. The results showed that the percentages of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells were significantly increased in activated human PBMC from both healthy donors and GVHD patients with hPMSCs and in the liver and spleen of hPMSC-treated GVHD mice, and the level of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells in the liver was greater than that in the spleen in hPMSC-treated GVHD mice. The serum level of IL-27 decreased and the symptoms abated in hPMSC-treated GVHD. Further, in vitro results showed that IL-27 promoted the regulatory effects of hPMSCs by enhancing the generation of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells from activated PBMC. Activation occurred through increases in the expression of programmed death ligand 2 (PDL2) in hPMSCs via the JAK/STAT signaling pathway. These findings indicated that hPMSCs

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DOI: 10.1002/eji.201970400

could alleviate GVHD mice symptoms by upregulating the production of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells in the spleen and liver and downregulating serum levels of IL-27. In turn, the ability of hPMSCs to induce the generation of $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T cells could be promoted by IL-27 through increases in PDL2 expression in hPMSCs. The results of this study will be of benefit for the application of hPMSCs in clinical trials.

P2802

Long noncoding RNA expression profile in fibroblast-like synoviocytes from patients with rheumatoid arthritis

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Background: Long noncoding RNAs (lncRNAs) have recently received wide attention as key molecules that mediate a variety of physiological and pathological processes by regulating gene expression; however, knowledge of lncRNAs in rheumatoid arthritis (RA) is limited. Thus, we investigated the lncRNA expression profile in fibroblast-like synoviocytes (FLSs) from patients with RA and explored the function of abundantly expressed lncRNAs.
Methods: LncRNA and mRNA microarrays were performed to identify differentially expressed lncRNAs in RA FLSs compared with normal FLSs. Quantitative polymerase chain reaction (qPCR) was used to validate the results, and correlation analysis was used to analyze the relationship between these aberrantly expressed lncRNAs and clinical characteristics. A receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic value of the lncRNAs identified.
Results: According to the gene expression profiles, 135 lncRNAs were differentially expressed between RA and normal FLSs. Furthermore, qPCR data showed that lncRNA ENST00000483588 was up-regulated and that three lncRNAs (ENST00000438399, uc004afb.1, and ENST00000452247) were down-regulated in RA FLSs. The expression level of ENST00000483588 was positively correlated with the level of C-reactive protein and the Simplified Disease Activity Index score. Moreover, the areas under the ROC curve were 0.85, 0.92, 0.97, and 0.92 for ENST00000483588, ENST00000438399, uc004afb.1, and ENST00000452247, respectively.
Conclusions: The results indicate that the dysregulation of ENST00000483588, ENST00000438399, uc004afb.1,and ENST00000452247 may be involved in the pathological processes of RA and that these lncRNAs may have potential value for the diagnosis and assessment of the disease activity of RA.

P2803

Circulating Exosome-derived lncRNA as potential biomarker for rheumatoid arthritis

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Objective: To investigate the different expression levels of Exosome-derived lncRNAs in serum of patients with rheumatoid arthritis (RA).
Methods: Exosomes were purified from RA patients, SLE patients and healthy controls(HCs). The present study selected four specific LncRNA (H19, MALAT1, Hotair and Xist) as possible biomarker. Exosomes were extracted from serum and characterized by nanoparticle tracking analysis and electron microscopy. The total number of exosomes was quantified by the membrane surface markers CD63 and CD81. RT-qPCR was performed to validate gene expression in exosomes and serum.

DOI: 10.1002/eji.201970400

Receiver operating characteristic curve (ROC) was applied to evaluate the potential diagnostic value of the lncRNAs.
Results: Detection of CD81 showed that the concentration of exosomes in RA decreased significantly than other groups (P< 0.05), CD63 was no statistical difference between three groups (P>0.05). The four lncRNAs, only lncRNA Hotair and XIST levels of serum exosomes in RA were significantly higher than SLE and healthy controls (P< 0.05), the areas under the ROC curve were 0.798 and 0.729 respectively. In addition, levels of two lncRNAs were related to the level of C-reactive protein, erythrocyte sedimentation rate and disease activity score-28 (DAS-28) values.
Conclusions: The results indicate that the increased level of lncRNA Hotair and XIST derived from serum exosomes may be involved in the pathological processes of RA. Exosomal lncRNA could be a biomarker for diagnosis, disease activity in RA.

P2804

Clinical characteristics and outcomes of lung-dominant rheumatoid arthritis

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Objectives: Anti-citrullinated protein antibody (ACPA) is an essential pathogenic mediator for rheumatoid arthritis (RA). ACPAs can be generated in the lung, and can be detected up to 10 years before clinical arthritis onset. This study aimed to characterize lung-dominant RA (LD-RA) cases, which are ACPA-positive interstitial lung disease (ILD) patients without arthritis.
Methods: We retrospectively enrolled ACPA-positive ILD patients in Peking Union Medical College Hospital from Jane 2008 to Dec 2017. RA was defined by the 2010 ACR-EULAR classification criteria. Patients with other known causes of ILD were excluded. Chest HRCT images were evaluated in a blinded manner and classified into “UIP”, “probable UIP” or “non-UIP” patterns. Patients were followed up every 3-6 months. The primary outcome was occurrence of arthritis in LD-RA cases.
Results: We enrolled 46 LD-RA patients without arthritis, and 42 definite RA-ILD patients with arthritis after ILD. During follow-up, arthritis occurred in 10 female LD-RA patients who were subsequently diagnosed as RA, with a median ILD-to-arthritis interval of 24 months (IQR 17-111 months). Compared with those who did not develop arthritis, Patients with arthritis after ILD presented with a higher frequency of positive RF (97.9% vs. 60.0%, p< 0.001), a higher anti-CCP level (mean, 1469 vs. 397 U/ml, p< 0.001), a higher ESR level (mean, 59 vs. 36 mm/h, p=0.005), and a higher frequency of UIP pattern (59.4% vs. 31.3%, p=0.066).
Conclusions: ACPA-positive ILD patients with positive RF, higher level of anti-CCP and ESR, and UIP pattern, were more likely to develop arthritis during follow-up.

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DOI: 10.1002/eji.201970400

P2805

Resolvin D1 programs inflammation resolution by increasing TGF-β expression induced by dying cell clearance in experimental autoimmune neuritis

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Experimental autoimmune neuritis (EAN) is the animal model of human acute inflammatory demyelinating polyradiculoneuropathies (AIDP), an auto-immune inflammatory demyelination disease of the peripheral nervous system (PNS) and the world´s leading cause of acute autoimmune neuromuscular paralysis. EAN and AIDP are characterized by self-limitation with spontaneous recovery; however, endogenous pathways that regulate inflammation resolution in EAN and AIDP remain elusive. **Here, we demonstrated that resolvin D1 (RvD1) induced transforming growth factor-β (TGF-β) expression through promoting dying cell clearance to program inflammation resolution in EAN.** The expression of resolvin D1 (RvD1), its synthetic enzyme and its receptor were greatly increased in PNS during the recovery stage of EAN. Both endogenous and exogenous RvD1 increased regulatory T (Treg) cell and anti-inflammatory macrophage counts in PNS, enhanced inflammation resolution and promoted disease recovery in EAN rats. Moreover, RvD1 up-regulated TGF-β level and pharmacologic inhibition of TGF-β signaling suppressed RvD1-induced Treg cell counts, but not anti-inflammatory macrophage counts, and RvD1-improved inflammation resolution and disease recovery in EAN rats. Mechanistically, the RvD1-enhanced macrophage phagocytosis of apoptotic T cells, leading to reduced apoptotic T cell accumulation in PNS, induced TGF-β production and Treg cells to promote inflammation resolution and disease recovery in EAN. Thus, these data highlight the crucial role of RvD1 as an important pro-resolving molecule in EAN and suggest its potential as a therapeutic target in human neuropathies.

P2806

Diagnostic value of multiple detections for Rheumatoid Arthritis

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Objective: To analyze diagnostic value of multiple detections for Rheumatoid Arthritis.
Methods: 150 Rheumatoid Arthritis patients were enrolled this research, they were detected type and quantity of RF, quantity of anti-CCP, AKA, APF, CRP, ASO and ESR in serum, then calculate the positive frequency of them in deferent classification diseases, to demonstrate the value of these detections for Rheumatoid Arthritis.
Result: The high positive frequency of these indicators were RF-IgA、RF-IgM、CCP、ESR, APF, AKA (96.36%, 98.18%, 94.55%, 92.73%, 80%, 60%), the diagnostic efficiency of RF typing test was better than total RF test (diagnostic sensitivity 96.36%, 87.27%, diagnostic specificity 81.82%, 78.18%). The value of Multiple detections was better than a single detection (diagnostic sensitivity 100%). Logistic regression analysis showed high level anti-CCP and CRP were related with severe Rheumatoid Arthritis (OR:12.28, 15.27).
Conclusion: RF typing test had better diagnostic value of Rheumatoid Arthritis, Multiple detection can improve detection rate of Rheumatoid Arthritis, the level of anti-CCP and CRP were related with severe Rheumatoid Arthritis.

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DOI: 10.1002/eji.201970400

P2807

The anti-inflammatory effects of statins on patients with rheumatoid arthritis

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Background: Over the past several years, numerous studies investigated the anti-inflammatory effects of statin on patients with RA. However, the findings of the individual studies were often inconsistent or conflicting.
Materials and Methods: The Pubmed, Web of Science, Embase, Cochrane Library and CNKI literature databases were searched in order to identify randomized controlled clinical trials where the association between the anti-inflammatory effect of statin and RA was investigated. Quality parameters and risk of bias in the included studies were assessed according to Cochrane's guidelines. The pooled Standardized Mean Difference (SMD) with a 95%CI was used to assess the anti-inflammatory effect of statin in patients with RA.
Results: Fifteen randomized controlled clinical, classified as “high quality” and with a relatively low risk of selection bias, were included in the meta-analysis . Nine studies reported a change in C-Reactive Protein (CRP) after atorvastatin treatment, and the pooled analysis showed that atorvastatin decreased CRP in RA patients. Seven studies investigated the change of Erythrocyte Sedimentation Rate (ESR), and the pooled analysis showed that atorvastatin decreased ESR . Nine studies reported the improvement of disease activity score in RA patients after taking atorvastatin for 12 weeks, and the pooled analysis showed atorvastatin could decrease the DAS28 score in RA patients.
Conclusions: Statins have a significant anti-inflammatory effect in RA patients. However, atorvastatin was superior to simvastatin both in terms of its anti-inflammatory and lipid-lowering activities.

P2808

Association of serum fibrosis-related biomarkers with disease activity in patients with IgG4-related disease

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Objective: The aim of this study was to identify fibrosis-related serological biomarkers in patients with immunoglobulin G4-related disease (IgG4-RD).
Methods: This was a clinical observational study of 72 patients with IgG4-RD from our hospital. The serum concentrations of growth differentiation factor 15 (GDF-15), CCL2, hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinases 1 (TIMP-1) were measured by enzyme-linked immunosorbent assays. The enhanced liver fibrosis (ELF) score was calculated from the TIMP-1, PIIINP, and HA values. We evaluated associations between the values of these biomarkers and other laboratory and clinical data.
Results: Compared with the 50 healthy controls, the patients with IgG4-RD had significantly elevated serum concentrations of GDF-15, HA, PIIINP, and TIMP-1 and ELF scores. The patients' serum concentrations of GDF-15, CCL2, HA, and TIMP-1 were positively correlated with each other. Serum GDF-15 most efficiently distinguished patients with IgG4-RD from healthy controls. Serum GDF-15 was not associated with the number of organ involvements.
Conclusions: We observed increased serological biomarkers measurements of fibrosis in IgG4-RD. GDF-15 may accurately reflect the fibrotic degree in patients with IgG4-RD.

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DOI: 10.1002/eji.201970400

P2809

Betaine ameliorates experimental autoimmune encephalomyelitis by inhibiting dendritic cell-derived IL-6 production and Th17 differentiation

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IL-17-secreting T cells (Th17 cells) play a pathogenic role in multiple autoimmune diseases, including multiple sclerosis (MS), and dendritic cell (DC)-derived cytokines play pivotal roles in promoting the differentiation of naive CD4+ T cells into Th cell subsets (Th1 and Th17). Therefore, small molecules blocking the key cytokines produced by DCs will be beneficial in MS. In this article, we report that betaine treatment ameliorates MS pathogenesis by inhibiting DC-derived IL-6 production and Th17 differentiation. Using experimental autoimmune encephalomyelitis, a widely used mouse model of MS, we found that, compared with the vehicle-treated group, betaine-treated mice exhibited less severe experimental autoimmune encephalomyelitis symptoms, including lower clinical scores, reduced leukocyte infiltration, and less extensive demyelination in the CNS. Moreover, a significantly lower percentage of Th17 cells, one of the major pathogenic effector cells in MS progression, was observed in the peripheral immune system and in the CNS. Interestingly, in the in vitro Th17-differentiation assay, no significant change in Th17 cells was observed between the vehicle- and betaine-treated groups, whereas in the in vitro DC culture experiment, betaine treatment significantly decreased DC-derived IL-6 production. In the DC-T cell coculture experiment, a significantly decreased Th17 differentiation was observed upon betaine treatment. All of these data demonstrated that betaine inhibited Th17 differentiation indirectly by reducing IL-6 production by DCs. In brief, our findings demonstrated the pivotal roles of betaine in modulating MS pathogenesis and suggested that it may serve as a potential novel drug candidate for the treatment of MS.

P2810

Spontaneous colitis in IL-10-deficient mice was ameliorated via inhibiting glutaminase1

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Immunity imbalance and barrier damage in the intestinal mucosa are the main pathogenic factors of Crohn´s disease (CD). BPTES is a glutaminase 1 (Gls1) inhibitor with the dual functions of increasing glutamine levels and immune regulation. Our study tends to examine the expression and role of Gls1 in CD and investigated whether BPTES can improve CD-like enteritis. Intestinal specimens with (n=13) and without (control, n=17) CD were obtained from patients who underwent intestinal resection. Immunohistochemistry was used to detect the expressions of Gls1 in the intestinal tissues of CD. *Il-10*$^{-/-}$ mice (15-week, male) with spontaneous enteritis were divided into the control and BPTES-treated groups. Gls1 expression showed significant increase in CD intestinal tissue than in control group. Treatment with BPTES ameliorated chronic colitis in the *Il-10*$^{-/-}$ mice significantly, as manifested in a decreased disease activity index, bodyweight change, histological inflammatory degree and inflammatory cytokines. BPTES-treatment showed protective effects on CD which associated with maintaining intestinal barrier integrity and Th/Treg balance. BPTES treatment may act in part through mTORC1 signaling. In conclusion, Gls1 expression was increased in CD intestines. Inhibition of Gls1 expression can attenuate chronic colitis by maintaining intestinal barrier integrity and the Th/Treg balance, thereby ameliorating CD-like colitis.

DOI: 10.1002/eji.201970400

P2811

Novel biomarkers containing citrullinated peptides for diagnosis of systematic lupus erythematosus using protein microarrays

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Objective: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by autoantibody production. This study aims to identify biomarkers involving citrullinated peptides that can be used for SLE diagnosis.
Methods: After a negative selection step with serum from healthy controls (HCs), a phage library of 12 peptides was used for three rounds of screening with sera from 30 SLE patients. After four rounds of biopanning, 21 positive peptides were sequenced. We produced 37-feature arrays containing 16 recombinant citrullinated peptides. The microarrays were tested with an independent validation set of serum samples from 50 HCs, 60 SLE patients, and 60 rheumatoid arthritis (RA) patients.
Results: Microarray analysis showed that the positive rates of 13S1212Cit3-IgM (60.0%), 13S1210-IgG (43.33%), and 13S1212Cit3-IgG (41.67%) were increased in SLE patients compared with HCs and RA patients. The area under the receiver operating characteristic curve (AUC) was 0.770, 0.687 and 0.698, respectively. The combination of 13S1212Cit3-IgM and 13S1210-IgG (termed COPSLE, for combination of peptides for SLE) was more efficient for SLE diagnosis, with a larger AUC (0.830) and a positive rate of 73.33%. COPSLE could be used to identify 80.0% of SLE patients who were negative for anti-Smith (Sm), anti-double-stranded DNA (ds-DNA), and anticardiolipin (ACA). The Spearman rank correlation indicated that COPSLE increased with albumin, serum level of C3 and platelet distribution width, but had negative correlations with decreased C3 and discoid lupus.
Conclusion: A citrullinated/non-citrullinated peptide panel is a valuable diagnostic marker of SLE, even for patients who are negative for anti-Sm, anti-ds-DNA and ACA.

Immunity to Viruses

O049

Viral-hijacked monocytic myeloid-derived suppressor cells promote clonal deletion of hepatitis B surface antigen-specific CD8$^+$ thymocytes

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Chronic infection of hepatitis B virus (HBV) is a major threat to human health worldwide. HBV exposure in adults often progresses to acute infection, whereas HBV exposure in newborns and infants is extremely inclined to develop into chronic hepatitis B (CHB). However, the mechanism involved in this age-related outcome of HBV exposure is undefined yet. We herein found that monocytic myeloid-derived suppressor cells (mMDSCs) carrying HBsAg could migrate to the thymic medulla, which was mediated by the interaction between thymic-derived CCL25 and HBsAg-enhanced CCR9 on mMDSCs. We further observed that thymic-recruited mMDSCs promoted deletion of HBsAg-specific CD8$^+$ thymocytes through activation of caspase-3. In addition, ovalbumin (OVA) peptide-loaded mMDSCs acquire the ability to delete OVA-specific CD8$^+$ T thymocytes. Finally, we proved that

DOI: 10.1002/eji.201970400

deletion of HBsAg-specific CD8$^+$ T thymocytes by mMDSCs could contribute to age-related outcome of HBV exposure in hydrodynamic injection-based HBV mouse model, and this was supported by our finding that ratio of HBsAg-specific CD8$^+$ T cells negatively correlated with the expension of mMDSCs in young patients, but not in adults. Taken together, our results uncovered a mechanism by which HBV-hijacked mMDSCs promote clonal deletion of HBsAg-specific CD8$^+$ thymocytes.

O050

Influenza A virus (IAV) infection in humans leads to expansion of highly diverse CD8 T cell repertoires cross-reactive with Epstein Barr virus (EBV)

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The competence of T cell responses predominantly depends on how efficient T cell receptors (TCRs) are at recognizing antigenic epitopes. We show here that during acute severely symptomatic IAV infection there was an expansion of IAV-M1/EBV-BRLF1 and IAV-M1/EBV-BMLF1 double-tetramer+ cells directly ex-vivo in 5 HLA-A2+ patients. We questioned whether this expansion specific to these two different cross-reactive responses would lead to alterations in the IAV-M1$_{58}$, EBV-BRLF1$_{109}$ and -BMLF1$_{280}$ TCR repertoires from before and during acute IAV infection. Using staining with VB mAb we found that T cell responses generated to these epitopes became surprisingly more polyclonal, with the sharing of Vb between M1, BMLF1 and BRLF1 populations which is not seen in healthy donor. Furthermore, by using next generation sequencing and single-cell analysis of TCRα and TCRβ repertoire of tetramer sorted IAV-M1 cells we showed dramatic changes in specific clonotype usage during acute IAV infection compared to before infection. In summary, these changes in TCR repertoire during acute symptomatic IAV infection suggest that during severe infection there is a preferential expansion of highly diverse cross-reactive responses between IAV and the persistent virus, EBV, which leads to permanent changes in TCR repertoires to both of these two viruses (NIH AI049320 and NIH AI109858).

O051

Human neutralizing antibodies against Zika virus preferentially bind to fully mature virions

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Zika virus (ZIKV) neutralizing antibodies hold a great promise for antibody-based interventions and the development of vaccine against ZIKV infection. However, how these neutralizing antibodies are generated and their epitope specificity on the fully mature virions remain unknown. We previously reported on the isolation of 17 monoclonal antibodies (mAbs) from the longitudinal samples of two ZIKV-convalescent individuals and characterized their binding and neutralizing activities against ZIKV. We showed that all isolated mAbs bound to ZIKV E protein but only 5 of them exhibited potent neutralization activity. While epitope specificity of these 5 neutralizing mAbs covers diverse regions on the E protein, the non-neutralizing mAbs predominantly recognize fusion loop epitope (FLE). Here we further characterize these mAbs in their binding affinity to mature and immature virions of ZIKV. We found that neutralizing antibodies preferentially bind to fully mature virions while non-neutralizing mAbs bound to immature ones. Neutralizing mAbs that strongly bound to mature virions generally show less

DOI: 10.1002/eji.201970400

cross-reactivity with other members of flavivirus family. In contrast, non-neutralizing mAbs demonstrated broad cross-reactivity and potential antibody-dependent enhancement *in vivo*. Collectively, our results showed neutralizing antibodies against Zika virus preferentially bind to fully matured virions. Future vaccine design aiming to induce neutralizing antibodies should therefore focus on mature rather than immature virions.

O052

A longitudinal investigative study of dengue-specific B cell responses during natural infection

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Dengue is a viral disease for which the immune response can be both protective and detrimental. T and B cells are highly activated in patients with severe disease, and this massive activation inevitably affects the immune repertoire. T and B cells are also important for the generation of immune memory and subsequent protection against re-infection. However, this anti-dengue adaptive immune response is not well characterized. To this end, we deeply investigated the cellular and molecular phenotype of dengue-specific B cell responses in a longitudinal cohort of 68 individuals infected by dengue virus for the first time or the second time.
We observed a dramatic increase of plasmablast frequency during acute infection and a diminution by the early convalescent phase. In addition, we identified at least 2 distinct populations of plasmablasts at the acute phase, with potentially different functions.
Study of anti-dengue antibodies in the serum of patients showed that titers drop rapidly between 1 and 6 months after fever. By investigating the neutralization ability of these antibodies, we observed a peak of neutralization at 1 month after fever, which is even higher in secondary-infected subjects. B-cell Elispot assays have been performed to assess the long-lasting B cell memory responses against dengue and highlight a maintenance of anti-dengue memory B cell responses at least 6 months after fever.
This work, completed by T cell response investigation, will lead to important insights in the field of dengue immunology and vaccinology.

O053

Staphylococcus aureus facilitates HIV-1 reservoir establishment in CD4+ T-cells by promoting RALDH activity in dendritic cells

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The human immunodeficiency virus type 1 (HIV-1) persists in antigen-specific memory CD4+T-cells despite viral-suppressive antiretroviral therapy (ART). Recent advances linked gut dysbiosis to HIV pathogenesis, with components of the microbiota sensed by dendritic cells (DC) boosting HIV replication in mucosal CD4+T-cells. Here, we used a model of HIV *trans*-infection in monocyte-derived DC (MDDC):CD4+T-cells co-cultures to investigate HIV reservoir establishment in CD4+T-cells reactive to *Staphylococcus aureus (SA)*, a promoter of Th17-differentiation and retinoic acid (RA)-synthesizing enzyme (RALDH) activity in DC.

DOI: 10.1002/eji.201970400

The immunogenic and HIV *trans*-infection potential of MDDC was evaluated upon exposure to SA lysates and co-culture with autologous T-cells. SEB and CMV-pp65 were used as controls for polyclonal and Th1-cell proliferation, respectively. The RALDH inhibitor DEAB and the RA receptor antagonist LE540 were used to block the RA pathway.
MDDC loaded with SA *versus* SEB or CMV exhibited a superior ability to transmit HIV to proliferating CD4+T-cells. Efficient HIV *trans*-infection by SA-loaded MDDC coincided with the highest levels of RALDH activity, as well as superior CCR5 and integrin beta7 expression on *SA*-specific CD4+T-cells. The RA pathway blockade significantly reduced the MDDC-mediated HIV *trans*-infection of SA-reactive CD4+T-cells.
In conclusion, specific components of the microbiota that promote RALDH activity in MDDC, such as SA, contribute to efficient DC:CD4+T-cell HIV dissemination during the antigen presentation process. These results point to RALDH as an important metabolic pathway hijacked by HIV for viral reservoir establishment in Th17 cells and residual transcription likely occurring during ART at mucosal sites rich in SA.

O054

AXL promotes Zika virus infection in astrocytes by antagonizing type I interferon signalling

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Zika virus (ZIKV) is associated with neonatal microcephaly and Guillain-Barré syndrome. While progress has been made in understanding the causal link between ZIKV infection and microcephaly, the life cycle and pathogenesis of ZIKV are less well understood. In particular, there are conflicting reports on the role of AXL, a TAM family kinase receptor that was initially described as the entry receptor for ZIKV. Here, we show that while genetic ablation of AXL protected primary human astrocytes and astrocytoma cell lines from ZIKV infection, AXL knockout did not block the entry of ZIKV. We found, instead, that the presence of AXL attenuated the ZIKV-induced activation of type I interferon (IFN) signalling genes, including several type I IFNs and IFN-stimulating genes. Knocking out type I IFN receptor α chain (IFNAR1) restored the vulnerability of AXL knockout astrocytes to ZIKV infection. Further experiments suggested that AXL regulates the expression of SOCS1, a known type I IFN signalling suppressor, in a STAT1/STAT2-dependent manner. Collectively, our results demonstrate that AXL is unlikely to function as an entry receptor for ZIKV and may instead promote ZIKV infection in human astrocytes by antagonizing type I IFN signalling.

O055

Expression of CD38 and MHC-II on CD8$^+$ T cells following severe influenza virus infection

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DOI: 10.1002/eji.201970400

Patients who succumbed to severe avian H7N9 influenza displayed impaired $CD8^+$ T-cell function. Our previous studies showed that expression of the activation markers, CD38 and HLA-DR (MHC-II) are increased on $CD8^+$ T-cells during the early stages of H7N9 infection, gradually declining during recovery. Conversely, patients hospitalised with H7N9 in China, who succumbed to disease displayed high and prolonged expression of CD38 and HLA-DR on $CD8^+$ T-cells. Here, we used mouse models of H7N9/H3N2 infection to investigate mechanisms underpinning expression of CD38 and MHC-II on $CD8^+$ T-cells. We observed co-expression of CD38/MHC-II on virus epitope-specific and bystander $CD8^+$ T-cells. This was demonstrated using endogenous D^bNP_{366} and $D^bPA_{224}{}^+CD8^+$ T-cells, as well as by transgenic OT-I cells adoptively transferred into C57B/L6 mice. In the latter model, OT-I cells upregulated similar level of CD38/MHCII after both X31-OVA and X31 virus infection, indicating that these $CD8^+$ T cells activation is more associated with disease severity rather than antigen-driven expansion during infection. Strikingly, we found that OT-I cells adoptively transferred into MHC-$II^{-/-}$ mice could not express MHC-II when infected with influenza virus, suggesting that MHC-II expression on $CD8^+$ T-cells occurs via trogocytosis from other immune cells, including B-cells and macrophages. Our study illustrates that $CD38^+MHCII^+$ $CD8^+$ T-cells can be activated by both virus-specific and non-virus specific manners, and trogocytosis of MHC-II molecules from other immune cells is the underlying mechanism. These activated T cells may contribute an important effector role following influenza infection or could be a potential biomarker for patients at risk of fatal disease.

O056

Hemagglutinin glycosylation modification of H5N1 subtype avian influenza virus regulates innate immune response of dendritic cells

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The outbreak of "cytokine storm" is the main reason for the high pathogenicity of H5N1 subtype avian influenza virus (AIV). Dendritic cells (DCs), serving as professional antigen presenting cells, also play a vital role in regulating the host innate immune response. Carbohydrate structure can regulate the innate immune of DCs, implying that AIV with glycosylation modification on surface hemagglutinin (HA) could regulate host innate immune via DCs. In this study, the effects of different hemagglutinin glycosylation-modified H5N1 subtype AIVs (rS-WT, rSΔ158, and rSΔ169 mutations) on the innate immune levels of mouse DCs were evaluated *in vitro*. We found that, compared with rS-WT, rSΔ158/rSΔ169 significantly increased the expression of phenotypic markers (CD80, CD86, CD40, and MHCII), early activation markers (CD69), and migration markers (CCR7) on DCs; promoted the secretion of pro-inflammatory cytokines (IL-1β, IL-6, IL-12, and TNF-α). *In vivo,* rSΔ158/rSΔ169 via nasal infection significantly promoted the migration of DCs to the submucosal mucosa of the nasal cavity and further into cervical lymph nodes compared to that of rS-WT. The main subtype of involved DCs was $CD103^+$. Furthermore, we found that H5N1 subtype AIVs with deletion of 158 or 169 glycosylation sites mainly through the SIGN-R1 and MR, but not MGL C-type lectin receptor (CLRs) dependent pathways to infect DCs, and further caused high levels of innate immune response *in vitro* and *in vivo*. This study provides a theoretical basis for the analysis of the pathogenic mechanism of H5N1 highly pathogenic AIV in mammals.

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DOI: 10.1002/eji.201970400

O193

Sequential IL-2 treatment improves immune function of non-response patients with hepatitis B virus infection after IFN-α therapy

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Background: Patients with chronic hepatitis B (CHB) undergoing interferon (IFN)-α-based therapies face the risk of a diminished HBeAg serological response. However, the mechanism underlying this unsatisfactory effect remains poorly understood, and there is an unmet need for new therapies.
Methods: Through the first clinical trial, we isolated peripheral blood mononuclear cells from chronic HBV-infected patients with or without Peg-IFN-α-2b therapy, and healthy donors, and systematically analyzed the immunology and the clinical features. In our second clinical trial, patients for whom IFN-α therapy had failed, were treated with sequential IL-2 for 24 weeks. We examined the HBV-specific $CD8^+$T cells and immune function, as well as the clinical outcomes.
Results: The majority of patients had unsatisfactory effect after Peg-IFN-α-2b therapy and were regarded as non-response patients. The IL-2Rα expression on $CD4^+$T cells decreased after these non-response patients, which had been treated with sequential IL-2 for 24 weeks. After IL-2 therapy for these non-response patients, on one hand, the percentage of regulatory T cells and programmed cell death protein 1 (PD-1) expression decreased. On the other hand, effective immune functions have been reconstituted, involving increased proportions of multi-functional effector $CD8^+$T cells and natural killer (NK) cells, the up-regulated percentage of HBV-specific $CD8^+$T cells and their cytotoxicity. Most importantly, it resulted in enhanced clinical outcomes, including HBeAg seroconversion.
Conclusions: Our findings suggest that sequential IL-2 therapy contributes to the restoration of immune function in non-response patients with refractory CHB.

O194

Combating herpesvirus encephalitis by potentiating a TLR3-mTORC2 axis

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Toll-like receptor 3 (TLR3) is a double-stranded RNA (dsRNA) sensor indispensable for defense against Herpes Simplex Virus (HSV-1) infection in the brain. We show here that TLR3 was required for innate immune responses to HSV-1 in neurons and astrocytes. In HSV-1 infection, TLR3 recruited the mammalian target of rapamycin complex 2 (mTORC2) that lead to induction of chemokines and trafficking of TLR3 to the cell periphery. TLR3 trafficking enabled activation of molecules required for type I interferon (type I IFN) induction including mTORC1. Intracranial HSV-1 infection in mice was exacerbated by impairing TLR3 responses with an mTOR inhibitor, and significantly rescued by potentiating TLR3 responses with an agonistic anti-TLR3 antibody. These results suggest that the TLR3-mTORC2 axis might be a therapeutic target to combat herpes simplex encephalitis.

DOI: 10.1002/eji.201970400

O195

Fundamental contribution and host range determination of ANP32 protein family in influenza A virus polymerase activity

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The polymerase of the influenza virus is key machinery for viral replication. Host factors may contribute to the influenza A virus RNA replication through interacting with viral RNA polymerase, although the key host factors involved in RNA replication and required for the polymerase activity remain largely unknown. The cellular protein ANP32A has been recently found to interact with viral polymerase, and to influence polymerase activity and interspecies restriction. Here we report that the contribution of ANP32B is equal to that of ANP32A, and together they play a fundamental role in the activity of mammalian influenza A virus polymerase, while neither human ANP32A nor ANP32B support avian viral polymerase. Interestingly, we found that chicken ANP32B is evolutionarily non-functional in supporting viral replication, revealing a common 129-130 domain of ANP32A and ANP32B necessary for viral polymerase activity, and these amino acids are also crucial in the maintenance of viral polymerase activity in other ANP32 proteins. Our findings strongly support ANP32A&B as key factors for both virus replication and adaption, and suggest these proteins could be potential targets for anti-influenza strategies.

O196

Characterization of anti-HCMV HLA-E-restricted unconventional CD8 T-cell responses in seropositive HCMV$^+$ hosts: frequencies, influencing factors and phenotype

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Human cytomegalovirus (HCMV) causes poor outcome in immunocompromised hosts such as transplant recipients. Cytotoxic CD8 T cells against HCMV antigens (pp65, IE1) presented by classical HLA class-I molecules are major cellular components of the protective anti-HCMV immunity. HLA-E-restricted CD8 T cells targeting HCMV UL40 leader peptides (Lp) have been recently reported as unconventional T-cell responses observed in few hosts but still need characterization. Our study provides an *ex vivo* quantification of HLA-E_{UL40} CD8 T-cell responses and a phenotype analysis in comparison to conventional anti-HCMV responses, in a large cohort of kidney transplant recipients and healthy volunteers using a dedicated detection assay by flow cytometry,. HLA-E_{UL40} CD8 T-cells were detected in >30% of seropositive HCMV$^+$ hosts and may represent >30% of total circulating CD8 αβT cells. Host-related genetic factors (HLA-A*02 and HLA-E genotype) and HCMV strain, determining the sequence of UL40Lp, are critical parameters for this response. HLA-E_{UL40} CD8T appear early post-infection and persist for life. A key feature of these cells is their potential ability to be responsive against self and allogeneic HLA-ILp homologous to UL40Lp. HLA-E_{UL40} CD8T are TEMRA cells (CD45RA+,CCR7-,CD27-,CD28-) that differ from HLA-A*02pp65CD8T by displaying low migratory properties (CD62L-, CXCR5-) but selective recognition of endothelial cells through CX3CR1. They are not exhausted (PD1-, Tim3-, Lag3-) but instead fully functional with high CD56 and CD57 levels. In conclusion, unconventional HLA-E_{UL40} CD8 T-cells belong to the common immune arsenal against HCMV. Their functions remain to be defined toward HCMV infection as well as their potential alloimmunity in transplantation.

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DOI: 10.1002/eji.201970400

O197

Subsequent malaria enhances virus-specific T cell immunity in SIV-infected Chinese rhesus monkeys

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How malaria affects the progression of human immunodeficiency virus (HIV) disease has been a controversial issue when coinfection of HIV and Plasmodium parasites occurs in HIV- and malaria-endemic areas. Here, we used a Chinese rhesus macaque model for co-infection with simian immunodeficiency virus (SIV) and *Plasmodium cynomolgi* (Pc) to address this issue under controlled conditions. The experiment consists two coinfection groups that Pc malaria initiated pre- or post-SIV infection, an SIV alone and a Pc alone group. The pre-existing and subsequent malaria produced different effects on SIV infection. Animals infected with malaria prior to SIV produced a greater number of CD4+CCR5+ target cells for SIV replication, which resulted in higher viral loads. Conversely, malaria after SIV infection induced sustainable higher proportion of CD4+CD28^{high}CD95^{high} central memory T cells and maintained the repertoire diversity of SIV-specific T cell receptors that was reduced considerably in SIV infected alone or pre-existing malaria-SIV coinfected animals. Not only more but also new SIV-specific clonotypes of T cells were continuously generated to trace the SIV antigen variation, and stronger SIV-specific cellular immune response was elicited in the subsequent malaria-SIV coinfected animals. As a result, subsequent malaria may improve the survival of the SIV infected animals. The complex outcomes in this study may have important implications for human HIV and malaria co-infection studies. The infection order of the two pathogens should be considered.

O198

ITK regulates antigen-specific CD8$^+$ T cell-derived IL-10 production and modulates influenza-induced pulmonary immunopathology

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Influenza (flu) infection is a leading cause of respiratory disease and death worldwide, causing 3-5 million cases of severe illness and more than 250,000 deaths during an average flu season. Severe life-threatening influenza infections in humans are accompanied by an aggressive pro-inflammatory response and an insufficient anti-inflammatory immunity. The production of the immunosuppressive cytokine IL-10 by virus-specific T cells is critical in limiting the immunopathology during flu infection, however, our understanding of how this immunomodulatory event is regulated at the molecular level is very limited. Using mouse models that report the production of IL-10 through green fluorescent protein, OTI CD8$^+$ T cells and influenza A viruses that express the OTI antigen ovalbumin (OVA), we find that the T cell receptor (TCR) responsive tyrosine kinase, Interleukin-2 Inducible T cell kinase (ITK), is a

DOI: 10.1002/eji.201970400

critical regulator of the ability of $CD8^+$ T cells to develop into IL-10-producing cells in an antigen specific manner. The absence of ITK resulted in increased morbidity and mortality in murine model post influenza A infection, accompanied by a severe impairment of IL-10 production by $CD8^+$ T cells in the airway. ITK kinase activity is required for its role in promoting IL-10-producing $CD8^+$ T cell differentiation, by regulating the levels of expression of transcription factors including IRF4, Blimp1, AHR and cMAF. Our data suggest that ITK regulates $CD8^+$ T cell production of IL-10 during influenza A infection, and modulates the development of pulmonary immunopathology. Targeting ITK signaling maybe a promising strategy in modulating airway inflammation during viral infections.

O199

The complex of MCMV proteins and MHC class I evades NK cell control *via* missing-self and drives the evolution of virus-specific activating Ly49 receptors

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Cytomegaloviruses (CMVs) are widespread dsDNA viruses that efficiently downregulate MHC I molecules to avoid recognition by cytotoxic T cells. However, absence of MHC I on the cell surface renders the infected cell susceptible to NK cell killing upon missing-self recognition. To counter this, mouse CMV (MCMV) rescues some MHC I molecules to engage inhibitory Ly49 receptors. Here we identify a new viral protein, MATp1, that is essential for MHC I surface rescue together with m04. Rescued altered-self MHC I molecules show increased affinity to inhibitory Ly49 receptors resulting in inhibition of NK cells despite substantially reduced MHC I surface levels. This enables the virus to evade recognition by licensed NK cells. Interestingly, MATp1 ORF is highly variable among different virus strains which has prompted us to investigate whether it has been under strong selection pressure by the immune system. Differential sensitivity of various mouse strains to MCMV has been linked to the capacity of NK cells to recognize infected cells via activating Ly49 receptors. We and others have previously shown specific recognition of MCMV-infected cells by activating Ly49 receptors that requires MHC I, m04 and additional virally encoded factor. Using multiple MCMV mutants we show that the unknown virus factor is also MATp1. Thus, MATp1-mediated MCMV evasion of inhibitory Ly49 receptor could have prompted the evolution of activating, virus-specific Ly49 receptors.

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DOI: 10.1002/eji.201970400

O200

The immunological roles of human γδ-T cells in Epstein-Barr virus infection-induced diseases

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Epstein-Barr virus (EBV) is a herpesvirus that latently infects human B cells in most individuals and a leading cause of several human malignancies, such as EBV-induced B cell lymphoproliferative disorders (EBV-LPD). Human Vγ9Vδ2-T cells represent a minor but crucial population in immune system and act as an important component of immune effector cells that contribute to immunosurveillance against viral infection. However, the immunological roles of Vγ9Vδ2-T cells in EBV infection and EBV-related disorders are not well studied and characterized. In our study, we demonstrated that phosphoantigen-activated human Vγ9Vδ2 -T cells have potent cytotoxic activity against EBV-transformed B lymphoblastoid cell lines (EBV-LCLs) through TCR γδ and NKG2D triggering, and FasL and TRAIL engagement. By targeting human Vγ9Vδ2-T cells, we further demonstrated that pamidronate can control EBV-LPD in humanized mice through boosting Vγ9Vδ2-T cell immunity. Furthermore, the human macrophage plays a very critical role in triggering the immune surveillance against the EBV infection. But, the relationship between the Vγ9Vδ2-T cells and macrophages is still unknown during EBV infection. Accordingly, our data showed that human Monocyte-driven-Macrophages (MDMs) could be infected by EBV. Subsequently, Vγ9Vδ2-T cells displayed potential cytotoxic activities against EBV-infected MDMs in vitro. Using the blocking experiment, the result demonstrated that special monoclonal antibodies against TCR γδ and NKG2D could significantly inhibit the killing of infected-MDMs by Vγ9Vδ2-T cells. Moreover, Vγ9Vδ2-T cells could impair viral replication in the infected-MDMs through producing IFN-γ. These findings provide proof-of-principle for a therapeutic approach using Vγ9Vδ2-T cells to control EBV infection and EBV-associated human malignancies.

O265

MiR-31 slows down HIV-1 disease progression by restraining $CD4^+$ T cell activation and differentiation

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It remains poorly defined whether there exist miRNAs playing protective roles during HIV infection. Here we investigated miRNA expression profile in HIV-infected long-term non-progressors in comparison to that in progressors, and identified that miR-31 was reversely correlated with disease progress in a cross-sectional study. We further proved that higher expression of miR-31 is correlated with a slower disease progress both in a 25-month prospective cohort study followed from acute to early chronic HIV-1 infection (n=54) and a 60-month prospective cohort study during chronic infection (n=50). *Ex vivo* experiments showed that miR-31 restrains $CD4^+$ T cell activation, proliferation and differentiation by down-regulating crucial transcriptional factors, such as T-bet, GATA3, RORC, Foxp3, and thereby reduces HIV-1 infection target cells to suppress HIV-1 spread. These results suggest that miR-31 is a novel predictor of disease progression during HIV-1 acute and chronic infection and could be potentially employed as a new HIV medicine in combination with ART regimen.

DOI: 10.1002/eji.201970400

O266

Activation of follicular helper T cells and tissue resident memory T cells in human nasopharynx-associated lymphoid tissue by novel influenza vaccines

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Both anti-hemagglutinin(HA) antibody and cytotoxic T cell responses are critical for anti-influenza immunity. T follicular helper cells (TFH) are considered important for antibody response, whereas tissue-resident memory T (T_{RM}) cells are critical for rapid protection against mucosal influenza reinfection. Most data supporting the role for TFH in the antibody response are from animal studies, and direct evidence from humans is limited. We studied the activation/induction of TFH and their role in the anti-influenza antibody response induced by a live-attenuated influenza vaccine (LAIV) as compared to inactivated vaccine in human nasopharynx-associated lymphoid tissue (NALT). We also studied the capacity of Modified Vaccinia Ankara-vectored vaccine MVA-NP+M1 to activate M1-specific CD8+ T cell response including T_{RM} cells. We showed LAIV induced TFH proliferation correlated with anti-HA antibody production, and TFH were critical for the antibody response. Induction of TFH from naive T cells by LAIV was shown in newly induced TFH expressing BCL6 and CD21, followed by the detection of anti-HA antibodies. Antigen specificity of LAIV-induced TFH was demonstrated by expression of CD154+ antigen-specific T cell upon challenge by H1N1 HA. TFH differentiation was inhibited by BCL6, IL-21, ICOS blocking and that diminished anti-HA antibody production. These support the induction of TFH in NALT by use of intranasal vaccines provides an effective vaccination strategy. We also demonstrated MVA-NP+M1 elicited a substantial M1-specific cytotoxic T cell response including mucosal T_{RM} cells, demonstrating its strong capacity to activate memory T cell pool therefore offering great potential for rapid and cross-reactive protection against influenza re-infection.

O267

TFF2-a novel immunomodulatory molecule in protection against influenza virus infection

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TFF2 is a member of a tripartite family of small proteins, mainly produced by stomach and colon. Consequently, recombinant TFF2 has been shown to be protective, in models of gastric and colonic injury, reducing inflammatory indices and promoting mucosal repair. In addition, TFF2 is also expressed in respiratory tract, implying a potential immunomodulatory role. However, whether TFF2 actually modulates the pathophysiology of respiratory tract upon external challenge remains elusive. In this study, we focused on the modulatory effect of TFF2 on infection of influenza A virus. Using murine infection model, we first discovered that the administration of TFF2 protein significantly reduced the mortality and morbidity caused by a variety of influenza A viruses, including H7N9, PR8 and H9N2. The examination of TFF2 knockout mice proved that endogenous TFF2 attenuated influenza induced pathogenicity. Our further analysis revealed that TFF2 did not affect the influenza virus replication per se, but rather inhibit the early inflammatory response. We also provided evidence that TFF2 was mainly produced in the airway epithelial cells. In addition, the binding of TFF2 to target cells appeared to be modulated by glycosyltransferase A4GNT, indicating that TFF2 engagement requires the

DOI: 10.1002/eji.201970400

glycosylation of its cell surface receptor. These findings laid the foundation for our ongoing identification of TFF2 receptor. Collectively, we demonstrated TFF2 as a novel immunomodulatory molecule key to the protection of respiratory tract against influenza evasion. TFF2 thus holds promising therapeutic potential in the prevention and treatment of inflammatory acute lungs injury.

O268

Combination anti-HIV antibody therapy is associated with increased virus-specific T cell immunity

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Antiretroviral therapy (ART) is highly effective in controlling HIV. However, life-long therapy is required as the virus rebounds from latent reservoirs. Broadly neutralizing anti-HIV antibodies (bNAbs) represent a potential alternative or adjuvant to ART. In addition to virus neutralization, antibodies can engage the immune system. BNAb administration to macaques elicited adaptive immune responses that were associated with long-lasting viral control. However, this has not been established in HIV-infected individuals.
We investigated participants enrolled in a clinical study assessing the combined infusion of bNAbs 10-1074 and 3BNC117 at weeks 0, 3 and 6. Analytical treatment interruption (ATI) was performed at day 2. Nine individuals demonstrated viral suppression for more than 15 weeks after ATI. For these participants, we assessed HIV-specific T cell responses prior to bNAb infusion and at week 12 after ATI during maintained viral suppression.
BNAb-infused individuals with suppressed viremia during ATI demonstrated increased HIV-specific CD8+ and CD4+ T cell responses by ICS and by activation-induced marker assays in blood when compared to baseline. Responses to nearly all tested HIV antigens were expanded in 2 individuals with long-term viral control (>30 weeks) but not in participants who rebounded between weeks 15-26. Increased T cell responses were due to both expansion of pre-existing responses and the emergence of responses to new epitopes. In contrast, HIV-specific T cell responses remained unchanged in ART-treated individuals who did not receive bNAb infusions.
These data demonstrate that bNAb treatment during ART interruption is associated with increased HIV-specific T cell responses while maintaining viral suppression.

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DOI: 10.1002/eji.201970400

O269

Design of innovative Hantaan virus vaccine based on novel CD8$^+$ T-cell epitopes and ThCTL/Th1 responses-inducing T-cell epitopes

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Hantaan virus (HTNV) could cause lethal hemorrhagic fever with renal syndrome in human. Although the potential roles of T cells in viral control have been extensively studied, the contribution of T cells to host protective response against HTNV infection remains unclear. We have identified several novel CD8$^+$ and CD4$^+$ T-cell epitopes restricted by different HLA molecules on HTNV structure proteins, among which, **the crystal structure of CD8$^+$ T-cell epitope/HLA-A2 or -B35 complexes were analyzed and displayed typical MHC class I folds.** Importantly, in HTNV challenge trials, we found that **the levels of HTNV RNA loads in liver, spleen, and kidneys were significant reduced in HLA-A2.1/K^b transgenic mice pre-vaccinated with HLA-A2 restricted CD8$^+$ T-cell epitopes**. Moreover, **HTNV CD4$^+$ T-cell epitopes could induce both effective Th1 responses with polyfunctional cytokine secretion as well as ThCTL responses with cytotoxic mediator production against HTNV infection**, which significantly correlated with milder disease in human. All of these immunodominant protective epitopes may function as good candidates for the development of HTNV peptide vaccines. Based on these, **we designed series connected epitope peptides with HTNV CD8$^+$ T-cell epitope restricted with HLA-A2, the HTNV neutralizing B-cell epitope and Pan-DR Th epitope as "B+Th+CTL",** which could induce higher frequencies of IFN-γ and cytolytic mediator production and stronger proliferation capacity of specific CD8$^+$ T cells in immunized HLA-A2.1/K^b transgenic mice. **Our study highlight the efforts of T-cell immunity to HTNV control and provided theoretical and practical basis for the design of innovative HTNV peptide vaccine.**

O270

Human immunity to chikungunya infection

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Chikungunyna virus is expanding globally and continue to cause major public health threat to Indian populations. Vaccine efforts are underway, and it is hoped that these will eventually progress to human evaluation. However, currently we have little understanding of the phenotypes and functions of the human T cells in chikungunya patients, a knowledge that is essential for improving vaccine design/ testing and evaluation efforts. Here, we provide a detailed analysis of the CD8 T cell responses in chikungunya patients from India. We found that CD38$^+$HLADR-38$^+$ CD8 T cell subset expanded dramatically in chikungunya febrile patients with frequencies averaging about 20% of the total CD8 T cells, and reaching as high as 50% of the CD8 T cells in some patients. The frequencies of these activated CD8 T cells were substantially low and barely above background levels in afebrile patients reporting to the clinic with persistent arthralgia/ arthritis that was lasting for more than 30 days. These massively expanding CD8 T cells observed in the acute febrile patients were highly proliferating (ki67$^+$),robustly expressing markers indicative strong Th1 differentiation (T-bet$^+$), cytotoxic functions (Perforin$^+$) and inflammatory/ synovial tissue homing characteristics (CX3CR1 and CXCR4).

DOI: 10.1002/eji.201970400

Interestingly, antigen-stimulation mediated IFN-g producing functions of these cells was highly compromized, reminiscent of the "cytokine stunned" phenotype. Taken together, these results suggest that these highly differentiated effector CD8 T cell that were massively expanding during acute chikungunya febrile infection might be involved in protection by homing to infected tissues and eliminating infected targets rather than causing inflammation.

O271

Downregulated NDR1 protein kinase inhibits innate immune response by initiating an miR146a-STAT1 feedback loop

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Interferon (IFN)-stimulated genes (ISGs) play crucial roles in the antiviral immune response; however, IFNs also induce negative regulators that attenuate the antiviral response. Here, **we show that both viral and bacterial invasion downregulate Nuclear Dbf2-related kinase 1(NDR1) expression via the type I IFN signaling pathway. NDR1 promotes the virus-induced production of type I IFN, proinflammatory cytokines and ISGs in a kinase-independent manner. NDR1 deficiency also renders mice more susceptible to viral and bacterial infections. Mechanistically, NDR1 enhances STAT1 translation by directly binding to the intergenic region of miR146a, thereby inhibiting miR146a expression and liberating STAT1 from miR146a-mediated translational inhibition.** Furthermore, STAT1 binds to the miR146a promoter, thus decreasing its expression. Together, our results suggest that NDR1 promotion of STAT1 translation is an important event for IFN-dependent antiviral immune response, and suggest that NDR1 has an important role in controlling viral infections.

O272

LPS is involved in liver inflammation of infectious mononucleosis-like disease in mice infected with MHV68

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IL-27 is an immune regulatory cytokine consisting of p28 and EBI3. Its receptor also has two subunits WSX1 and gp130. IL-27 promotes Th1 differentiation in naïve T cells and induces IL-10 production in effector Th1 cells, curtailing excessive Th1 responses. We employed p28- and WSX1-deficient mice (collectively called IL-27-KO mice) to examine the role of IL-27 in primary infection of murine γ-herpesvirus 68 (MHV68), a murine model of human Epstein-Barr virus. Upon airway infection with MHV68, IL-27-KO mice had much aggravated lung inflammation, although the mice cleared MHV68 more efficiently than wild-type mice. IL-27-KO mice had increased IFN-γ-producing $CD8^+$ T cells and decreased IL-10-producing effector $CD8^+$ T cells in the lung compared with WT mice. IL-27-KO mice also displayed an aggravated infectious mononucleosis (IM)-like disease with prominent splenomegaly and hepatitis compared with wild-type mice. In the liver, infiltration of IFN-γ-producing NK cells and $CD8^+$ T cells was associated with upregulation of CXCL9/10/11, which attract Th1-type effector cells via CXCR3. Oral neomycin as well as C34, an inhibitor of Toll-like receptor 4 signaling, ameliorated liver inflammation with decreased production of the CXCR3 chemokines in both IL-27-KO and WT

DOI: 10.1002/eji.201970400

mice, suggesting that LPS produced by the intestinal microbiota plays a role in liver inflammation through upregulation of the CXCR3 chemokines in the liver. Collectively, in primary infection with MHV6, (1) IL-27 plays a critical role in induction of IL-10-producing Th1 effector cells, and (2) LPS is involved in liver inflammation of the IM-like disease.

O353

NLRP3 inflammasome facilitates CD4+ T cell loss during chronic HIV infection

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Chronic HIV-1 infection is generally characterized by progressive CD4+ T-cell depletion and persistent inflammation. Recent studies have suggested that abortive infection of quiescent CD4+ T cells results in pyroptosis, a highly inflammatory form of cell death, which accounts for the massive CD4+ T cell loss and potentially further perpetuates chronic inflammation. However, the clinical relevance and the underlying mechanism of CD4+ T cell pyroptosis in chronic HIV infection are not well understood. By detection of caspase-1 activation and GSDMD cleavage in CD4+ T cells of PBMC and lymph nodes, we found that the pyroptotic levels of CD4+ T cells were elevated during chronic HIV infection, with correlations with viral load, CD4 counts and CD4/8 ratio. Mechanismly, inflammasome-related genes were significantly upregulated in CD4+ T cells during chronic HIV infection when compared to healthy controls. HIV infusion induced ROS production in both productively infected and bystander CD4+ T cells, which resulted in NLRP3/Caspase-1/GSDMD dependent pyroptosis. This study contributed to the understanding of CD4+ T cell loss and might be leveraged to develop novel interventions to HIV treatment.

O355

Triggering CD8+ T cell immunity by liver sinusoidal endothelial cells during intrahepatic HBV clearance is HBeAg dependent and is mediated by TNF-α and IL-27

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As an important population of liver resident antigen presenting cells, liver sinusoidal endothelial cells (LSECs) help to shape the immune function of intrahepatic T cells. Little is known about how HBV infection influences the function of LSECs in regulating T cell immunity. Here, we investigated the function and mechanism of LSECs regulating CD8+ T cell immunity during HBV infection using HBV-replication mouse models and human liver samples. We found that LSECs switch from tolerogenic to immunogenic property in mediating CD8 T cell immunity during the course of HBV clearance in the HBV hydrodynamic injection (HDI) mouse model. Both in vitro and in vivo HBeAg stimulation could induce the functional maturation of naive LSECs and enable them to promote the IFN-γ production of TCR-activated CD8+ T cells. Consistently, HDI of HBV strain with HBeAg expression deficiency could not induce the functional maturation of LSECs and resulted in decreased intrahepatic HBV specific CD8 T cell responses. Deep sequencing analysis demonstrated that both intrahepatic HBV replication and HBeAg stimulation could induce TNF-α and IL-27 production of LSECs. Blockade of TNF-α or IL-27 function could partially abolish the ability of HBV- or HBeAg-stimulated LSECs to promote effector T cell response. In addition, in vivo TNFα blockade significantly delayed HBV clearance in HBV HDI mice. In conclusion, our data suggest that intrahepatic HBV replication can revert LSECs suppressive

DOI: 10.1002/eji.201970400

properties to promote T cell immunity, which is partially mediated by HBeAg induced TNF-α and IL-27 production by LSECs.

O356

BCL-2 antagonism sensitizes CTL-resistant HIV reservoirs to elimination ex vivo

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Curing HIV infection will require the elimination of a reservoir of infected $CD4^+$ T-cells that persists despite HIV-specific CTL responses. While viral latency is a critical factor in this persistence, recent evidence also suggests a role for intrinsic resistance of reservoir-harboring cells to CTL killing. We explored the hypothesis that this resistance is mediated by BCL-2 family proteins, which can antagonize CTL-induced apoptosis. We show that the reactivatable HIV reservoir is disproportionately present in BCL-$2^{hi}CD4^+$T-cells, which are relatively resistant to CTL. BCL-2/BCL-$X_L$antagonists were sufficient for inducing the elimination of HIV-infected cells from a primary-cell model of latency, but did not drive reductions in *ex vivo*viral reservoirs when tested either alone or with a latency reversing agent (LRA). The triple combination of LRAs, HIV-specific T-cells, and a BCL-2 antagonist uniquely enabled depletions in *ex vivo*viral reservoirs, providing rationale for novel therapeutic approaches targeting HIV cure.

Highlights

· The intact-inducible HIV reservoir in *ex vivo* $CD4^+$ T cells resists elimination by cytotoxic T-lymphocytes
· The reactivatable HIV reservoir is present in cells over-expressing the pro-survival factor BCL-2
· BCL-2/BCL-X_L antagonism reduced infected cells from a model of HIV latency
· BCL-2/BCL-X_L antagonism did not reduce infected cells from *ex vivo* CD4 reservoirs
The combination of HIV-specific T-cells + latency reversal agent + a BCL-2 antagonist reduced *ex vivo* HIV reservoirs

O357

The gut microbiota plays a protective role in the host defence against Hepatitis B virus (HBV) infection

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Background: Gut microbiota helps to shape the immune system and its depletion prolonged HBV infection in mice model, but the mechanisms are not well known.
Method: C57BL/6 mice were treated with broad-spectrum antibioticsto deplete the gut microbiota, and thenwere injected hydrodynamically with pSM2-HBV. Liver infiltrated lymphocytes and splenocytes were analysed by FACS. Liver tissues were homogenized and plated onto blood agar plates for bacteria culture.
Results: ABX treatment had no significant effect on the frequencies and numbers of CD4+T cells,

DOI: 10.1002/eji.201970400

CD8+ T cells, Tregs, and MDSCs in the liver and spleen. But the frequencies of activated T cells were increased in the liver in the ABX treated mice. The production of TNF- α and IL-2 was decreased in both CD4+ T cells and CD8+ T cells via TCR stimulation. The bacteria in liver homogenates from ABX treated mice was abundant, while the liver homogenatesofcontrol mice were kept sterile. In the ABX treated mice, HBV replication was prolonged significantly. Furthermore, the expression of CTLA-4 and PD-1 in HBV specific T cells were enhanced, and the production of IFN-γ or IL-2 were reduced significantly.
Conclusion: Our results indicated that the depletion of gut microbiota leads to the microbiota translocation to liver, which may inhibit HBV specific T-cell response by up-regulating CTLA-4 and PD-1 expression in T cells, and finally prolonged HBV replication in the mice model. This data implied that the normal gut microbiota may help the host to protect from HBV infection.

O358

Development of ELISA-based surrogate assays for studying the rhinovirus-receptor interactions

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Rhinoviruses (RVs) are the major cause of the common cold, a usually mild disease, but they can also trigger severe respiratory illnesses such as exacerbations of asthma and chronic obstructive pulmonary disease (COPD). So far, no prophylactic and/or therapeutic vaccines have been developed for RV infections. Conventional neutralization assays used to determine the ability of antibodies to inhibit the binding of RVs to their human target cells are laborious and time-consuming because they require human target cells and infectious virus. Therefore, our goal was to develop an ELISA-based surrogate assay to study the interaction between RV strains representative for the major and minor RV groups and their corresponding cellular receptors, ICAM-1 and LDLR, respectively. For that, ELISA plates were coated with recombinant human ICAM-1 or LDLR, respectively, and incubated with purified RV strain preparations representative for major and minor groups. The binding of RVs to their receptors was detected by RV-strain specific antisera. We found that major and minor RV strains can specifically bind to their corresponding cellular receptors in an ELISA-based assay and that the binding can be inhibited using anti-ICAM-1 and anti-LDLR specific antibodies. Thus, the developed surrogate ELISA assay can be used to test the ability of RV-specific antibodies to inhibit the attachment of RV strains to their receptors which is a crucial first step during RV infection. The ELISA assay may therefore be useful for the development of passive and active vaccination strategies for RV infections and the identification of anti-RV compounds.

O359

m6A modification regulates the Epstein-Barr virus-host interactions

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N6-methyladenosine (m6A) is the most common mRNA modification. Recent studies have revealed that m6A modification is involved in virus infection process. Here we used MeRIP-seq to examine the cellular and virus m6A epitranscriptome changes upon Epstein-Barr virus infection of BJAB cells (an EBV-negative human B lymphoma cells). EBV infection increased the whole m6A modification levels and altered the distribution of m6A peaks in host cells epitranscriptome. The highly m6A modified host genes upon EBV infection were involved in apoptosis, ubiquitin mediated proteolysis, p53 signaling

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DOI: 10.1002/eji.201970400

pathway, RNA transport and viral-associated signalings. Two important EBV viral genes, *EBNA2* and *BHRF1*, contained abundant m6A modifications during infection process. Knockdown of *METTL3* gene (a "writer") or treated with 3-DAA (a m6A modification inhibitor) increased the *EBNA2* expression, suggesting the m6A modification system played a protection role through repressing the virus *EBNA2* expression. *METTL3* Knockdown did not affect the virus binding to the host cells, but facilitate the virus entering the cells through upregulating the surface expression of CD21 (the EBV receptor) on the membrane of the host cells. A panel of host genes involved in the immune modulation had been confirmed to have increased m6A modifications upon EBV infection. Knockdown of *METTL3* gene also modulated hundreds of host genes transcription which are involved in energy metabolism, signal transduction, antigen extraction and cell differentiation. These results revealed a critical role of m6A modifications in regulating the Epstein-Barr virus-host interactions and provided rich resources for future investigations.

O360

Regulation of adaptive immunity via autophagy in myeloid cells upon MVA vaccination

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Modified vaccinia virus Ankara (MVA) has been broadly used as vaccine vector for its effective T cell activation, antibody production and protective long-term memory induction. Macrophages as antigen scavengers play an important role in pathogen uptake. Here, we demonstrate that autophagy negatively regulated innate immune responses in macrophages. In addition, it had a significant impact on T cell and antibody responses.
Interestingly, mice in which autophagy is selectively deficient in myeloid cells showed a basal inflammation status in the spleen. Upon viral vector immunization, elevated levels of different cytokines and chemokines were found in knockout mice which resulted in lower target antigen amounts and reduced T cell responses. On the other hand, antibody titers from knockout mice were increased and germinal center (GC) cells were found in higher frequencies, probably due to elevated cytokine levels promoting B cell differentiation.
Our data show the important role of autophagy at balancing the immune environment upon virus infection/vaccination and will help to optimize future vaccine design by targeting autophagy for a better immune protection.

LB018

Peptide presentation by bat MHC class I provides new insight into the antiviral immunity of bats

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Bats harbor many zoonotic viruses, including highly pathogenic viruses of humans and other mammals. The infections caused by these viruses are typically asymptomatic in bats, raising questions about immune differences which might exist between bats and other mammals. Though the critical cellular and molecular components of the adaptive immune system are conserved and functional in bats, a large number of unique amino acid (AA) substitutions or insertions occur in bat immune genes, including MHC I. We screened bat MHC I Ptal-N*01:01 binding peptides from different bat-borne viruses (HeV, EBOV, MERS-CoV, and H17N10 influenza-like virus) and for the first time, determined the structures of bat MHC class I based on these viral peptides. The structures of Ptal-

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DOI: 10.1002/eji.201970400

N*01:01 display unusual peptide presentation features in that the bat-specific sequence combination of the 3-AA insertion at the N-terminus of the α1 helix and the charge matching residues enable the tight anchoring of the P1-Asp in pocket A of bat MHC I. Interestingly, this uncommon peptide presentation features of bat MHC I may be shared by the MHC I from various marsupials. Our study may help to understand the greater capacity of bats with 3AA insertion to co-exist with a variety of viruses from the perspective of adaptive immunity. What's more, our study sheds light on bat adaptive immunity and may benefit future vaccine development against bat-borne viruses of high impact on humans.

LB056

Immunotherapy with the preS-based grass pollen allergy vaccine BM32 induces hepatitis B-specific protective antibody responses: kinetics and epitope specificity of antibody responses

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There are several unsolved issues in the management of hepatitis B infection (HBV). Approx. 10% of vaccinees are non-responsive to HBsAg-containing vaccines, and therapeutic vaccines for the treatment of chronic infection are not available. Conventional HBV vaccines are based mainly on HBsAg, although the other surface protein preS has been shown to be crucial for the virus´ entry into hepatocytes via NTCP. We recently found that immunotherapy with the preS-containing recombinant grass pollen allergy vaccine BM32 induced antibodies which inhibit HBV infection *in vitro*. To further investigate the vaccine´s anti-HBV potential, we produced preS and three preS-derived peptides: peptide A, comprising the epitope, involved in liver cell attachment; peptide B - the epitope, thought to be required for inhibition of infection; and peptide C, including both regions. Sera from 130 grass pollen allergic patients, who had received 3, 4 or 5 injections of BM32 or placebo in monthly intervals, were tested in ELISA assays. We analyzed levels of IgG as well as IgG isotypes and subclasses. We detected the induction of a robust preS-specific IgG antibody response, consisting mostly of IgG_1 and IgG_4 subclasses; IgG_1 appeared early, while IgG_4 appeared later and was more sustained. Serum levels of IgG_1 and IgG_4 antibodies against preS and preS-derived peptides were quantified. PreS-specific IgG_1 and IgG_4 reacted with all three peptides and thus were directed against the regions of preS important for liver cell attachment and inhibition of infection, which supports our previous findings and indicates that BM32 indeed may protect against HBV.

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DOI: 10.1002/eji.201970400

LB092

Translational control of the antiviral immune signaling by the cap-binding protein 4EHP

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Type I interferon is essential for host defense against invading pathogens. However, IFN must be tightly controlled because the sustained IFN production is detrimental to host resulting in either tissue damage or autoimmune diseases. Although transcriptional control of type I interferon expression has been extensively studied, its translational control is not well understood. Of note, we recently found that the loss of eIF4E Homologous Protein (4EHP) both in vitro and in vivo models result in an enhanced type I interferon immune response concomitant with decreased viral replication. We further showed that 4EHP translationally controls IFN-ß production through its association with the miRNA34a-mediated silencing machinery directly targeting the *Ifnb1* 3'UTR. Meanwhile, the miR-34a can be upregulated by both RNA virus infection and rh-IFN-β-induced JAK-STAT signaling pathway, suggesting a negative feedback regulatory mechanism governing IFN-β expression. These findings demonstrate the direct involvement of 4EHP in viral pathogenesis and host defense; which underscore the translational repression mechanism mediated by 4EHP that impedes type-I IFN production. Our study unveiled a previously unappreciated regulatory function of 4EHP in host intrinsic negative modulation system of IFN, highlighting its essential role in dampening virally induced sustained innate immune response to maintain host homeostasis.

P0290

Screening, identification and basic research for application of $CD8^+$ CTL epitopes of EBV membrane protein LMP2A/2B in nasopharyngeal carcinoma

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EBV (Epstein-Barr virus) shows clonal amplification in nasopharyngeal carcinoma (NPC) cells, which is a type II recessive infection mode and mainly expresses viral latent membrane proteins. In previous studies, we predicted and screened the candidate virus antigen LMP2A/2B encoded by EBV gene in NPC through single-cell sequencing and bio-information technology, which was highly expressed in almost all carcinoma cells and involved in the transformation, occurrence and development of NPC. However, the screening, identification and application of specific epitopes of LMP2A/2B antigen in NPC remain unclear. In this study, we systematically intend to screen and identify the HLA-I type restricted associated $CD8^+$ CTL epitopes from the LMP2A/2B. And then using tetramer staining, we identify T cells specifcally recognizing LMP2A/2B epitopes from PBMCs and clone the T cell receptor (TCR) α and β chains using a single-cell cloning technique. By expressing the paired TCRα/β genes in primary T cells, we generate TCR-T cells for verification of the specificity and affinity of epitopes. After, we carry out the animal tumor model experiments to verify and discuss its killing effects and mechanisms. Finally, we plan to develop the screened LMP2A/2B epitopes into a personalized EBV vaccine in the future, explore and evaluate its immune defense function and mechanism in EBV and NPC. Therefore, the screening and identification of EBV-specific epitopes and the design of TCR-T have profound significance for the research on how to identify, prevent and treat NPC associated with EBV infection, and provide sufficient scientific basis for the development of new vaccines.

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DOI: 10.1002/eji.201970400

P0291

Circulating CXCR3$^+$ Tfh cells positively correlate with neutralizing antibodies responses during HCV infection

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Circulating T follicular helper (cTfh) cells have been identified as counterparts of germinal center Tfh (GC Tfh) cells in humans and can support T-dependent B cell maturation and antibody production in vitro. However, the role of cTfh cells in neutralizing antibody (nAb) responses during HCV infection remains unclear. Here, we characterized the phenotype and function of cTfh cells and demonstrated associations of cTfh cells and their subsets with nAb responses during HCV infection. A total of 38 HCV-infected individuals and 28 healthy controls were enrolled from a pool of injection drug users (IDUs). The frequency and function of blood Tfh cells were analyzed by flow cytometry. The titers and breadths of serum neutralizing antibodies were measured using HCV pseudo-particle neutralization assays. Herein, we report several key observations. First, HCV infection skewed cTfh toward CXCR3$^+$ cTfh cell differentiation. Second, the frequency of CXCR3$^+$ cTfh cells was positively correlated with HCV nAb titers and breadths. Third, CXCR3$^+$ cTfh cells showed higher expression of Tfh-associated molecules compared with CXCR3$^-$ cTfh cells from individuals with HCV infection. Coculture of cTfh cells and autologous memory B cells in vitro indicated that CXCR3$^+$ cTfh cells show a superior ability to support HCV E2-specific B cell expansion compared with CXCR3$^-$ cTfh cells from individuals with HCV infection. HCV infection skews cTfh cells toward CXCR3-biased Tfh cell differentiation, which positively correlates with the magnitude and breadth of the HCV nAb response. These findings will provide insights for the rational design of a neutralizing antibody-based HCV vaccine.

P0292

HBV coinfection with HCV alters circulating Tfh cell distribution and impairs HCV neutralizing antibody responses

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Hepatitis C virus (HCV) and hepatitis B virus (HBV) coinfection reciprocally influences viral replication and host defense responses. This study aimed to investigate the impact of HBV coinfection on circulating T follicular helper cell (cTfh) distribution and the HCV neutralizing antibody (nAb) response. HCV nAb responses were measured in individuals with HCV monoinfection (n=83) and HBV/HCV coinfection (n=78) using neutralization assay. The frequencies of cTfh cells and their subsets in HCV monoinfection (n=34) and HBV/HCV coinfection (n=30) were analyzed by flow cytometry. The correlations of clinical parameters, cTfh cells and nAb responses were analyzed. Compared with HCV monoinfection, the HBV coinfection group showed significantly lower HCV nAb responses (P< 0.001) and a decreased frequency of circulating Th1-like Tfh cells (Tfh1) (P=0.004). In HCV monoinfection, the frequency of the Tfh1 subset was positively correlated with HCV nAb responses (R=0.378, P=0.03), but this correlation was lost under HBV/HCV coinfection (R=0.115, P=0.551). In contrast, the frequency of circulating Th2-like Tfh cells (Tfh2) was negatively correlated with the HCV nAb responses (R=0.404, P=0.003). Further analysis showed that HBV coinfection enhanced the Tfh2 subset composition within cTfh cells (P< 0.001), which was associated with serum HBsAg in HBV/HCV coinfection (R=0.521, P=0.003). As expected, HBsAg also exhibited an inverse association with HCV nAb responses in HBV/HCV coinfection (R=0.59, P< 0.001). In contrast to HCV monoinfection, HBV/HCV coinfection leads to altered cTfh cell distribution and impaired HCV nAb

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DOI: 10.1002/eji.201970400

responses, which are associated with HBsAg. These findings will be helpful for better understanding the immunopathogenesis of HBV/HCV coinfection.

P0293

Elevated LAG-3 on CD4$^+$ T cells negatively correlates with neutralizing antibody response during HCV infection

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Lymphocyte activation gene-3 (LAG-3), an inhibitory molecule, which has been shown co-expressed with multiple inhibitory receptors on CD8$^+$ T and natural killer (NK) cells and negatively regulates T and NK cell responses during hepatitis C virus (HCV) infection. However, whether LAG-3 is involved in the regulation of the antibody response remains unclear. This study aims to investigate the relationship of LAG-3 with neutralizing antibody (nAb) response during HCV infection. A total of 66 HCV-infected individuals and 36 sex- and age-matched healthy controls from a population of intravenous drug users were recruited. Circulating follicular helper T (cTfh) cells and LAG-3-expressing CD4$^+$ T cells, type 1 regulatory T (Tr1) cells, and regulatory T (Treg) cells were characterized by flow cytometry. Serum nAb response of HCV-infected individuals was determined using pseudoparticle neutralization assays. We found that HCV infection enhanced LAG-3 expression on CD4$^+$ T cells and exhibited regulatory T cell-like phenotype and inversely associated with the HCV nAb response. Further analysis showed that frequency of CXCR3$^+$ cTfh cells positively correlated with nAb response, however LAG-3$^+$ CD4$^+$ T cells inversely associated with CXCR3$^+$ cTfh cells. This study observed that LAG-3$^+$ CD4$^+$ T cells exhibit a regulatory cell phenotype and negatively associate with the HCV nAb response, implying that LAG-3 may be involved in the negative regulation of humoral immunity during HCV infection.

P0294

NK cell-derived IFN-γ promotes hepatocellular carcinoma through EpCAM-EMT axis in hepatitis B virus transgenic mice

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Hepatitis B virus (HBV) is a major risk factor for development of hepatocellular carcinoma (HCC) at least partially due to dysfunctional anti-HBV adaptive immunity; however, the role of innate immune response to HBV in this process is not well understood. In this study, low dose poly (I:C), an NK cell activator (3 µg/g bw, twice/week for 8 weeks) induced HCC in HBV transgenic (HBs-Tg) mice, with an incidence of 100% after 6 months; while HBs-Tg mice without treatment only had HCC with an incidence of 16.7%. In HBs-Tg mice, poly (I:C) induced liver inflammation with markedly increased infiltrating lymphocytes, along with the concurrently increased apoptosis and proliferation of hepatocytes, leading to the accelerated epithelial-mesenchymal transition (EMT) of hepatocytes. The EMT and tumorigenesis depended on the presence of NK cells since depletion of these cells significantly reduced HCC rate to 28.6%. Further, intrahepatic NK cells highly expressed IFN-γ, anti-IFN-γ neutralizing mAb obviously alleviated the hepatitis, and hepatocyte-specific IFN-γ over-expression promoted HCC. Moreover, IFN-γ deficiency in HBs-Tg mice prevented HCC occurring though hepatic NK cells existed and could be activated, suggesting the critical role of IFN-γ in NK cell-mediated tumorigenesis. *In vitro* experiment, IFN-γ up-regulated epithelial cell adhesion molecule (EpCAM) expression *via* p-STAT1 pathway, which was followed by EMT, and p-STAT1 inhibitor might

DOI: 10.1002/eji.201970400

absolutely abolish the expression of EpCAM and EMT in HBsAg-positive hepatocytes.
Conclusion: This work demonstrates NK cell-derived IFN-γ promotes HCC through EpCAM-EMT axis in HBs-Tg mice, revealing the importance of innate immunity in pathogenesis of HBV-associated HCC.

P0295

Aberrant alteration of circulating follocular helper T cell subsets in children with enterovirus 71 infection

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Follicular helper T (Tfh) cells and their subsets are pivotal for generation of antibody, which play critical roles in viral diseases. Enterovirus 71 (EV71) is one of the most common viruses of hand, foot and mouth disease (HFMD), suggesting a potential role for Tfh cell subsets in children with EV71-associated HFMD. Our aim was to explore the potential role of circulating Tfh cell (cTfh) subsets in children with EV71-associated HFMD. In this study, compared to HC, the frequencies of $CD45RA^-$ $CXCR5^+CD4^+$T (cTfh) with PD-1 or ICOS expression, cTfh2 ($CXCR3^-CCR6^-$), cTfh17 ($CXCR3^-CCR6^+$) cell subset, and plasmablasts ($CD19^+CD138^+IgD^-$) were significantly expanded, as well as elevated serum concentrations of IL-4, IL-17A, and IL-21 in children with EV71-associated HFMD. A positive correlation was found between the frequency of PD-1^+cTfh cells (Tfh2 or plasmablasts) and serum IL-21 concentration (or specific neutralizing antibodies against EV71, NAb-EV71), respectively. Moreover, The mRNA levels of transcription factors (GATA-3 and RORγt) were significantly increased, but T-bet and Bcl-6 mRNA expression was not obviously varied in $CD4^+$T cells from these patients. Additionally, aberrant alteration of cTfh cell subsets and associated molecules levels were significantly different between mild and severe patients. Interestingly, the frequencies of cTfh cell subsets were partly normalized by treatment. Taken together, the aberrant alteration of cTfh cell subsets and associated molecules play an important role in the pathogenesis of EV71-associated HFMD, which are potential clues for children with EV71-associated HFMD' interventions.

P0296

Association of HLA-G 3′ UTR polymorphism and expression with the progression of cervical lesions in human papillomavirus 18 infections

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Human leukocyte antigen (HLA)-G is an immune checkpoint molecule, which expression in cervical cancer cells enables them to escape immunosurveillance. To date, limited information has been published on the association of HLA-G genetic background in malignant cells with levels of HLA-G expression and the clinical outcome of patients. In this study, we investigated the influence of the HLA-G *14bp In/Del* (rs66554220) and +3142*C/G* (rs1063320) polymorphisms in 130 cases of HPV16 infection, 130 cases of HPV18 infection and 185 age-matched, unrelated, HPV-negative, and cytologically normal Chinese Han women. Case-matched cervical biopsy tissues were evaluated by immunohistochemistry. Our findings show that the frequency of alleles, *14bp In* (38.5% vs 29.2%, OR=1.52, $P< 0.05$) and +3142*G* (72.7% vs 57.0%, OR=2.01, $P< 0.05$), were significantly increased in the HPV18-infected group compared with the control group. The *HLA-G* polymorphisms (alleles *14bp In* and +3142*G*) are also associated with the progression of HPV18-related cervical lesions. Moreover,

DOI: 10.1002/eji.201970400

HLA-G expression increased from CIN1 to CIN2/3 lesions and was highest in patients with adenocarcinoma; however, a significant association between these characteristics and the HLA-G polymorphisms was not observed. Our results support that the *HLA-G 14bp In* and +3142*G* alleles are risk factors for HPV18 infections and influence the progression of HPV18-related cervical lesions. This suggests that HLA-G-driven immune mechanisms play an important role in cervical carcinogenesis.

P0297

A novel mouse model of hepatocellular carcinoma mediated by hepatitis B virus-specific CD8$^+$ T cells

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It is widely believed that hepatitis B virus (HBV) infection-induced cirrhosis and hepatocellular carcinoma (HCC), the most critical human health problems, is mediated principally by the destruction of infected hepatocytes by anti-HBV CTLs in human, however, this immunopathogenic process was never been clearly observed in vivo since lack of mouse model. In this report, we use immunocompetent Fah$^{-/-}$ mice as the recipients and adoptively transfer HBsAg$^+$ hepatocytes from HBs-Tg mice to replace the recipient hepatocytes (HBs-HepR). The HBs-HepR mice maintained HBV persistence with chronic hepatitis and spontaneous liver fibrosis, and eventually developed HCC with an incidence of 100%, and importantly, **HCC in this model had the same molecular features as human HBV$^+$HCC** but different from other four extensively used mouse HCC models and human HCV+ or alcoholic HCC samples. **Further, HBsAg-specific CD8$^+$T cells were gradually generated to specifically mediate hepatocyte apoptosis,** and depletion or deficiency of CD8$^+$T cells might prevent HCC, which could be reproduced by transfer back of HBsAg-primed CD8$^+$T cells in HBs-HepR. **This mouse model of HCC would be useful for understanding HBV-specific immunopathogenesis.**

P0298

Arginine methyltransferase PRMT5 negatively regulates cGAS- triggered antiviral immune response

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Cyclic GMP-AMP synthase (cGAS) functions as an essential DNA sensor, which senses cytoplasmic double strand DNA (dsDNA) and activates antiviral response. However the post translational modification of cGAS has not been fully understood and whether cGAS has any methylation modification is not known. Here we demonstrated that cGAS could be methylated at arginine, and mass spectrometry identified PRMT5 as a potential cGAS interacted methyltransferase. We further verified the interaction between cGAS and PRMT5 by co-immunoprecipitation assay and demonstrated their endogenous colocalolization after HSV-1 infection in macrophage. Both gain of function and loss of function assay demonstrated PRMT5 negatively regulated cGAS-induced *Ifnβ* expression. We further demonstrated that PRMT5 significantly inhibited cGAS-STING pathway and its downstream TBK1 and IRF3 phosphorylation upon HSV-1 infection. After pretreatment with PRMT5

DOI: 10.1002/eji.201970400

specific inhibitor EPZ015666 in macrophage, *Ifnβ* expression was significantly increased upon HSV-1 infection, while *Ifnβ* level was not changed after transfection of 2'3'-cGAMP, which indicated that PRMT5 negatively regulated antiviral immunity through cGAS but not STING. Further analysis indicated that symmetrical dimethylation of cGAS catalyzed by PRMT5 could barrier the binding between cGAS and cytoplasmic dsDNA, which further blocked cGAS-triggered type I IFN production and anti-viral response. Altogether, our study demonstrated a novel post translational modification of cGAS and revealed a possible mechanism of viral escape. Importantly, it provided a potential therapeutic target for effective manipulation of virus infection.

P0299

The ubiquitin E3 ligase TRIM65 is necessary for MDA5-mediated antiviral innate immunity

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MDA5 as a cytoplasmic dsRNA sensor that activates the type I interferon signaling pathway and plays a key role in antiviral innate immunity. However, little is known about the mechanisms of MDA5 activation. Here, we indenfy that TRIM65 specifically interacts with MDA5 and boosts K63-linked ubiquitination of MDA5 at lysine 743, which is essential for MDA5 oligomerization and activation. Ablation of *Trim65* eliminates MDA5 agonist or encephalomyocarditis virus (EMCV)-induced IRF3 phosphorylation and type I interferon production, but has no effect for RIG-I, TLRs or cGAS signaling pathway. Significantly, *Trim65*$^{-/-}$ mice sensitive to EMCV infection produce little type I interferon *in vivo.* In summary, our research confirms that TRIM65 as an indispensable factor for MDA5 signaling pathway and provide physiological evidence showing that K63-linked ubiquitination is critical for MDA5 oligomerization and activation.

P0300

Nonstructural protein NS1 of porcine parvovirus activated NF-KB signaling pathway through TLR2-MyD88/NF-KB induce host innate immune response

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Porcine parvovirus (PPV), a parvovirus, is a single-stranded DNA etiologic agent causing porcine reproductive failure. It is a great significance to study the pathogenesis of PPV for the prevention and control of the disease. PPV nonstructural protein NS1 is involved in viral DNA replication, transcription regulation and cytotoxicity. Previous investigation showed that PPV infection could significantly up-regulate the level of IL-6 through activating NF-κB signaling pathway. Here we investigated whether PPV non-structural protein NS1 modulate IL-6-mediated activation of the NF-κB signaling pathway. Our research showed that PPV NS1 had up-regulated IL-6 and TNF-α expression in a dose-dependent manner. It also showed NS1 protein induced p65 nuclear translocation, p65 phosphorylation and IκBα degradation. Furthermore, the upstream pathways of NF-κB signaling pathway was activated by pEGFP-N1-NS1. In addition, We also demonstrated that TLR2 contribute to NF-κB activation in response to PPV-NS1 transfection. The results indicated that NF-κB signaling pathways was activated by PPV nonstructural protein NS1 through TLR2 and then induced the expression of IL-6 secretion. Collectively, these findings provide new avenues of investigation into the molecular mechanisms of PPV infection.

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DOI: 10.1002/eji.201970400

P0301

USP14 promotes K63-linked RIG-I deubiquitination and suppresses antiviral immune responses

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RIG-I is a critical RNA virus sensor that initiates antiviral immune response through K63-linked ubiquitination. In this study, we demonstrated USP14, a deubiquitinating enzyme, as a negative regulator in antiviral responses by directly deubiquitinating K63-linked RIG-I. USP14 knockdown significantly enhanced RIG-I-triggered type I IFN signaling and inhibited VSV replication both in mouse peritoneal macrophages and THP1 cells. USP14 overexpression in HeLa cells attenuated RIG-I-triggered IFN-β expression and promoted VSV replication. Besides, USP14 specific inhibitor-IU1 increased RIG-I-mediated type I IFN production and antiviral responses in vitro and in vivo. In addition, USP14 could interact with RIG-I and remove RIG-I K63-linked polyubiquitination chains. This paper is the first to report that USP14 acts as a negative regulator in antiviral response through deubiquitinating K63-linked RIG-I. These findings provide insights into a potential new therapy targeting USP14 for RNA virus related diseases.

P0302

HBV infection-induced liver cirrhosis development in dual-humanised mice with human bone mesenchymal stem cell transplantation

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Developing a small animal model that accurately delineates the natural history of hepatitis B virus (HBV) infection and immunopathophysiology is necessary to clarify the mechanisms of host-virus interactions and to identify intervention strategies for HBV-related liver diseases. This study aimed to develop an HBV-induced chronic hepatitis and cirrhosis mouse model through transplantation of human bone marrow mesenchymal stem cells (hBMSCs). Transplantation of hBMSCs into *Fah$^{-/-}$Rag2$^{-/-}$IL-2Rγc$^{-/-}$ SCID* (FRGS) mice with fulminant hepatic failure (FHF) induced by hamster-anti-mouse CD95 antibody JO2 generated a liver and immune cell dual-humanized (hBMSC-FRGS) mouse. The generated hBMSC-FRGS mice were subjected to assessments of sustained viremia, specific immune and inflammatory responses and liver pathophysiological injury to characterize the progression of chronic hepatitis and cirrhosis after HBV infection. The implantation of hBMSCs rescued FHF mice, as demonstrated by robust proliferation and transdifferentiation of functional human hepatocytes and multiple immune cell lineages, including B cells, T cells, NK cells, dendritic cells (DCs) and macrophages. After HBV infection, the hBMSC-FRGS mice developed sustained viremia and specific immune and inflammatory responses and showed progression to chronic hepatitis and liver cirrhosis at a frequency of 55% after 54 weeks. This new humanized mouse model recapitulates the liver cirrhosis induced by human HBV infection, thus providing research opportunities for understanding viral immune pathophysiology and testing antiviral therapies *in vivo*.

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DOI: 10.1002/eji.201970400

P0303

Study on the anti-influenza virus efficacy of extracts from the the medicinal plant Bletilla Striata in mice

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This study aimed to evaluate the anti-influenza viral activity in mice of ethanol extracts from the medicinal plant Bletilla striata.
84 female BALB/c mice were intranasal infected by influenza virus A/Sydney/5/97(H3N2), 2h later B. striata extracts were intragastrical administrated at doses of 8g/kg·d, 4g/kg·d, 2g/kg·d,Tamiflu as control. The mice were killed on 1st, 3rd, 5th and 7th days. The lungs were weighed and the lung index were calculated. The pathological changes of lung tissue were observed by HE staining. The viral load in lung tissue was detected by real time quantitative PCR. The contents of IL-2, IFN-α and IFN-β in serum were determined by ELISA. Comparing with the model mice, the pathological section of lung tissue showed that the inflammatory symptoms improved, the number of inflammatory infiltrating cells decreased, the virus amount in lung decreased gradually, after treated with B. striata extract, especially in the high dose.The levels of IL-2 on days 1, 3, 5 and 7 were higher than those in the model group, IFN-α and IFN-β levels were significantly increased on the 1st to 3rd day after infection, and gradually increased to the 5th day. Compared to the model group, the levels of IFN-α in the high dose on 1st, 3rd and 5th days, were different significantly ($P<0.01$).Thus, B. striata extract has anti-influenza virus activity in mice significantly, which may be related to the increase of IL-2, INF-α, INF-β .

P0304

Regulation of molecular chaperone GRP78 by HBV: control of viral replication and cell survival

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Chronic hepatitis B (CHB) remains a global healthy problem, carrying a high risk for progression into cirrhosis and liver failure. Molecular chaperones are involved in diverse physiopathological processes including viral infection. However, the role of molecular chaperones in HBV infection and its underlying mechanisms remain still unclear. Herein, we identified GRP78 as one of the most strongly induced molecular chaperones by HBV in human hepatocytes. Gain and loss of functional analysis demonstrated GRP78 exerted inhibitory effect on HBV transcription and replication. Further study showed that GRP78 did not activate innate immune responses in HBV-replicating hepatocytes, and the activation of AKT/mTOR signaling might contribute to GRP78-mediated inhibition of HBV. Of note, HBV-upregulated GRP78 was found to play a crucial role in maintaining the survival of hepatocytes via facilitating a mild ER stress. Together, our findings suggest that HBV may sacrifice part of its replication for facilitating a persistent infection through induction of GRP78, a master ER stress regulator. Targeting GRP78 may help develop to design novel therapeutic strategies against chronic HBV infection and the associated hepatocellular carcinoma (HCC).

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DOI: 10.1002/eji.201970400

P0305

KAT8 attenuates antiviral immune response via directly mediating K359 acetylation of IRF3

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KAT8 (also known as MOF or MYST1) is a major enzyme that catalyzes H4K16 acetylation in mammalian cells and is ubiquitously expressed in immune cells such as macrophages, dendritic cells, T cells, B cells, and et al. KAT8 participates in diverse biological processes, including embryonic development, DNA repair, autophagy, chromatin architecture, cellular lifespan, and mitochondrial DNA transcription, and has been implicated in cancer development and T cell differentiation. However, a regulatory function for KAT8 in host defense against infectious agents and the associated signal transduction pathways have not been reported. In this study, we found that KAT8 selectively inhibits RNA and DNA virus-triggered IFN-I production in macrophages and dendritic cells. In addition, KAT8 deficiency protects mice against viral infection by enhancing IFN-I production. Mechanistically, KAT8 directly interacts with IRF3 and promotes IRF3 acetylation at lysine 359 via its MYST domain. Furthermore, KAT8 decreases the abundance of IRF3 at IFN-I gene promoters and the transcriptional activity of IRF3. Our study reports an important role for KAT8 in down-regulating virus-triggered IFN-I production and an unconventional PTM of IRF3 that directly inhibits its transcriptional activity and attenuates antiviral immune response.

P0306

High glucose induces reactivation of latent Kaposi's sarcoma-associated herpesvirus

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High prevalence of Kaposi's sarcoma (KS) is seen in diabetic patients. It is unknown if the physiological condition of diabetes contributes to KS development. We found elevated levels of viral gene expression when Kaposi's sarcoma-associated herpesvirus (KSHV) infected cells were cultured in high glucose medium. To demonstrate the association between high blood glucose and KSHV replication, we xenografted telomerase-immortalized human umbilical vein endothelial cells that are infected with KSHV (TIVE-KSHV) into hyperglycemic and normal nude mice. The injected cells expressed significantly higher levels of KSHV lytic genes in hyperglycemic mice than in normal mice. We further demonstrated that high glucose induced production of hydrogen peroxide (H_2O_2), which down regulated silent information regulator 1 (SIRT1), a class-III histone deacetylase (HDAC), resulting in epigenetic transactivation of KSHV lytic genes. These results suggest that **high blood glucose in diabetic patients contributes to development of KS by promoting KSHV lytic replication and infection.**

DOI: 10.1002/eji.201970400

P0307

Novel immunological mechanism of thrombocytopenia in HFRS patients

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Hemonrrhagic fever with renal syndrome (HFRS) caused by Hantaan virus (HTNV) infection claims 100,000 cases per year worldwide, of which more than 90% occured in China with clinical manifestation of thrombocytopenia, hypovolemia, and hypotensive shock. However, the mechanism of thrombocytopenia is still unclear. Growth arrest specific protein 6 (Gas6) and milk fat globule epidermal growth factor 8 (MFGE-8) belong to the opsonin family and facilitate phagocytic removal of apoptotic cells by acting as a bridging molecule between phosphatidylserine(PS) exposed on the apoptotic cells and receptors on phagocytes. Interestingly, PS also become externalized during platelet activation. In view of this, we found that the proportion of CD62P positive platelets among the PS^+ platelets in the acute phase of HFRS patients was significantly increased compared with that in the recovery and the normal control group. The plasma levels of Gas6 and MFG-E8 in HFRS patients were significantly higher and were related to the course of disease and negatively correlated with platelet count (PLT). The frequency of Gas6 receptor Tyro3, MFG-E8 receptor integrin αvβ3 and αvβ5 in HFRS patients was significantly increased especially on the nonclassical monocytes. The phagocytosis rate was enhanced in THP-1 cells to thrombin-activated platelets system in the addition of MFG-E8, while decreased after the addition of αVβ3 or αVβ5 neutralizing antibody, with synergistic effect on the inhibition of phagocytosis. Our study demonstrates that Gas-6 and MFG-E8 involved in the phagocytic removal of both apoptotic and activated platelets which may cause thrombocytopenia in HFRS.

P0308

HBV core antigen impairs M2 macrophages polarization and promotes pro-inflammation cytokine production through the TLR2 signaling pathway

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Chronic Hepatitis B virus (HBV) infection is a major public health problem affecting about 350 million people worldwide. Adaptive and innate immunity both play primary roles in eliminating HBV while the virions may inhibit immune response to escape from eradication. Although macrophages can retrieve anti-viral activity and facilitate elimination of HBV, the exact mechanism is unclear. Here, after successfully stimulated monocytes from healthy human PBMCs into M1/M2 macrophages, HBcAg was added simultaneously to detect the role of HBcAg in macrophages polarization. It has been found that HBcAg had negligible impact on M1 polarization while it effectively impaired M2 polarization and promoted the production of pro-inflammatory cytokines such as IL-6 and TNF-α. Mixed lymphocyte reaction implied that HBcAg-stimulated M2 macrophages reinforced their ability to activate CD8+ T cells to secrete higher level of IFN-γ production compared with that of M2 macrophages. Furthermore, HBcAg-treated M2 macrophages up-regulated TLR2 expression and anti-TLR2 antibody treatment could abolish the effects of HBcAg on the impairment of CD206 or CD163 expression and the production of inflammatory cytokines of M2 macrophages. The signaling pathway analysis revealed that NF-κB phosphorylation was up-regulated after HBcAg treatment both in M1/M2 macrophages. Finally, we found the expression level of M2-related molecules were decreased and the production of pro-inflammation cytokine of M2 macrophages was increased after the monocyte-derived M1/M2 macrophages from AsC and CHB patients were treated with HBcAg. In conclusion, our study reveals

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DOI: 10.1002/eji.201970400

that HBV core antigen impairs M2 macrophage polarization and promotes pro-inflammatory cytokine production through the TLR2 signaling pathway.

P0309

Analysis of the effect of hepatitis C chemiluminescence antibody on blood screening strategy

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Objective: To evaluate the effect of chemoluminescence antibody detection of hepatitis C on blood screening strategy by analyzing the results of hepatitis C antibody, in combination with laboratory enzyme-linked immunosorbent assay.
Methods: The serum disk of HCV antibody was detected by chemiluminescence detection system and enzyme-free detection system (two kinds) to evaluate the influence of the chemiluminescence detection system on the blood screening test strategy.
Results: The specificity of the three reagents (one chemiluminescence and two enzyme-linked immunosorbent assays) of HCV antibody were 89%, 71% and 87.7%, respectively; the sensitivity was 98.4%, 96.5% and 99.7%, respectively; 95.3%, 88.2%, and 95.8%, respectively.
Conclusion: The detection of HCV antibody has a great difference in specificity. In addition, the enzyme-linked immune system and luminescent system have their own characteristics. Therefore, the different performance of the reagents should be comprehensively evaluated. The detection strategy should be more in line with the laboratory requirements according to the characteristics of different systems.
Key words: hepatitis C chemiluminescence antibody; Analysis of the effect; blood screening strategy

P0310

USP27X deubiquitinates and stabilizes the DNA sensor cGAS to regulate cytosolic DNA-mediated signaling

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Cyclic GMP-AMP synthase (cGAS), a cytosolic DNA sensor, catalyzes formation of the second messenger 2'3'-cGAMP that binds to STING and triggers the type I interferon (IFN) signaling pathway. Activation of cGAS can be regulated by several protein post-translational modifications such as ubiquitination. However, the cGAS activation regulated by protein deubiquitination remains poorly understood. In this study, we identified a deubiquitinase USP27X could interact with cGAS and cleave K48-linked ubiquitination chain from cGAS, leading to cGAS stabilization. Consistently, Knockout of USP27X resulted in accelerated turnover of cGAS, decreased cGAMP production, phosphorylation of TBK1 and IRF3, and IFN-β production. Further, USP27X knockout macrophages showed impaired innate antiviral responses against herpes simplex virus type 1 (HSV-1) infection. Our data suggest that USP27X is a novel regulator of the cGAS-STING cytosolic DNA sensing pathway.

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DOI: 10.1002/eji.201970400

P0311

NLRP3 contributes to inflammation, pyropotic cell death and mucin production in human airway epithelial cells in response to rhinovirus

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Background: The airway epithelium maintains the mucosal homeostasis and effectively responds to pathogens. Although NLRP3 plays critical roles in orchestrating the innate immune response in a variety of immune cells to pathogens, the function and the mechanism mediated by NLRP3 in airway epithelium upon virus infection and its role related to virus-induced exacerbation in chronic airway inflammation remain poorly understood.
Objective: We investigate the mechanisms and the cellular function of epithelial NLRP3 inflammasome upon human rhinovirus (RV) infection.
Methods: By using the primary human nasal epithelial cells (hNECs) under RV infection, the key factors for NLRP3 inflammasome activation pathway and cell death/mucus production were functionally examined using immunoblot, immunofluorescence staining, flow cytometry and ELISA respectively.
Results: RV could induce inflammasome activation and cell death in hNECs. The inflammasome-mediated IL-1β secretion upon virus stimulation was dependent on NLRP3 and its upstream regulators (DDX33 and DDX58). We also identified that the inflammasome-mediated pyroptosis was dependent on NLRP3-caspase-1-GSDMD axis. In addition, RV could induce mucus production in hNECs, which was dependent on NLRP3 inflammasome activation.
Conclusion: NLRP3 inflammasome could mediate respiratory rhinovirus-induced inflammation, pyroptosis and mucus production in human airway epithelial cells, which would be associated with the virus-induced airway remodeling and disease exacerbation.
Keywords: nasal epithelial cells, NLRP3 inflammasome, rhinovirus, inflammation, mucus, pyroptosis

P0312

Analysis of cervical local immune microenvironment in cervical lesions

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Objective: Analysis of the number and proportion of immune cells by detecting CD4, CD8, CD56, CD83 Immune cell markers in cervical lesions.
Methods: The expressions of CD4, CD8, CD56, CD83 were detected by immunohistochemical method in HPV persistent infection cervical tissus, CINI, CINII, CINIII tissues.
Results: The average number of CD4 positive cells per high-power field (x400) in HPV persistent infection cervical tissus are, CINI, CINII, CINIII tissues are 1.12±1.22, 1.58 ±0.92, 2.32 ±0.87, 7.24±6.33, there is a difference between groups.The average number of CD8 are 5.21±2.21, 3.22±0.88, 3.01±0.97, 2.22±2.10, there is a difference between groups.The average percentage of CD56 are 3.51±0.48, 3.89±0.77, 3.71±0.88, 2.77±0.32, there are difference between CINI and CINIII.The average percentage of CD83 are 0.02±0.11, 0.57±0.74, 2.29±1.65, 2.33±0.15, there are difference between HSIL and LSIL.
Conclusion: The expression of CD4 increased significantly with the progression of cervical squamous

DOI: 10.1002/eji.201970400

epithelial lesions.The expression of CD8 decreased significantly with the progression of cervical lesions, and CD4/CD8 was increased.The expression of CD56 decreased significantly with the progression of cervical lesions.The expression of CD83 decreased significantly with the progression of cervical lesions.The number and proportion of immune cells of cervical tissues changed with the progress of cervical lesions.The changes in the number and proportion of cervical tissues immune cells lead to changes in the local microenvironment of the cervix, which can promote the irreversible progression of the cervical lesions

P0313

$CD4^+$ T cells play a critical role in microbiota-maintained anti-HBV immunity in a mouse model

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The ability of the host to clear hepatitis B virus (HBV) is closely correlated to the establishment of commensal microbiota. However, how microbiota affects anti-HBV immunity is still unclear. Using a well-known hydrodynamical HBV transfection mouse model and treatment with antibiotics (Atb), we explored the change in adaptive immunity ($CD4^+$ cells, germinal center B cells and anti-HBs Ab). In our setting, adult B6 mice exhibited complete clearance of HBV within 6 weeks post-hydrodynamic injection of HBV-containing plasmid, whereas Atb-treated mice lost this capacity, showing high serum level of hepatitis B surface antigen (HBsAg) without hepatitis B surface antibodies (anti-HBs), similar as what happened in *Rag1*$^{-/-}$ mice or *CD4*$^{-/-}$ mice, suggesting that microbiota may influence the function of $CD4^+$ T cells. Furthermore, the numbers of splenic and hepatic effector $CD4^+$ T cells ($CD44^{hi}CD62L^-CD4^+$ T cells) both decreased with impaired function (IFN-γ synthesis), resulting in lower frequency of germinal center B cells and $CD4^+$ follicular helper T cells, and impaired anti-HBs production. We further tried to find the bacterial species responsible for maintaining anti-HBV immunity, and found that each antibiotic alone could not significantly influence HBV clearance compared to antibiotic combination, suggesting that global commensal microbial load is critical for promoting HBV clearance. We also confirmed that TLRs (e,g. TLR2, 4, 9) are not major players in immune clearance of HBV using their agonists and knock-out mice. These results suggest that commensal microbiota play an important role in maintaining $CD4^+$ T cell immunity against HBV infection.

P0314

Occult Hepatitis B Virus Infection after haploidentical haematopoietic stem cell transplantation (HSCT) patients: incidence and characterization of Pres/S gene mutations

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Background and Aims: Occult hepatitis B virus (HBV) infection (OBI) is found in allogeneic haematopoietic stem cell transplantation (HSCT) patients. However, incidence and characterization of HBV preS/S gene mutations in patients with OBI infection receiving haploidentical HSCT (haplo-HSCT) has not been prospectively studied.
Method: From January 2014 to September 2018, 227 patients who received haplo-HSCT at Peking University Institute of Haematology were screened for hepatitis B surface antigen (HBsAg), antibodies to HBV core (anti-HBc). Serum samples negative for HBsAg and positive for anti-HBc were subjected to nucleic acid extraction and HBV DNA detection by real-time PCR. PreS/S gene mutants were analyzed by clonal sequencing in cases with occult HBV infection and 30 control chronic carriers.

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DOI: 10.1002/eji.201970400

Results: 189/227 patients were HBsAg-negative. The 15/189 (7.94%) HBsAg-negative patients with haplo-HSCT tested positive for HBV-DNA in serum. A total 13 OBI-patients and 30 control patients were analyzed for the the preS gene and S gene through sequencing simultaneously. 45 preS/S gene mutations were cloned from 13 OBI sequential serum samples, including 10 mutations that were not previously documented. Of 13 OBI sequences, 9 harboured MHR mutations.
Conclusion: This is the first report demonstrating OBI after haplo-HSCT confirmed by molecular analysis. We found a high prevalence (7.94%) of OBI in haplo-HSCT patients suggesting that periodic HBV DNA screening for HBV-DNA should be considered in preventing HBV-related complications. Our data suggest that preS mutations and "a" determinant mutations are associated with OBI. This has implications for HBV diagnosis and vaccine improvement.

P0315

Immune-modulatory role of conjugated bilirubin during hepatitis A virus infection: potential contribution of AhR in a mechanism dependent on the expression of TIM-3 on Tregs

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We previously reported that conjugated bilirubin (CB) plays an anti-inflammatory role during acute hepatitis A virus (HAV) infection by modulating T cell function. However, how CB may induce the modulatory effect remains to be elucidated. It has been suggested that the T cell immunoglobulin and mucin domain containing protein 3 (TIM-3) increases T-regulatory cells (Tregs) suppressor function via its interaction with galectin-9 (GAL-9). Herein, we show that *in vitro* stimulation of Tregs from healthy donors with CB resulted in enhanced Tregs activity and TIM-3 expression. Likewise, HAV-infected patients with abnormal CB values relative to healthy individuals, showed a greater proportion of $CD4^+CD25^+$ T cells expressing TIM-3 and $CD4^+$ T lymphocytes expressing GAL-9. When Tregs from healthy donors were induced to over-express TIM-3 following CB treatment, their ability to suppress CD4+ T cell proliferation increased, along with the upregulation of the aryl hydrocarbon receptor (AhR). This observation suggest that interaction between CB and the AhR may occur and allow for TIM-3 function in Tregs. Furthermore, treatment of Tregs with an anti-TIM-3 antibody previously described to have blocking functions *in vivo*, resulted in a significantly lower suppression of $CD4^+$ T cell proliferation. Taken together, our data suggest that CB may have an important immunological role during HAV infection. Thus, CB interaction with AhR in Tregs may enhance Tregs function through a TIM-3/GAL-9 dependent mechanism.

P0316

Viral restriction by IFITM3 - is it all down to expression?

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DOI: 10.1002/eji.201970400

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The mechanism of IFITM3-mediated viral restriction is still unknown. We have evidence suggesting that the expression level of IFITM3 can directly alter the number of virally infected cells, suggesting that high expression of IFITM3 is required for viral control. To investigate this, we used CyTOF to look at the expression level of IFITM3 in several immune cell subsets from whole blood-derived PBMC, cord blood-derived PBMC and lung tissue cells. We find that constitutive IFITM3 expression varies dramatically within immune cell populations, with monocytes having significantly higher expression than T cells, and cord blood-derived $CD34^+$ haematopoietic stem cells expressing the highest level of IFITM3 expression. Interestingly, some subsets of cord blood-derived immune cells express significantly higher levels of IFITM3 than equivalent populations isolated from adult blood. As IFITM3 is an interferon-stimulated gene, we next investigated the induction of IFITM3 by interferon and find that Type I interferon induces the greatest increase in IFITM3 expression, followed by Type III. However, lymphocytes exhibit an impaired ability to induce additional IFITM3 upon interferon stimulation. Additionally, we find that it takes ~36 hours to reach maximum expression of IFITM3. Variability in IFITM3 expression levels may have an impact on viral tropism and be an important factor in determining the severity of infection as lower natural expression may give the virus an advantage in the early stages of infection. The time it takes for maximal IFITM3 expression is considerably slow for an interferon-stimulated gene reinforcing the advantage to the virus in the early stages of infection.

P0317

Assessment of the contribution of HIV patient serum antibodies that recognize lymphocyte antigens to the inhibition of the HIV-1 envelope-dependent membrane fusion

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Efficiency of the HIV-1 envelope (Env) to induce membrane fusion is an important determinant of HIV-1 pathogenicity. Besides virus receptors, other adhesion/signaling molecules on infected, target cells and virus particles can enhance fusion. The presence of anti-lymphocyte autoantibodies (ALA) in HIV patients' serum points out their possible contribution to the inhibition of the Env-mediated membrane fusion. Here, we evaluated the contribution of ALA to the patient's serum activity on the HIV-envelope dependent cell-cell fusion. The effect or sera on fusion of $CD4^+$ with Env^+ Jurkat cells was tested before and after adsorption on CD4-negative Jurkat cells to remove ALA. Sera from 38 HIV-1 infected treatment-naïve men and 30 heathy donors were analyzed. IgG and IgM binding to Jurkat CD4-negative cells was detected in 74% and 84% of HIV-positive sera, respectively. Inhibition of fusion decreased in 58% of serum samples after adsorption, reflecting that ALA contributed to fusion inhibition in these sera. Fusion increased in 31.6%, and did not change in 10.5% of other serum samples after adsorption. The extent of the contribution of ALA to the effect of sera on fusion was highly variable, being 33% on average. Thus, fusion-inhibitory ALA other than anti-CD4 antibodies may contribute significantly to the inhibition of the Env-mediated cell-cell fusion. Only detection of fusion-inhibitory ALA, but not total ALA levels, associated with patient's low plasma viral loads, suggesting that autoantibodies with particular specificities may participate in virus containment during HIV-1 infection. Fusion-inhibitory ALA may be relevant in the antiviral humoral immune response to HIV.

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DOI: 10.1002/eji.201970400

P0318

IL-33 promotes antiviral immunity and protects against congenital Zika disease in mice

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Zika virus (ZIKV) infection during pregnancy can cause congenital Zika syndrome and other birth defects. The alarmin IL-33 can evoke strong antiviral immune responses during viral infection. However, whether IL-33 is involved in immune responses during ZIKV infection is not clear. In this study, we infected three-week-old wild-type (WT) and IL-33 knockout (KO) mice with ZIKV and analyzed the ZIKV-specific cytotoxic T lymphocyte (CTL) responses at 6 days post infection. We found that IL-33 deficiency resulted in decreased ZIKV-specific CTL responses as evidenced by lower numbers of IFN-g^+TNF-a^+ $CD8^+$ T effectors. To determine if IL-33 plays a protective role in congenital Zika disease, we infected pregnant WT and IL-33 KO mice by s.c. injection of ZIKV at E6.5 and found higher percentages of fetal demise and absorption in IL-33 KO mice compared with that in WT mice (16.7 % vs. 3.3 %) from necropsy on E13.5. In addition, the fetuses from IL-33 KO mice displayed smaller sizes than those of WT fetuses (55 mm^2 vs. 48 mm^2). Moreover, IL-33 deficient mice had lower numbers of virus-specific $CD8^+$ T cells in the placenta, uterine and decidua. Importantly, IL-33 deficiency resulted in higher viral loads in both the placenta and fetus. In summary, our study highlights IL-33 as a key immune regulator in inducing anti-ZIKV CTL responses and protecting against congenital Zika disease.

P0319

Low-dose interleukin-2 treatment inhibits follicular cytotoxic CD8+ T cells and deteriorates CD8+ T cell-mediated immunopathology during viral infection

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Cytotoxic CD8 T cells have long been considered as a key population in modulating infectious and inflammatory diseases. Recent studies from us and others revealed a novel subpopulation of CD8 T cells specializing in controlling infection in B cell follicles. This subset, termed as follicular cytotoxic CD8 T (Tfc) cells, is shown to possess a similar profile to Tfh cells, where CXCR5, Bcl-6, Blimp1 and TCF-1 play important roles in shaping the population. However, unlike Tfh cells, it remains poorly understood that how Tfc cell fate is regulated by environmental cytokine milieu. Here we report interleukin-2 administration impaired Tfc cells, viral control in follicles and promoted immunopathology during infection. In chronic LCMV infection, low-dose IL-2 treatment significantly diminished Tfc differentiation and their Bcl-6 expression. Limited entry of CD8 T cells into follicles was also noticed in this setting. Mechanistic studies further revealed that IL-2-mediated suppression of Tfc cells is in a STAT5 dependent while Blimp-1 independent manner. Intriguingly, using acute LCMV and influenza infection mouse model, we further found, but not noticed in the CD8a knock-out mice or low-dose IL-2/anti-IFNg combined treatment setting, that low-dose IL-2 treatment not only inhibited Tfc cells but promoted severe immunopathology, characterized by significantly reduced body weight, declined survival rate and enhanced inflammation in organs such as lung, kidney and liver. Together, this study for the first time identified IL-2 as a key regulator of Tfc cells and suggested that low-dose IL-2 treatment can be risky to promote CD8 T cells-mediated pathology during viral infection.

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DOI: 10.1002/eji.201970400

P0320

Anti-spike IgG causes severe acute lung injury during acute SARS-CoV infection

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Emerging respiratory pathogen, such as SARS-CoV,MERS-CoV and H7N9, cause fatal acute lung injury (ALI) by driving hypercytokinemia and aggressive inflammation through mechanisms that remain elusive. Here, in SARS-CoV/macaques models, we show that anti-Spike IgG (S-IgG) causes severe ALI by skewing inflammation-resolving response in productively infected lungs. Alveolar macrophages underwent functional polarization demonstrating wound-healing characteristics in acutely infected macaques to facilitate inflammation-resolving. The presence of S-IgG prior to viral clearance, however, abrogatedwound-healing responses, promoted MCP-1 and IL-8 production and pro-inflammatory monocytes/macrophage accumulation. Critically, deceased patients displayed similarly accumulated pulmonary pro-inflammatory, absence of wound-healing macrophages, and faster neutralizing-antibody response. Their sera enhanced SARS-CoV-induced MCP-1 and IL-8 production by human monocytes-derived wound-healing macrophages whereas blockade of Fcγ-receptor reduced such effects. Our findings reveal a mechanism responsible for virally mediated ALI, defines a pathological consequence of viral specific antibody response, and provide a potential target for treatment of SARS-CoV or other virus-mediated lung injury.

P0321

Analysis of the chikungunya virus-induced inflammatory process

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Introduction: Chikungunya fever disease is caused by the chikungunya virus (CHIKV) transmitted by the *Aedes genus* mosquitoes. The disease can be characterized by high fever, pain in the muscles and joints that can progress to a chronic stage presented up to ten years or more, affecting normal activities of people and causing serious public health and economic problems.
Aim: The aim of this work was to study the correlation between the genotype and the inflammatory process with severity of CHIKF manifestations of people infected with CHIKV during the 2014-2016 outbreak in Mexico.
Methods: samples of 101 cases between 0 and 5 days of the evolution of the disease and clinically clasified under the criteious as: arthralgia, severe polyarthralgia, and arthritis-like syndrome were diagnosed by qRT-PCR, sequenced to determine the genotype and submitted to a cytokines analysis.
Results: 79 out of the 101 samples were positive for CHIKV. The genotype identified in these samples was the Asian. When samples were analysed for a cytokine array, an increased concentration for IL-8, IL-6 y TNF-a as soluble immunological mediators were detected. Altogether, these results indicated a close correlation between the high levels of IL-8, IL-6 y TNF-a soluble cytokines with this genotype, and these two parameters with the severity of the disease. Soluble proteins could be useful as biomarkers for diagnosis of CHIKV. Research in epidemiology and the immunopathogenesis needs to be supported for Governments in endemic areas for CHIKV and other arboviruses, for opportune economic and health policies implementation.

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DOI: 10.1002/eji.201970400

P0322

Platycodin D inhibits IAV-induced lung injury by suppressing excessive autophagy and apoptosis in macrophage cells

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Background: Platycodin D comes from the main triterpenoid saponins in the dry roots of Platycodon grandiflorum, which has been used in traditional Chinese medicine in the treatment of respiratory disease. This study aimed to evaluate the therapeutic effects of Platycodin D on mouse alveolar macrophages induced by IAV *in vitro* and to investigate the Platycodin D of its action.
Material and methods: Raw264.7 mouse alveolar macrophages were grown in culture and the CCK8 assay determined cell viability. Cells were treated for 24 h with increasing doses of Platycodin D (0,5,10,20,40,60,80,100μM). The apoptosis was detected by Annexin V-FITC/PI and Tunel assay. Levels of IL-1β, IL-6 and TNF-α of supernatants were assessed using ELISA assay. The numbers of phagophores, autophagosomes and autolysosomes and the ultrastructure of cells were assessed using TEM assay. The relative protein expression of LC3I, LC3II, Beclin1 and Bcl2 were evaluated by western blotting. The localizations of Beclin1 and Bcl2 were viewed with a fluorescence microscope.
Results: Platycodin D treatment resulted in significant dose-dependent inhibition of the growth of Raw264.7 cells with an IC50 of 115.74μM at 24 h. After Raw264.7 cells infected by influenza virus, Platycodin D(2.5, 5 and 10μM) can improve the activity of cells, reducing apoptosis of cells, decreasing the concentrations of IL-1β, IL-6 and TNF-α in the supernatant of cells, reducing autophagic injury of cells and regulating the protein expression of Beclin1 and Bcl2.
Conclusions: Platycodin D could inhibit excessive apoptosis and autophagy of macrophage cells induced by IAV in a dose-dependent manner and then inhibit the secretion of inflammatory factors caused by influenza virus infection caused by apoptosis and autophagy and then ultimately regulate the pathogenesis of influenza virus.

P0878

Tissue-resident memory T Cells protect against influenza through interferon-stimulated genes responses at an early infection stage

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CD8+ T cells play an important role in protection against influenza A virus (IAV). Previously, we discovered that an effective and timely CD8+ T cell response was critical for overcoming H7N9 infection in humans. Here we used murine infection models to elucidate the contribution of lung-resident memory CD8+ T cells (TRMs) to anti-influenza immunity and the underlying mechanism. We showed that the infection with a lowly pathogenic IAV, namely H9N2, could raise protective immunity against a subsequent challenge of heterologous H7N9, which was largely dependent on the engagement of lung TRMs. In a second cross-immune infection model, we further studied the antigen-specific memory CD8+ T cell response mounted by PR8-GP33, an engineered PR8 IAV expressing GP33 epitope of lymphocytic choriomeningitis virus (LCMV). When the PR8-GP33-immunized mice were subjected to a later LCMV infection, GP33-specific T cells were found to be populated in the lung airway within two days after viral challenge. Their expression of low levels of CD11a indicated that they likely did not originate from circulating memory T cells but rather from lung TRMs. Gene profiling analyses revealed these early appearing lung CD8+ cells had an activated status with significant induction of IFN-γ expression, concomitant with upregulation of interferon-stimulated genes (ISG) in

DOI: 10.1002/eji.201970400

the lung epithelial tissues. Thus, we proposed that one major function of lung TRM cells in influenza-protective immunity, preceding the recruitment of circulating memory T cells, is to elicit earlier lung-localized ISG responses to achieve timely control and clearance of virus via IFN-γ secretion.

P0879

HFE-dependent MAVS degradation restricts innate immunity caused by RNA virus infection

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Iron metabolism is involved in numerous physiological processes such as erythropoiesis, oxidative metabolism. However, the *in vivo* physiological functions of iron metabolism-related gene *Hfe* in viral infection remain poorly understood. Here, we show the association of host iron homeostasis with antiviral immune response. Influenza A virus (H7N9) infection caused the significant reduction of free iron, the elevation of ferritin, hepcidin and C-reactive protein in serum, and a high-iron diet significantly aggravated the H7N9 viral load and increased mortality. A high-throughput assay identified the gene *Hfe* as a negative regulator of RNA virus sensor MAVS stability. *Hfe*-knockout mice were more resistant to H7N9 infection and enhanced type I interferon expression compared to wild-type littermates. In mechanism, HFE interacted with MAVS and catalyzed its degradation. These findings establish a novel mechanism of iron homeostasis in regulating the innate immune response and suggest that HFE might a therapeutic target for RNA viral infections.

P0880

Wolbachia wAlbB regulate microRNAs pathway contributing to dengue virus inhibition in the Aedes aegypti

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The endosymbiont *Wolbachia* induces the pathogen interference in novel host *Ae.aegypti*, which is one of the best known phenotype. Recently, we found *Wolbachia* utilizes ROS to activate Toll pathway, contributing to the dengue virus inhibition in *Ae.aegypti*. However, *Wolbachia* exploits multiple antiviral mechanisms for pathogen interference, which is still not well known. Here, utilizing microarray and real-time PCR assay, we showed miRNA pathway and piRNA pathway are activated by *Wolbachia w*AlbB upon the DENV-2 infection. Moreover, the suppression of miRNA pathway significantly increased DENV-2 replication in *Wolbachia w*AlbB infected *Ae.aegypti*, suggesting miRNA pathway plays important role in DENV-2 inhibition. Our findings showed *Wolbachia w*AlbB employs miRNAs pathway and immune Toll pathway in pathogen interference. It is important for providing a new mechanism for the immune priming model as well as for better understanding the multiple molecular mechanisms underlie the Wolbachia induced pathogen interference.

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DOI: 10.1002/eji.201970400

P0881

Expression of HLA restricted HPV16 E6 and E7 antigen-specific CTL in advanced cervical squamous cell carcinoma patients

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Objective: To screen the human leukocyte antigen (HLA) restricted HPV16 E6 and E7 antigen specific peptides from advanced cervical squamous cell carcinoma (CSCC), and provide the target peptides for further clinical research.
Methods: Specific immune response level of peripheral T cells in Peripheral blood mononuclear cells (PBMC) of 67 CSCC patients and 92 control subjects were measured by using ELISpot assay. Meanwhile, stimulate the CSCC patients to produce IFN-γ by the synthetic E6 and E7 antigen peptide. Combined with the results of HLA, the HLA restrictive HPV16 E6 and E7specific peptides were screened among the patients.
Results: 1.The response frequency of specific CTL cells stimulated by HPV antigen peptides E6 and E7 pools in peripheral blood of advanced CSCC patients were both significantly higher than control subjects(*P*=0.028/0.016,respectively). 2.The response intensity of specific CTL cells stimulated by HPV antigen peptides E7 pools in PBMC of advanced CSCC patients was significantly higher thancontrol subjects(*P*=0.011). 3.The CTLs response frequency of E6 antigenic peptide in CSCC patients with the allele A*24:02 was statistically different from that of control subjects (*P*=0.018). 4.The positive reactions were shown in HPV16 E6 “Peptide-14 DLLIRCINCQKPLCPEEK” and E7 “Peptide-9 LRLCVQSTHVDIRTLEDL” restricted by the allele A * 24:02.
Conclusion: HPV antigen E6 and E7 proteins may be tumorigenic proteins that cause the occurrence and development of CSCC.The allele HLA-A*24:02 restrictive HPV16 E6 “Peptide-14 DLLIRCINCQKPLCPEEK” and E7 “Peptide-9 LRLCVQSTHVDIRTLEDL” may provide a research direction for the development of cervical cancer vaccines.
Keywords: CSCC；HLA；HPV16；CTL

P0882

Identification and characterization of GLDC as host susceptibility gene to severe influenza

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Influenza viruses affect the human population, leading to hospitalization and death. However, not all persons exposed to the same influenza virus developed a similar disease outcome since the pathogenesis of influenza viruses could be determined by viral virulence factors and host immunity. Therefore, it is conceivable that the differential expression of the cellular gene involved in viral growth or host immune response against viruses may contribute to the disease severity of human influenza. Here, we integrated the GWAS from the A(H1N1)pdm09 cohort and the cohort of A(H7N9) with expression quantitative trait loci (eQTL) analysis. Glycine decarboxylase (GLDC), the P protein of the glycine cleavage system, emerged as a potential susceptibility gene in both study cohorts.

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DOI: 10.1002/eji.201970400

In the present study, from *in vitro* results, we demonstrated that AOAA-mediated GLDC inhibition and siRNA-mediated depletion amplified IFN β and IFN-stimulated genes (ISGs) in combination with Poly I:C stimulation. Upon H1N1 or H7N9 viral infection, GLDC inhibition and depletion suppressed the viral replication via boosted antiviral response of type I IFNs and ISGs. Conversely, GLDC overexpression attenuated the type I IFN immune response, promoting the viral growth. Moreover, We found that GLDC inhibitor AOAA increased type I IFNs and ISGs, thus restricting the viral replication in H1N1-infected BALB/c mice. Interestingly, the survival rate of mice given AOAA at 14 days post-lethal infection was 100%.
Overall, our data suggest that GLDC modulates host anti-viral response to affect the viral replication, supporting its important role as a new host gene to severe influenza in humans.

P0883

Cell-cell contact promotes ebolavirus GP-mediated infection

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Ebola virus (EBOV) is a highly pathogenic filovirus that causes hemorrhagic fever in humans and animals. Here we provide evidence that cell-cell contact promotes infection mediated by the glycoprotein (GP) of EBOV. Interestingly, expression of EBOV GP alone, even in the absence of retroviral Gag-Pol, is sufficient to transfer a retroviral vector encoding Tet-off from cell to cell. Cell-to-cell infection mediated by EBOV GP is blocked by inhibitors of actin polymerization, but appears to be less sensitive to KZ52 neutralization. Treatment of co-cultured cells with cathepsin B/L inhibitors, or an entry inhibitor 3.47 that targets the virus binding to receptor NPC1, also blocks cell-to-cell infection. Cell-cell contact also enhances spread of rVSV bearing GP in monocytes and macrophages, the primary targets of natural EBOV infection. Altogether, our study reveals that cell-cell contact promotes EBOV GP-mediated infection, and provides new insight into understanding of EBOV spread and viral pathogenesis.

P0884

Delayed oseltamivir plus sirolimus treatment attenuates H1N1 virus-induced severe lung injury correlated with repressed NLRP3 inflammasome activation and inflammatory cell infiltration

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Severe influenza A virus infection causes high mortality and morbidity worldwide due to delayed antiviral treatment and inducing overwhelming immune responses, which contribute to immunopathological lung injury. Sirolimus, an inhibitor of mammalian target of rapamycin (mTOR), was effective in improving clinical outcomes in patients with severe H1N1 infection; however, the mechanisms by which it attenuates acute lung injury have not been elucidated. Here, delayed oseltamivir treatment was used to mimic clinical settings on lethal influenza A (H1N1) pdm09 virus (pH1N1) infection mice model. We revealed that delayed oseltamivir plus sirolimus treatment protects mice against lethal pH1N1 infection by attenuating severe lung damage. Mechanistically, the combined treatment reduced viral titer and pH1N1-induced mTOR activation. Subsequently, it

DOI: 10.1002/eji.201970400

suppressed the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome-mediated secretion of interleukin (IL)-1β and IL-18. It was noted that decreased NLRP3 inflammasome activation was associated with inhibited nuclear factor (NF)-κB activation, reduced reactive oxygen species production and increased autophagy. Additionally, the combined treatment reduced the expression of other proinflammatory cytokines and chemokines, and decreased inflammatory cell infiltration in lung tissue and bronchioalveolar lavage fluid. Consistently, it inhibited the mTOR-NF-κB-NLRP3 inflammasome-IL-1β axis in a lung epithelial cell line. These results demonstrated that combined treatment with sirolimus and oseltamivir attenuates pH1N1-induced severe lung injury, which is correlated with suppressed mTOR-NLRP3-IL-1β axis and reduced viral titer. Therefore, treatment with sirolimus as an adjuvant along with oseltamivir may be a promising immunomodulatory strategy for managing severe influenza.

P0885

Porcine circovirus type 2 infection promotes M1 macrophage polarization via targeting signaling pathway and histone modification

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Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus-associated disease (PCVD) that leads to immense economic losses in the global swine industry. Macrophages are the major target cells of PCV2, which are typically polarized by the dichotomy of classical (M1) and alternative (M2) activation statuses. However, the role of PCV2 in macrophage polarization remains unclear. Here we found that PCV2 infection strikingly induced expression of a subset of M1-associated genes including *Cxcl10,Socs3,iNos, Tlr2,Stat1*, and *Hif1a* in primary macrophages. Moreover, PCV2 infection also decreased expression of M2-associated genes such as *Irf4, Klf4,Pparg* and *Ccr2*. Interestingly, induction of M1 genes by PCV2 infection is dependent on activation of NF-κB and JNK signaling pathways while decrease of M2 genes by PCV2 is related to histone deacetylase and methylation. Thus, our study suggested that PCV2 infection skewed macrophages toward a M1 status by promoting M1 gene expression as well as suppressing of M2 gene expression *in vitro*. Further study will be performed to address the underlying mechanisms that how PCV2 infection drives M2 gene expression via histone modification and the role of PCV2-mediated M1 macrophages in the pathogenesis of PCVD *in vivo* .Supported by Shandong"Double Tops"program.

P0886

Tandem bispecific neutralizing antibody prevents fully and induces prolonged T cell immunity against pathogenic SHIV in monkey models

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Passive immunization of highly potent and broadly neutralizing antibodies (bNAbs) have been shown to prevent and suppress viremia in animal models and human clinical trials. Our previous study has proved that the tandem bi-specific bNAbs, BiIA-SG, was highly effective in protection and treatment settings in humanized mice. Here, we further investigate the prophylactic and therapeutic potential of BiIA-SG against pathogenic simian-human immunodeficiency virus (SHIV) challenge in Chinese-origin rhesus macaques.
A single intramuscular injection of BiIA-SG one day before $SHIV_{SF162P3}$ infection conferred full

DOI: 10.1002/eji.201970400

protection in all rhesus macaques tested. In addition, with a single injection of BiIA-SG after 1 day or 3 days of $SHIV_{SF162P3}$ challenge, the peak viremia occurrence was significantly postponed, followed by undetectable setpoint viral loads from 2 months post infection onwards. Importantly, the antibody infusion vastly reduced the chance of rapid progression to AIDS as compared with control animals. Mechanistically, CD8+ T cells were involved in long term viral suppression. Rapid viral rebound was induced in animals treated with T-cell-depleting anti-CD8β antibody, suggesting that early administration of BiIA-SG may induce long-lasting $CD8^+$T cell immunity to durably suppress $SHIV_{SF162P3}$ replication.
In summary, our study demonstrates the efficacy of BiIA-SG in controlling SHIV infection. Our findings strongly support the investigation of BiIA-SG immunotherapy against HIV-1 in humans.

P0887

Monoclonal antibody against inflammatory cytokines ameliorates influenza A (H1N1) virus induced acute lung injury

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Most patients infected with influenza A (H1N1) virus suffered respiratory illness. Some severely infected patients developed acute lung injury (ALI) and even acute respiratory distress syndrome (ARDS), which is the predominant cause for reported deaths. Previous studies showed that excessive early cytokine responses with early recruitment of inflammatory immune cells to the lung were key contributors to the morbidity. In this report, we are the first to describe a mouse model of H1N1 virus infection with acute lung injury and immune responses that reflect human clinical disease. Elevated levels of IL-17, IP-10 and CCL2-responsive cytokines were found in serum samples of H1N1 infected patients in Beijing. IL-17, IP-10 and CCL2 deficiency or treatment with monoclonal antibodies against IL-17, IP-10 and CCL2 ameliorated acute lung injury induced by the H1N1 virus in mice. These results suggest that inflammatory cytokines such as IL-17, IP-10 and CCL2 play an important role in influenza H1N1 virus induced acute lung injury and that monoclonal antibodies against these inflammatory cytokines could be useful as a potential therapeutic remedy for future influenza pandemics.

P0888

Epidemiological analysis on an suspect aseptic meningitis outbreak

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Objective: To investigate pathogens in an outbreak of suspect aseptic meningitis in a kindergarten in Jinhua City , Zhejiang.
Methods: Viral RNA was extracted and reverse-transcripted, followed by PCR amplification of the partial sequences of the 5'-UTR and VP1 gene of enteroviruses. The serotypes of viruses were determined by the comparison of the partial VP1 sequences. Phylogenetic tree of the partial VP1 sequence was analyzed by MEGA6.
Results: CV-A10 were detected in 50 % of samples, while Echo 6 were found in 19.4 % of samples. Besides, co-infection may exist in 11.1% of samples. Comparing to cerebrospinal fluid and throat swab samples, it was quick and convincent to detect enterovirus in blood samples, with a higher positive rate. According to the phylogenetic analysis, CV-A10 detected in this study was closely related to CV-

DOI: 10.1002/eji.201970400

A10 isolated in China in recent years, such as the strains isolated in Xiamen in 2015 and in Yunnan in 2017. In addition, Echo 6 detected in this study was closely related to those isolated in Yunnan, Guangzhou, Shandong in 2014.
Conclusion: The outbreak of aseptic meningitis is caused by CV-A10 and Echo 6. Blood sample is suitable for the pathogenic detection of HFMD and aseptic meningitis in clinical research.

P0889

Effect of hepatitis C virus on the expression of lipid metabolism related index

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Objective: To investigate the effect of hepatitis C virus (HCV) on the expression of lipid metabolism related index in vitro and in vivo.
Methods: A total of 114 samples of HCV infected patients and 96 samples of healthy individuals in Renmin Hospital of Wuhan University from September 2017 to September 2018 were collected. Automatic biochemical analyzer was used for following lipid metabolism parameters:TG, TCH, HDL, LDL, sdLDL, L(a), ApoA1 and ApoB detection.The expression of fatty acid synthase (FASN), acetyl CoA carboxylase (ACACA), hydroxymethylglutarate mono-acyl CoA reductase (HMGR), ApoA1, ApoB and low density lipoprotein receptor (LDLR) mRNA in Huh7.5.1 cells infected with HCV and control cells were measured by real-time fluorescent quantitative polymerase chain reaction (RT-qPCR).
Results: Serum TCH, HDL, LDL, ApoA1 and ApoB levels of HCV infected patients were all lower than those of healthy control group(2.98±0.51 vs 4.24±0.43, 0.87±0.16 vs 1.24±0.21, 1.75±0.24 vs 2.64±0.37, 0.94±0.18 vs 1.47±0.26, 0.67±0.31 vs 0.98±0.14 mmol/L respectively), and the difference was statistically significant(all $P< 0.05$). There was no significant difference in serum TG, sdLDL and L(a) levels between the two groups. The expression level of ApoA1 mRNA in Huh7.5.1 cells infected with HCV was lower than that in control cells, and the mRNA expression levels of FASN, ACACA, HMGR, ApoB and LDLR mRNA were higher than those of control cells, and the differences were statistically significant(all $P< 0.05$).
Conclusion: HCV infection can up-regulate mRNA levels of fatty acids and cholesterol synthesis related enzymes in hepatocytes, and affect lipid metabolism in vivo and in vitro.

P0890

The failure of immune blockade of vertical transmission is associated with viral load and PD-L1 expression on monocytes in pregnant women

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Chronic hepatitis B virus (HBV) infection is a major public health concern. Mother-to-child vertical transmission has become another major pathway for HBV infection, especially for mothers who are hepatitis B e antigen (HBeAg)- and hepatitis B surface antigen (HBsAg)-positive with high viremias. Routine hepatitis B vaccine (HepB) combined with hepatitis B immune globulin (HBIG) has significantly reduced mother-to-child transmission rate of hepatitis B, but there remains 0.75-9.66% of infants infected with hepatitis B virus (HBV) due to failure of immune blocking. We recruited 776 pregnant women with positive hepatitis B surface antigen (HBsAg) and 776 infants for this study.

DOI: 10.1002/eji.201970400

Hepatitis B vaccine were injected into infants and 100 IU for HBIG according to the status of pregnant women. The first injection should be completed within 2 hours of the birth of infants and hepatitis B vaccine should be inoculated at 1 and 6 months of age respectively. HBV infection status in infants was detected after 7 months. Among 776 infants, 14 failed to block immunity and was infected with HBV. The rate of HBV infection in infants was 1.81% (14/776). Statistical analysis results show that high viral load in pregnant women, inadequate vaccines and delayed first injection were associated with failure of immune blockade. The high PD-L1 expression on monocytes in peripheral blood of pregnant women was also a key factor for failure of immune blockade. This study remind us paying attention to the immune cell function of pregnant women for the maternal and infant blockade of HBV.

P0891

The responses of follicular cytotoxic T (TFC) cells involved in the pathogenesis of pneumonia induced by human adenovirus (HAdV) type 7

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TFC cells were a subset of CD8 T cells which exhibit more potent cytotoxic function and involve in the viral clearance. The role of TFC cells in the pathogenesis of acute HAdV infection was not clear. During an HAdV-7 outbreak in a military camp, forty-eight male military staffs at the age between 18 and 27 years old were enrolled, including HAdV-7 infected patient, silent infection individuals (SIs, individuals with HAdV-7 infection, but without fever) and close contacts (used to be infected by HAdV-7 during this outbreak, but no fever presented). Healthy controls (HC) were also included in this study. During the acute period of this outbreaks, a slightly decreased frequency of TFC cells (CXCR5+CD8 T cells) of treatment naïve patients compared to 3 other groups was observed. The IFN-γ and TNF-α secretion of TFC cells of contacts and SIs were higher than HCs and patients. This result suggested that adequate responses of TFC cells were important for the control of adenovirus. We also found that TFC cells of patients with pneumonia after HAdV-7 infection expressed lower level TNF-α compared to those without pneumonia. After treatment, TFC frequency of patients elevated significantly but IFN-γ and TNF-α secretion of TFC cells of them did not changed till 4 weeks after the termination of outbreaks. Taken together, our results suggested that differential responses of TFC cells determined the fate of patients after HAdV-7 infection and this subset involved in the pathogenesis of HAdV-7 infection.

P0892

TFH responded to the infection of HAdV infection which correlated to the antibodies production

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The mechanism of HAdV induced humoral immunity was not clear by now. Follicular T helper cells (TFH cells) are specialized T cell subset which is involved in the regulation of humoral immunity. To figure out how HAdV regulates humoral immunity via TFH cells, we enrolled a group of patients infected by HAdV type 7d, a group of subjects who were infected but did not show any symptom (silent infected subjects, SIs), and close contacts (used to be infected but no symptoms presented), from an outbreak in a military camp. Healthy controls (HCs) were also included. Our results showed that the

DOI: 10.1002/eji.201970400

frequency of TFH cells was not changed significantly by HAdV-7d infection, but PD-1 expression on TFH cells of treatment-naïve patients was higher than SIs, contacts and HCs, and this indicator did not decrease when they discharged from hospital. Until about 1 month after the end of this outbreak, this indicator decreased to the comparable levels of SIs, contacts and HC. HAdV-7d infection evoked higher productions of HAdV-7-specific IgG (HAdV-7-IgG), pan-HAdV-specific IgM (HAdV-IgM) and IgG (HAdV-IgG) of treatment naïve patients compared to contacts and SIs. Correlation analysis revealed that PD-1 expression of TFH cells was positively correlated with production of HAdV-IgM and HAdV-7-IgM in treatment naïve patients. And for SIs, PD-1 expression of TFH cells was positively correlated with HAdV-7-IgG levels. These results would deepen the understanding of HAdV associated humoral immunity and promote the researches on vaccine against HAdV.

P0893

Investigation of clinical prevalence of occult HBV infection with viral immune escape-related mutations in a large cohort of Chinese chronic HBV-infected patients

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Background and aims: Clinical occult HBV infection (OBI) with related mechanism remains a concern. This study aimed to investigate clinical OBI prevalence and characterize immune escape-related mutations in major hydrophilic domain (MHR) of HBV S region from the OBI patients.
Methodology: A total of 14,815 chronic HBV-infected patients who visited Beijing 302 Hospital from 2007 to 2016 and taken in HBV gene database of the Hospital were enrolled for investigation. Serum HBV DNA was extracted for sequence analysis. Vectors with individual MHR mutants were constructed and transfected to HepG2 cells. HBV replication, HBsAg expression, N-glycosylation and antigenicity were analyzed.
Results: Twenty-five (0.175%) patients were identified as OBI. In MHR, 19 kinds of immune-escape-related mutations were detected, including five mutations that have not been documented before. Nine representative mutations including the novel five were further studied. In comparison to wild-type strain, HBV DNA level was comparable; but extracellular and intracellular HBsAg levels of the nine mutants were reduced by 56.39%-99.09% and 42.76%-99.77%, respectively. Confocal immunofluorescence revealed that relative intensity ratios of HBsAg-myc-His fusion protein detected by anti-HBs and anti-His-tag were lower by 11.87%-76.27% for the nine mutants compared to the wild-type strain. Specifically, Consistent results were obtained using Western blotting and double-antibody sandwich ELISA assays (anti-myc + anti-HBs *vs.* anti-myc + anti-His).
Conclusions: Clinical prevalence of OBI was 0.175% in the studied patient cohort. Most OBI patients were detected with viral immune escape-related mutations in MHR, and antigenicity reduction could contribute mostly to poor detectability of HBsAg for the OBI patients.

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DOI: 10.1002/eji.201970400

P0894

Human bocavirus 1 (HBoV1) inhibits TNF-α-mediated activation of NF-κB signaling pathway

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HBoV1 is a DNA etiologic agent causing acute respiratory tract infections in young children worldwide and associated with cancers. Nuclear factor kappa B (NF-κB) transcription factors play crucial roles in the clearance of invading viruses through activation of many physiological processes. The previous investigation showed that HBoV1 infection could significantly upregulate the level of TNF-α a strong NF-κB stimulator. Here we investigated whether HBoV1 proteins modulate TNF-α-mediated activation of the NF-κB signaling pathway. We showed that HBoV1 NS1 and NS1-70 proteins blocked NF-κB activation in response to TNF-α. Overexpression of TNF receptor-associated factor 2 (TRAF2)-, IκB kinase alpha (IKKα)-, IκB kinase beta (IKKβ)-, constitutively active mutant of IKKβ (IKKβ SS/EE)-, or p65-induced NF-κB activation was inhibited by NS1 and NS1-70. Furthermore, NS1 and NS1-70 did not interfere with TNF-α-mediated IκBα phosphorylation and degradation, nor p65 nuclear translocation. Coimmunoprecipitation assays confirmed the interaction of both NS1 and NS1-70 with p65. Of note, NS1 but not NS1-70 inhibited TNF-α-mediated p65 phosphorylation at ser536. Our findings together indicate that HBoV1 NS1 and NS1-70 inhibit NF-κB activation. NP1 protein which is unique for Bocaparvovirus, also inhibits the activation of NF-κB. Coimmunoprecipitation assays confirmed the interaction of NS1 and NP1. The mechanism of NP1 inhibits the activation of NF-κB is still unclear. Whether the crosstalk between NS1 and NP1 can inhibit the NF-κB activation in a new method need further study. All in all, HBoV1 has been shown to inhibit NF-κB activation, revealing a potential immune-evasion mechanism that is likely important for HBoV1 pathogenesis.

P0895

Transmission routes of dengue virus bypassing mosquito vectors

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Currently, an estimated 50 million people worldwide are infected with the dengue virus (DENV). As the primary and secondary vectors of DENV are the *Aedes aegypti* and *Aedes albopictus* mosquitoes, respectively, most human cases occur in the tropical regions of Asia, Oceania, Africa and the Americas and result from horizontal transmission. In both adults and children, DENV infection leads to a high rate of hospitalizations. The severe form of infection manifests as dengue hemorrhagic fever (DHF), and pediatric and perinatal (infant and fetus) infections are particularly prone to this life-threatening outcome. *Aedes* eradication programs based on the theory of DENV mosquito-borne transmission have proved successful in controlling DENV epidemics; yet, when the programs were discontinued, reinvasions of *Aedes* spp. were always followed by epidemic DENV outbreaks. It is likely that atypical transmission routes contribute to these, and as such should be considered in the ongoing research to develop more effective eradication programs. In this review of the atypical transmission routes, including mucocutaneous contact, mother-to-fetus vertical transmission and accidental exposures (such as needlestick injury or receipt of contaminated blood or organs), we discuss in detail the two unevaluated: aerosol and sexual. Future eradication programs should include monitoring of populations at higher risk for these atypical routes of infection to expand the overall efficacy of their implementation. Approaches to creating such comprehensive public programs will be most productive by including a coordinated network of epidemiologists, hospitals, researchers and patients in both endemic and non-endemic areas.

DOI: 10.1002/eji.201970400

P0896

Respiratory syncytial virus replication is promoted by autophagy-mediated inhibition of apoptosis

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Respiratory syncytial virus (RSV) is the main cause of acute lower respiratory tract infection (ALRI) in children worldwide. Virus-host interactions affect the progression and prognosis of the infection. Autophagy plays important roles in virus-host interactions. Respiratory epithelial cells serve as the front line of host defense during RSV infection, However, it is still unclear how they interact with RSV. In this study, we found that RSV induced autophagy that favored RSV replication and exacerbated lung pathology *in vivo*. Mechanistically, RSV induced complete autophagy flux through reactive oxygen species (ROS) generation and activation of the AMP-activated protein kinase/mammalian target of rapamycin (AMPK-MTOR) signaling pathway in HEp-2 cells. Furthermore, we evaluated the functions of autophagy in RSV replication and found that RSV replication was increased in HEp-2 cells treated with rapamycin but decreased remarkably in cells treated with 3-methylademine (3-MA) or wortmannin. Knockdown key molecules in the autophagy pathway with short hairpinp RNA (shRNA) against autophagy-related gene 5 (*ATG5*), autophagy-related gene 7 (*ATG7*), or *BECN1/Beclin 1* or treatment with ROS scavenger N-acetyl-L-cysteine (NAC) and AMPK inhibitor (compound C) suppressed RSV replication. 3-MA or sh*ATG5/BECN1* significantly decreased cell viability and increased cell apoptosis at 48 hours postinfection (hpi). Blocking apoptosis with Z-VAD-FMK partially restored virus replication at 48 hpi. Those results provide strong evidence that autophagy may function as a proviral mechanism in a cell-intrinsic manner during RSV infection.

P0897

Discovery of RNA virus G-quadruplex structure and its ligand and their significance in viral replication

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Hepatitis C virus (HCV) infection is a major cause of human chronic liver disease and hepatocellular carcinoma. G-quadruplex (G4) is an important four-stranded secondary structure of nucleic acids. Recently, we discovered that the core gene of HCV contains a G4 RNA structure. We identified a cellular protein, nucleolin (NCL), which bound and stabilized the HCV core RNA G4 structure. We demonstrated the direct interaction and colocalization between NCL and wild-type core RNA G4 at both in vitro and in cell physiological conditions of the alive virus; however no significant interaction was found between NCL and G4-modified core RNA. HCV infection induced NCL mRNA and protein expression, while NCL suppressed wild-type viral replication and expression, but not G4-modified virus. Silencing of NCL greatly enhanced viral RNA replication. Our findings provide new insights that NCL may act as a host factor for anti-viral innate immunity, and binding of cellular NCL with the viral core RNA G4 structure is involved in suppressing HCV replication.

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DOI: 10.1002/eji.201970400

P0898

TRIM22 promotes RSV replication through enhancing autophagy level by binding ULK1 and Beclin 1

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Respiratory syncytial virus (RSV) is one of the most important pathogens causing respiratory viral infection in children. Transcriptome analysis shows that RSV increases a variety of TRIM proteins expression, including TRIM22, TRIM14, TRIM21 TRIM25, andTRIM38. In this study, we focused on TRIM22 to explore its role in RSV-host interaction. qPCR and western blot analysis showed that TRIM22 mRNA and protein expression were increased significantly in RSV-infected A549 cells. Immunofluorescence staining were used to determine the localization of trim22 protein and the result showed that TRIM22 localized in the cytoplasm with a diffuse and/or a speckled pattern. Knockdowning TRIM22 expression could inhibit RSV gene expression and viral titers in A549 cells, and vice versa. Mechanistically, TRIM22 promotes RSV replication through enhancing RSV-induced autophagy level. Furthermore, Coimmunoprecipitation assay confirmed that TRIM22 enhanced autophagy by binding autophagy-related proteins ULK1 and Beclin1. These results suggest that RSV can increase TRIM22 protein expression and TRIM22 promotes RSV replication by acting as an platform assembling autophagy-related proteins ULK1 and Beclin 1 to enhance autophagy.

P0899

Generation and characterization of potent neutralizing nanobody for Hand-foot-mouth disease therapy

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Hand-foot-mouth disease (HFMD) is mostly caused by Coxsakie virus A16 (CVA16) and Enterovirus 71 (EV71). Outbreaks of EV71 infection was associated with serious meningitis, polio-like syndrome with life threatening complications. To date, there are not specific anti-enterovirus drugs to treat EV71 infection. Our previous data indicated that antibodies derived from HFMD convalescent sera could prevent EV71 infection. compare to conventional IgG antibodies, camelid nanobodies lack of light chain present various advantages for their small size with high solubility, stability and affinity. Thus, we hypothesized that potent neutralizing nanobodies could prevent EV71 infection. To develop neutralizing nanobodies against EV71 infection, Llama was immunized with EV71 licensed vaccine. VP1, VP2 and VP3 were applied to screen nanobodies via phage display nanobodies library. In addition, neutralization assay and EV71 infected humanized NCG mice were exploited to evaluate the candidate nanobodies. Here, anti-sera with high titer against the protein of EV71 was induced. Several potent neutralizing nanobodies against EV71 infection were isolated from phage display nanobodies library. Furthermore, One humanized nanobodies present robust inhibition of EV71 infection in humanized mice model. One potential nanobody against EV71 infection will be provided.

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DOI: 10.1002/eji.201970400

P0900

Clinical efficacy of Qinggan Huashi Huoxue decoction combined with liver - protecting and enzyme - lowering drugs in treatment of alcoholic liver disease

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Objective: To investigate the clinical efficacy and safety of Qinggan Huashi Huoxue decoction combined with liver - protecting and enzyme - lowering drugs in the treatment of alcoholic liver disease (ALD).
Methods: A total of 175 ALD patients who were admitted to The Second People´s Hospital of Tangshan from January 2012 to December 2015 were enrolled and randomly divided into treatment group (87 patients) and control group (88 patients).
Results: After treatment, all patients achieved varying degrees of improvements in clinical symptoms and signs, which showed significant differences between the two groups at weeks 4, 8 and 12 of treatment ($t = 14.390$, 10. 487 and 13. 547, all $P < 0.05$). The liver function parametersin the two groups showed varying degrees of improvements at weeks 4, 8 and 12 of treatment, and ALT, AST, and GGT showed significant differences between the two groups at weeks 4, 8, and 12 of treatment ($F = 21.050$, 8. 108, and 12. 038, all $P < 0.01$) . In the control group, HDL showed a significant change after treatment ($t = 3.101$, $P < 0.05$). After treatment, HDL showed a significant difference between the two groups ($t = 2.784$, $P < 0.05$).
Conclusion: Qinggan Huashi Huoxue decoction combined with liver - protecting and enzyme - lowering drugs can improve the clinical outcome of ALD patients and holds promise for clinical application.
Keywords: liver disease, alcoholic; Qinggan Huashi Huoxue decoction; therapy

P0901

Alterations of the frequency and function of T follicular regulatory cells and its role in HIV infection

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The recently discovered T follicular regulatory cells (Tfr) have the ability to regulate Tfh cells through direct or indirect interactions, and then regulate the germinal center responses. The role of Tfr cells in disturbance of humoral immunity of HIV infected pateints needed to be studied further. T follicular helper cells（Tfh）are the most important cells that support the maturation and antibody secretion of B cells. This study found that the frequency of Tfh cells in treatment naïve group (tn-group) was significantly lower than that of the treated group (t-group) and healthy control (HC) group, and the frequency of PD-1+IL-21+Tfh cells in tn-group was lower than the HC group. Otherwise, the frequency of Tfr cells was significantly upregulated in HIV-infected patients than HCs, and the expression of IL-10 and CTLA-4 were also significantly upregulated and gradually declined to normal level after treatments. Blockade of CTLA-4 and IL-10 attenuated the inhibitory effect of Tfr and augmented the ability of Tfh to secrete IL-21. Tfr cells were not sensitive to CD95 (Fas) induced apoptosis, and the apoptotic rate and caspase-3 expression of Tfr were downregulated in HIV-infected patients after Fas ligand (FasL) stimulation. IFN-α sensitized CD4+T cell subsets to CD95-mediated apoptosis except Tfr cells. Blocking the PI3K-AKT pathway and ERK pathway restored the sensitivity of Tfr to CD95-mediated apoptosis, but had no effect in Tfh, Th and Treg cells. Tfr might be the important therapeutic target and IL-10 and CTLA-4 were the appropriated candidates for HIV immunotherapy.

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DOI: 10.1002/eji.201970400

P0902

Seroprevalence of Wenzhou virus in China

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Arenaviruses are enveloped, single-stranded RNA viruses. To date, only two arenaviruses have been described in Asia, lymphocytic choriomeningitis virus (LCMV) and Wenzhou virus (WENV). WENV was first identified in WENZHOU, CHINA in 2015. Although the prevalence of WENV in humans has been studied in Southeastern Asia, it has not been well addressed globally.
Here we established a competitive ELISA method to analyze the antigenic relationships between nucleocapsid proteins (N proteins) of WENV and LCMV. Further, we evaluated the serum epidemiology of WENV in the healthy population by detecting the IgG antibodies against N proteins. Meanwhile, we tested WENV RNA of the respiratory samples derived from the patients who had acute lower respiratory infections by a nested RT-PCR method.
Our results suggest that WENV and LCMV share cross-reactive epitopes between their N proteins. Then anti-WENV IgG antibodies were detected in 830 serum samples from healthy individuals. A total of 32/830 (3.86%) serum samples were positive for WENV. The seroprevalence rates in children group is 0%, 0%, 2.9%, and 2.2% in 0~0.5, 0.5~2, 2~5, 5~14 age group respectively. In adults group, the seroprevalence rate is 3.6%, 5.4%, and 4.1% in 15~44, 45~59, ≥60 age group respectively. However, we did not detect WENV RNA in 1000 acute lower respiratory infection samples by the RT-PCR
These data indicate the low transmission level of WENV in the Chinese population and help us understanding the interspecies transmission of WENV.

P0903

Triggering complement receptors on DCs shapes cellular immune responses during HIV-1 infection

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Immediately upon pathogen entry, non-specific immune responses are triggered in the host and within a very short time innate immunity is fully activated. Among these first innate components activated is the systemic complement system. Viruses such as HIV-1, spontaneously activate the complement system and therefore HIV-1 is already coated with complement fragments at the initial stages of infection.
Here we described a novel way of dendritic cell (DC) modulation during acute HIV-1 infection by triggering integrin receptor signaling. We could show that complement-opsonized HIV-1 (HIV-C) is sensed by dendritic cells, the sentinels and most important antigen-presenting cells of our immune system, compared to non-opsonized HIV. This recognition goes along with higher activation and maturation of dendritic cells and therefore causes stronger induction of cytotoxic T cells as well as T helper cells. We furthermore found that complement opsonization of HIV-1 induces a T helper 17 polarizing cytokine profile in DCs and polarized naïve T helper cells into the T helper 17 lineage. Additionally stronger maturation of DCs exposed to HIV-C induced higher type-I interferon responses, increased production of inflammatory cytokine and cellular complement components. These observed

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DOI: 10.1002/eji.201970400

inflammatory responses and DC maturation was completely abrogated when complement component 3a receptor (C3aR) was blocked.
Our data indicate that there is a clear relation between activation of complement receptors and DC maturation along with induction of cellular immune responses. Thus, investigating the complement receptor signaling pathway in more detail could lead to the development of a novel DC-based vaccination therapy against viral infections.

P0904

Investigating the dynamics of skin immune responses in arbovirus infection and disease

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The skin is the first line of defence against most infections, including those by **ar**thropod-**bo**rne viruses (arboviruses). The skin is a compartmentalised organ populated by a variety of immune cells that play a critical role in coordinating immune responses after skin infection. Arboviruses such as Ross River virus (RRV) and Chikungunya virus (CHIKV) cause debilitating musculoskeletal and arthritic disease and are considered significant public health threats of major global concern due to the scale of outbreaks, and the limited treatment options available. To understand the dynamics of arthritogenic arbovirus dissemination following skin infection, we developed a pathogenic model of RRV infection to mimic a mosquito bite. We found that inflammatory monocytes, neutrophils and dermal macrophages dominated the early inflammatory response in the skin. In addition, we found that RRV-infected $CD11b^+$ migratory cells translocate to the skin draining lymph nodes. Importantly, we unveiled a critical role for viral envelope glycosylation in driving viral dissemination kinetics and disease severity. Intradermal infection with RRV derived from mosquito cells resulted in more severe disease compared to RRV derived from mammalian cells. This difference in disease was accompanied by glycosylation-specific transcriptional response in the skin after intradermal infection. Interestingly we also show evidence indicating that viral envelope glycosylation dictates the dissemination of RRV to target tissues in muscle and joint. Using a new model of cutaneous arboviral infection, we show previously unappreciated mechanisms of immune response and viral dissemination that, in future, will help develop novel approaches to limit arboviral dissemination and ameliorate disease outcomes.

P0905

Defective hepatitis B specific immunity of pregnant females enhance the chances of HBV vertical transmission to their newborns

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Background: Vertical transmission of Hepatitis B virus (HBV) from infected mother to the newborn is the main cause of chronicity of HBV infection. However, there is rather scanty information on the role of maternal immunity in maternofetal transmission. transmission.
Methods: A total of 18461 pregnant females were screened for HBsAg. Fifty HBsAg+ve pregnant mothers and their newborns were recruited and tested for transmission of HBV; 22 were positive and rest were negative. PBMCs from the transmitting (Gr. A, n=22) and non-transmitting (Gr. B, n=28) mothers were analyzed for different immune cell subsets including dendritic cells (DCs), CD4, CD8, TFh and B cells. The HBV-specific responses of different cells were assessed using pooled surface and core overlapping HBV peptides. NGS was performed in PBMCs; qRT-PCR and ELISA was

DOI: 10.1002/eji.201970400

performed to validate the flow data.
Results: Mothers in Gr. B then A had higher frequencies and greater HBV specific DC, CD4, CD8, TFh and B cell-mediated protective responses. RNAseq showed increased expression of CD4, CD8, B, TFH, and Immune-related genes in Gr. B compared to Gr. A. Frequency of TFh cells inversely correlated with HBV DNA and IL-21. ROC analysis gave the cut-off values with the highest specificity and sensitivity to predict the involvement of TFh and B cells in HBV vertical transmission.
Conclusions: Serum IL-21 levels, TFh, and plasma B cells are useful predictors of vertical transmission of HBV from infected mothers to the newborns. Our study first time showed defective maternal immunity influences the chances of HBV transmission into their newborns.

P0906

γδ T cells protect against lung infection by promoting innate B cell response *via* an interleukin-17A-dependent mechanism

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γδ T cells contribute uniquely to immune competence at barrier sites. However, exactly how the unique inflammatory milieu in the lung shapes the effector function of lung-infiltrating γδ T cells remains largely unknown. Here we found that thymus-derived γδ T cells dominated lung interleukin 17A (IL-17A) production in mice at early stage of influenza infection. We further demonstrated that the induction of IL-17A in γδ T cells was dependent upon T cell receptor (TCR)-mediated signaling. The abundance of the transcriptional factor IRF4 was associated with γδTCR signaling strength and acted as a key regulator for IL-17A expression in γδ T cells. A comparison between virus-infected wild-type and IL-17A deficient (*Il17a*$^{-/-}$) mice identified IL-17A-dependent protection afforded by γδ T cells. In infected lungs, γδ T cells promoted plasmacytic differentiation of innate B-1a cells in an IL-17A dependent manner. Both biochemical and molecular analyses revealed that B-1a cell differentiation was significantly promoted by IL-17A signaling *via* induction of Blimp-1 expression and NF-κB activation in B-1a cells. Consistent with this observation, B-1a-derived antibody production was severely impaired in the respiratory tract of *Il17a*$^{-/-}$ mice associated with a deficiency in viral clearance, while transfer of B-1a-derived natural antibodies rescued *Il17a*$^{-/-}$ mice from otherwise lethal infections. Thus, we identified a protective role of lung IL-17A-producing γδ T (Tγδ17) cells against influenza virus by promoting plasmacytic differentiation and natural antiviral antibody production. These findings may underlie the therapeutic potential of Tγδ17 cell-mediated pulmonary immune responses.

P1483

Genome-wide gain-of-function screen reveals a restrictive role of platelet factor 4 on respiratory syncytial virus replication

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DOI: 10.1002/eji.201970400

Respiratory syncytial virus (RSV) is the leading cause of childhood respiratory tract infections worldwide. Despite a substantial disease burden, there are no vaccines available for the prevention of RSV except the passive immunoprophylaxis with a humanized neutralizing antibody, palivizumab. A better understanding of the interactions between RSV and the host is necessary for the development of anti-RSV therapies. In this study, we carried out a genome-wide human cDNA library screening and identified nine host factors that enhance RSV reproduction and 49 host factors that inhibit RSV replication, including platelet factor 4 (PF4), a new host restriction factor against RSV replication. PF4 inhibits RSV infection by blocking viral attachment to heparin and heparan sulfate (HS), the well-known RSV cellular receptors. In RSV-infected mice, intranasal pretreatment with PF4 significantly alleviates inflammatory cytokine levels and reduces the RSV load in the lungs, providing evidence that PF4 exhibits anti-RSV effects in vivo. Further, we observed obviously increased concentration of endogenous PF4 protein in the plasma from both RSV-infected children and mice compared with the controls. Interestingly, PF4 also shows antiviral activity against enterovirus and herpes virus, suggesting a potential broad anti-viral activity of PF4 against heparan sulfate-dependent viruses. Collectively, these findings suggest that PF4 may play a protective role during RSV infection, providing new insights into the virus replication and informs the development of novel prophylactics and therapeutics against RSV infections.

P1484

Antibody response and neutralizing mechanism against Zika virus during natural infection

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Zika virus (ZIKV), a mosquito-borne flavivirus, has generated worldwide concern for its linkage to microcephaly in fetuses and Guillian-Barre syndrome in adults. Here we examined longitudinal antibody response against ZIKV in two convalescent individuals by evaluation of binding specificity and neutralizing activity of polyclonal plasma and isolated mAbs from B cells. Plasma binding to DI/DII appeared to peak and wane during early infection with extensive cross reactivity with DI/DII of DENV. Binding to DIII, however, peaked later but persisted months. Monoclonal antibodies (mAbs) isolated during early infection are largely DI/DII specific, weakly neutralizing and highly cross-reactive with DENV while those from later on infection are more diverse in recognition, potently neutralizing and ZIKV-specific. One of the most potent neutralizing mAbs, ZK2B10, provided 100% protection in mice from lethal ZIKV infection. By Crystal structure determination, its epitope is confined to the edge of the lateral ridge on DIII and is constituted largely by ZIKV unique residues in BC and DE loop. Cryo-EM structure of ZK2B10 Fab in complex with the mature ZIKV showed that all the epitopes were covered by ZK2B10 Fab including those at the five-fold vertex of the virion. Modeling the binding of ZK2B10 to the post-fusion E trimer spike showed severe clashes between the bound antibody and the viral membrane, revealing a unique hot spot on the ZIKV E protein for inducing ZIKV-specific antibodies. All these findings will assist us to better understand the pathogenesis of ZIKV infection and inform rational design of vaccines.

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DOI: 10.1002/eji.201970400

P1485

Aedes salivary specific rAlb-34k2 protein promotes DENV-2 infection by adhering to BHK21 cell in early stage

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Saliva of the *Aedes* mosquito is known to facilitate transmission and infection of dengue virus (DENV) to target cells. Mosquito saliva contains more than one hundred proteins, in which specific protein component on DENV infection is still not well understood. 34k2 protein has been identified highly and specifically expressed in the saliva of *A. albopictus* mosquitoes and its function remains unknown. This study investigated the possible effects of recombinant *A. albopictus* salivary specific 34k2 protein (rAlb-34k2) on DENV-2 infection by adhering various target cells. The results showed that rAlb-34k2 protein could promote DENV-2 infection in BHK-21 cells within 24 hours compared with the group containing heat-inactivated rAlb-34k2 or not, respectively, by indirect immunofluorescence. Moreover, rAlb-34k2 protein was found to adhere to BHK-21, HepG2, Raw264.7 and HaCaT cells by immunoblotting and indirect immunofluorescence, which showed a dose-dependent manner and the adhere effects on BHK-21 and HepG2 could not be blocked by specific rabbit anti-rAlb-34k2 antibody. It is revealed that **rAlb-34k2 protein can adhere to several kinds of target cells and has the potential to mediate DENV-2 adsorption and invasion into host cells,** which provides further understanding of the functions of salivary specific 34k2 protein on DENV infection.
Keywords: *Aedes albopictus*; recombinant 34k2 protein; dengue virus; target cell; adhesion

P1486

Antiviral role of IFITM proteins in pseudorabies virus Bartha-61 infection

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The interferon-inducible transmembrane proteins IFITM1, IFITM2 and IFITM3 are host restriction factors against a broad range of viruses including Influenza A and B viruses (IAV, IBV), West Nile virus (WNV), Dengue virus (DENV), SARS coronavirus (SARS CoV) and hepatitis C virus (HCV). But there was limited reports available regarding their effects on pseudorabies virus (PRV) replication. We investigated the effect of these proteins on PRV replication in mammalian cells. These proteins could be induced by interferon stimulated and PRV infection. Then overexpression of IFITMs were obtained by transient transfection. IFITM1, IFITM2 or IFITM3 overexpression significantly inhibited PRV replication. Through RT-qPCR and western blot analysis revealed that overexpression of IFITMs could trigger the activation of IFN pathway. And IFITMs mediated inhibition of PRV replication was found to involve the JAK-STAT pathway: IFN-stimulated genes (ISGs) such as OAS1, ISG15 were augmented by IFITMs overexpression. Collectively, these results provide insights into the possible mechanism associated with the anti-PRV role of IFITMs.

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DOI: 10.1002/eji.201970400

P1487

Paradoxical role of the NLRP3 inflammasome during influenza virus infection

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Influenza virus infects more than 4 million people and causes 300,000 deaths in the world every year. As a critical component of the innate immune system, NLRP3 inflammsome is essential for host survival upon H1N1 influenza virus infection. Mice deficient for NLRP3 inflammasome component proteins showed higher mortality compared with wild type littermates during H1N1 influenza A virus (IAV) challenge. With the gain-of-function mutant *Nlrp3*^{R258W} mice, our study demonstrated the cellular mechanism for NLRP3 inflammasome in response to H1N1 influenza virus infection. The *Nlrp3*^{R258W} mutation protected mice against H1N1 infection through IL-1β signaling-dependent neutrophil recruitment. However, NLRP3 inflamamsome was detrimental for highly pathogenic avian influenza virus H7N9 infection. *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$, *Casp1*$^{-/-}$, *Il1r1*$^{-/-}$ mice all resisted H7N9 infection, with less pro-inflammatory cytokines and chemokines secretion, as well as decreased inflammatory cell infiltration and tissue damage compared with WT controls. Recent work demonstrated that inhibition of NLRP3 in the early phase of H3N2 IAV infection was detrimental, while such inhibition was beneficial in the late phase of the same IAV infection. Since NLRP3 is expressed in multiple types of cells, we aim to address the special requirement for NLRP3 in responses to different strains of IAV infections by use of conditional NLRP3 knock out mice (*Nlrp3*$^{f/f}$). We plan to cross *Nlrp3*$^{f/f}$ mice with cell type specific Cre transgenic mice to demonstrated cell linage specific role of the NLRP3 inflammasome upon infection with certain IAV strains. Our study provides references for the development of influenza virus vaccines and more efficient anti-viral drugs.

P1488

Murine cytomegalovirus immune evasion targeting the MEK-IRF1 connection

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Murine cytomegalovirus (MCMV) modulates host immune system for their propagation. Although MCMV immune evasion against NK cells or CD8 T cells has been extensively studied, less is known about modulation of the infected cells innate immunity by MCMV. Recently, we found that activation of mitogen-activated protein kinase kinase (MEK) suppresses antiviral functions of interferon regulatory factor 1 (IRF1) (ref.1). As MCMV infection is known to activate MEK (ref.2), we hypothesized that MCMV suppresses antiviral functions of IRF1 by promoting MEK activation during infection. When NIH3T3 cells or mouse embryonic fibroblasts (MEF) were infected with MCMV, we observed increased phosphorylation of extracellular signal-regulated kinases (ERKs), downstream elements of MEK, at 1, 2, 3 and 4 days after infection, and decreased expression of IRF1 at 2, 3 and 4 days after infection. Treatment of the MEK inhibitor U0126 restored IRF1 expression and further inhibited MCMV infection. We are currently investigating whether MEK activation regulates IRF1 functions by modulating one of its posttranslational modifications, SUMOylation, during MCMV infection. This study will identify a novel strategy of MCMV immune evasion that targets the potent host antiviral protein IRF1.
1. Oncogenic Ras inhibits IRF1 to promote viral oncolysis. (2015) Komatsu Y, Christian SL, Nhu Ho, Pongnopparat T, Licursi M and Hirasawa K. Oncogene, 34(30):3985-93.

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DOI: 10.1002/eji.201970400

2. cAMP response element of murine cytomegalovirus immediate early gene enhancer is transactivated by ras oncogene products. Gaboli M, Angeretti A, Lembo D, Gariglio M, Gribaudo G, Landolfo S. J Gen Virol. 1995 Apr;76(Pt 4):751-8.

P1489

CD74 interacts with 2B and 3C proteins of human enterovirus 68

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Human enterovirus 68 (EV-D68) is a member of the EV-D species, which belongs to the EV genus of the Picornaviridae family. Over the past several years, clusters of EV-D68 infections have occurred worldwide. A recent outbreak in the United States is the largest one associated with severe respiratory illness and neurological complication. Although clinical symptoms are recognized, the virus remains poorly understood. CD74 forms complex with MHCII in antigen presentation. In the present study, we found CD74 reduced human enterovirus 68 (EV-D68) infections in 293T cells. EV-D68 infections were enhanced in CD74 knock out THP-1 cells. We showed that CD74 interacted with 2B and 3C proteins of EV-D68. 2B has important role in EV-D68 replication and interacts with CD74 in it's hydrophobic region. 3C cleaved CD74 at Q125 site. These results suggest that a dynamic interplay between EV-D68 and CD74 may determine the outcome of EV-D68 infection.

P1490

Higher viral loads and genetic diversities of HIV-1 in seminal compartments than in bloods of seven Chinese men with MSM who have early HIV-1 infection

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To date, there have been no reports characterizing HIV-1 in the semen of Chinese men who have sex with men (MSM) with early infection. In this study, genetic diversity and viral load of HIV-1 in the seminal compartments and blood of Chinese MSM with early HIV-1 infection were examined. Viral load and genetic diversity of HIV-1 in paired samples of semen and blood were analyzed in seven MSM with early HIV-1 infection. HIV-1 RNA and DNA were quantitated by real-time PCR assays. Through sequencing the C2-V5 region of the HIV-1 env gene, the HIV-1 genotype and genetic diversity based on V3 loop amino acid sequences were determined by using Geno2pheno and PSSM programs co-receptor usage. It was found that there was more HIV-1 RNA in seminal plasma than in blood plasma and total, and more 2-LTR circular and integrated HIV-1 DNA in seminal cells than in peripheral blood mononuclear cells from all seven patients with early HIV-infection. There was also greater HIV-1 genetic diversity in seminal than in blood compartments. HIV-1 in plasma displayed higher genetic diversity than in cells from the blood and semen. In addition, V3 loop central motifs, which present some key neutralizing antibody epitopes, varied between blood and semen. **Thus, virological characteristics in semen may be more representative when evaluating risk of transmission in persons with early HIV infection.**

DOI: 10.1002/eji.201970400

P1491

HIV-specific CXCR5$^+$CD8$^+$ T cells can be enhanced by TGF-β

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The persistence of HIV reservoirs represents a major barrier to curing human immunodeficiency virus (HIV) infection. **CXCR5$^+$CD8$^+$ T cells (TFC) can inhibit HIV replication and migrate into B-cell follicles containing latent HIV in follicular CD4 T helper (TFH) cells.** However, the mechanism of TFC differentiation is unclear. Therefore, gaining full understanding of TFC differentiation may be an important strategy toward to curing HIV infection. The development and trafficking of TFC and TFH cells use similar mechanisms; therefore, we hypothesized that cytokines TGF-β, IL-21, and IL-6, which are essential for TFH differentiation, might contribute to TFC differentiation. In this study, cytokines TGF-β, IL-21, and IL-6 were co-cultured with peripheral blood mononuclear cells (PBMCs) from patients with HIV. The TFC percentage and their functions were investigated. High-throughput analysis of T cell receptor (TCR) repertoire profiles was conducted to explain the diversity between CXCR5$^+$ and CXCR5$^-$CD8 T cells at a deeper level. **The results showed that TGF-β enhanced the expression of CXCR5 on CD8 T cells from PBMCs and LNMCs from patients with HIV. TGF-β increased the expression of IFN-γ, TNF-α, and CD107a from CXCR5$^+$CD8 T cells, which was associated with decreased HIV DNA in PBMCs from patients with HIV.** TGF-β stimulation could also enhance the diversity and HIV-associated CDR3 sequences of TCR repertoires. **This study indicated that using TGF-β to increase the expression of CXCR5 on CD8$^+$ T cells could be a new immunotherapeutic regimen to cure HIV infection.**

P1492

PD-1 exhibits different function on HIV specific CXCR5+ and CXCR5-CD8+ T cells during chronic HIV infection

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CXCR5$^+$CD8$^+$ T cells have been demonstrated to play an important role in the control of chronic viral replication; however, the relationship between CXCR5$^+$CD8$^+$ T cells, HIV disease progression, and PD-1 expression profile on CXCR5$^+$CD8$^+$ T cells during HIV infection remain poorly understood. We enrolled a total of 101 HIV patients, including 62 typical progressors (TPs), 26 complete responders (CRs), and 13 immune non-responders (INRs). Flow cytometric analysis, immunohistochemical staining, and relative function assays were performed to analyze the properties of CXCR5$^+$CD8$^+$ T cells. **HIV-specific CXCR5$^+$CD8$^+$ T cells in the peripheral blood and distribution of CXCR5$^+$CD8$^+$ T cells in the lymph node (LN) were negatively correlated with disease progression during chronic HIV infection.** PD-1 was highly expressed on CXCR5$^+$CD8$^+$ T cells and positively associated with peripheral CD4$^+$ T cell counts. **Functionally, IFN-γ and TNF-α production of CXCR5$^+$CD8$^+$ T cells were reduced by PD-1 pathway blockade, but the production of IFN-γ and TNF-α from CXCR5$^-$CD8$^+$ T cells increased in response to TCR stimulation.** Interestingly, PD-1 expression was constantly retained on CXCR5$^+$CD8$^+$ T cells while significantly decreased on CXCR5$^-$CD8$^+$ T cells after successful antiretroviral treatment in chronic HIV-infected patients. PD-1$^+$CXCR5$^+$CD8$^+$ T cells may represent a novel therapeutic strategy for chronic HIV infection.

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DOI: 10.1002/eji.201970400

P1493

Loss of CD127+KLRG1- CD8+T cells is associated with incomplete immune recovery in HIV infection

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The differentiation and functional status of CD8+ T cells is significantly influenced by specific and ongoing congenetic antigen recognition. Importantly, the expression profiles of the interleukin-7 receptor alpha chain (CD127) and the killer cell lectin-like receptor G1 (KLRG1) have been shown to be differentially influenced by repetitive T-cell receptor interactions. Profound dysfuntion of CD8+ T cells has been described for HIV infection, such as over-activation, expansion, immune senescence and exhaustion. Here, we analyzed the surface phenotype and its association with disease in different CD8 T cell subsets before and after antiviral therapy in HIV-infected patients. Surprisingly, we found that CD127+KLRG1- CD8+T was significantly depleted in treatment naïve patients, which showed significant correlation with CD4 counts, VL and CD4/CD8 ratio. After ART treatement, this group of cells were partially recovered in immunological responders (IR) but not in immunological non-responders (INR). Notably, the decrease in KLRG1-CD127+ subset after HIV infection accounted for the dramatic decrease of the naïve compartment in CD8+T cells. Differentiation of CD127+ KLRG1-CD8 T cells is regulated by mTOR and AMPK pathway, which may be potential intervention targets for HIV infected patients with incomplete immune recovery.

P1494

Co-inhibition of adenosine signalling and PD-1 restores exhausted CD8 T cells during chronic HIV infection

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The recent successes of immune checkpoint (IC) targeting therapies in treating cancer patients has driven a resurgence of interest in targeting these pathways in HIV-infected patients. Recent studies have implicated that ICs facilitate viral latency and can be used as reservoir markers. Additionally, targeting ICs for treatment reactives the resting infected cells and at the same time restores cytotoxic function of virus-specific CD8 T cells. However, there are several limitations of IC therapy such as low efficiency and toxicity, so further research is necessary.
CD39+ CD8 T cells are characterized by terminal exhaustion, immunoregulatory activity and enrichment of antigen-specific T cells. Here We show HIV infection induce upregulation of PD-1+, CD39+ as well as PD-1 CD39 double positive CD8 T cells, which showed correlations with CD4 counts, viral load and CD4/8 ratio. *In vitro* combined blocking of PD-1 and CD39/adenosine signalling showed synergic effect on restoring CD8 T cell function. These findings may be important for developing novel checkpoint therapies against HIV and other chronic viral infections.

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DOI: 10.1002/eji.201970400

P1495

Prolonged evolution of virus-specific memory T cell immunity after severe avian influenza A (H7N9) virus infection

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Since 2013, influenza A H7N9 virus has emerged as the most common avian influenza virus subtype causing human infection, and it is associated with a high fatality risk. However, the characteristics of immune memory in patients who have recovered from H7N9 infection are not well understood. We assembled a cohort of 45 H7N9 survivors followed for up to 15 months after infection. Humoral and cellular immune responses were analyzed in sequential samples obtained at 1.5 to 4 months, 6 to 8 months, and 12 to 15 months postinfection. H7N9-specific antibody concentrations declined over time, and protective antibodies persisted longer in severely ill patients admitted to the intensive care unit (ICU) and patients presenting with acute respiratory distress syndrome (ARDS) than in patients with mild disease. Frequencies of virus-specific gamma interferon (IFN-γ)-secreting T cells were lower in critically ill patients requiring ventilation than in patients without ventilation within 4 months after infection. The percentages of H7N9-specific IFN-γ-secreting T cells tended to increase over time in patients ≥60 years or in critically ill patients requiring ventilation. Elevated levels of antigen-specific CD8+ T cells expressing the lung- homing marker CD49a were observed at 6 to 8 months after H7N9 infection compared to those in samples obtained at 1.5 to 4 months. Our findings indicate the prolonged reconstruction and evolution of virus-specific T cell immunity in older or critically ill patients and have implications for T cell-directed immunization strategies.

P1496

Immune cellular effectors required for the clearances of different circulating and intrahepatic HBV antigens

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Both HBsAg and HBeAg serve as important serologic markers for diagnosis and monitoring of patients with chronic hepatitis B. A cure for HBV infection is most specifically indicated by HBsAg seroconversion, which usually occurs later than HBeAg seroconversion. In clinical practice, however, some patients with HBsAg seroconversion were found to be persistently positive for circulating HBeAg and HBV DNA, as well as intrahepatic HBV antigens and DNA. These observations suggest that different immune effectors are responsible for the clearances of different circulating and intrahepatic HBV antigens. We could demonstrate that in the HBV HDI mouse model, serum HBV DNA clearance is associated with serum HBeAg clearance but not HBsAg seroconversion. Intrahepatic HBsAg remains positive after HBsAg seroconversion, while the clearances of intrahepatic HBcAg and serum HBeAg are synchronous. Depletion of CD8 T cells resulted in persistence of serum HBeAg and HBV DNA, but only a slight delay of HBsAg seroconversion. In contrast, depletion of CD4 T cells or B cells resulted in persistence of serum HBsAg, HBeAg and HBV DNA. When CD8 T cells, CD4 T cells or B

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DOI: 10.1002/eji.201970400

cells were depleted, HBsAg-positive and HBcAg-positive hepatocytes could be detected at 22dpi post HI. Taken together, these results reveal that apart from CD8 T cells and CD4 T cells, B cells serve as master effectors for circulating HBsAg, HBeAg and HBV DNA clearances, and B cells are responsible for intrahepatic HBcAg and HBsAg clearance in the HBV HDI mouse model.

P1497

NMHC-IIA recognizes sialic acids on sialylated RNA viruses to suppress pro-inflammatory responses via DAP12-Syk pathway

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Viral infections induce pro-inflammatory signaling cascades and inflammatory cytokine production, which is precisely regulated for host benefits. In the current study, we unravel a previously unappreciated role of non-muscle myosin heavy chain IIA (NMHC-IIA) as a negative regulator in inflammatory responses. We identified that cell membrane NMHC-IIA recognized sialic acids on sialylated RNA viruses during early infection, and interacted with an immune adaptor DNAX-activation protein of 12 kDa (DAP12) to recruit downstream spleen tyrosine kinase (Syk), leading to suppressed virus-triggered pro-inflammatory responses. More importantly, recognition of sialylated RNA viruses and sialic acid mimics by NMHC-IIA was shown to inhibit lipopolysaccharide (LPS)-induced pro-inflammatory responses via DAP12-Syk pathway. These findings uncover a novel negative regulation mechanism of pro-inflammatory responses, and provide a molecular basis to design anti-inflammatory drugs.

P1498

Control of NK cell activation by immune checkpoint for HBV persistence

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Chronic viral infection is featured in natural killer (NK) cell and CD8+T cell dysfunction, but how dysfunction is induced remains to be explored. In this study, we analyzed the phenotype and function of NK cells in the peripheral blood of patients with chronic hepatitis B (CHB) infection and healthy donors, and found that an increased proportion of NK cells expressing inhibitory receptor NKG2A along with decreased secretion of IFN-γ in peripheral blood in patients suffering from CHB. Meanwhile, the percentage of IL-10 produced by Treg cells was increased along with a high level of IL-10 in the plasma of CHB patients. More importantly, NKG2A expression on NK cells was positively correlated with IL-10 produced by Treg cells in CHB patients. Antibody target NKG2A prevented NK cell dysfunction and promoted IFN-γ secretion by NK cells. Blocking IL-10 could also inhibit NKG2A expression on NK cells and enhance NK cell activity. Moreover, reactivated NK cells provided sufficient IFN-γ that enhanced the activity of anti-viral CD8+T cells. Therefore, these findings indicated that **NKG2A as an important immune checkpoint for HBV-induced NK cell dysfunction contributes to HBV-mediated liver tolerance, and that NKG2A blockade sequentially restores NK cell and CD8+T cell activity**, providing new insights into prevention and treatment of CHB.

DOI: 10.1002/eji.201970400

P1499

Identification of HBV-specific HLA-DRB1*0803/HLA-DRB1*1202-restricted CD4 T-cell epitopes

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Background and aims: Hepatitis B virus (HBV)-specific CD4 T cells plays important roles in both viral clearance and liver damage, while the lack of verified epitopes prevents from detailed functional dissection of those cells.
Methods: Using blood samples from 27 HLA-DRB1*0803 or HLA-DRB1*1202 chronic HBV infection patients, peptides targeted by patients were screened out based on HBV core and S antigen derived peptides matrix stimulation. Peptide-pulsed HLA-DRB1 matched allogeneic B lymphoblastoid cell lines (BLCLs) were used to test the HLA class II restriction of candidate peptides. Another 13 HLA-DRB1*0803 or HLA-DRB1*1202 samples were used to test the candidate epitope peptides by single peptide/pair stimulation.
Results: Peptide $core_{46-60}$, $core_{56-70}$, $core_{66-80}$, $core_{136-150}$, $core_{161-175}$, B&C_S_{81-95}, B&C_$S_{156-170}$, B&C_$S_{186-200}$ were verified to stimulate peptide-specific CD4 T cells in a HLA-DRB1*0803 restricted way. Peptide $core_{116-130}$, $core_{166-180}$, B&C_S_{6-20}, B&C_S_{36-50}, B&C_S_{51-65}, B&C_S_{56-70}, B&C_S_{76-90}, B&C_$S_{141-155}$, C_$S_{191-205}$ were verified to stimulate peptide-specific CD4 T cells in a HLA-DRB1*1202 restricted way. Compared to using autologous cells as antigen presentation cells (APCs), using allogeneic BLCLs as APCs usually stimulated more peptide-specific CD4 T cells, possibly as a result of high HLA-DR, CD80, and CD86 expression on allogeneic BLCLs.
Conclusions: The identification of HBV-specific CD4 T-cell epitopes might facilitates our understanding of HBV-specific CD4 T cells. Higher HBV-specific CD4 T-cell response might be detected using potent APCs.

P1500

Down-regulation of lncRNA-GMR facilitates viral escape through modulating TBK1 modification and signaling

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Despite vigorous innate and adaptive, cellular and humoral immune responses against virus infection, viruses have evolved numerous mechanisms to evade the host immune surveillance. However, whether long non-coding RNAs play a role in immune escape of virus remains largely unknown. Through transcriptome sequencing and RNA interference (RNAi)-mediated functional screen, we identified lncRNA-GMR (GSH-Modification related RNA) as a key regulator of antiviral innate immunity. RNA-seq analysis of macrophages from lncRNA-GMR-deficient mice, combined with gain-of-function and rescue experiments, revealed a specific role for this lncRNA in promoting type I interferon production. And lncRNA-GMR-deficient mice manifested higher viral burden and exhibited more susceptibility to virus challenge consistently. Mechanistically, lncRNA-GMR is highly expressed in resting macrophages and blocks the interaction between TBK1, a kinase that plays an essential role in antiviral signaling, and GSTM1. Viral infection dramatically down-regulates the expression of

DOI: 10.1002/eji.201970400

lncRNA-GMR, resulting in reduced kinase activity of TBK1 through S-glutathionylation, an unconventional post-translational modification (PTM). Furthermore, we found the metabolite glutathione might also participate in this regulatory network. Our results have provided a novel mechanistic insight into the lncRNA modulation of antiviral responses and identified a previously unknown PTM of TBK1, providing evidence for viruses could manipulate lncRNA to facilitate viral escape. LncRNA-GMR may serve as a potential biomarker and therapeutic target in viral infectious diseases.

P1501

Antigen-specific T-cell immunity to emerging and re-emerging viruses in human

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The emerging and re-emerging viruses with high pathogenicity pose a great threaten to public health. The host T-cell immunity not only provides a protection role during the virus clearance, but also can lead to an effect of immunopathogesis. We established a specific T-cell test and research platform to investigate the mechanism by which the human T-cell immunity reacts to the highly-pathogenic viruses. 1) For the healthy population, we investigated the pre-existing cellular and humoral immune responses against the newly emerging viruses (such as H7N9 and MERS-CoV) in the healthy population who were recovered from the genetic related virus-infection (such as pH1N1 and SARS-CoV). 2) For the virus-infected patients, we determined their T-cell immune features during the acute infection and the convalescence phase. We found that the dysfunctional immunity were not only associated with disease progression but also correlated to the human-to-human transmission of the avian influenza virus. 3) We also utilized the virus-specific T-cell test to evaluate the vaccine efficacy. $CD8^+$ and $CD4^+$ T-cell responses were indispensable for the protection role of the newly developed vaccines to ZIKV, CHIV, and other viruses. Our study will benefit the understanding of immune mechanism of the emerging highly-pathogenic virus infections and the development of the vaccines.

P1502

Autophagy negatively regulates antiviral innate immunity through selectively degardation of IRF3

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Autophagy controls the homestasis of eukaryotic cells and play a key role in antiviral innate immune response. The published *in vitro* data showed the contrary effects of autophagy on innate immunity after RNA virus infection, however, the *in vivo* effect of autophagy remain unknown. Here we found autophagy negatively regulated an anti-VSV (vesicular stomatitis virus) innate immunity *in vivo*. The $Atg7^{f/f}$ Lysm-Cre or $Atg3^{f/f}$ Lysm-Cre mice with autophagy-deficient myeloid cells exhibit higher overall survival, more production of type I IFN and a dimished viral load after infected with lethal dose of VSV compared with wild-type mice, however, production of cytokines TNFα and IL-6 in lung remained unchanged. Autophagy inpeded phohorylated of transcription factor IRF3 and translocation of IRF3 to the nucleus after viral infection. Notably, VSV infection promoted autophagy flux and degradation of IRF3 protein, even using CHX as an inhibitor of protein synthese. IRF3 form complex with TRIM21 and SQSTM1, which target IRF3 to autophagosome for degradation. Furthermore, deficiency of autophagy also attenuated IRF3 activation and innate immune reponses to TLR3/TLR4 ligation and DNA virus

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DOI: 10.1002/eji.201970400

infection, indicating autophagy as a general regulator for IRF3 activation and type I IFN production. These findings indentify that autophagy negatively regulates antiviral innate immune response through selectively degardation of IRF3.

P1503

Serum levels of trypsin inhibitor Kazal1 (SPINK1) increased in patients with HBV-related hepatocellular carcinoma, predicts poor prognosis of HBV-related HCC

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Background and Aim: Hepatocellular carcinoma (HCC) is the second leading causes of cancer-related deaths. Aim to investigate the predictive value of serum SPINK1 in the prognosis of HBV-related HCC.
Methods: Detected the serum SPINK1 levels of 98 HC, 102 CHB, 95 LC, 45 ICC and 108 HBV-related HCC, and the expression levels of four cell lines (Hep2.2.15, HepG2, Hun7, and LO2). Estimate the diagnostic value of SPINK1 by ROC analysis, Kaplan-Meier analysis and Cox regression analysis.
Results: Serum SPINK1 levels was highest in the HBV- related HCC group compared to other three groups (1.09 ± 0.49, 1.61 ± 0.47, 1.96 ± 0.53, 2.35 ± 0.86 vs. 3.08 ± 1.16, P < 0.05). Serum SPINK1 levels was highest in Hep2.2.15, compared to other three cell lines (0.99 ± 0.04, 1.56 ± 0.05, 1.50 ± 0.04 vs. 2.83 ± 0.03, P < 0.05). In the HBV- related HCC, patients with larger tumor, multiple and worse Child-Pugh grades and BCLC stages, had higher Serum SPINK1 levels. By the Cox analysis, higher SPINK1 levels, worse BCLC stages, and larger tumor size showed shorter DFS and OS (HR=1.19, 95% CI, .87-5.76; *P* < 0.05), (HR =1.41, 95% CI, 2.29-7.30; *P* < 0.05).
Conclusion: SPINK1 may be a useful marker for stratification of HBV-related HCC prognosis.

P1504

Tim-3 targets NF90 for ubiquitination and degradation to inhibit antiviral immune response

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Nuclear factor NF90 is considered to be a novel virus sensor that plays an important role in initiating antiviral innate immunity. However, the post-translational modification mechanisms of NF90 has not been fully understood so far. We report here that VSV, a RNA virus, specifically up-regulates the expression of Tim-3, an immune checkpoint inhibitor, in macrophages and promotes phosphorylation of the tyrosines within the Tim-3 tail. Tim-3 in turn promotes the expression of E3 ubiquitin ligase TRIM47 and recruits TRIM47 to the zinc finger domain of NF90, and leads to NF90 degradation via K48-linked ubiquitination at Lys297 by TRIM47, thus initiating the degradation of NF90 via proteasome-dependent pathway. Generally, following infection, NF90 triggers the formation of stress granule (SGs) in cytoplasm which is a hallmark in initiating antiviral innate immunity by inhibiting the post-transcriptional translation of the virus. Targeted inactivation of Tim-3 increases the expression of two stress granule markers G3BP1 and TIA-1, and protects mice from lethal VSV challenge. In summary, we have revealed a novel pathway of immune evasion mediated by Tim-3, exploited by RNA viruses, which has potential antiviral applications. These findings also provide insights into the functions of Tim-3, a negative immune checkpoint molecule, in innate immunity.
Keywords Tim-3, NF90, ubiquitination, TRIM-47, immune evasion

DOI: 10.1002/eji.201970400

P1505

RNA m^{6}A demethylase FTO facilitates viral replication through inhibiting IRF3 pathway and antiviral immune response

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Backgrounds: Innate immune system plays essential roles in the recognition and elimination of invading viruses. N6-methyladenosine (m^{6}A) RNA modification is involved in various cellular functions. However, the regulation of host innate response to viral infection via m^{6}A modification remains unclear.
Research Objective: The primary aim of our study is to investigate the role of m^{6}A RNA modification-associated enzymes in the interaction of host and virus, and explore the underlying mechanisms for the antiviral innate response.
Methods and Results: Dot blot detection shows that viral infection increases m^{6}A RNA modification in mouse primary macrophages. RNAi functional screening of m^{6}A writer complex (METTL3, METTL14 and WTAP) and erasers (ALKBH5 and FTO) identifies FTO as an important molecule in promoting viral replication. We found that knockdown of FTO significantly reduces viral infection in macrophages by increasing IFN-β and IFN-α expression. Mechanistically, FTO reduces the phosphorylation and dimerization of transcription factor IRF3 in the antiviral innate response.
Conclusion: FTO promotes viral replication by inhibiting IFN-I expression through suppressing IRF3 activation. We demonstrate the inhibitory role of FTO in antiviral immune response, adding new insight into the epigenetic regulation of innate signaling and response.
Keywords: Viral replication, m^{6}A RNA modification, FTO, IRF3, IFN-I

P1506

Neutrophil extracellular traps level in circulation correlates with poor prognosis of severe viral infection

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Neutrophil extracellular traps (NETs) could prevent viral infection in acute stage and also implicated in the pathogenesis of organ dysfunction. Through the clinical studies in severe infection of H1N1 and severe fever with thrombocytopenia syndrome virus (SFTSV), we analyzed the relationship between NET level and disease prognosis. In the H1N1 cohort 2009 (n=66) and 2017 (n=30), we found that the plasma NET levels in patients with severe H1N1 infection were significantly elevated compared with

DOI: 10.1002/eji.201970400

those in mild patients and healthy donors.The NET levels were positively correlated with APACHE II scores and MODS. In the cohort 2017, NET levels in bronchoalveolar lavage fluid (BALF) were much higher than those in plasma, however, they showed no correlation with disease severity. Receiver-operating characteristic analysis revealed that NETs in plasma, rather than in BALF, could discriminate the severe H1N1 individuals from mild cases with high specificity and sensitivity in both cohorts.
In the multicentre cohort of SFTS (n=112), we found that severe SFTSV infection was accompanied by pronounced NET formation in the serum. The serum levels of NET were dynamically changed during disease progression, showing an opposite pattern compared with the trend of platelets and neutrophils. The cfDNA levels were strongly associated with MODS. High level of cfDNA (>711.7ng/mL) at initial diagnosis predicted significant severe illness in SFTS (odds ratio, 8.285; 95% confidence interval, 2.049-33.503; P = 0.003). Collectively, we proposed that NET (cfDNA) was a powerful marker for multiple pathological lesions and could predict severity and prognosis of severe viral infections.

P1507

Development of a 2-in-1 vaccine for dengue and measles by a recombinant measles virus expressing tetravalent dengue envelope protein domain III

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Dengue virus (DENV) infection has a major impact on global public health and current vaccine application is restricted in some countries and only recommended for children older than 9 years. In this study, we have demonstrated that a recombinant measles virus (MV) carried dengue envelope protein domain III (ED3) genes could infect and replicate in the susceptible transgenic mice expressing MV receptor human CD46. After the MV-vectored dengue vaccine immunization, mice developed specific interferon-gamma responses to MV and DENV. Between 4 serotypes of DENV, a dominant DENV-3-specific T-cell response was observed, possibly due to more T-cell epitopes located in DENV-3 ED3 than other serotypes. Neutralizing antibodies against MV and DENV were also induced in the MV-vectored dengue vaccine immunized mice, and protective levels of FRNT50>10 were detected to all serotypes of DENV. We also evaluated the protection against dengue infection. After inoculation with DENV-2, a detectable but significant lower viremia was observed in MV-vectored dengue vaccine immunized mice versus the vector control. In addition, the inflammatory cytokines associated with severe dengue were also significant lower in MV-vectored dengue vaccine immunized mice. Our results with regard to immunogenicity and protection in murine model provide clues for the future application of a 2-in-1 vaccine to against both measles and dengue.

P1508

Seroprevalence of subclinical HEV infection in healthy pregnant urban Dwellers of Bangladesh: identification of possible risk factors

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Hepatitis caused by hepatitis E Virus (HEV) is not uncommon in developing countries. It is usually a self-limiting conferring immunity against subsequent infection. However, HEV infection during pregnancy results in varying degree of morbidity, often fatal. The present study was designed to find

DOI: 10.1002/eji.201970400

out the seroprevalence of subclinical HEV infection during pregnancy at different trimesters without history of hepatitis. A total 255 asymptomatic healthy pregnant women of three trimesters (85×3=255) with no history of jaundice were included in this cross-sectional study. The subjects were sub-grouped according to socioeconomic status and education level. HEV IgG antibody in serum was determined by enzyme linked immunosorbent assay (ELISA). Results were expressed as number (percent). Chi-square, Odds Ratio and 95% CI were calculated as applicable. Data analyses were carried out using statistical package for social science for Windows Version 15.0. A p< 0.05 was taken as level of significance. Seropositivity for HEV IgG was 38% (96/255) in pregnant women; the higher percentages were recorded in the 2nd and 3rd trimesters − 41% and 46% respectively. The seropositivity of HEV IgG was significantly high in pregnant women with low education level ((p=0.001; OR=2.70, 95% CI=1.602−4.575) and low socioeconomic status (OR=7.54, 95% CI=4.118−13.029) having monthly income below 27,000 taka (p=0.001).
Conclusion: Data concluded that seroprevalence of anti-HEV IgG is higher at third trimester in pregnant women in Bangladesh where low socio-economic status and less education level were identified as possible risk factors. Appropriate measures may diminish the possible exposure to infection and reduce maternal mortality.

P1509

Cannabis plus cocaine use increases HIV-specific IL-17 producing T cells in HIV infected individuals

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Background: The use of drugs is common amongst individuals infected with HIV. This study aimed to evaluate the influence of the use of cannabis and/or cocaine in HIV- and CMV-specific T-cell responses of HIV-infected individuals under antiretroviral therapy.
Methods: Cryopreserved PBMCs from thirty-seven HIV-infected subjects undergoing antiretroviral therapy using cannabis, cocaine or cannabis-plus-cocaine were thawed and evaluated for IFN-γ- and IL-17A-producing CD4+ and CD8+ T-cells in response to in vitro stimulation with HIV-1 Gag or Cytomegalovirus pp65 peptide pools or Staphylococcal Enterotoxin B (SEB) using flow cytometry.
Results: In all drug users taken together, it was found a high percentage of IL-17-producing HIV Gag-specific CD8+ T-cell compared to HIV-infected non-drug users, while increased percentages of IL-17-producing HIV Gag-specific CD4+ and CD8+ T-cell were found in HIV-infected cannabis plus cocaine users. Among HIV-infected cocaine users, higher IL-17-producing CMV-specific CD8+ T-cell percentage were showed compared to HIV non-drug users, while no differences in the percentages of IFN-γ- and double (IL-17 and IFN- γ)-producing HIV- and CMV-specific CD4+ and CD8+T-cells were found among groups. Moreover, considering all drug users together, a higher percentage of SEB-stimulated IL-17-producing CD4+ T cells were found in relation to HIV-infected non-drug users, while cannabis users presented higher percentage of IL-17-producing CD4+ T cells in relation to cocaine or non-drug users.
Conclusion: The concomitant use of cannabis plus cocaine by HIV-infected individuals preferentially increases IL-17-specific T-cell responses, which may favor the pathogenic conditions associated with IL-17 overproduction.
Financial Support: FAPEG, CNPq, CAPES.

DOI: 10.1002/eji.201970400

P1510

Defining correlates of protection against viral reinfection

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Induction of an immune response that can prevent re-infection is the basis behind all vaccines. But induction of a protective immune response against many of the RNA viruses that circulate with large genetic diversity has been problematic. We studied, in a rare prospective cohort of 11 subjects that were naturally followed through multiple infections of hepatitis c virus (HCV), the B cell and antibody characteristics that correlated with protection against chronic re-infection. HCV will develop chronic disease in 75% of patients and so we were able to compare individuals that were able to clear their second infection to those that developed a second or third chronic infection. We performed pseudoparticle assays to measure autologous binding and neutralising breadth and also phenotyped and single cell RNA sequencing on B cells sorted for HCV envelope specificity. We found that the breadth of the antibody response did not necessarily protect from chronic re-infection, with evidence of antigenic sin from the conservation of the epitope hierarchy and clonal recall. However, a higher ratio of IgD+ to IgG+ HCV E2 positive cells and also a less 'activated' transcriptomic profile did associate with protection from chronic disease. Understanding what constitutes a protective response for highly variable RNA viruses is extremely important for both vaccine design and also for evaluating vaccine efficacy.

P1511

Sensing of HIV-1 nucleic acids by TLR8 in human CD4+ T cells

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During HIV-infection, cell-to-cell transmission results in endosomal uptake of the virus by target CD4+ T cells and potential exposure of the viral ssRNA genome to endolysosomal Toll-like receptors (TLRs). TLRs are instrumental in activating inflammatory responses in innate immune cells, but their function in adaptive immune cells is less well understood.
Here we show that combined TLR8 ligand and T cell receptor (TCR) stimulation boosted CD4+ T cell activation by enhancing NF-kB and MAPK signaling. This led to increased production of inflammatory cytokines and surface activation marker expression. Adjuvant TLR8 stimulation, but not TLR7 or TLR9, promoted interleukin (IL)-17 and interferon (IFN)-g effector cytokine production, indicating lineage commitment towards the pro-inflammatory T helper (Th)1 and Th17 axis.
Using a cell-to-cell transmission model, we found that endosomal HIV-1 induced cytokine secretion from CD4+ T cells, which could be significantly reduced by a TLR8 specific inhibitor. TLR8 engagement also enhanced HIV-1 replication in CD4+ T cells and potentiated the reversal of latency

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DOI: 10.1002/eji.201970400

in patient-derived CD4+ T cells.
Taken together, our results reveal a previously unknown function of TLR8 in human primary CD4+ T cells in shaping immunity and modulating HIV-1 infection. This adjuvant TLR8 activity can contribute to low-grade inflammation seen in HIV patients and may have broad implications for therapeutic targeting and vaccine development.

P1512

A salivary protein of *Aedes aegypti* promotes Dengue-2 virus replication and transmission

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Dengue is the most prevalent arthropod-borne viral disease in humans, yet still no effective medication or vaccine is available. Previous studies indicated that mosquito salivary proteins may influence the infection of dengue virus in the mammalian host. However, the function of specific salivary proteins on DENV replication in *Aedes aegypti* mosquito remains largely unknown. In this study, we investigated the effect of a salivary protein, named AaSG34, on DENV serotype 2 (DENV2) replication and transmission. We reveal that transcripts of AaSG34 were upregulated in the mosquito salivary glands after an infectious blood meal with DENV2. We further disclosed that transcripts of the dengue viral genome and envelop protein in the mosquito salivary glands were significantly diminished after infectious blood meal in the absence of AaSG34. To clarify the effect of AaSG34 on DENV2 transmission, *Stat1*-deficient mice were used. We reveal that while intradermal inoculation of infectious mosquito saliva induced hemorrhage development in *Stat1*-deficient mice, saliva from the AaSG34-silenced mosquitoes lost the ability to induce hemorrhage, suggesting that the AaSG34 enhances DENV2 transmission. Taken together, this is the first report to reveal that the AaSG34 promotes DENV2 replication in the mosquito salivary glands as well as enhancing transmission of the virus to mammalian host.

P1513

IFITM3 regulates CMV-induced TLR-mediated inflammatory cytokine production by myeloid cells and restricts viral pathogenesis

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IFN-induced transmembrane protein 3 (IFITM3) is an antiviral restriction factor that limits cellular entry of numerous viruses. Using the murine cytomegalovirus (mCMV) model of viral infection, we have previously shown that IFITM3 additionally functions as a checkpoint regulator that controls pro-inflammatory cytokine production and restricts viral pathogenesis independently of direct antiviral control. However, the mechanisms by which IFITM3 regulates CMV-induced inflammation are poorly understood.Using *Ifitm3*$^{-/-}$ bone marrow-derived DCs, we now show that Ifitm3 limits mCMV-induced pro-inflammatory responses by restricting TLR7- and TLR9-mediated cytokine production. Importantly, genetic deletion of the adaptor protein MyD88 that acts downstream of TLR7 and TLR9 alleviates mCMV-induced pathogenesis in *Ifitm3*$^{-/-}$mice. To investigate the role that IFITM3 plays in regulating cytokine responses in human cells, we differentiate healthy control hIPSCs +/- biallelic mutations in *IFITM3*into iPS-Dendritic Cells (iPS-DCs) and iPS-derived macrophages (iPSDMs). We found that

DOI: 10.1002/eji.201970400

iPS-DCs and iPSDMs generated from *IFITM3*$^{-/-}$iPSCs produced significantly more IL-6 than control cells in response to HCMV infection and various ligands of TLRs, including TLR2. Furthermore we found that TLR2, which requires MyD88 for signalling, is pivotal in IFITM3-regulated cytokine production triggered by HCMV. Overall, these data demonstrate that IFITM3 regulates virus-induced cytokine secretion by murine and human myeloid cells via modulation of TLR-mediated cytokine production.

P2071

MMP2/MMP9-mediated CD100 shedding is crucial for inducing intrahepatic anti-HBV CD8 T cell responses and HBV clearance

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Background and aims: CD100 is constitutively expressed on T cells and can be cleaved from the cell surface by matrix metalloproteases (MMPs) to become soluble CD100 (sCD100). Both membrane-bound CD100 (mCD100) and sCD100 play important immune regulatory functions that promote immune cell activation and responses. The aim of this study was to investigate the expression and role of mCD100 and sCD100 in regulating antiviral immune responses during HBV infection.
Methods: mCD100 expression on T cells, sCD100 levels in the serum and MMPs expression in the liver and serum were analyzed in patients with chronic hepatitis B (CHB) and HBV replicating mice. The ability of sCD100 in mediating antigen-presenting cell maturation, HBV-specific T cell activation and HBV clearance were analyzed in HBV replicating mice and CHB patients.
Results: CHB patients had higher mCD100 expression on T cells and lower serum sCD100 levels than healthy controls. Therapeutic sCD100 treatment resulted in the activation of dendritic cells and liver sinusoidal endothelial cells, enhanced HBV-specific CD8 T cell response and accelerated HBV clearance, while blockade of its receptor CD72 attenuated the intrahepatic anti-HBV CD8 T cell response. Together with MMP9, MMP2 mediated mCD100 shedding fromT cell surface. CHB patients had significantly lower serum MMP2 levels, which positively correlated with serum sCD100 levels. Inhibition of MMP2/9 activity resulted in an attenuated anti-HBV T cell response and delayed HBV clearance in mice.
Conclusion: MMP2/MMP9-mediated sCD100 release plays an important role in regulating intrahepatic anti-HBV CD8 T cell response, thus mediating subsequent viral clearance during HBV infection.

P2072

Kruppel-like factor 10 protects against acute viral myocarditis by negatively regulating cardiac MCP-1 expression to decrease inflammatory monocytes infiltration

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Viral myocarditis (VMC) is a cardiac disease associated with inflammation and injury of myocardium induced by virus infection. Cardiomyocytes have recently been regarded as key players in eliciting and

DOI: 10.1002/eji.201970400

modulating inflammation within the myocardium. Monocyte chemotactic protein-1 (MCP-1), which is one of the best-studied chemokines secreted by infected-cardiomyocytes, binds to CCR2 and results in accumulation of monocytes to inflammation sites. There is overwhelming evidence on the essential roles of MCP-1 in cardiovascular diseases. However, the underlying regulatory mechanism of its expression in cardiomyocytes remains poorly understood. Here, we report that KLF10 acts as a novel transcriptional regulator to inhibit MCP-1 expression in cardiomyocytes and protects against Coxsackievirus B3 (CVB3) -induced myocarditis. Murine heart tissues and primary cardiomyocytes showed downregulated KLF10 expression upon CVB3 infection. KLF10 deficiency aggravated murine VMC progress, due to massive Ly6C^{high} monocytes/macrophages infiltration into myocardium. We further identified MCP-1 as a novel target of KLF10 in cardiomyocytes and KLF10 cooperated with HDAC1 to negatively regulate MCP-1 expression via binding its promoter. Finally, KLF10 overexpressed mice via AAV9 infection could significantly retarded VMC progress. These data indicated that KLF10 inhibits MCP-1 transcriptional activity in resting cardiomyocytes to sustain myocardium homeostasis, while CVB3 infection suppresses KLF10 expression, leading to the activation of MCP-1 transcription and the recruitment of monocytes/macrophages into myocardium, which might give new insights into the pathogenesis of VMC and propose a potential therapy target for VMC.

P2073

A human herpesvirus 6 gene product inhibits innate antiviral immune response

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Human herpesvirus 6(HHV-6) is an enveloped DNA virus, belonging to roseolovirus genus of the betaherpesvirus subfamily. HHV-6 U26 is a roseolovirus genus specific gene with unknown function. pU26 is predicted as a membrane protein containing 8 transmembrane helices with a portion of the protein located in the mitochondria. RIG-1/MAVS/IRF signaling pathway plays an important role via recognizing and response to the RNA antigen (PAMP or DAMP). Recently, collective evidence showed that RIG-1 was also involved in the host innate immune to DNA virus infection. Here, we showed that HHV-6 pU26 regulated the RIG-1/MAVS/IRF signal pathway. It inhibited the phosphorylation of IRF3 and subsequently the production of type I interferon by targeting the mitochondrial antiviral-signaling protein (MAVS) through the proteasome degradation pathway. Inhibition of U26 expression resulted in the decreased HHV-6 propagation. These findings indicated that HHV-6 U26 was a novel antagonistic viral factor to host innate antiviral immunity.

P2074

USP1-UAF1 deubiquitinase complex facilitates innate antiviral immune responses

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Type I interferons (IFNs, IFN-α/β) play fundamental roles in innate antiviral responses and its expression must be fine-tuned during viral infection. Ubiqutination is an important protein posttranslational modification (PTM) for the control of antiviral immunity. Although several E3 ubiquitin ligases have been reported to regulate ubiquitination and protein degradation of TBK1 and cGAS, the functions of deubiquitinating enzymes on TBK1/cGAS activity remain largely unclear. Here, we

DOI: 10.1002/eji.201970400

identified a deubiquitinase complex, which is formed by ubiquitin specific peptidase 1 (USP1) and USP1-associated factor 1 (UAF1), as a viral infection-induced physiological enhancer of antiviral innate immunity. USP1-UAF1 complex enhanced TLR, RLR and cGAS induced IFN regulatory factor 3 (IRF3) activation and subsequent IFN-β secretion. Mechanistically, USP1 and UAF1 bound to TBK1, removed its K48-linked polyubiquitination, and then reversed the degradation process of TBK1. Interestingly, we found that USP1 and UAF1 interacted with cGAS, indicating that cGAS may be another target of the USP1/UAF1 complex. Furthermore, we found that ML323, a specific USP1-UAF1 inhibitor, attenuated IFN-β expression and enhanced viral replication both in vitro and in vivo. Therefore, our results outline a novel mechanism for the control of TBK1/cGAS activity, and suggest USP1-UAF1 complex as a potential target for the prevention of viral diseases.

P2075

TNF-α/IFN-γ profile of HBV-specific CD4 T cells is associated with liver damage and viral clearance in chronic HBV infection

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Background and aims: The role of Hepatitis B virus (HBV)-specific CD4 T cells in chronic HBV infection patients is not clear.
Methods: Through intracellular IFN-γ and TNF-α staining, HBV-specific CD4 T cells were analyzed in 65 chronic HBV infection patients with alanine aminotransferase (ALT) < 2×upper limit of normal (ULN) and 20 hepatitis B flare patients (ALT > 10×ULN).
Results: For patients with ALT < 2×ULN, HBV-specific CD4 T-cell response was dominated by TNF-α producing CD4 T cells in HBeAg positive patients, while the dominance of HBV-specific IFN-γ producing CD4 T cells increased in patients with HBeAg or HBsAg seroclearance.
For hepatitis B flare patients, HBV-specific TNF-α producing CD4 T cells was associated with liver damage: 1) the frequency of HBV-specific TNF-α producing CD4 T cells was positively correlated with patients' serum ALT level; 2) multiple sampling from the same patients showed that the frequency of HBV-specific TNF-α producing CD4 T cells decreased simultaneously with the declination of serum ALT level. Multiple sampling for the same patients also indicated a TNF-α producer to IFN-γ producer differentiation process for HBV-specific CD4 T cells during flare. Compared to patients without HBeAg or HBsAg seroclearance shortly after flare, patients with HBeAg or HBsAg seroclearance showed higher frequency of HBV-specific IFN-γ producing CD4 T cells.
Conclusions: HBV-specific TNF-α producing CD4 T cells is associated with liver damage, while HBV-specific IFN-γ producing CD4 T cells is associated with viral clearance in chronic HBV infection patients.

DOI: 10.1002/eji.201970400

P2076

IFN-κ suppresses the replication of influenza A virus through IFNAR-MAPK-Fos-CHD6 axis

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Type I interferon is the first line of defense against infectious virus, but its action mechanism remains not fully understood. By using both *in vivo* and *in vitro* influenza infection models, we identified that IFN-κ, a type I interferon factor, is capable to efficiently contain the replication of both group 1 and group 2 influenza viruses through MAPK pathway. Interestingly, Zika virus is also highly sensitive to IFN-κ-mediated suppression. Our data revealed that IFN-κ is the earliest responding type I interferon among all known members and appeared as early as six hours in mice after H9N2 infection, a low-pathogenic Avian Influenza, but not after highly pathogenic H7N9 infection. Its ectopic expression significantly up-regulated C-Fos and CHD6 genes, two interferon simulating genes, and was associated with significant suppression of influenza viruses and ZIKV whereas knock-out of CHD6 aborted IFN-κ-mediated suppression. Further dissection identified that CHD6 is regulated through IFNAR-p38-C-Fos pathway. The intranasal administration of IFN-κ conferred protection of mice from lethal PR8 challenge and resulted in less loss of the body weight, reduced inflammatory responses and attenuated pathological damages. Overall, our study unveiled a novel pathway for IFN-κ in fighting against influenza virus, which expanded our knowledge on anti-viral mechanism of type I interferon. In addition, our results supported the application of IFN-κ in developing new prevention and treatment agents against emerging viral infection, such as influenza and ZIKV.

P2077

PGE2 ameliorated viral myocarditis development by inducing IL-10 producing regulatory B cells expansion via MAPKs/AKT-AP1 axis or AhR signaling

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B10 cells, a specific subset of B cells, are capable to regulate immune function and ameliorate inflammation and autoimmune diseases progression by producing IL-10. B10 cells frequency is significantly changed during inflammation and autoimmunity. However, it remains unclear how B10 cells is changed and the underlying mechanism in viral myocarditis (VMC). Therefore, the present work was addressed to clarify the B10 cells changes and potential mechanisms. Our results showed a significant change in the frequency of B10 cells in VMC model. The changes of PGE2 level in the heart of VMC model mice were consistent with the changes of B10 frequency. PGE2 induced the expansion of B10 cells and the production of IL-10 dependent on MAPKs/AKT-AP1 axis or AhR signaling. In addition, B10 cells pretreated with PGE2 inhibited the differentiation of naïve $CD4^+$ T cells into Th17 cells. *In vivo*, PGE2 treatment significantly ameliorated VMC development. Our results provide a highlight role that inducing the expansion of B10 cells by PGE2 may become a promising therapeutic approach for VMC and acute inflammatory injury.

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DOI: 10.1002/eji.201970400

P2078

Characterization of ZIKV EIV9 epitope-specific CD8 T-cells in the immuneprivilege organs in virus-infected mice

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ZIKV is an enveloped non-segmented, single-stranded, positive-sense RNA virus and belongs to the Flavivirus genus. ZIKV can induce inflammation in immunoprivileged organs (e.g., the central nervous system and testicle), leading to the Guillain-Barre syndrome and damaging the testicle. T cell immunity plays an important role in the control of ZIKV infection and virus clearance. However, the characterization and mechanisms of T cells, especially ZIKV specific CD8 T-cells infiltrated into the immune privilege organs have not been found completely.
For detecting the ZIKV specific CD8 T-cells, we use EIV9 (IGVSNRDFV)-Tetramer staining technique which our previous study reported. With detecting the ZIKV RNA and specific CD8 T-cells in the spleen, central nervous system and testicle at different time-points of ZIKV post-infection, we found the occurrence and disappearance of virus and T cells have positive correlation. Importantly, we found the CD8 T-cells have infiltrated through the blood-brain and blood-testis barrier in early infection stage (near day 4). However, on day 90, The proportion of EIV9 epitope-specific CD8 T-cells was still significantly higher in the central nervous system. It suggests that residual CD8+ T cells may also be associated with the autoimmune nervous system disease such as GBS. Moreover, we found that the infiltrated CD8 T cells in spleen, central nervous system and testicle, are majority of effective memory T cells (TEM), especially EIV9-specific CD8 T-cells. Interestingly, these ZIKV-specific T cells are PD-1 positive T cells. These functional ZIKV-specific CD8 T-cells may not only have virus clearance, but induce pathological characteristics in immunoprivileged organs.

P2079

RNAI screening identifies methyltransferase SETD2 as a critical amplifier of interferon antiviral activity by methylating STAT1

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Interferon-α (IFNα) signaling is a key component of cellular responses to infection by inducing IFN-stimulated genes (ISGs). However, how epigenetic enzymes-mediated epigenetic changes and post-translational modification regulate the antiviral responses of interferons is still unclear. We performed a high-throughput RNAi screening which targets 711 epigenetic regulators and identified methyltransferase SETD2 as a critical amplifier of IFNα-mediated inhibition of HBV replication. Specific deficiency of *Setd2* in mouse hepatocyte showed more serious HBV infection in vivo. Mechanistically, SETD2 directly catalyses methylation of STAT1 at lysine 525 via its methyltransferase activity, which reinforces IFN-activated STAT1 phosphorylation and antiviral cellular response. In addition, SETD2 selectively mediates the tri-methylation of H3K36 on promoters of some ISGs such as ISG15 to promote their transcription, enhancing interferon's antiviral activity. Our study illustrates methyltransferase SETD2 catalysed-K525 methylation of STAT1 is an essential modification for IFNα-

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DOI: 10.1002/eji.201970400

dependent antiviral immunity and indicates potential application of SETD2 in controlling viral infections.

P2080

Basic fibroblast growth factor protects against influenza A virus-induced acute lung injury by recruiting neutrophils

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Influenza virus (IAV) infection is a major cause of severe respiratory illness that affects almost every country in the world. IAV infections result in respiratory illness and even acute lung injury and death, but the underlying mechanisms responsible for IAV pathogenesis have not yet been fully elucidated. In this study, the basic fibroblast growth factor2 (FGF2) level was markedly increased in H1N1 virus-infected humans and mice. FGF2, which is predominately derived from epithelial cells, recruits and activates neutrophils via the FGFR2-PI3K-AKT-NFκB signaling pathway. FGF2 depletion or knockout exacerbated influenza-associated disease by impairing neutrophil recruitment and activation. More importantly, administration of the recombinant FGF2 protein significantly alleviated the severity of IAV-induced lung injury and promoted the survival of IAV-infected mice. Based on the results from experiments in which neutrophils were depleted and adoptively transferred, FGF2 protected mice against influenza virus infection by recruiting neutrophils. Thus, FGF2 plays a critical role in preventing IAV-induced lung injury, and FGF2 is a promising potential therapeutic target during IAV infection.

P2081

Human cases of Wenzhou virus infection confirmed by pseudo-virus neutralization test in Yunnan province, China

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Mammarenaviruses are associated with human hemorrhagic fever diseases in Africa and America. Recently, a rodent mammarenavirus, Wenzhou virus (WENV) and related viruses, have been reported in China, Cambodia, and Thailand. But human cases infected by WENV have been not confirmed. In this paper, a total of 828 serum samples from patients with fever of unknown origin were detected in Yunnan Province, which contained 23 positive samples (cut off value > 0.1) by Enzyme-linked immunosorbent assay, further screened by Western blot and pseudo-virus neutralization test with WENV. The distribution and infection of WENV have been finally determined.

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DOI: 10.1002/eji.201970400

P2082

Determination of HSV-2-specific cellular responses by IFN-γ ELISpot assay in HIV-1 heterosexual discordant couples in Dakar, Senegal

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Background: Herpes simplex virus type 2 (HSV-2), the most frequent cause of genital ulcer disease (GUD), has been shown to play a more important role than any other sexually transmitted infections (STIs) in driving HIV prevalence in Africa. HSV-2 infection increases the risk of HIV-1 acquisition and also enhances the onward transmission of HIV among discordant couples.
Methods: Nineteen HSV peptide pools (gB and gD) were first used to analyze the frequency and the magnitude of HSV-2-specific cellular responses in 30 HSV-2 seropositive patients and 17 HSV-2 seronegative individual in a cohort of heterosexual Senegalese HIV-discordant couples by using IFN-γ ELISpot assay. We further compared HSV-2-specific T cell responses between 21 HSV-2 co-infected with HIV-1 and 9 HSV-2 mono-infected individuals.
Results: Twenty one (70%) out of 30 HSV-2 seropositive patients showed detectable HSV-2-specific cellular responses compared with 2 (12%) out of 17 HSV-2 negative subjects ($p < 0.001$), in concordance with higher magnitude T cell responses observed among HSV-2 infected patients (median, 61 vs. 0 spots/10^6 PBMC, $p = 0.001$). Interestingly, HSV-2 mono-infected patients showed significantly higher magnitude of HSV-2-specific T cell responses than did HSV/HIV co-infected patients (median, 140 vs. 42 spots/10^6 PBMC, $p = 0.024$).
Conclusion: Our finding suggest that co-infection with HIV-1 in HSV-2-infected patients might be associated with reduced HSV-2 cellular immune responses. However, the interaction between HIV and HSV-2 appears complex, and precise longitudinal studies will be required to dissect their exact temporal relationship.

P2083

Selective autophagy controls innate antiviral immunity via cargo receptor networks

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Autophagy is a highly selective degradation process, which depends on cargo recognition by the cargo receptors. Cargo receptors often contain ubiquitin-binding domains, which could recognize the substrates with ubiquitination modification. To gain insights into the detail functional mechanism of selective autophagy in antiviral immune responses, we generated selective autophagy-deficient cells by depletion the expression of cargo receptors p62/SQSTM1, NDP52 and OPTN. Upon RNA virus infection, we observed that the activation of type I interferon (IFN) signaling was largely increased in *SQSTM1*, *NDP52* and *OPTN* deficient cells, suggesting that selective autophagy contributes to the tolerance of antiviral innate immune responses. Mechanistically, we demonstrated that IFN-induced Tetherin/BST2 recruits the E3 ligase MARCH8 to catalyze the lysine 27 (K27)-linked ubiquitin chains on MAVS at K7, thus providing a recognition signal of MAVS for NDP52-dependent selective autophagic degradation. Tetherin suppresses antiviral immune signaling through a negative feedback loop, which in turn confers tolerance on innate antiviral responses. Furthermore, we identified RIG-I, TBK1, and IRF3 are sequestered by p62, hence to be delivered to autophagosome for degradation. To ensure the specificity and availability, ubiquitin and ubiquitin-like modifier ISG15 play important

DOI: 10.1002/eji.201970400

roles in the recognition of RIG-I, TBK1 and IRF3 by p62. The negative regulatory function of selective autophagy supports the immune tolerance in innate antiviral responses, which identified novel therapeutic targets for developing antiviral therapies.

P2084

25-hydroxyvitamin D3 inhibits HBV replication via upregulating TREM-1

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Vitamin D3 is an immune regulator that plays important roles in both innate immune and adaptive immune. Vitamin D3 has been reported to suppress hepatitis C virus (HCV) production through affecting the assemble of viral particles. However, the role of vitamin D3 in HBV replication remains unclear. In this study, we confirmed that HCC cell lines and human liver tissues had the potential to convert vitamin D3 into its active form 1,25-dihydroxyvitamin D3 (1α,25(OH)2D3). Furthermore, 25-hydroxyvitamin D3 (25(OH)D3) treatment remarkably inhibited the expression of HBV antigens, the production of HBV pregenomic RNA (pgRNA) and covalently closed circular DNA (cccDNA) in vitro and in vivo. In mechanism, 25(OH)D3 strongly increased the expression of triggering receptor expressed on myeloid cells 1 (TREM-1), which was necessary for 25(OH)D3 mediated inhibition on HBV replication. In conclusion, we identify vitamin D3 as a novel anti-HBV agent and preliminary reveal the important role of VD3-TREM1 pathway in regulating HBV replication.

P2085

RIG-I pathway is suppressed by viral 3D polymerase via impacting on mitochondrial MFN2 and Fis1 expression dynamics during enterovirus-D68 infecting human bronchial epithelial cell

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Enterovirus-D68（EV-D68）belongs to Picornaviridae family, can lead to severe clinical manifestation in respiratory system. 3D polymerase is one of important non-structure proteins during the EV-D68 replication, but there are still few research about its function of interacting with host antiviral response during the process of the EV-D68 infection. Here we try to investigate 3D polymerase impacting on mitochondrial dynamics and interfering innate immune response in human bronchial epithelial cells. According to the results of sub-cellular analysis of immunofluorescence, TEM and the Western blot , we find EV-D68 duplication is correlated with the structure and number of mitochondria dynamics in the early stage of infection in 16HBE cells, including regulation of the transmission of mitochondrial DNA, and cellular apoptosis. Importantly, we observed that viral 3D polymerase promotes mitochondrial expression of the MFN2 and Fis1, inhibits RIG-I signaling, suggesting the 3D polymerase functioning in the modulation of innate proinflammatory cytokines.Collectively, our findings suggest intracellular viral protein interfere with the mitochondrial function during EV-D68 infection, thereby 3D polymerase of EV-D68 can suppressed the RIG-I pathway through upregulating the expression of the MFN2 and the Fis1.

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DOI: 10.1002/eji.201970400

P2086

UBE2S negatively regulates type I inteferon production through recruiting USP15 to deubiquitin TBK1

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Type I interferon (IFN) signaling plays an essential role in host innate immune response against RNA and DNA virus infection. TANK-binding kinase 1 (TBK1) is an important enzyme in the regulation of type I IFN production and innate antiviral immune response. Here we reported the ubiquitin-conjugating enzyme E2S (UBE2S) acts as a novel negative regulator of TBK1 activation and type I IFN signaling pathway. Ectopic expression of UBE2S suppressed poly(I:C)- and virus-induced type I IFN production, whereas deficiency of UBE2S expression enhanced antiviral immune responses to RNA and DNA viruses and decreased viral replication both in vitro and in vivo. UBE2S recruited USP15 to TBK1 to cleave Lys63 (K63)-linked polyubiquitin from TBK1. Knockdown of either UBE2S or USP15 enhanced K63-linked ubiquitination of TBK1 and attenuated the phosphorylation of TBK1 and the transcription factor IRF3. Our results identify a previously unrecognized role for UBE2S in the regulation of type I interferon signaling and provide a new molecular insight into the regulation of innate immunity.

P2087

Foot-and-mouth disease virus activates RIG-I/STING-mediated ER stress response to facilitate its replication

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Interferon gene stimulator (STING) is an important immune-related protein located in the endoplasmic reticulum (ER) of mammalian cells. Although STING has been elucidated as a key molecule involved in anti-DNA virus infection in the cells, recent studies showed that STING also plays an important role in restricting RNA virus in cells, but the mechanism beyond remains unclear. Foot-and-mouth disease virus (FMDV) is a single-stranded positive-stranded RNA virus, which causes foot-and-mouth disease in cloven-hoofed animals. Our studies showed that FMDV infection resulted in degradation of STING in PK15 cells and induced cellular autophagy via activating ER stress response. In further exploring how FMDV initiates the signaling cascades, it was found that FMDV induced ER stress responses originates from cellular pattern recognition receptor RIG-I, which transmits signals to the ER anchored adaptor protein STING to specifically activate PERK-EIF2alpha signaling pathway, and finally leads to autophagy and degradation of STING itself and then resolve ER stress. Knockdown of STING suppressed FMDV replication, but had little effect on the IFN I response. The results illustrated the signaling cascades that mediates cellular stress response to FMDV and uncovered the link between innate immune responses and cellular stress responses.
Keywords: stimulator of interferon genes; RNA virus; foot-and-mouth disease virus; stress response; innate immunity

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DOI: 10.1002/eji.201970400

P2088

ATP1B3: a virus-induced host factor against EV71 replication by up-regulating the production of type-I interferons

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Enterovirus 71 (EV71) infection can cause severe diseases, and is becoming increasingly common in children. In the current study, we first carried out yeast two-hybrid assays to screen human proteins that could interact with 3A protein of EV71 using human homogenization cDNA library. Next we use both confocal and co-IP to identify the interaction between two proteins. Finally, human β3 subunit of Na^+/K^+-ATPase (ATP1B3) protein was demonstrated to interact with the 3A protein of EV71. We transfect different dosage of pCMV-3A vector to overexpression 3A protein and detect the expression of ATP1B3 protein. Although 3A protein had no effect on the expression of ATP1B3, EV71 infection resulted in elevated expression of ATP1B3 in RD cell line, both on messenger RNA (mRNA) and protein levels along with infection MOI and time. Interestingly, knockdown of ATP1B3 using ATP1B3 siRNA could significantly increase the replication of EV71, whereas overexpression of ATP1B3 by transfecting pCMV-ATP1B3 vector significantly suppressed the replication of EV71 in RD cells. Furthermore, we demonstrated that the expression of ATP1B3 could induce the production of type-I interferons which can influence EV71 virus replication. Our study demonstrated that ATP1B3 inhibit EV71 replication by enhancing the production of type-I interferons, which could act as a potential therapeutic target in EV71 infection.

P2089

Fiber2 protein of serotype 4 fowl adenovirus hijacks host KPNA3/4

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The outbreak of the hepatitis-hydropericardium syndrom (HHS) caused by serotype 4 fowl adenovirus (FAdV-4) has resulted in huge economic loss in the poultry industry globally since 2013. Although the fiber proteins of the virus play vital roles in mediating the infection and carry protective antigenic epitopes for FAdV-4, little is known about the molecular mechanism for the pathogenesis of FAdV-4. In this study, through coimmunoprecipitation, mass spectrum and western blot assay, fiber2, not fiber1, was identified to be efficiently interacted with the karyopherin alpha 3 and karyopherin alpha 4 protein (KPNA3/4). Truncation analysis revealed the N-terminus (61-114aa) of fiber2 was the efficient interacted domain with KPNA3/4. Moreover, the overexpression of the KPNA3 in LMH cells could assist the virus replication at early time points whereas the knockout of KPNA3 in LMH cells using CRISPR-Cas9 could slightly block the virus replication at early time points and significantly promote the virus replication at late time points. Poly(I:C) stimulation analysis further revealed that the levels of IFN-β and OASL in KPNA3-KO LMH cells were significantly lower than those in the wild type LMH cells. All these data not only demonstrate that FAdV-4 may hijack host KPNA3/4 through fiber2 to favorite its infection and pathogenesis, but also provide potential antiviral targets for FAdV-4.

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DOI: 10.1002/eji.201970400

P2090

IFIT5 inhibit PEDV replication via phosphorylation of the STAT1

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The IFN-induced protein with tetratricopeptide repeats (IFITs) family is characterized by interferon stimulating and involving in a multitude of cellular and viral regulations. In this study, we investigated the functions and associated molecular mechanism of IFIT5, a novel member in IFITs family, on the infection and replication of porcine epidemic diarrhea virus (PEDV). Our preliminary results showed that PEDV infection upregulated the expression of IFIT5 on both mRNA and protein levels, detected by real-time RT-qPCR and western blotting respectively. However, overexpression of IFIT5 significantly inhibited PEDV replication. In addition, the results from dual luciferase assay demonstrated that PEDV-induced IFIT5 increased promotor activity of interferon stimulated response element (ISRE). Further investigations suggested that IFIT5 promoted the phosphorylation of STAT1 and interacted with STAT1, STAT2, and IRF9. Additionally, knocking-down of IFIT5 facilitated PEDV replication and downregulated the phosphorylation of STAT1. In conclusion, the results from this study revealed that IFIT5 was a positive regulator of type I interferon signaling pathway, demonstrating a suppression effect on PEDV replication by interacting with STAT1 and promoting the phosphorylation of STAT1.
Keywords: IFIT5, PEDV, STAT1

P2091

The effect of probiotic Bifidobacterium lactis Bl-04 on host antiviral responses in vitro

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Several studies report that ingestion of probiotics may be beneficial for reducing the risk of upper respiratory tract illnesses. These effects most likely relate to the ability of probiotic bacteria to modulate host immune responses by affecting immune cell gene expression. *Bifidobacterium animalis* subspecies *lactis* Bl-04 (Bl-04) has been reported to significantly reduce the risk of respiratory illness episodes by 27% over 5-month winter season in healthy active adults. In addition, in another clinical trial the ingestion of Bl-04 was shown to affect the baseline state of innate immunity in the nasal mucosa and the subsequent response of the host to rhinovirus challenge. Ingestion of Bl-04 for one month prior to rhinovirus challenge maintained higher IL-8 levels in nasal lavage compared to placebo. Administration of Bl-04 was also associated with a reduction in nasal lavage virus titer. The mechanisms behind the probiotic's effects in these studies are mostly unknown. To assess the mechanisms in more detail we investigated the effects of Bl-04 on human monocyte-derived macrophages and dendritic cells *in vitro*. We analyzed the pro-inflammatory, anti-inflammatory, and immunomodulatory characteristics of Bl-04-treated cells challenged with Toll-like receptor ligands (PolyI:C and R848) which mimic viral infection. To obtain information on the transcriptional changes in immune cells during probiotic treatment, we analyzed the cytokine profiles from cell culture supernatants after challenge and performed transcriptomics analysis for both cell types. The findings will provide information on the immunomodulatory mechanisms behind reduced upper respiratory illness risk and reduced viral replication by the probiotic Bl-04.

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DOI: 10.1002/eji.201970400

P2092

Arterivirus nucleocapsid protein binds to protein inhibitor of activator STAT1 (PIAS1) and triggers NF-κB activation to produce proinflammatory mediators during viral infection

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Porcine reproductive and respiratory syndrome virus (PRRSV) activates NF-kB during infection. We examined the ability of all 22 PRRSV genes for NF-kB regulation and found the nucleocapsid (N) protein was the NF-kB activator. The N protein of PRRSV is a small phosphoprotein of 123 amino acids and is the most abundant viral protein expressed during infection. N is localized in the cytoplasm of infected cells but is also specifically distributed in the nucleus and nucleolus. Protein inhibitor of activated STAT1 (signal transducer and activator of transcription 1) (PIAS1) was identified as a cellular protein binding to N. PIAS1 is known to bind to p65 (RelA) in the nucleus and blocks its DNA binding, and thus functions as a repressor of NF-kB. Binding of N to PIAS1 released p65 for NF-kB activation. The N-terminal half of PIAS1 was mapped as the N-binding domain, and this region overlapped its p65-binding domain. For N, the region between 37 and 72 aa was identified as the binding domain to PIAS1, and this domain alone was able to activate NF-kB. A nuclear localization signal (NLS) knock-out mutant N did not activate NF-kB, and this is mostly likely due to the lack of its interaction with PIAS1 in the nucleus, demonstrating the positive correlation between the binding of N to PIAS1 and the NF-kB activation. Our study reveals a role of N in the nucleus for NF-kB activation and proinflammatory cytokine production during infection.

P2093

The susceptibility of primary dermis fibroblasts from the Chinese tree shrew to human cytomegalovirus infection

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As a universal pathogen leading to neonatal defects and transplant failure, human cytomegalovirus (HCMV) has strict species specificity and this has prevented the development of a suitable animal model for the pathogenesis study. The mechanism of cross-species barrier remains elusive and there are so far no non-human cell culture models that support HCMV replication. The Chinese tree shrew (*Tupaia belangeri chinensis*) is a small laboratory animal and evolutionary closely related with primates. We investigated the susceptibility of primary tree shrew dermis fibroblasts (TSDF) to HCMV infection. Infection with a GFP-expressing HCMV virus resulted in green fluorescence in infected cells with the expression of IE1, UL44 and pp28. The titers of cell-free viruses reached 10^3 PFU/mL at 96 hpi, compared to titers of 10^4 PFU/mL observed in primary human foreskin fibroblasts. Our results suggested that TSDF was semi-permissive for HCMV infection. The TSDF model could be further used to investigate key factors influencing cross-species multiplication of HCMV.

DOI: 10.1002/eji.201970400

P2094

Tree shrew as a new animal model for the study of dengue fever

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Tree shrew, a small primate-like animal belongs to the family Tupaiidae. Phylogenetically, it is evolutionally closest to primate than rodents at whole genomic level. Tree shrew animal models have been established in several disease studies, such as HCV, HEV and glioblastoma. This study aims to develop a new animal model for dengue infection using tree shrews. 45 healthy Chinese tree shrews were intravenously (IV) and subcutaneous multi-point injection with dengue virus (DENV) serotype 2 or 3 at 1×10^4 PFU/animal. Dengue fever (DF) developed 2 to 33 days after infection in 8 of 36 animals (△2℃> Normal body temperature). Blood chemistry appeared to be normal with exception of aspartate transaminase, alanine aminotransferase and alkaline phosphatase, which peaked at 12 days after infection. Viremia peaked from the 2nd to the 7th day, and neutralizing titer peaked at 7 to 15 days after infection. Viral copies could be detected in the liver and brain after 60 days. The tree shrews showed similar manifestations of disease to human, for example, fever, viremia and elevated glutamate transaminase. These findings are believed to provide a basis for the future to establish a mature dengue animal model, which benefit for in-depth understanding of the pathogenesis and the development of effective drug and vaccines of dengue.

P2095

Evaluation of the immune status against the Hepatitis B virus of a population living in Senegal

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In this study, we aimed to evaluate the immune status against the hepatitis B virus (HBV) in a population living in Senegal.
This is a prospective, descriptive and analytical study that took place in June 2018 at the Medical Biology Laboratory of the General Hospital Grand Yoff (LBM-HOGGY). The inclusion criteria were the presence of patients at LBM-HOGGY for HBV Ag testing. In HBs Ag-positive subjects, HBe antigen (HBe Ag) and anti-HBe antibody (Anti-HBe Ab) were investigated. Anti HBsAg were titrated in HBsAg negative individuals. A microparticular immunological technique was used as analytical method. Data was processed using the software Stat View version 5.0.
This study included 97 patients (71% were women). The median age at the start was 30 years with extremes of 3 and 79 years. The most represented age group was 20 - 29. The prevalence of HBsAg carriage was 15.46%. No significant association was found between the risk factors studied and the carriage of HBsAg. Among the HBs Ag-positive patients, none expressed HBe Ag in their serum, the marker of active multiplication of the virus; however, all of them were Ac anti-HBe positive.
Considering the study population, 41.23% were unprotected (HBsAg < 10 mIU/ml) whilst 43.29% HBV remained protected (HBsAg > 10 mIU/ml).
Our data have shown a high prevalence of HBV infection in the study population as well as a large percentage of unprotected subjects. Therefore, vaccination remains the best means of prevention against HBV
Keywords: HBV - Serological markers - Immune status - Senegal

DOI: 10.1002/eji.201970400

P2096

HIV-1 infected individuals are at a higher risk of HPV 16 associated oral and oropharyngeal squamous cell carcinoma than HPV 18

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Background: Recent studies suggest HIV-infected individuals have an overall oral HPV DNA prevalence between 20% and 45% (in the alpha genus), and an oncogenic oral HPV DNA prevalence between 12% and 26%. This study aimed at determining the prevalence of HPV 16, 18 and factors associated with oral and oropharyngeal squamous cell carcinoma (OPSCC) among HIV-1 infected patients attending Mulago Hospital, Uganda 2010-2015 in comparison to HIV-1 uninfected individuals.
Methods: This was a cross sectional study in which 174 HPV DNA positive tissue blocks diagnosed with oral and oral pharyngeal squamous cell carcinoma were stained with Hematoxylin and Eosin (H&E) to confirm the diagnosis. They were genotyped for HPV 16, 18 using conventional SPF10 PCR techniques. The study site was Mulago Hospital, Uganda.
Results: 200 tissue blocks were diagnosed with Squamous cell carcinoma, 174 were positive for HPV DNA. HPV genotypes 16 and 18 were identifiable in 165 cases: HPV 16 occurred in 92 cases (52.8%) and HPV 18 occurred in 41 cases (41.95%). The overall HPV prevalence was 73.16%. HIV was the only risk factor associated with HPV 16 (p=0.018).
Conclusion: We confirmed a role of HPV16 and 18 in OPSCC pathogenesis in HIV-1 infected individuals in the Ugandan population. The results suggest HPV16 is an etiological factor responsible for the high occurrence of oral and oral pharyngeal squamous carcinoma among HIV-1 infected people in this population.

P2097

Intrinsic and extrinsic cellular regulation of influenza-specific CD8+ T cell biology via CISH

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CISH is a member of the SOCS family of proteins. SOCS proteins are intracellular negative regulators of JAK/STAT signalling. To date, limited studies have investigated the direct cellular contribution of CISH to infectious disease. Using *Cish-/-* mice, we establish that CISH has a significant role in intrinsic and extrinsic regulation of $CD8^+$ T cell responses to influenza A virus (IAV) infection. Infection in the absence of CISH results in enhanced recovery mediated by an elevated IAV-specific CD8+ T cell response. This is supported by intrinsic modification of the proliferative capacity of *Cish-/-* $CD8^+$ T cells. Changes in homeostatic $CD8^+$ T cell proliferation and clonal expansion of effector $CD8^+$ T cells is likely associated with CISH regulation of IL-2 cytokine receptor signalling. In conjunction with the intrinsic regulatory function of CISH in CD8+ T cells, we also observed significant changes in conventional dendritic cell (cDC) development and tissue distribution particularly in the lung. CISH regulation of cDC development contributes to increased numbers of activated lung-resident cDCs following infection. Yet, CISH also appears to directly regulate co-stimulatory molecule expression post antigen exposure. We observed increased expression of CD80 and CD86 following IAV stimulation in *Cish-/-* cDCs. Robust activation led to increased trafficking of *Cish-/-* lung-resident cDCs to the lymph node followed by increased activation of lymph node-resident cDCs. Our work highlights that the absence of Cish results in increased expansion of IAV-specific $CD8^+$ T cells mediated by

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DOI: 10.1002/eji.201970400

intrinsic regulation of IL-2 cytokine receptor signalling and extrinsic regulation of cDC development and activation.

P2098

PD-1^{hi} exhausted-like CD8^{+}resident memory T cells balance immunity and fibrotic sequela

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CD8^{+}tissue-resident memory T cells (T_{RM}) provide frontline immunity in mucosal tissues. The mechanisms regulating CD8^{+}T_{RM}maintenance, heterogeneity, protective and pathological functions are largely elusive. Here we identify a CD8^{+}T_{RM}population that is maintained by MHC-I and B7 signaling following acute influenza virus infection. These T_{RM}co-exhibit exhausted-like phenotypes and memory features yet provide heterologous immunity against secondary infection. PD-L1 blockade at the memory stage promotes the rejuvenation of the exhausted-like $T_{RM}$and protective immunity against secondary heterologous influenza virus infection, but at the cost of developing post-infection fibrotic sequelae. Thus PD-1 serves to limit pathogenic capacity of T_{RM} following acute respiratory viral infection. Increased numbers of CD8^{+}T_{RM}were observed in the lungs of pulmonary fibrosis patients compared to control patients. Therefore T_{RM}exhaustion is the result of a tissue-specific cellular adaptation that balances fibrotic sequelae with protective immunity.

P2099

Does living in the rural environment protect children against rhinovirus infections? A study from South Africa

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Allergens and infections by rhinovirus (RV), one frequent cause of common cold, are major factors triggering wheezing/asthma attacks in children. However, there are currently no serological tests available which would allow detecting specificities of antibody responses against RV epitopes as a result of infection. We, therefore, developed a high resolution antibody assay based on micro-arrayed recombinant proteins and peptides from the most common RV strains and demonstrated that extremely small sample volumes were sufficient to detect RV-specific IgG antibodies to a broad panel of micro-arrayed RV antigens and that it was possible to discriminate between group- and partially strain-specific antibody responses. In this study, we used the “PreDicta” chip to analyze RV-specific antibody responses in serum samples from 167 Black African children aged 12-36 months, who were either recruited from randomly selected creches in the Cape Town metropole (n=72) or from the areas surrounding 10 community health clinics in the rural Mqanduli district of the Eastern Cape (n=95). We found IgG antibody levels specific not only for RV-A and RV-C species, but also for RV-B, which had not been previously observed in children from European countries. Furthermore, RV-specific IgG antibody levels were significantly higher in urban children compared to children from the rural areas. No relevant differences with regard to age, gender and allergic sensitizations were found between the urban and rural groups. Our data thus suggest that living in the rural environment might protect children from recurrent RV infections and subsequently from virus-induced wheezing attacks.

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DOI: 10.1002/eji.201970400

P2100

IFITM3 rs12252 genotype is associated with IFITM3 expression levels and immune responses

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The rs12252-*CC* genotype of the viral restriction protein IFITM3 increases the risk of severe influenza infection. *IFITM3* rs12252-*CC* patients have a higher level of pre-existing antibodies to pandemic H1N1 influenza compared to -*TT* donors, suggesting that rs12252-*CC* patients produce stronger immune responses. How this synonymous change alters IFITM3 function to effect immune responses is unclear.Combining RNA and protein data from cell lines and primary cells we have determined the effect of *IFITM3* rs12252 genotype on IFITM3 expression levels and immune responses. Using a CRISPR-generated clones of cell lines and primary cells from different genotypes, we find lower expression of IFITM3 in -*CC* cells, both at a protein and mRNA level. Analysis of heterozygote (-*CT*) patients shows a clear bias towards overexpression of the T allele. We find a differential interferon-stimulated gene signature in rs12252-*CC* patients compared to -*TT*. We also find that a subset of interferon stimulated genes are highly correlated to IFITM3 expression. From a large cohort of Gambian children vaccinated with Live Attenuated Influenza Vaccine, we find that in -*CC*donors there is more viral shedding at day 7 compared to -*TT* and stronger $CD4^+$ T cell responses to H1N1 in -*CC*. *IFITM3* rs12252-*CC* genotype is associated with reduced expression of IFITM3, compromising its viral restriction function. Lower expression could lead to reduced expression of other interferon stimulated genes, further lowering the cell's defences against viruses. The induction of stronger immune responses may correlate with increased severity of infection.

P2101

Role of gamma delta (γδ) T cells in human metapneumovirus infection

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Gamma delta (γδ) T cells are 'unconventional' small subgroup of T cells in the circulation and are distinct with the expression of γδ T cell receptor (TCR). Our previous studies have demonstrated that human metapneumovirus (HMPV) infection in mice induces the recruitment of γδ T cells into the lungs and that these cells were regulated by neutrophils. Furthermore, absence of γδ T cells during HMPV infection led to significantly reduced lung inflammation, demonstrating the important role played by γδ T cells during HMPV induced inflammatory responses. Those findings led to us to hypothesize that γδ T cells play a crucial role in HMPV-induced pathogenesis. In further understanding the role of γδ T cells during HMPV infection, we assessed their effect on lung viral replication, as well as the

DOI: 10.1002/eji.201970400

recruitment of immune cells and the cytokine/chemokine production in the lung. Our findings demonstrate that absence of γδ T cells altered lung cytokine/chemokine profile and reduced infiltration of neutrophils into the lung. Hence we observed that γδ T cells and neutrophils act interdependently during lung inflammatory response to HMPV infection. However, lack of γδ T cells did not have any effect on viral replication, suggesting their role in lung inflammation but not viral clearance. Overall, these findings indicate that γδ T cells play an important role in HMPV-induced inflammatory responses and further our knowledge on the role of these cells in respiratory viral infections.

P2102

Suppression of type I IFN production by Middle-East respiratory syndrome coronavirus ORF8b accessory protein through inhibition of HSP70-dependent activation of IKKε

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Middle-East respiratory syndrome coronavirus (MERS-CoV) is another human coronavirus causing severe disease in addition to severe acute respiratory syndrome coronavirus. A robust IFN response is not induced by MERS-CoV, indicating that it has developed countermeasures to evade host innate immunity. Here we report on the type I IFN antagonism of MERS-CoV ORF8b accessory protein. Anti-ORF8b antibodies were raised to show the abundant expression of ORF8b in MERS-CoV-infected cells. ORF8b-deficient MERS-CoV was constructed and compared with wild-type virus for replication dynamics and type I IFN induction patterns. This mutant virus was capable of inducing IFN-β more robustly. Enforced expression of ORF8b resulted in the suppression of IRF3-dependent induction of IFN-β production by Sendai virus and poly(I:C). Mechanistically, ORF8b operated prior to the phosphorylation and activation of IRF3. It interacted preferentially with chaperone protein HSP70 and prevented it from interacting with and activating IKKε that phosphorylates IRF3. In other words, ORF8b competed with IKKε for interaction with HSP70. Overexpression of HSP70 reversed the suppression of IFN-β production by ORF8b and this effect required full activity of IKKε. Our findings suggest an essential role for HSP70 chaperon in the activation of IKKε activation, which is suppressed by MERS-CoV accessory protein ORF8b. Supported by Hong Kong Research Grants Council (T11-707/15-R).

LB117

Heme oxygenase-1 induction modulates dendritic cell function in response to herpes simplex virus infection in vitro

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Herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) are highly prevalent worldwide and induce both, mild and severe disease. The most common clinical manifestation caused by these viruses is cutaneous lesions, although most individuals are asymptomatic. HSVs are capable of negatively modulating the host antiviral response and establishing persistence and latent infection. Importantly, these viruses interfere with dendritic cell (DC) function, which are key immune cells necessary for mounting and modulating effective adaptive immune responses. Recently, we reported that the host

DOI: 10.1002/eji.201970400

enzyme heme oxygenase-1 (HO-1) reduces HSV infection in epithelial and neuronal cells *in vitro*. HO-1 is normally induced in response to stress stimuli to counteract cell damage provoked by reactive oxygen species, among others, and catalyzes the degradation of iron-containing free heme into biliverdin, carbon monoxide and ferrous iron, all of which display cytoprotective effects. Here, we assessed whether HO-1 expression in DCs positively modulates their function, such as the capacity to activate naïve T cells after HSV infection. Our results indicate that HO-1 expression in DCs recovers some of their functions, despite poor maturation. Interestingly, HSV-infected DCs expressing HO-1 elicited FOXP3+ virus-specific $CD4^{+}$T cells. Taken together, our findings suggest that HO-1 expression in DCs infected with HSVs may promote an anti-inflammatory response to these viruses.

LB119

TRABD2A restricts HIV-1 progeny production in resting CD4+ T cells

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Resting CD4+ T cells are highly resistant to Human Immunodeficiency Virus type 1 (HIV-1) progeny production. It is elusive how resting CD4+ T cells restrict HIV-1 progeny production and release in the late viral replication phase of infection. We report that cell membrane metalloprotease TraB domain-containing protein 2A (TRABD2A) is responsible for inhibition of HIV-1 progeny production in resting CD4+ T lymphocytes by directly cleaving Gag polyprotein precursor. TRABD2A depletion or inhibition of metalloprotease activity profoundly enhanced HIV-1 release from resting CD4+ T cells. TRABD2A expression was higher in resting than activated CD4+ T lymphocytes and dramatically reduced by activation. Delivery of TRABD2A reinforced activated CD4+ T cells restriction to HIV-1 progeny production.In addition, human TRABD2B as well as chimpanzee TRABD2A strongly inhibited HIV-1 infectivity, suggesting a conserved inhibitory mechanism among TraB family proteins in primates. The TraB family also restricted HIV-1 CXCR4, HIV-1 CCR5, HIV-2 and SIVmac239 virion infectivities. Resting CD4+ T cells exhibited viral progeny production and release when treated with Co2+ and Ni2+ to inhibit TRABD2A protease activity. We propose that metalloprotease TRABD2A cleaves polyprotein Gag at the plasma membrane to restrict HIV-1 progeny assembly, production in resting CD4+ T lymphocytes. These results not only elucidate the molecular mechanism resting CD4+ T employ to potently restrict HIV-1 progeny assembly and production but also suggest a new strategy for anti-HIV therapeutics as well as a novel experimental technique for allowing HIV-1 to complete its replication cycle in resting CD4+ T cells.

LB120

USP5 negatively regulates antiviral innate immunity by targeting IRF3

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Innate immunity is the first line of host cells against virus invasion. Type I interferons and its downstream antiviral genes are the core of the innate immune response following viral infection. However, the excessive production of IFN-I leads to the occurrence of many autoimmune diseases and autoinflammatory diseases. Therefore, the expression and activation of IFN-I need to be precisely regulated. It has been established that post-translational modifications, particularly ubiquitination modifications, play key roles in modulating IFN-I production and IFNAR downstream antiviral signaling. Deubiquitinases (DUBs) are a class of proteases related to ubiquitination that reverse the ubiquitination of substrate proteins and regulate cell function. During antiviral innate immunity, there

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DOI: 10.1002/eji.201970400

are many DUB molecules involved in the regulation of antiviral immunity haven't been discovered. We found a deubiquitinase USP5 was significantly downregulated in the cells infected with VSV, SeV, and WSN. USP5 overexpression, knockdown, and knockout experiments demonstrated that USP5 significantly promotes the replication of VSV, SeV and WSN by inhibiting the production of IFN-I. Finally, we proved that IFN-I regulates the expression of USP5 which simultaneously inhibits the expression of IFN-I via specific deubiquitination of K48-linked IRF3, thus suppressing its nuclear translocation after activation and promoting infection by RNA viruses. In summary, our study demonstrated that IFN-I is involved in regulating the expression of USP5, and USP5 can precisely regulate IFN-I and participate in the antiviral immune response. This study has described the role of DUB in antiviral innate immunity, provided potential targets for the treatment of autoimmunity and infectious diseases.

LB126

An inflammatory status in the brain causes behavioral alterations after infection by the human respiratory syncytial virus

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The human respiratory syncytial virus (hRSV) is the most common infectious agent that affects children before two years of age. Outbreaks due to hRSV cause a significant increase in hospitalizations during the winter season associated with bronchiolitis and pneumonia. Despite the devastating effects of this virus, an effective vaccine has not been approved yet. Recently, neurologic alterations have been associated with hRSV infection in children, which include seizures, central apnea, and encephalopathy. Also, hRSV RNA has been detected in cerebrospinal fluids (CSF) form patients with neurological symptoms. Furthermore, previous work demonstrated that hRSV can be detected in lungs and brain of mice exposed to the virus. The effects of hRSV infection within the central nervous system (CNS) are unknown. In this work using a murine model of hRSV-infected mice, we show that hRSV infection cause the permeability alteration of the blood brain barrier (BBB) which allow the immune cell infiltration and the expression of pro-inflammatory cytokines in the CNS. Additionally, we demonstrate that the virus infects murine astrocytes *in vivo* and *in vitro*. Murine astrocytes hRSV-infected presented an increased production of nitric oxide (NO) and TNF-α. hRSV infection caused an acute and chronic behavior impairment (up to two months) and altered expression of cytokines such as IL-4, IL-10 and CCL2. This work suggests that hRSV-infection can impair the proper CNS function and induces local inflammation. Furthermore, this study provides a better understanding of the neuropathy caused by hRSV in humans and the possible detrimental consequences on behavior.

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DOI: 10.1002/eji.201970400

P2812

Hantaan virus induced ER stress in differentiated THP-1 cells

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Background: Since ER stress plays an important role in viral infection and immune escape, there are few reports of HTNV-induced ER stress and apoptosis.
Objectives: We explored whether HTNV infection could induce ER stress in differentiated THP-1 (dTHP-1) cells to reveal the pathogenic mechanism of HTNV infection.
Methods: First, the dTHP-1 cells were infected with HTNV and detected by immunofluorescence. Real-time quantitative PCR was performed to detect mRNA expression levels of glucose regulatory protein (GRP78), Glucose-regulated protein 94(GRP 94), X-box binding protein 1 (XBP1s), activative factor factor 6(ATF6), PKR-like ER kinase(PERK), inositol-requiring enzyme1(IRE1) and C/EBP homologous protein (CHOP). Western blot was used to detect the response of ER stress marker protein GRP78. Annexin V-FITC /PI apoptosis assay kit and flow cytometry were used to detect the apoptosis status of dTHP-1 cells 12h after infection with 100TCID50 HTNV.
Results: It showed that the mRNA and protein levels of GRP78 and mRNA level of XBP-1, ATF6 and PERK after HTNV infection were significantly higher than that in uninfected control group. However, the mRNA level of CHOP, GRP 94 and IRE1 was not significantly different between the infected group and the untreated group in 2h after virus infection. Meanwhile, dTHP-1 cells infected with HTNV at 12h did not show obvious apoptosis.
Conclusion: These results indicated that the HTNV infection could induce ER stress in dTHP-1 cells, but not directly lead to cell apoptosis during 12 h after virus infection.
Keywords: HTNV, HFRS, ER stress, GRP78, dTHP-1

P2813

MOV10 sequesters the RNP of influenza A virus in the cytoplasm and is antagonized by viral NS1 protein

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MOV10 has emerged as an important host antiviral factor. MOV10 not only inhibits various viruses including human immunodeficiency virus type 1, hepatitis C virus and vesicular stomatitis virus, it also restricts the activity of retroelements LINE-1, Alu, SVA and IAP. Here we report that MOV10 suppresses influenza A virus infection through interacting with viral nucleoprotein, sequestering viral RNP in the cytoplasm and causing the degradation of viral vRNA. This antiviral activity of MOV10 depends on the integrity of P-bodies. We also found that the antiviral activity of MOV10 is partially countered by viral NS1 protein that interferes with the interaction of MOV10 with viral nucleoprotein and causes MOV10 degradation through the lysosomal pathway. Moreover, NS1 defective IAV is more susceptible to MOV10 restriction. Our data not only expand the antiviral spectrum of MOV10, but more importantly reveal the NS1 protein as the first viral antagonist of MOV10.

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DOI: 10.1002/eji.201970400

P2814

CRISPR/Cas9 inhibits multiple steps of HIV-1 infection

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CRISPR/Cas9 is an adaptive immune system that bacteria and archaea have evolved to resist the invading viruses and plasmid DNA by creating site-specific double-strand breaks in DNA. In this study, we have tested this gene editing system in inhibiting HIV-1 infection by targeting the viral long terminal repeat and the gene coding sequences. We observed strong inhibition of HIV-1 infection by Cas9/gRNA, which resulted not only from the insertions and deletions (indels) that were introduced into viral DNA due to Cas9 cleavage, but also from the marked decrease in the levels of viral cDNA products and the integrated viral DNA. This latter defect might have reflected the degradation of viral DNA that has not been immediately repaired after Cas9 cleavage. We further observed that Cas9, when is solely located in the cytoplasm, inhibits HIV-1 as strongly as the nuclear Cas9, only that the cytoplasmic Cas9 does not act on the integrated HIV-1 DNA, thus cannot be used to excise the latent provirus. Together, our results suggest that Cas9/gRNA is able to target and edit HIV-1 DNA both in the cytoplasm and in the nucleus. The inhibitory effect of Cas9 on HIV-1 is attributed to both the indels in viral DNA and the reduction in the levels of viral DNA.

P2815

CCL3 exacerbates enterovirus 71-induced acute brain injury by interactions with neutrophils

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Enterovirus 71 (EV71) infection has become a major threat to infants and young children worldwide, which is responsible for critical and fatal cases of hand, foot and mouth disease (HFMD) that are characterized by acute brain injury including common brainstem encephalitis. The cytokine storm has been confirmed in the process of severe EV71 infection, but its exact contribution and underlying mechanism to the progression of HFMD remains unclear. In this study, the CCL3 level was markedly increased in severe EV71-infected patients and mice, which is expressed by neutrophils and regulated mostly through the PI3K-AKT-NFκB signaling pathway. Deletion and depletion experiments verified that furious CCL3 exacerbated the clinical symptoms and central nervous system damages of EV71-infected mice by recruiting and activating neutrophils. Based on the results from experiments in which antibody-mediated depletion of neutrophils led to a marked amelioration of the clinical symptoms in EV71-infected mice, CCL3 exacerbated EV71 infection by interactions with brain-infiltrated neutrophils through CCR5 in mice. Collectively, our data uncover an indispensable role of CCL3 in EV71-induced acute brain injury and provide a potential diagnostic and therapeutic target for human EV71 infection.

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DOI: 10.1002/eji.201970400

P2816

The long noncoding RNA lnc-NONH upregulates miR-34c-5p and Tas expression to promote the early transcription of human foamy virus.

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Background: Human foamy virus (HFV) is a complex and unique retrovirus with the longest genome among the retroviruses and is used as a vector for gene therapies. The viral Tas protein transactivates the viral long terminal repeat (LTR) promoter and is required for viral replication. We have utilized RNA sequencing (RNA-seq) to characterize and identify the long noncoding RNA (lncRNA) NONHSAG000101 (lnc-NONH), which markedly increases in HFV-infected 293T cells. However, little is known about the function of lnc-NONH.
Objectives: We aim to explore the role of lnc-NONH during HFV infection.
Methods: To assess the lnc-NONH role during HFV infection, the siRNAs were used to silence the lnc-NONH expression. The microRNA (miRNA) mimic and inhibitor were employed to explore the function of lnc-NONH-related miRNA miR-34c-5p. Quantitative real-time polymerase chain reaction (qRT-PCR) assay and Western blotting were applied to measure the mRNA and protein levels of HFV transactivator Tas. Luciferase assay was used to determine the transcriptional activity of the HFV unique internal promoter (IP). Western blotting was used to detect the autophagy markers P62 and LC3.
Results: The interaction between lnc-NONH and miR-34c-5p enhances the transcriptional activity of the HFV IP. Moreover, increased lnc-NONH is involved in the induction of autophagy in 293T cells, indicating the multiple function of lnc-NONH.
Conclusions: In the current study, we report a novel mechanism for the lnc-NONH-mediated upregulation of Tas expression. Our findings contribute to the understanding of regulatory network of Tas expression and HFV replication.

P2817

Effects of interaction between DENV-2-infected HUVECs and macrophages on expression of adhesion molecules

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The severe pathological manifestation of Dengue virus (DENV) infection is dengue hemorrhagic fever / dengue shock syndrome (DHF/DSS), which is proposed to cause endothelial dysfunction resulting in increased vascular leakage. However, little is known about the effects of DENV-infected macrophages interaction with DENV-infected primary human umbilical vein endothelial cells (HUVECs) on adhesion molecules (ICAM-1, VCAM-1, E-selectin) in predicting the severity of DENV infection. In this study, in vitro DENV-2-infected HUVECs co-cultured with DENV-2-infected human monocyte-derived macrophages were included, and the level of DENV-2 NS1, ICAM-1, VCAM-1, E-selectin were determined at 4h, 8h, 12h, 24h, 48h and 72h. The results indicated that the expression of DENV-2 NS1, ICAM-1, VCAM-1 and E-selectin mRNA increased rapidly to peak at 24h then declined in DENV-2 infected HUVECs co-cultured with DENV-2-infected macrophages. Moreover, the level of soluble ICAM-1 and E-selectin reached peak at 24h then deceased in culture supernatants of infected-HUVECs with infected-macrophages, but the secretion of soluble VCAM-1 were not detected. In conclusion, DENV-2-infected macrophages promote upregulation of DENV-2 NS1 mRNA and

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DOI: 10.1002/eji.201970400

adhesion molecules of infected-HUVECs, these parameters may correlate with impairment of endothelial cells to induce vascular leakage. It is significant for further understanding of the mechanism of DENV-infected macrophages how to enhance adhesion molecules of DENV-infected HUVECs.

P2818

TRIM21 promotes respiratory syncytial virus (RSV) replication by interferon-independent mechanisms in A549 cells

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Respiratory syncytial virus (RSV) is one of the most common pathogens causing viral pneumonia in children. The Tripartite motif (TRIM) protein, which belongs to the E3 ubiquitin ligase family, play important and controversial roles in innate antiviral immune. Transcriptome analysis showed that RSV infection increased a variety of TRIM proteins expression, including TRIM21. In this study, we aim to survey the role of TRIM21 in type Ⅰ IFN regulation and RSV replication. qPCR and western blot analysis showed that RSV increased TRIM21 mRNA and protein expression in A549 cells. Immunofluorescence and Western blot analysis of cytoplasmic and nucleoprotein were used to determine TRIM21 subcellular localization and the result showed that it is distributed in the cytoplasm. qPCR and dual luciferase reporter assay showed that overexpression of TRIM21 could increase IFNβ and ISGs gene expression and IFNβ promoter activity in presence or absence of RSV, and transcriptional silencing of TRIM21 gene decreased their expression. Exogenous IFNβ increased TRIM21 expression in presence or absence of RSV, indicating TRIM21 maybe act as an ISGs gene. Interestingly, TRIM21 can promote RSV gene expression and viral titers accompanied with IFNβ increasement. Furthermore, we found that TRIM21 also promotes RSV replication in IFN deficient Vero cells, suggesting that TRIM21 promotes RSV replication in an interferon-independent way. **These results indicates that RSV infection increases TRIM21 expression, which is important for efficient RSV replication and that the effects of TRIM21 on promoting RSV replication prevails over its role in antagonizing virus through type Ⅰ IFN system.**

P2819

Novel role of autophagic adaptor p62 in antiviral innate immunity by inhibiting PP2A-mediated dephosphorylation of TBK1

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The scaffold or adaptor protein,p62(also known as sequestosome1,SQSTM1), functions as a hub for various signaling pathways, including autophagy and innate immunity. Previous studies have shown that p62 regulates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation by interacting with multiple factors. In addition, p62 is phosphorylated by critical kinase protein TANK-binding kinase 1 (TBK1), which is essential for autophagic clearance to restrict bacteria invasion; however, the role of p62 in virus-triggered innate immunity has not been fully elucidated. In this study, we found that p62was involved in the RNA virus-induced type I interferon (IFN) pathway. Silencing p62 reduced TBK1 kinase activity, which in turn impaired IFN regulatory factor3 (IRF3) activation and IFN-β production. Further studies showed that the knock down of p62 enhanced protein phosphatase2A (PP2A)-induced dephosphorylation of TBK1 Ser^{172}. Moreover, p62 interacted with TBK1 in multiple

DOI: 10.1002/eji.201970400

regions, including kinase activity-related domains. Lastly, the knockdown of p62 promoted virus replication probably due to the impairment of the host antiviral immune response. In conclusion, our study suggests that p62 maintains antiviral innate immunity by stabilizing TBK1 kinase activity, and thus, providing mechanistic insights into the physiological function of p62 ininnate immunity.

P2820

Immunogenicity of influenza vaccine in elderly people: a systematic review and meta-analysis of randomized controlled trials

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Background: Older people are particularly vulnerable to the adverse consequences of influenza in the short- and long-term, but it is unclear whether their antibody response is lower than that of adults. We thus did the meta-analysis to assess the immunogenicity of influenza vaccines in the two age groups.
Methods: We conducted a broad literature retrieve to identify relevant studies: the randomized controlled trials (including older adults sample) assessed the immunogenicity of inactivated quadrivalent influenza vaccine that produced in embryonated egg.
Findings: 6 studies were eventually included in our meta-analysis(7976 participants). For seroprotecton rate(SPR), the pooled risk ratios(RRs) were 0.92(95%CI: 0.90-0.94, $p<0.0001$) for A/H1N1 and 0.94(95%CI: 0.90-0.98, p=0.002) for B/Victoria, with the A/H3N2 and B/Yamagata had similar antibody response in the two age groups. For seroconversion rate(SCR), the pooled RRs were 0.84(95%CI: 0.76-0.94, p=0.003) , 0.77(95%CI: 0.66-0.91, p=0.002),and 0.83(95%CI: 0.71-0.96, p=0.02) for A/H1N1, B/Victoria and B/Yamagata respectively, while the antibody responses of A/H3N2 in the two groups were similar. Additionally, we found little variation in antibody responses across virus types and subtypes after influenza vaccination.
Interpretation: The SPR and SCR of older adults were lower than adults for A/H1N1 and B/Victoria, and the elderly had lower SCR for B/ Yamagata, while the two age groups had similar antibody responses for A/H3N2. Secondly, antibody responses to vaccine were not significantly associated with real-world VE. Hemagglutination inhibition assay(HAI) might not fully reflect the vaccine effectiveness of A/H3N2, and valuable additional information may need to further improve the accuracy of HAI.

P2821

PRV infection triggers innate antiviral immune responses via cGAS-STING axis and TLR9-MyD88 axis mediated DNA-sensing signaling pathway

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Pseudorabies virus (PRV) is an important DNA virus that infects numerous species of mammals, including livestock, pets, and wildlife, resulting in devastating disease and great economic and public health losses in the worldwide. However, the identity of the host innate immune sensor is unknown. Here we show that PRV infection elicits the production of type I IFN and inflammatory cytokines in murine *in vivo*. Transcription factors IRF3 and IRF7, and the positive feedback loop mediated by IFNAR1, are required for the induction of type I IFN and inflammatory cytokines. In addition, we found that WT mice were resistant to PRV infection compared with the IFNAR1$^{-/-}$, IRF3$^{-/-}$ and IRF7$^{-/-}$ mice,

DOI: 10.1002/eji.201970400

which was associated with lower viral loads and milder pathology in the brain of WT mice. Furthermore, mice defcient in STING and MyD88 exhibited lower type I IFN and inflammatory cytokines levels and higher viral loads, and are more susceptible to PRV infections. Meanwhile, PRV also induces type I IFN and inflammatory cytokines levels and lower viral loads in wild-type mice, but not in mice lacking cGAS or TLR9, and mice deficient in cGAS and TLR9 were more susceptible to lethality caused by infection with PRV. Therefore, our study indicates that the cGAS-STING axis and TLR9-MyD88 axis play pivotal roles in the host defense against PRV infection in murine *in vivo*.
Acknowledgments: The work was supported by the Gansu Natural Science Funds (17JR5RA325) and the China National Natural Science Funds (31302072, 31372423 and 30871884).

P2822

Enterovirus 71 inhibit the cellular endoplasmic reticulum-associated degradation (ERAD) pathway and hijacks ERAD component p97 to promote its replication

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Endoplasmic reticulum-associated degradation (ERAD) is an important function for cellular homeostasis. The mechanism of how picornavirus infection interferes with ERAD remains unclear. In this study, we demonstrated that enterovirus 71 (EV71) infection significantly inhibits cellular ERAD by targeting multiple key ERAD molecules with its proteases 2Apro and 3Cpro using different mechanisms. Ubc6e was identified as the key E2 ubiquitin-conjugating enzyme in EV71 disturbed ERAD. EV71 3Cpro cleaves Ubc6e at Q219G, Q260S, and Q273G. EV71 2Apro mainly inhibits the de novo synthesis of key ERAD molecules Herp and VIMP at the protein translational level. Herp differentially participates in the degradation of different glycosylated ERAD substrates α-1 antitrypsin Null Hong Kong (NHK) and the Cterminus of sonic hedgehog (SHH-C) via unknown mechanisms. p97 was identified as a host factor in EV71 replication; it redistributed and co-exists with the viral protein and other known replication-related molecules in EV71-induced replication organelles. Electron microscopy and multiple-color confocal assays also showed that EV71-induced membranous vesicles were closely associated with the endoplasmic reticulum (ER), and the ER membrane molecule RTN3 was redistributed to the viral replication complex during EV71 infection. Therefore, we propose that EV71 rearranges ER membranes and hijacks p97 from cellular ERAD to benefit its replication. These findings add to our understanding of how viruses disturb ERAD and provide potential anti-viral targets for EV71 infection.

P2823

Mechanism research on type I IFN production suppression through m^6A modification induced by MERS-CoV NS4b in the nucleus

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MERS-CoV has been posed a serious threat to global public health and social stability because of its high mortality. The lack of understanding of its viral pathogenesis impeded the development of drugs against MERS-CoV. Pathogenic viruses have developed strategies to escape from the host innate immune response. NS4b predominantly localizes to the nucleus and inhibits the induction of the type I IFN in the nucleus. However, the underlying mechanism is poorly understood. Our previous studies

DOI: 10.1002/eji.201970400

have found that NS4b interacts with DDX1 and hnRNP C in the nucleus. It has been reported that DDX46 inhibits innate immunity by entrapping m^6A-demethylated antiviral transcripts in the nucleus. DDX1 and DDX46 may have similar functions due to their similar structures. It has also been reported that hnRNP C is related to m^6A modification of RNA. Thus, how NS4b inhibits the production of IFN through DDX1 and hnRNP C deserves further study. This project aims to clarify the mechanism of NS4b inhibit the generation of IFN through m^6A modification in the nucleus. These studies would be helpful to enrich of our understanding of the pathogenesis of MERS-CoV, further to direct us finding new potential drug targets against MERS-CoV.

P2824

Identification of occult hepatitis B infection in HBsAg-negative/HBcAb-positive people

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Background and aims: Knowledge on occult hepatitis B virus (HBV) infection in serum HBsAg-negative/HBcAb-positive people is still poor. This study aimed to reveal characteristics of intrahepatic HBV total DNA (tDNA), covalently closed circular DNA (cccDNA) and RNA, and serum HBcAb levels in HBsAg-negative/HBcAb-positive people to further the knowledge.
Methodology: A total of 130 adult people were enrolled in this study, including 100 HBsAg-negative/HBcAb-positive individuals without illness history of hepatitis B, twenty with chronic hepatitis B (CHB) as positive control, and ten without any HBV serum markers as negative control. All subjects received liver biopsy. Intrahepatic HBV tDNA, cccDNA and RNA and serum HBcAb levels were quantitated measured. Immune escape-related mutations in major hydrophilic region (MHR) of HBV S gene were sequenced.
Results: The tDNA was detected in 22 HBsAg-negative/HBcAb-positive individuals [0.37 (0.26−1.63) copies/cell] and all 20 CHB patients [103.85 (35.75−231.56) copies/cell]. The cccDNA were detected in 9 of the 22 HBsAg-negative/HBcAb-positive individuals [0.04 (0.02−0.05) copies/cell] and all 20 CHB patients [1.98 (0.95−8.02) copies/cell]. A lower level of HBV RNA was detected from two cccDNA-positive HBsAg-negative/HBcAb-positive individuals. HBcAb was 300.0 (163.4, 442.5) IU/mL in the cccDNA-positive individuals, significantly higher than that in the cccDNA-negative individuals. No significant difference in immune escape-related mutations in the MHR was observed between HBsAg-negative/HBcAb-positive individuals and CHB patients.
Conclusions: HBV tDNA and cccDNA were detectable in some HBsAg-negative/HBcAb-positive individuals and a relatively higher HBcAb level was closely related to cccDNA positivity. Therefore, quantitative detection of HBcAb may help noninvasively identify occult HBV infection from HBsAg-negative/HBcAb-positive population.

P2825

Transduction with lentiviral vectors altered the expression profile of host MicroRNAs

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DOI: 10.1002/eji.201970400

RNA interference (RNAi) is widely used in gene knockdown analysis and as a tool to screen host genes involved in viral infection. Owing to the limitations of transducing cells with synthetic small interfering RNAs (siRNAs), lentiviral short hairpin RNA (shRNA) vectors are more widely used. However, we found that stable transduction with lentiviral shRNA vectors inhibited hepatitis C virus (HCV) propagation in human hepatoma cells. We found by microRNA (miRNA) microarray analysis that this inhibition was induced by the alteration of host miRNA expression. In addition to one miRNA (miR-196b-5p) previously reported to be involved in HCV infection, other miRNAs (miR-216a-5p, -216b-5p, 217, and -30b-5p) were found to influence HCV infection in this study. Further studies suggested that this effect was independent of the transcription of shRNAs. The lentiviral vector itself and the integration site of the lentiviral vector might determine the change in miRNA expression. Moreover, the upregulation of JUN contributed to the dysregulation of miR-216a-5p, -216b-5p, and -217 in stably transduced cells. Although the changes in miRNA expression were beneficial for inhibiting HCV infection in our
study, this off-target effect should be considered when transduction with lentiviral vectors is performed for other purposes, especially in therapy.

P2826

Mutation and recombination of coxsackievirus A2 isolated in patients with hand, foot and mouth disease in Xiangyang city in 2017

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Abstract: Objective To understand the hand, foot and mouth disease (HFMD) associated coxsackievirus A2 (CV-A2) isolates circulating in Xiangyang city in 2017. Methods Clinical samples were cultured in cells. The sequence of CV-A2 cell isolates were amplified by RT-PCR. The VP1 gene homology was analyzed by Megalign. The phylogenetic tree was constructed by MEGA6, and the recombination analyzed with SimPlot software. Amino acid sequence alignment of the VP1 of isolates derived from patients with acute flaccid paralysis, HFMD and respiratory infection (RTI) was performed. Results The phylogenetic tree of the VP1 gene of CV-A2 strains in Xiangyang city, shows that it belongs to genotype D. The nucleotide and amino acid sequence identities of the VP1 were 96.5% to 99.5% and 98.0% to 100.0% among CV-A2 Xiangyang isolates. It was found that differences in amino acid residues existed at positions 21, 60, 82 and 215 between strains with acute flaccid paralysis and strains with HFMD, while there is only one difference at position 167 when strains causing HFMD and RTI were compared. The CV-A2 recombination analysis indicated that the Xiangyang strains were in the same branch in the P1 region as the Fleetwood strain, while it had the highest similarities with the Coxsackievirus A5 Swartz strain in the P2 region, and with the Coxsackievirus A16 G-10 in the P3 region. Conclusion The amino acid sequence analysis of the VP1 gene and the complete genome suggests mutations and interserotype recombination of CV-A2 may result in changes in pathogenicity.

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DOI: 10.1002/eji.201970400

P2827

Immunogenicity and efficacy of Coxsackievirus A16 vaccine candidate formulations in a mouse model

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Objective: To evaluate the immunogenicity and protective effect of CVA16 vaccine candidate strain in a mouse model.
Methods: Different doses of the full virus particles or empty virus particles of CVA16 vaccine candidate strain which were inactivated or not inactivated by formalin were formulated with different adjuvants into vaccine preparations. After immunization, the anti-CVA16 IgG antibody and neutralizing antibody levels in serum of female mice were detected. The pups from immunized female mice were challenged with homologous and heterologous viruses.
Results: It was showed that the L731 vaccine induced robust immune responses in mice. Mouse antisera contained the highest titers of neutralizing antibodies reported to date and prevented cell from infected by prototype A and subtype B2b of CVA16 in vitro. Pups born to immunized mice were protected from disease and death after challenged with 10 000 LD_{50} of homologous and heterologous viruses.
Conclusion: L731 is a promising vaccine candidate that can be used alone or as a component of multivalent or combined vaccines.

P2828

The association between MICA/MICB polymorphism and respiratory syncytial virus infection in children

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MICA/MICB gene polymorphisms are related to several cancers and infectious diseases, but there are no reports on the association between MICA/MICB gene polymorphisms and respiratory syncytial virus (RSV) infection. To clarify the association between MICA/MICB gene polymorphisms and infection of RSV in children, we collected fresh blood samples from paediatric patients with and without pneumonia after RSV infection. The MICA/MICB alleles were characterized by PCR sequence-specific primers (PCR-SSP) and PCR sequence-based genotyping (PCR-SBT), and then, the frequency of the MICA/MICB alleles and haplotypes was calculated. The results showed that the frequencies of MICA*002:01 and MICA-A9 in RSV-infected patients were significantly lower than in controls (9% vs. 20%, pc = 0.04). The allele frequency of MICA*002:01 in pneumonia patients (8%) and nonpneumonia patients (9%) was significantly lower than in controls (20%, pc = 0.02). MICA*002:01-MICB*008(Δrel = 0.616), MICA*009-MICB*016 (Δrel = 0.506), and MICA*045-MICB*014 (Δrel = 0.700) showed linkage disequilibrium in patients infected with RSV. The haplotype frequency of MICA*002:01-MICB*005:02 in RSV-infected patients was significantly lower than in controls (10% vs. 16%, pc = 0.033). In conclusion, allele MICA*002:01/A9 and haplotype MICA*002:01-MICB*005:02 were negatively associated with RSV respiratory tract infections.

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DOI: 10.1002/eji.201970400

P2829

Research progress on the prevalence and control of avian leukosis in China

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The exogenous viruses of Avian leukosis virus(ALV) have been prevalent and have caused huge economic losses and damage in the poultry markets of China. ALV could be transported into China with the importation of the white feather broilers and it has expanded its scope of infection by generations via vertical transmission of breeding eggs, as a result, it has spread widely in different kinds of hens and broiler chickens. While in many investigations of local varieties it has been found that ALV strains has constantly mutated, chicken is still the natural host of the ALV as it has not been detected in the tested many kinds of poultry so far. In the study of ALV inducing tumors, immunosuppression and growth retardation, ALV has been found closely connected with mircoRNA and signaling pathway. It often occurs that different subgroups of Avian leukosis can be isolated from the same one clinical specimen, from which we concluded that ALV can have co-infection with other viruses and we have found that co-infection cause more serious symptoms and pathological changes. Various kinds of detective methods have merits and defects and it's the incomplete detection that becomes the insurmountable obstacle for purification work. There is no efficient vaccine for promotion at present. Hard as it is, scientists still continuously spare no efforts to explore variety of preventive treatments. We are supposed to take corresponding techniques to strive to do the purification work aimed at different feeding patterns and scales existing in one area.

P2830

ZAP-70 phosphorylation level of $CD8^+$ T cells as an indicative marker decreased in AIDS patients

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AIDS is a serious global disease caused mainly by HIV-1. $CD8^+$ T cells as a major player of immune cells can eliminate HIV-1. Meeting with HIV-1 triggers $CD8^+$ T cells activated and ZAP-70 phosphorylated to deliver a signal to downstream. ZAP-70 phosphorylation level decreased in AIDS patients compared to healthy donors. While patients receiving HAART present a similar ZAP-70 phosphorylation level to healthy donors. PD-1 normally expresses on activated $CD8^+$ T cells but it highly expresses in AIDS patients in this study. PD-L1 protein binds to PD-1 on $CD8^+$ T cells would promote ZAP-70 phosphorylation level and the correlation of PD-1 expression and ZAP-70 phosphorylation level is not significant. As a result, AIDS patients have lower ZAP-70 phosphorylation level which is an indicative marker for AIDS severity, meanwhile PD-1 expresses highly on $CD8^+$ T cells of AIDS patients but no correlation between them.

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DOI: 10.1002/eji.201970400

P2831

Transcriptome analysis of duck spleen infected with duck enteritis virus

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In order to exploring the impact of infection induced by DEV on transcriptome of duck spleen, this study chose 30 50-day-old healthy ducks balanced by body weight, which were randomly divided into 2 groups. The ducks in test group were inoculated intramuscularly in left leg with DEV of TCID50, while with sterilized normal saline (NS) in control group. After 66 h, 90 h and 114h of inoculation, spleen tissue samples of the infected ducks were collected to extract tissue RNA and sequence transcriptomics by high-throughput RNA-Seq technology, then the differentially expressed gene (DEGs) were screened and analyzed by GO and KEGG bioinformatics, and identified by the real-time PCR technology. The results showed as the follows: the amounts of DEGs were respectively 685, 685 and 580 in the 66h, 90 and 114h of the infected duck spleen, mainly involved in immune response, complement activation, cell adhesion, translation, antigen processing and presentation of peptide antigen via MHC class II and other biological processes, which significantly enriched in ECM-receptor interaction, cell adhesion molecules, ribosome, phagosome, intestinal immune network for IgA production and other signal pathways. And there are respectively 70, 64 and 66 of DEGs corresponding the immune response after inoculated for 66 h, 90 h and 114 h respectively. These provide a basic information for the elucidation of DEV pathogenesis and duck antivirus mechanism.

P2832

Viral replication competence and innate host responses of HAdV-7: an analysis of ex vivo cultures of human lung

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Background: Epidemiologic reports have suggested that most of highly severe or fatal adenoviral diseases in children are associated with HAdV-7 .However, the underlying pathogenesis of HAdV-7 caused more severe disease remain poorly understood.
Methods: During 2014 June to 2018 December, 471 HAdV-positive specimens were collected and available for genotyping by qPCR or Sanger sequencing. Clinical data were collected retrospectively. The virus replication kinetics and host response elicited by HAdV-3 and HAdV-7 in *ex vivo* cultures of human lung were compared.
Results: Of the 471 HAdV-positive specimens, 447 (94.9%) were single HAdV strain infections and 24 (5.1%) were co-infections. The predominant genotype in 2018 was HAdV-7, which differed from 2014 、2015 and 2016,when HAdV-3 was the most prevalent genotype. The incidence of severe pneumonia was higher in HAdV-7(66/219, 30.1%) than in HAdV-3(10/222, 4.5%) ($p< 0.001$) . HAdV-7 caused longer length of hospital stay, higher rates of pleural effusion, dyspnea, mechanical ventilation and higher mortality than HAdV-3 ($p< 0.05$). HAdV-7 replicated more efficiently than HAdV-3 in *ex vivo* cultures of human lung. The levels of IP-10, IL-10, MIP-1α, MIP-1β and MIG in HAdV-7 infection group were higher than those in HAdV-3 infection group.
Conclusions: A marked increase of HAdV-7 infection associated with severe disease was documented among pediatric patients in Guangzhou city, China. The pathogenesis of HAdV-7 virus caused severe disease was most likely associated with viral replication and production of pro-inflammatory mediators.

DOI: 10.1002/eji.201970400

Keywords: Severe infection; HAdV-7; innate immune responses; viral replication

P2833

Antibodies against H1N1 influenza virus cross-react with a-cells of pancreatic islets

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Epidemiological studies have documented that the incidence of human type 1 diabetes was significantly increased after H1N1 epidemic. However, a direct link between human type 1 diabetes and virus infection remains elusive. We generated 84 clones of murine monoclonal antibodies against the H1N1, and carried out immunohistochemistry in normal human tissue microarray. The results showed that two clones specifically cross-reacted with human a-cells of pancreatic islets. Reverse transcription polymerase chain reaction and deoxyribonucleic acid sequencing showed that the amino acid sequences of light and heavy chains of these clones were different. Importantly, the expression profiles of two monoclonal antibodies were individual different. For the first time, we provide direct evidence that monoclonal antibodies against H1N1 can cross-react with human pancreas a-cells, another source of b-cells, suggesting a-cells might be a novel target to be investigated in diabetes research.

P2834

Rabies virus infection induces microtubule depolymerization to facilitate viral RNA synthesis by upregulating HDAC6

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Rabies virus (RABV) is the cause of rabies, and is associated with severe neurological symptoms, high mortality rate, and a serious threat to human health. Although cellular tubulin has recently been identified to be incorporated into RABV particles, the effects of RABV infection on the microtubule cytoskeleton remain poorly understood. In this study, we show that RABV infection induces microtubule depolymerization as observed by confocal microscopy, which is closely associated with the formation of the filamentous network of the RABV M protein. Depolymerization of microtubules significantly increases viral RNA synthesis, while the polymerization of microtubules notably inhibits viral RNA synthesis and prevents the viral M protein from inducing the formation of the filamentous network. Furthermore, the histone deacetylase 6 (HDAC6) expression level progressively increases during RABV infection, and the inhibition of HDAC6 deacetylase activity significantly decreases viral RNA synthesis. In addition, the expression of viral M protein alone was found to significantly upregulate HDAC6 expression, leading to a substantial reduction in its substrate, acetylated α-tubulin, eventually resulting in microtubule depolymerization. These results demonstrate that HDAC6 plays a positive role in viral transcription and replication by inducing microtubule depolymerization during RABV infection.

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DOI: 10.1002/eji.201970400

P2835

Influence of protein prenylation on antiviral innate immune response

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The threat of a pandemic spread of highly virulent influenza A viruses currently represents a top global public health problem. During pathogenic influenza virus infection, robust cytokine production (cytokine storm), excessive inflammatory infiltrates, and virus-induced tissue destruction all contribute to morbidity and mortality. The modification of eukaryotic proteins by isoprenoid lipids, which is known as prenylation, controls the localization and activity of a range of immune cells. Viperin is an interferon-inducible protein that acts as a broadspectrum antiviral protein via the inhibition of farnesyl pyrophosphate synthase (FPPS), but the molecular mechanism remains unclear. Here, we determined that statin and farnesyltransferase inhibitors, but not squalene synthase inhibitor, down-regulated the production of inflammatory factors. Knockout of farnesyltransferase subunit beta dramatically impaired the activation of RIG-I signaling pathway. These results suggest that protein prenylation play a key role in antiviral innate immune response.

P2836

TRIP negatively regulates IFN-β production and antiviral response by promoting proteasomal degradation of TBK1

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TANK-binding kinase 1 (TBK1) plays an essential role in Toll-like receptor (TLR)-and retinoic acid-inducible gene I (RIG-I)-mediated induction of type I interferons and host antiviral responses. TBK1 activation must be tightly regulated to maintain immune balance in the organism. How TBK1 activity is negatively regulated remains largely unknown. Here, we demonstrate that TNF receptor-associated factor (TRAF)-interacting protein (TRIP) promotes proteasomal degradation of TBK1 and inhibits TLR3/4- and RIG-I-induced IFN-β signaling. TRIP knockdown resulted in augmented activation of IFN regulatory factor 3 (IRF3) and enhanced expression of IFN-β in TLR3/4- and RIG-I-activated primary peritoneal macrophages, whereas overexpression of TRIP had opposite effects. Consistently, TRIP impaired Sendai virus (SeV) infection-induced IRF3 activation and IFN-β production and promoted vesicular stomatitis virus (VSV) replication. As an E3 ubiquitin ligase, TRIP negatively regulated the cellular levels of TBK1 by directly binding to and promoting K48-linked polyubiquitination of TBK1. Therefore, we identified TRIP as a negative regulator in TLR3/4- and RIG-I-triggered antiviral responses.

P2837

TRIM38 targets NAP1 to negatively regulate TLR3/4- and RIG-I-mediated IFN-β production and antiviral response

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DOI: 10.1002/eji.201970400

Recognition of RNA virus through TLR and RIG-I-like receptor results in rapid expression of type I IFNs, which play an essential role in host antiviral responses. However, the mechanisms to terminate the production of type I IFNs are not well defined. Here, we identified a member of the tripartite motif (TRIM) family, TRIM38, as a negative regulator in TLR3/4- and RIG-I-mediated IFN-β signaling. Knockdown of TRIM38 expression by small interfering RNA resulted in augmented activation of IFN regulatory factor 3 and enhanced expression of IFN-β, whereas overexpression of TRIM38 had opposite effects. Coimmunoprecipitation and colocalization experiments demonstrated that TRIM38 interacted with NF-κB-activating kinase-associated protein 1 (NAP1), which is required for TLR-induced IFN regulatory factor 3 activation and IFN-β production. As an E3 ligase, TRIM38 promoted K48-linked polyubiquitination and proteasomal degradation of NAP1. Thus, knockdown of TRIM38 expression resulted in higher protein level of NAP1 in primary macrophages. Consistent with the inhibitory roles in TLR3/4- and RIG-I-mediated IFN-β signaling, knockdown of TRIM38 significantly inhibited the replication of vesicular stomatitis virus. Overexpression of TRIM38 resulted in enhanced replication of vesicular stomatitis virus. Therefore, our results demonstrate that TRIM38 is a negative regulator for TLR and RIG-I-mediated IFN-β production by targeting NAP1 for ubiquitination and subsequent proteasome-mediated degradation.

P2838

Studies on molecular design, chemical synthesis and immunogenicity of novel antigenic peptides against the capsid protein of porcine circovirus type 2

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Porcine circovirus associated disease (PCVAD) encompasses a group of syndromes linked to infection with porcine circovirus type 2 (PCV2).This study aimed to synthesize and identify the antigenic peptides of B cell epitope on the capsid protein (CAP) of porcine circovirus type 2 (PCV2). The prediction of B cell epitopes of PCV2 CAP was conducted by DNAStar and BcePred software combining with amino acid average antigenic index method of WU Yu -zhang. A four -branch form of multiple antigen peptide (MAP) was synthesized which was linked a novel promiscuous T helper (Th) site for broad immunogenicity in multiple species. The four-branch MAP was synthesized by Fmoc method and analyzed by RP-HPLC and Mass- spectrum (MS). Then, Outbred rabbit and Kunming mice were immunized with the synthesized MAP, respectively. And their humoral immune response was observed to evaluate the effect of the novel antigens in animals. The amino acids of N-terminal number 98-103, 156-162 and 228-233 were predicted as the most potential epitopes and synthesized as immunogen. RP -HPLC revealed that the purity of synthetic MAP reached to 92.49%. The molecular weight was identified by MS, and the error was less than 0.1%. High titer of anti PCV2 CAP antibody was both obtained in rabbit and mice immunized with the synthesized MAP.
The new epitopes B98, B156 and B228 confirm the MAP of B cell epitope on PCV2 CAP has immunogen effect, which will lay a foundation for the study of antigenic peptide vaccine of PCV2 CAP.

DOI: 10.1002/eji.201970400

P2839

Usp2b negatively regulates IFN-β production and antiviral activity by targeting TBK1

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TANK-binding kinase 1 (TBK1) is essential for IFN-β production downstream of various innate receptors. However, how TBK1 activation is terminated is not well defined. In this study, we showed ubiquitin-specific protease (USP) 2b as a new negative regulator for TBK1 activation. Overexpression of USP2b inhibited RIG-I mediated IFN-β signaling; in contrast, knockdown of USP2b expression by small interfering RNA enhanced RIG-I mediated IFN-β signaling. Coimmunoprecipitation experiments demonstrated that USP2b interacted with TBK1. As a deubiquitinating enzyme, USP2b was demonstrated to cleave K63-linked polyubiquitin chains from TBK1 to inhibit TBK1 kinase activity. Consistent with the inhibitory roles of USP2b on TBK1 activation, knockdown of USP2b significantly inhibited the replication of vesicular stomatitis virus, whereas overexpression of USP2b resulted in enhanced replication of vesicular stomatitis virus. Therefore, our findings demonstrated that USP2b deubiquitinates K63-linked polyubiquitin chains from TBK1 to terminate TBK1 activation and negatively regulate IFN-β signaling and antiviral immune response.

P2840

TRIM26 negatively regulates interferon-β production and antiviral response through degrading nuclear IRF3

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Virus infection leads to the activation of transcription factor IRF3 and subsequent production of type I inteferons, which induce the transcription of various antiviral genes called interferon stimulated genes (ISGs) to eliminate viral infection. IRF3 activation requires phosphorylation, dimerization and nuclear translocation. However, the mechanisms for the termination of IRF3 activation in nucleus are elusive. Here we report the identification of TRIM26 to negatively regulate IFN-β production and antiviral response by targeting nuclear IRF3. TRIM26 bound to IRF3 and promoted its K48-linked polyubiquitination and degradation in nucleus. TRIM26 degraded WT IRF3 and the constitutive active mutant IRF3 5D, but not the phosphorylation deficient mutant IRF3 5A. Furthermore, IRF3 mutant in the Nuclear Localization Signal (NLS), which could not move into nucleus, was not degraded by TRIM26. Importantly, virus infection promoted TRIM26 nuclear translocation, which was required for IRF3 degradation. As a consequence, TRIM26 attenuated IFN-β promoter activation and IFN-β production downstream of TLR3/4, RLR and DNA sensing pathways. TRIM26 transgenic mice showed much less IRF3 activation and IFN-β production, while increased virus replication. Our findings delineate a novel mechanism for the termination of IRF3 activation in nucleus through TRIM26-mediated IRF3 ubiquitination and degradation.

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DOI: 10.1002/eji.201970400

P2841

Cytosolic DNA sensors activation inhibits HIV infection of macrophages

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The cytosolic DNA-sensing pathway is critical in the activation of the IFN-dependent antiviral innate immune response. We here examined whether activating the DNA sensors can inhibit HIV infection of macrophages. We observed that the stimulation of human primary monocyte-derived macrophages with poly(dA:dT) or poly(dG:dC), the synthetic ligands for the DNA sensors, induced the expression of both type I and III interferons (IFNs). In addition, the DNA sensors activation resulted in induction of the multiple intracellular HIV-restriction factors, including IFN-stimulating genes (ISGs: ISG15, ISG56, Viperin, OAS2, GBP5, MxA, MxB and Tetherin) and the HIV restriction microRNAs (miR-29C, miR-138, miR-146a, miR-155, miR-198 and miR-223). In addition, the DNA sensor signaling of macrophages upregulated expression of the CC chemokines (MIP-1α, MIP-1β and RANTES), the ligands for HIV entry coreceptor CCR5. More importantly, poly(dA:dT) or poly(dG:dC)-treated macrophages were more resistant to HIV infection and replication than untreated cells. These observations for the first time demonstrated the importance of cytosolic DNA-sensing signaling pathway in the macrophage innate immunity against HIV. Further studies are necessary in order to confirm that the DNA sensors are a potential target for immune activation-based anti-HIV therapy.

P2842

Data analysis reveals interference of HBeAg in anti-HBe measurement on the Abbott Architect i2000 analyzer

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Background: Quantitative measurement of serum markers play an important role in the diagnosis and treatment monitoring of hepatitis b virus (HBV) infection. In the present study, we analyzed the data from Abbott Architect i2000 immunoassay analyzer and investigate the relationship between HBeAg and anti-HBe.
Methods: Quantitative data of HBV serum markers between January first 2013 and March twenty-eight 2016, which are measured by Abbott Architect i2000, were exported from laboratory information system. Data cleaning and analysis were performed by R language. Records contain unprecise value were omitted and the rest were divided into groups according to the HBeAg state. The relationship between HBeAg and anti-HBe was explored by plotting and linear regression.
Results: Anti-HBe was correlated with HBeAg and linear regression showed R^2 equal to 0.92. Only in HBeAg positive data could this correlation be observed, whereas no pattern was observed from HBeAg negative ones. HBeAg and anti-HBe double positive can onley be seen in low HBeAg samples.
Conclusions: This study suggests that HBeAg partly interfere with the measurement of anti-HBe, which could lead to unprecise results of the patients who are HBeAg and anti-HBe double positive.

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DOI: 10.1002/eji.201970400

P2843

The ubiquitin E3 ligase TRIM31 promotes aggregation and activation of the signaling adaptor MAVS through Lys63-linked polyubiquitination

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Mitochondria function as platforms for innate antiviral signal transduction through the adaptor protein, MAVS. Viral infection could induce a conformational change in MAVS, which leads to the formation of prion-like functional aggregates that provide a sensitive trigger for antiviral signaling. However, the molecular mechanisms that regulate MAVS aggregation are poorly understood. Here, we report a mitochondria-localized protein, the E3 ubiquitin ligase TRIM31, as a positive regulator of MAVS. TRIM31 deficiency attenuated the innate antiviral response to infection with an RNA virus both in vitro and in vivo. TRIM31 interacted with MAVS and promoted K63-linked polyubiquitination on Lys10, Lys311 and Lys461 of MAVS after viral infection. Notably, TRIM31-mediated K63-linked polyubiquitination facilitated the formation of prion-like MAVS aggregates. Thus, our data provides novel insights in the molecular regulation of MAVS aggregation and the cellular antiviral response through TRIM31-mediated K63-linked polyubiquitination of MAVS.

P2844

The HMGB1/RAGE pathway promotes HTLV-1 replication by regulating autophagy

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High-mobility group box 1 protein (HMGB1), which previously was thought to function only as a nuclear factor that enhances transcription, was recently discovered to be a crucial cytokine that mediates the response to viral infection and replication especially carcinogenicity pathogens, such as HBV, HCV, HPV, etc. These observations have led to the emergence of a new field in cancer that is focused on understanding the mechanisms of HMGB1 release, its biological activities and its pathological effects in cancer. Autophagy is an intracellular degradation pathway that provides a host defense mechanism against intracellular pathogens. However, many viruses exploit this mechanism to promote their replication. This study shows that Cell culture supernatant dosing with HMGB1 recombinant protein can enhance cell autophagy and virus replication in MT2 HTLV-1-positive cell MT2. In contrast, HMGB1 neutralizing antibody inhibits cell autophagy and virus replication. The inhibition of autophagic activity by an autophagy inhibitor, 3-methyladenine, reduces the expression of HTLV-1 encoding proteins and the production of viral particles, revealing that autophagy is critical to HTLV-1 progression. But rapamycin does not enhance the autophagy induced by rhHMGB1. The activation involves Atg5 and Beclin-1, the key component of autophagy, but not the mTOR pathway. It shows that the receptor for advanced glycation endproducts (RAGE) and one of its primary ligands, high-mobility group box 1 (HMGB1), are required for optimal signal generator within tumors but not TIM-3 or TLR4. We found that RAGE is present in the cytoplasm of cultured HTLV-1 cell.

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DOI: 10.1002/eji.201970400

P2845

Expression and immunogenicity of severe fever with thrombocytopenia syndrome bunyavirus envelope glycoproteins Gn and Gc

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Severe fever with thrombocytopenia syndrome (SFTS) is caused by the SFTS virus and with a high fatality rate. No specific vaccines or antibodies against the disease were described so far. In our study, the recombinant plasmid, which express SFTS viral envelope glycoproteins rGn and rGc protein were transformed into the BL21 E.coli cells for its prokaryotic expression. The recombinant proteins rGn and rGc were used to immunize rabbits, after three vaccination, the antibody titers against recombinant proteins rGn and rGc could reach 1:512000. ELISA showed that the immune sera had binding affinity with rGn and rGc. *In vitro*, immune sera from immunized rabbits could effectively protect vero cells from viral attack compared with control groups ($P< 0.05$). SFTS virus envelope glycoproteins rGn and rGc has been expressed successfully and possessed high immunogenicity. These data suggest that recombinant protein rGn and rGc are vaccine candidates for future applied researches and potential antigens for monoclonal antibody researches.

P2846

Graphen oxide as “Nano-Trojan Horse” cargoing oncolytic measles virus for retargeted cancer therapy

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Replication-competent oncolytic viruses selectively infect and kill malignant cells, and this process, oncolytic virotherapy, has been proven to be a potent anticancer weapon for clinical therapy. However, pre-existing antibodies in patients may neutralize viruses and limit the oncolytic efficacy of this treatment. Cell carriers have been employed to protect oncolytic viruses from immune clearance and to deliver viruses to targeted tumor loci. Nevertheless, this technique is limited by the fact that carriers would have to be designed specifically and a general agent that's effective for major population is still unavailable. Graphene oxide nanoparticles (GOS) have been found to be potential biological carriers due to their excellent biological compatibility and targeting movement after decoration. In this study, we developed a novel strategy to achieve both virus protections and targeted release in the tumor by encapsulating oncolytic measles virus and conjugating pegylated folate. We loaded oncolytic measles virus (MV-Edm) into PEI and pegylated folate double conjugated GOS (PEI-GOS-PEG-FA) to protect the viral particles from neutralization of anti-virus antibodies in human blood and to efficiently deliver the particles to the tumor mass. We compare PEI-GOS-PEG-FA decorated MV-Edm (GOS/MV-Edm) to nano-trojan horse. We systemically evaluated the practicality, effectiveness and mechanisms of “nano-trojan horse” both in vitro and in vivo. Moreover, we optimized the loading strategy for GOS/MV-Edm, elucidated their infection pathway, revealed their virus protecting and tumor-targeting efficacy, and determined their efficacy and the biodistribution in vivo. This study provided a theoretical basis and a novel strategy for oncolytic viral therapy against malignancies.

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DOI: 10.1002/eji.201970400

P2847

Herpes simplex virus 1 tegument protein UL46 inhibits TANK-binding kinase 1-mediated signaling

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TANK-binding kinase 1 (TBK1) is a key component of antiviral immunity signaling pathway. It activates downstream interferon regulatory factor 3 (IRF3) and subsequent type I interferon (IFN-I) production. Herpes simplex virus type 1 (HSV-1) can antagonize host antiviral immune responses and lead to latent infection. Here, HSV-1 tegument protein UL46 was demonstrated to downregulate TBK1 dependent antiviral innate immunity. UL46 interacted with TBK1 and reduced TBK1 activation and its downstream signaling. Our results showed that UL46 impaired the interaction of TBK1 and IRF3, and downregulated the activation of IRF3 by inhibiting the dimerization of TBK1 to reduce the IFN-I production induced by TBK1 and immunostimulatory DNA. The IFN-I and its downstream antiviral genes induced by UL46-deficient HSV-1 (ΔUL46 HSV-1) were higher than those of wild-type HSV-1 (WT HSV-1). In addition, the stable knockdown of TBK1 facilitated the replication of ΔUL46 HSV-1, but not WT HSV-1. Together, these findings reveal a novel mechanism of immune evasion by HSV-1.

P2848

An intensive immune response to multi viral peptide by direct binding of Ii vector to H2-Ab

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Invariant chain (Ii) is used as immune vector based on its role in assisting MHC class II molecules to present antigen peptides. In the previous work we found that its cytoplasm/transmembrane domains (C/T) as vector could boost a specific antibody production. To explore its effect on immune response to a multi viral peptide we constructed recombinant genes, encoding the recombinant protein containing $Ii_{(C/T)}$ and two viral peptides (IBDV-VP2/NDV-F2) and immunized animals. We found that the antibody titers induced by $Ii_{(C/T)}$//VP2/F2 were significantly higher those induced by the single VP2 or F2, which ranged from 2.2 to 5.6 times in the animal serums ($P < 0.01$). Furthermore, the laser confocal detection results showed that $Ii_{(C/T)}$/VP2/F2 could enter into endosomes like H2-Ab and co-located with H2-Ab in the 293T cells, but VP2/F2 could not enter into endosomes and co-located with H2-Ab. Moreover, By the observation in western blots $Ii_{(C/T)}$/VP2/F2 could also bind to H2-Ab in co-expressed cells, but VP2/F2 could not. Together, these results suggest that $Ii_{(C/T)}$ as immune vector is able to enhance immune response to multi viral peptides via direct binding to MHC class II molecules and entering into antigen transport way in the cells.

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DOI: 10.1002/eji.201970400

P2849

Very early treatment during SIVmac239 acute infection achieves functional cure in Chinese rhesus macaque

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Although treatment in acute infection can result a few patients to function cure in clinic, the mechanisms of it are still not clearly elucidated. In this study, we used SIVmac239-infected Chinese rhesus macaque with treatment at acute infection to construct functional cure animal model and explored mechanisms of virus control after therapy discontinued. It was showed that 66.67% individuals achieved functional cure with persistently control of virus and normal immune during 122 weeks after cART interruption (PTC), while 33.33% individuals with virus rebound and persist viral replication (R). Although equivalent proviral load in PBMCs of PTC and R group during treatment, proviral load in PBMC and released particles of CD4+ T cells were significant lower in PTC group than R group after cART interruption, meanwhile lower virus isolation rate in PTC group. Furthermore, although SIV specific CD4+ T and CD8+ T response were induced in PTC and R group, obvious higher cytotoxic CD8+ T cell response existed in PTC group than R group. Moreover, anti-SIV antibodies were under-detectable in most PTC group individuals and they also appeared later than virus rebound. Interestingly, detection rate of plasma virus during treatment significantly correlates with detection rate of plasma virus, viral release capability and CD8+ T cell proliferation after treatment interruption. These results suggest that very early treatment in acute infection can achieve function cure in Chinese rhesus macaque and implied that therapeutic effect of treatment, cytotoxic CD8+ T cell response, virus reservoir size may be associated with virus control after treatment interruption.

P2850

The association between atypical chemokine receptor 1 polymorphism and hepatic injury in hepatitis C virus infected patients

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Background: Hepatitis C virus (HCV) infection is a major health problem worldwide and it has infected 71 million individuals globally. Atypical chemokine receptor 1 (ACKR1) has two main functions, one is as a sink or scavenger receptor for chemokines and the other is involved in the transcytosis of chemokines across endothelial cells and presents them to leukocytes. Chemokines have become increasingly recognized as important mediators of hepatic inflammation and injury. Some indicators such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB) and AST/ALT ratio can reflect hepatic injury degree.
Aims: To investigate the relationship between ACKR1 polymorphism and hepatic injury in HCV-infected patients.
Methods: We recruited 245 HCV-infected patients. TaqMan probe method was used for ACKR1 polymorphism analysis, and hepatic injury indicators above mentioned were collected. The correlation between ACKR1 polymorphism and hepatic injury in HCV-infected patients was analyzed by comparing hepatic injury indicators in patients with different ACKR1 genotypes.

DOI: 10.1002/eji.201970400

Results: In 245 HCV infected patients, GG was most predominant (n=212), AG was less common (n=33), and AA was not found. No significant deviation was found from HWE (*P*>0.05). By comparing hepatic injury indicators of patients with GG and AG genotypes, we found that ALT (*P*=0.025) and AST/ALT (*P*=0.004) were statistical differences between the two groups.
Conclusion: This study indicated that the degree of hepatic injury can be regulated by ACKR1 polymorphism. Carrying G allele has a certain protective effect on hepatic injury in HCV-infected patients.

P2851

HIV-1 can infect northern pig-tailed macaques (*Macaca leonina*) and form viral reservoirs *in vivo*

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Establishing a novel animal model that can be directly infected with HIV-1 will greatly contribute to develop effective strategies for eradicating HIV-1 infections. Among Old world monkeys, the pig-tailed macaque (*Macaca nemestrina*, PTM) is the only type of macaque that can be infected with HIV-1. Northern pig-tailed macaques (NPMs) is one species of PTM and distribute in China.
We inoculated 4 NPMs with a HIV-1 strain HIV-$1_{NL4.3}$ and monitored the infection for approximately 5 years. The HIV-1-infected NPMs showed transient viremia for about 10 weeks after infection. However, cell-associated proviral DNA and viral RNA persisted in the peripheral blood and lymphoid organs for about 3 years. These proviruses could be reactivated both *ex vivo* and *in vivo* in response to co-stimulation with the latency-reversing agents JQ1 and prostratin, suggesting HIV-1 can replicate at a low level and formed a long-term viral reservoir in NPMs.
To optimize HIV-1 infection in NPMs, we then generated 2 HIV-1-derived constructs, HIV-$1_{NL4-R3A}$ and stHIV-1sv, and infected 4 NPMs, respectively. These two viruses can replicate persistently in NPMs during 41 weeks of acute infection stage and showed a higher level of plasma viral loads than that of HIV-1_{NL4-3}. Compared to the HIV-$1_{NL4-R3A}$, stHIV-1sv showed a notably higher level of replication and fewer G-A hyper-mutations, suggesting that *vif* gene of SIV could antagonize the antiviral effects of APOBEC3 proteins in NPMs. These results demonstrate NPM is a potential HIV/AIDS animal model.

P2852

Decreasing microRNA-147 sustains HBV persistent infection by reducing inhibition of Foxp3

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Chronic hepatitis B (CHB) is closely associated with liver cirrhosis and hepatocellular carcinoma (HCC). Persistent replication of hepatitis B virus (HBV) is a key role of onset and deterioration of CHB. Immune tolerance of effector T cells (Teff) mediated by regulatory T cells (Treg) plays an important role in HBV persistent replication, and micorRNA (miRNA) regulates the function of Treg, but the mechanism remains unclear. With plasma exosome miRNA-seq analysis, we find that miRNA-147 is the most downregulated microRNA in patients with CHB infection. Further molecular biology, immunology, biological software (Targetscan, miRBase) studies demonstrate that miRNA-147 targets FoxP3 and CXCL10 in Treg subset, which is related to HBV persistent infection and replication in

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DOI: 10.1002/eji.201970400

patients with CHB. Furthermore, in CHB patients, we explore and confirm that miR-147 is from the hepatocellular exosome, and find that miR-147-FoxP3 signaling pathway increases the functional activity of Treg, and in turn inhibiting the cytotoxic effect of Teff cells, without repressing HBV replication. Finally, pretreatment with a miR-147 mimic delays HBV reproduction in the mouse model with HBV continued infection. Our study demonstrated the new mechanism of decreasing miR-147 promoting HBV persistent infection through the cooperative regulation of Treg/Teff by downstream signals of miR-147-FoxP3, and provided a promising target and theoretical basis for the prevention and treatment of chronic hepatitis B.

P2853

LRRFIP1 inhibits HBV replication in hepatocytes by strengthening type I interferon signaling

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Interferon alpha (IFN-α) is an attractive treatment option for hepatitis B patients, however, the clinical effectiveness, varied among individuals, needs to be further improved. The factors hampering IFN-α therapeutic responsiveness need further identification. Type I interferon (IFN-I) elicits expression of “interferon-stimulated genes” (ISGs) to perform antiviral functions in target cells. The poor responsiveness to IFN-α treatment in certain patients may be correlated with the IFN-I effector signaling and the level of ISGs activation in hepatocytes. We found that leucine-rich repeat flightless-interacting protein 1 (LRRFIP1) promoted IFN-α-mediated inhibition of HBV replication in hepatocytes. The hepatocyte-specific LRRFIP1-deficient mice exhibited longer persistence of HBV infection and more severe liver damage. Mechanistically, LRRFIP1 becomes dimerized after IFN-α treatment, then binds Janus kinase 1 (JAK1) and signal transducer and activator of transcription 1 (STAT1) as a scaffold protein. **LRRFIP1 promotes JAK1-STAT1 interaction and enhances STAT1 phosphorylation, empowering ISGs transcription in hepatocytes for more efficient IFN-α-mediated virus clearance.**

P2854

Porcine epidemic diarrhea virus membrane protein suppresses the activation of IRF7 to decrease type I interferon production

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Porcine epidemic diarrhea virus (PEDV) is a highly pathogenic porcine enteropathogenic coronavirus causing severe enteritis and lethal watery diarrhea in piglets. PEDV infection suppresses the synthesis of type I interferons (IFNs) and multiple viral proteins of PEDV have been shown to target the adaptors of type I IFN pathway to inhibit type I IFN production. The nsp1, papain-like protease 2, nsp5, and N protein have been identified as type I IFN antagonists. Here, we identified PEDV membrane (M) protein also inhibits type I IFN production and determined a novel antagonistic mechanism by M protein to target interferon regulatory factor 7 (IRF7), an important regulator of type I IFN production.

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DOI: 10.1002/eji.201970400

IRF7 is phosphorylated and activated by IKKε and TBK1 in response to viral infection. We found that M protein interacted with IRF7 and significantly suppressed IKKε- and TBK1-induced IRF7 phosphorylation and dimerization of IRF7, leading to the decreased expression type I IFN, and as expected, overexpression of M protein significantly increased PEDV replication in porcine cells. Taken together, our data identified a new IFN antagonist of PEDV, as well as a novel mechanism evolved by PEDV to inhibit type I IFN production.

P2855

Current research on diagnosis and control technology of African swine fever

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African swine fever (ASF) is an acute, hemorrhagic and highly contagious disease. African swine fever virus (ASFV) can infect domestic pigs of all breeds and ages as well as wild boars with high mortality rate. In August 2018, the first ASF case in China was diagnosed in Shenyang, Liaoning Province. ASF will inevitably have a huge impact and influence on the healthy development of China´s pig industry. Due to the huge genomic structure and complex immune evasion mechanisms of ASFV, no effective vaccine is currently available against the disease. A review of the diagnosis, prevention and control of ASF is expected to provide a reference for the effective comprehensive prevention and control of ASF.
The study was partly supported by National Key Research and Development Program（2017YFD0502304-4）
Keywords African swine fever; diagnostic techniques; prevention and control

P2856

Host cell interactome with oncoprotein LMP1 encoded by EBV reveals new targets for the treatment of diseases

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Epstein-Barr virus (EBV), which establishes a persistent infection over 90% of the world population, could lead to the development of a variety of diseases. The viral latent membrane protein 1 (LMP1), acts as a critical oncoprotein when immortalizing B lymphocytes, and activates host signaling pathways to stimulate cell proliferation, down-regulates tumor suppressor genes. Furthermore, LMP1 has been discovered interaction with host factors, which are drawing extensive attention because of potential targets for the treatment of diseases. In this study, the interaction landscape of LMP1was described by identifying host proteins co-immunoprecipitated with LMP1 through mass spectrometry. The overall identified 1,434 proteins were analyzed by enriched pathways, protein-protein interactions and pan-virus membrane proteins' interactome with GO, KEGG, STRING and ClueGO. Specific interactions between Sec61 or CANX and LMP1 were validated by co-immunoprecipitation. Besides, we also have provided strong evidence that LMP1 inhibits Sendai virus mediated type I interferon production and downregulates RIG-I signaling pathway by promotion RIG-I degradation dependent on proteasome. Nineteen kinds of E3 ligase are identified as LMP1-interactors, which are possibly recruited by LMP1 to mediate RIG-I degradation. CHIP is among these E3s, which has been reported to lead RIG-I degradation. Notably, we find C666-1, an EBV-positive nasopharyngeal carcinoma cell line, expresses low level of RIG-I, even treated with IFN-α, RIG-I expression could not be induced. This evidence indicates that EBV employs a unique strategy to evade RIG-I mediated immune

DOI: 10.1002/eji.201970400

responses and provide new insights for further investigations on EBV pathogenesis and therapeutic strategy.

P2857

Specific and stable suppression of hepatitis B virus expression by DNA methyltransferase1 and methyltransferase3a via specific zinc-finger-induced methylation of the X promoter

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Background: Hepatitis B virus（HBV）infection is a chronic liver disease resulting in hepatocellular carcinoma. Current therapies fail to provide effective long-term control of viral replication in patients. DNA methylation is a potential therapeutic approach to suppress DNA replication. Our previous study established a fusion protein integrating a C-terminal Zinc-finger domain of Dnmt3a and Dnmt1 that could specifically bind to the X gene promoter (XP) of the HBV genome.
Methods: In this work, we further packed these XP targeting genes into replication-defective adenoviral vectors, to establish an HBV XP specific methylation adenoviral gene therapy in the cell and mouse model.The targeting Dnmts specifically bound to HBV XP and inhibited the replication of HBV and expression of HBX. The combined Dnmts i.e., Ad-XPDnmt3a-1C inhibited the replication of HBV and expression of HBX more efficiently than the Dnmt3aC or Dnmt1C in the cell models. In the HBV transgenic（TgHBV）mouse model, the injection of Ad-ZFXP-Dnmt3aC-1C showed a significant reduction of the expression of HBV surface protein (HBsAg) till 14 weeks.
Conclusion: HBV X promoter specific ZNF-Dnmt integrated adenovirus is capable of silencing Hepatitis B virus replication for a long duration. This study establishes the foundation of an epigenetic way of controlling HBV-related diseases and the potential of a novel anti-HBV therapeutic approach.

P2858

Induction of UBL4A by viral infection augments type I IFN signaling by promoting the K63-linked ubiquitination of TRAF6

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UBL4A/GdX, encoding a ubiquitin-like protein, here was shown to be upregulated by viral infection and interferon stimulation. Then the functions of UBL4A in antiviral immune response were characterized. Overexpression of UBL4A promoted both RNA and DNA virus-induced ISRE or IFN-β or NF-κB activation and led to enhanced type I IFN transcription and reduced virus replication. Consistently, knockdown of UBL4A inhibited both RNA and DNA virus induced type I IFN transcription and enhanced virus replication. Concurrently, overexpression of UBL4A promoted virus induced phosphorylation of TBK1, IRF3, IKKα/β. Knockdown of UBL4A inhibited virus induced phosphorylation of TBK1, IRF3, IKKα/β. Coimmunoprecipitation showed that UBL4A interacted with TRAF6 and this interaction was enhanced upon viral infection. Ubiquitination assay showed that UBL4A promoted the K63-linked ubiquitination of TRAF6. Therefore, we identified a novel positive regulator in antiviral signaling by enhancing the K63-linked ubiquitination of TRAF6.

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DOI: 10.1002/eji.201970400

P2859

Foot-and-mouth disease virus 3B protein inhibits type I interferon pathway signaling by blocking the interaction of RIG-I with TRIM25

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Foot-and-mouth disease is a highly contagious disease of pigs, sheep, goats, bovine and various wild cloven-hoofed animals that has caused significant economic loss to global livestock industry. The causative agent of the disease is foot-and-mouth disease virus (FMDV), which belongs to the genus of *Aphthovirus* in family of *Picornaviridae*. FMDV suppresses host innate immune system by a variety of mechanisms to evade clearance by the host and facilitate viral replication. The viral protein 3B is an important determinant of the virulence of FMDV. Modifications in viral 3B protein of FMDV considerably decrease virus yield. Here we demonstrate the significant role of 3B in suppression of type I interferon (IFN) response. We found that 3B interacted with the viral RNA sensor RIG-I to impair type I IFN pathway activation. 3B did not affect RIG-I expression, but interacted with the CARD domain of RIG-I to block the RIG-I-TRIM25 interaction, resulting in the inhibition of the K63-linked ubiquitination of RIG-I and RIG-I-VISA complex formation. This inhibition of type I IFN pathway signaling by 3B decreased IFN-β, IFN-stimulated genes and proinflammatory cytokines expression, which in turn promoted FMDV replication. Together, our results indicate the role of 3B in suppression of host innate immune response, and reveal a novel antagonistic mechanism of FMDV that is mediated by 3B protein.

P2860

HIV-1 Vif suppresses IFNβ expression by targeting STING to evade host immune responses

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AIDS is an elusive world-wide problem, it is urgent to study its pathogenic mechanism. cGAS-STING-TBK1 axis plays an important role in anti-viral immune response. Despite the importance of protein Vif in HIV-1 replication, relatively little is known about the role of Vif in the regulation of host innate immune response. Here, we demonstrated that the HIV-1 protein Vif shares sequence similarity with the host immunoreceptor tyrosine-based inhibitory motif (ITIM). Further experiments showed that Vif interacted with the host cellular tyrosine phosphatase SHP-1 in an ITIM phosphorylation-dependent manner. The association of Vif with SHP-1 facilitated the recruitment of SHP-1 to STING and inhibited phosphorylation of STING at tyrosine 162 to negatively regulated K63-linked ubiquitination of STING at lysine 337, and then inhibited downstream signal transduction. Moreover, the ITIM of Vif suppressed HIV-1-stimulated expression of IFNβ and proinflammatory cytokines in macrophages. Our findings provide a new molecular mechanism for HIV-1 immune evasion and suggest ITIM-containing Vif is a potential target for the treatment of HIV-1 infection.

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DOI: 10.1002/eji.201970400

P2861

Parkin impairs antiviral immunity by suppressing the mtROS-NLRP3 axis and anti-viral inflammation

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Although mitochondria are known to be involved in host defense against virus infection, the physiological role of mitophagy, a crucial mechanism for maintaining mitochondrial homeostasis, in antiviral immunity remains poorly defined. Here, we show that Parkin, a central player in mitophagy, has a vital function in regulating host antiviral responses. Parkin-knockout mice exhibit improved viral clearance and survival after virus infection. However, Parkin deficiency doesn't affect antiviral signaling and interferon (IFN) production. Instead, the absence of Parkin augments innate antiviral inflammation by enhancing mtROS-mediated NLRP3 inflammasome activation and promoting viral clearance. Loss of NLRP3 can reverse the enhanced antiviral responses in Parkin knockout mice. Furthermore, we find that Parkin expression is down-regulated in PBMC of patients infected with virus. Collectively, our results suggest that regulation of Parkin plays an important role in antiviral immunity by controlling mtROS-NLRP3 axis-mediated inflammation. These findings provide the first physiological insight of the importance of mitophagy in regulating host antiviral response.

P2862

Macaca leonina: a unique animal model for HIV/AIDS research

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Pig-tailed macaque is one the three Asian macaques used in HIV/AIDS research. They are further divided into three species: *Macaca nemestrina* (southern pig-tailed macaques, SPMs), *Macaca leonina* (northern pig-tailed macaques, NPMs) and *Macaca pagensis* (Mentawai macaques) based on the most widely accepted taxonomy.Pig-tailed macaques are distinctive HIV/AIDS animal models due to their susceptibility to HIV-1, although it is still difficult to fully replicate AIDS in HIV-1-infected pig-tailed macaques. Meanwhile, SIV-infected SPMs show similar virological and immunological characteristics as rhesus macaques, such as $CD4^+$T cell depletion and immune activation, although it seems that SPMs progress to AIDS more quickly. Intriguingly, there were some unique characteristics in SIV-infected NPMs. First, the $CD4^+$T cell count in most SIV-infected NPMs recovered to the pre-infection level during the chronic infection phase and both naïve and memory $CD4^+$T cells kept relatively stable frequencies throughout the infection, which is seldom found in SIV-infected monkeys. In addition, unlike SPMs suffering from increased microbial translocation in the absence of SIV infection, NPMs kept lower levels of microbial translocation due to the superior intestinal integrity even after infection, which may further contribute to the lower levels of T cell activation. The mechanisms under these unique features are still unclear, nonetheless, NPMs are proved to be a good choice to study HIV or SIV-related $CD4^+$T cell reconstitution and microbial translocation.

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DOI: 10.1002/eji.201970400

P2863

The 7.5 kDa nonstructural protein of human parvovirus B19 triggers autophagy in UT7/EPO-S1 cells

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Human parvovirus B19 (B19V) is a common human pathogen which is associated with a broad spectrum of clinical manifestations. Although B19V infection has been shown to induce cellular autophagy, the viral proteins responsible for inducing autophagy are unknown. In this study, we examined the small 7.5kDa nonstructural protein of B19V for its function in inducing autophagy in transfection of megakaryoblastoid cell line UT7/EPO-S1, which is semipermissive to B19V infection. Our results showed that the protein expression ratio for LC3-Ⅱ/LC3-Ⅰ increased significantly in 7.5kDa-expressing UT7/Epo-S1 cells compared to control cells. Fluorescence confocal microscopy analyses revealed 7.5kDa protein induced an intracellular autophagosome as judged by LC3 staining. These results indicated possible involvement of 7.5kDa protein in cellular autophagy induction. We further analyzed whether 7.5kDa protein-induced autophagy was activated via endoplasmic reticulum (ER) stress. Western blot results demonstrated that 7.5kDa expression did not result in increased levels of ER stress markers GRP78/Bip and CHOP, thus implied 7.5kDa did not induce ER stress. Taken together, these results suggested that 7.5kDa protein of B19V could trigger autophagy in UT7/EPO-S1 cells independent of ER stress pathway activation. Further experiments are needed to explore the potential mechanisms by which 7.5kDa protein induces cellular autophagy.

P2864

Porcine parvovirus infection activates inflammatory cytokine production through Toll-like receptor 9 and NF-κB signaling pathways in porcine kidney cells

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Porcine parvovirus virus (PPV) is an animal virus that has caused high economic losses for the swine industry worldwide. Previous studies demonstrated that PPV infection induced significant production of interleukin 6 (IL-6) in vitro and in vivo. However, the inflammatory cytokines and specific signaling pathways induced during PPV infection remain largely unknown. In the present study, we analyzed the expression levels of IL-6 in PPV infected porcine kidney 15 (PK-15) and the results showed that PPV infection induced the increase of IL-6 mRNA expression in a dose-dependent manner. We also detected the expression of toll-like receptor 9 (TLR9) signaling proteins in the mRNA expressing level, when compared with the control group, the TLR9 expression in mRNA level increased at 24 h in PK-15 cells after PPV infection and reached the peak level at 48 h. In addition, the transcript profile of nuclear factor kappa B (NF-κB) signal pathway relating genes (MyD88, IRAK1, TRAF6, TAK1α, IκBκB and NF-κB) expression levels increased at different times. Furthermore, to verify the IL-6 expression was specific with the TLR9 expression and then by activating the NF-κB signal pathway, TLR9 and NF-κB specific inhibitors were applied during PPV infection, separately, the result indicated that the expression of IL-6 was decreased after inhibitor treatment. Taken together, PPV infection significantly induced IL-6 expression and this induction depended on NF-κB activation and TLR9 signaling pathways in PK-15 cell.

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DOI: 10.1002/eji.201970400

P2865

Ineffective immune regulation driven by immunosenescence causes rapid disease progression and robust immune activation in aged sivmac239-infected Chinese rhesus macaques

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Recent studies have shown that immunosenescence is the main cause of rapid disease progression and poor prognosis in elderly HIV-infected patients, but the mechanism remains unclear. Here, we studied young and old Chinese rhesus macaques (ChRM) through one year post simian immunodeficiency virus (SIV) SIVmac239 infection. Old ChRM showed a higher risk of accelerated AIDS development than did young macaques, owing to rapidly elevated plasma viral loads and decreased levels of CD4+ T cells. The low frequency of naïve CD4+ T cells before infection was strongly predictive of an increased disease progression. Moreover, in old ChRM, a robust innate host response with defective regulation was associated with the homeostatic proliferation of naïve T cell and a high level of immune activation. Transcriptome analysis of peripheral blood mononuclear cells validated the above results. SIV triggered the alterations in the expression of a number of genes involved in type-I interferon response, immune response, metabolic pathway and signaling pathway. Aged macaques showed a stronger perturbation in genes linked to type-I interferon response, DNA replication, cell cycle and positive regulation of transcription. Specifically, young macaques expressed their own unique gene module which enriching amount of regulated genes in immune activation. Our results support a model where rapid disease progression of SIV-infected aged macaques is driven by the loss of effective immune regulation from immunosenescence, leading to elevated immune activation and homeostasis proliferation, possibly contributing to increased exhaustion of immune system.

P2866

A nano silicon adjuvant enhances inactivated transmissible gastroenteritis vaccine through activation the Toll-like receptors and promotes humoral and cellular immune responses

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Inactivated transmissible gastroenteritis virus (TGEV) vaccines are widely used in swine herds in China. These are limited, however, by the need to elicit both humoral and cellular immunity, as well as the efficiency of adjuvants. In this study, a 70-nm nano silicon particle was applied with inactivated TGEV vaccine in mice, and its immune-enhancing effects and mechanism of action investigated. We found that nano silicon applied with inactivated TGEV vaccine induced high antibody titers, increase IL-6, TNF-α and IFN-γ expression, and stimulate CD3+ T cell proliferation with a high CD4+/CD8+ T lymphocyte ratio. Nano silicon could quickly activate innate and adaptive immunity by stimulating Toll-like receptor signaling pathways, indicating that the nano silicon adjuvant enhanced long-term humoral and early cellular immune responses when combined with inactivated TGEV vaccine. Nano silicon could be considered for use as an antigen- carrier and adjuvant for veterinary vaccines.

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DOI: 10.1002/eji.201970400

P2867

Screening and identification of linear B-cell epitopes within the non-structural proteins of enterovirus 71

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Enterovirus 71 (EV-A71) has attracted widespread attention in the past decade because of its higher neurotropicity in addition to causing hand, foot and mouth disease (HFMD). Mapping epitopes of viral proteins may promote our understanding of antiviral humoral immunity, and is useful for clinical application. In this study, the linear B-cell epitopes within non-structural proteins of EV-A71 were identified using bioinformatics methods combined with peptide ELISA. Twenty epitopes were predicted and located at 2ABC (7), 3ABC (5) and 3D (8) protein respectively. Of 20 epitope-containing peptides, 14 were verified by ELISA (S/CO>2.1), 9 of which had higher reactivity (S/CO>5.0). Furthermore, synthetic peptide SP09 (EV-A71-3ABC$_{26-41}$) can react with healthy children sera, and its immunoreactivity was closest to that of EV-A71-VP1 protein. The protein BLAST analysis revealed that SP09 may contain a common epitope due to the high homology of amino acid sequences with other members of human *Enterovirus* species A. These findings may be useful in the development serological tests for the diagnosis of HFMD caused by a broad range of human *Enterovirus* species A.

P2868

Quantitative proteomic analysis of duck ovarian follicles infected with duck tembusu virus by label-free LC-MS

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Duck Tembusu virus (DTMUV) is a newly emerging pathogenic flavivirus that has caused massive economic losses to the duck industry in China. DTMUV infection mainly results in significant decreases in egg production in egg-laying ducks within 1-2 weeks post infection. However, information on the comparative protein expression of host tissues in response to DTMUV infection is limited. Now the cellular protein response to DTMUV infection in duck ovarian follicles was analyzed using nano-flow high-performance liquid chromatography-electrospray tandem mass spectrometry. Quantitative proteomic analysis revealed 131 differentially expressed proteins, among which 53 were up regulated and 78 were down regulated. **The identified proteins were involved in the regulation of essential processes, interestingly associated with innate immune response, such as interferon regulatory factor 3, two ISGs, 2′ -5′ -oligoadenylate synthase-like protein (OASL) and interferon-induced protein with tetratricopeptide repeats 5 (IFIT5). These two ISGs have not been reported previously.** Next, we identified and cloned the OASL gene (named duOASL) from healthy adult Cherry Valley ducks. Full-length duOASL cDNA (1630 bp) encoded a 504-amino acid polypeptide containing three conserved domains, namely, nucleotidyltransferase domain, 2′-5′-oligoadenylate synthetase domain, and two ubiquitin-like repeats. Results of qRT-PCR showed that duOASL was broadly expressed in all examined tissues, with the highest mRNA expression in the large intestine. Antiviral activity of duOASL revealed that duOASL overexpression slightly inhibited DTMUV replication, whereas duOASL knockdown increased DTMUV replication. **In end, our study may be helpful to understand the host response to DTMUV infection and the inherent mechanism of DTMUV replication and pathogenicity.**

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DOI: 10.1002/eji.201970400

P2869

γδ T cells provide protective function in highly pathogenic avian H5N1 influenza A virus infection

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Given the high mortality rate (>50%) and potential danger of intrapersonal transmission, highly pathogenic avian influenza (HPAI) H5N1 epidemics still pose a significant threat to humans. γδ T cells, which participate on the front line of the host immune defense, demonstrate both innate, and adaptive characteristics in their immune response and have potent antiviral activity against various viruses. However, the roles of γδ T cells in HPAI H5N1 viral infection remain unclear. In this study, we found that γδ T cells provided a crucial protective function in the defense against HPAI H5N1 viral infection. HPAI H5N1 viruses could directly activate γδ T cells, leading to enhanced CD69 expression and IFN-γ secretion. Importantly, we found that the trimer but not the monomer of HPAI H5N1 virus hemagglutinin (HA) proteins could directly activate γδ T cells. HA-induced γδ T cell activation was dependent on both sialic acid receptors and HA glycosylation, and this activation could be inhibited by the phosphatase calcineurin inhibitor cyclosporin A but not by the phosphatidylinositol 3-kinase (PI3-K) inhibitors wortmannin and LY294002. Our findings provide a further understanding the mechanism underlying γδ T cell-mediated innate and adoptive immune responses against HPAI H5N1 viral infection, which helps to develop novel therapeutic strategies for the treatment of H5N1 infection in the future.

P2870

Elevated plasma interleukin 34 level correlates with disease severity-reflecting parameters of patients with haemorrhagic fever with renal syndrome

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Haemorrhagic fever with renal syndrome (HFRS) is characterised by an uncontrolled cytokine storm that causes vascular leakage and kidney injury. The cytokine interleukin 34 (IL-34) enhances proliferation and differentiation of myeloid cells and secretion of pro-inflammatory cytokines, which is involved in the pathogenesis of some inflammatory and infectious diseases. However, to date, the role of IL-34 in patients with HFRS is unclear. To investigate the possible effects of IL-34 in the pathogenesis of HFRS, IL-34 levels in the plasma from 52 patients with HFRS and 20 healthy controls were quantified using enzyme-linked immunosorbent assay (ELISA). Compared with healthy controls, the plasma IL-34 levels in patients were significantly elevated at acute phase [37.92 (10.58-73.86) pg/ml vs. 7.13 (3.99-11.31) pg/ml, $p < 0.0001$], and decreased to the normal levels at convalescent phase. Importantly, IL-34 levels positively correlated with white blood cell count and monocyte count ($r = 0.503$, $p < 0.0001$ and $r = 0.367$, $p = 0.0003$, respectively), and negatively correlated with platelet count and serum albumin levels ($r = -0.614$, $p < 0.0001$ and $r = -0.598$, $p = 0.0003$, respectively). Since these clinical parameters could reflect the disease severity of HFRS, potential roles of IL-34 in pathogenesis of HFRS need to be further investigated.

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DOI: 10.1002/eji.201970400

P2871

Molecular mechanism of EV71 induced autophagy in mouse hippocampal cells

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Objective: Enterovirus 71 (EV71) is the main pathogenic pathogen causing the death of children with hand, foot and mouth disease (HFMD). Fatal neurological complications can be induced by EV71, but the pathogenesis of EV71 is still unclear. Autophagy is a conservative, lysosomal-dependent catabolism pathway in evolution that remove pathogenic microorganism through autophagy pathways to exert a natural immune effect. This paper focuses on the molecular mechanism of autophagy induced by EV71 in mouse hippocampus cells, so as to find new therapeutic targets for anti-EV71 therapy.
Methods: Mouse hippocampal cells were used as normal nerve cells of EV71 infection, and their biological characteristics in these cells were studied by electronic microscope, flow cytometry, Western-blot and Laser scanning confocal microscope.
Results: The autophagosomes was detected in these cells by electron microscopy, and a sustained high expression of LC3II protein with the prolongation of infection time was detected by flow cytometry and Western-blot. These findings verified that EV71 could induce autophagy in mouse hippocampal cells. Furthermore, the aggregation of EV71 protein molecules on autophagosomes was detected by immunofluorescence, and the co-localization of EV71-RNA and autophagy molecules was observed under laser scanning confocal microscope. Therefore, we speculated that EV71 might produce immune escape using the mechanism of autophagy in hippocampal cells, which may be closely related to the causes of neurological complications after EV71 infection.
Conclusions: EV71 can induce autophagy and may use this mechanism to escape from the innate immune response in mouse hippocampus cells.

P2872

Autophagy is required in plasmacytoid dendritic cell dysfunction following EV71 infection

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Previous studies showed that the replication of Enterovirus 71 (EV71) could occur in DCs and the pathogenicity of EV71 may be related to its ability to evade host innate immunity through inhibiting cellular type I interferon signaling. As one type of DCs and type I IFN producing cells, Plasmacytoid dendritic cells (pDCs) are important drivers of both innate and adaptive immune responses, but the role of it against EV71 is unknown. Here, we aimed to study the function changes of pDCs from different condition HFMD patients infected by EV71 and further verify the role of autophagy in these pDCs against EV71 in vitro experiments. Our results revealed that critical HFMD patients exhibited the lowest percentage of pDCs in the peripheral blood lymphocytes compared to other patients. Decreased live cell numbers, obvious apoptosis of pDCs, reduced levels of pro-inflammatory

DOI: 10.1002/eji.201970400

cytokines, and increased IL-10 and TNF-α released from pDCs were observed over time post EV71 infection. It noted that EV71 could trigger the occurrence of autophagy in pDCs, moreover, the autophagy effect in pDCs from critical HFMD cases increased more evidently than other cases, and the JNK signal pathway seems could replace NF-κB pathway in this event. After added inhibitors to block the pDCs activation or autophagy, it suggested that autophagy was required following the blockade of pDCs activation. Thus, we predicted that the immune defect of pDCs accompanied with the exhausted CD4+T cells occurred in severity HFMD patients. Meanwhile, the autophagy plays a great role in recovering the immunity of pDCs.

Immunity to Bacteria

O217

Inhibition of transmembrane TNF-α shedding by a specific antibody protects against septic shock

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Tumor necrosis factor-α (TNF-α) is first synthesized as transmembrane TNF-α (tmTNF-α) and cleaved by the TNF-α converting enzyme (TACE) to release secretory TNF-α (sTNF-α). tmTNF-α and sTNF-α display opposite effects in septic shock. Targeting tmTNF-α shedding is able to decrease the detrimental effects of sTNF-α and increase the beneficial effect of tmTNF-α. Previously, we developed an antibody specific to tmTNF-α without cross-reaction to sTNF-α. We found that this antibody specifically suppressed tmTNF-α shedding by competing with TACE. tmTNF-α antibody significantly inhibited LPS-induced secretion of interlukine (IL)-1β, IL-6, interferon-β and nitric oxide by monocytes/macrophages, and protected mice from lipopolysaccharide (LPS)- or cecal ligation and puncture-induced septic shock, meanwhile reduced bacterial load. The mechanism study revealed that tmTNF-α antibody facilitated LPS-induced toll-like receptor 4 (TLR4) internalization and degradation by recruiting Triad3A to TLR4. Moreover, the antibody inhibited LPS-induced activation of nuclear factor-κB and interferon regulatory factor 3 pathways by upregulating A20 and monocyte chemotactic protein-induced protein 1. Similarly, treatment of macrophages with exogenous tmTNF-α suppressed LPS/TLR4 signaling and release of proinflammatory cytokines, indicating that increased tmTNF-α expression by the antibody contributes to its inhibitory effect and utilizing tmTNF-α antibody to specifically suppress tmTNF-α shedding may be a promising strategy for the treatment of septic shock. (This research was funded by the National Natural Science Foundation of China, 81471899 and 31671470)

O218

Using neutrophil specific biomarkers as proxy for Mtb burden, lung damage and treatment response kinetics

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DOI: 10.1002/eji.201970400

Validated biomarkers of early TB treatment response could significantly aid patient management and improve outcomes. Preliminary data from a longitudinal cohort study of 75 individuals with microbiologically confirmed TB, collected under the RePORT network, identified surface CD15 expression on neutrophils as a potential biomarker for TB disease. Expression of this carbohydrate adhesion molecule was significantly reduced at baseline and increased rapidly with treatment initiation, irrespective of HIV status. Baseline CD15 levels correlated with both neutrophil counts and sputum bacterial load. We hypothesised that reduced CD15 expression is related to continual recruitment of immature neutrophils from the bone marrow due to ongoing lung inflammation. Using stored plasma samples, we measured a panel of neutrophil associated cytokines/chemokines and other soluble factors to investigate the biological mechanisms involved. These were correlated with clinical markers of TB disease including longitudinal sputum bacterial load, differential blood counts and baseline chest x-rays. Among the 25 analytes, 10 involved in neutrophil recruitment, activation and immune response modulation were significantly increased in active TB, resolved during treatment and were unaffected by HIV co-infection. Correlation with clinical signs of disease revealed associations between plasma levels of IL-2Ra, IL-6, S100A9, TREM-1 and CD15 expression levels, in addition to neutrophil count and sputum bacterial load. Interestingly, baseline bacterial load did not predict treatment response, but baseline neutrophil count, CD15 expression, IL-2Ra, IL-6, S100A9 and TREM-1 are significantly different in early TB treatment responders' and may represent useful markers for treatment shortening.

O219

TLR2 is critically required for shaping CD4 T cell responses and preventing oviduct tissue pathology during genital Chlamydia infection

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Chlamydia trachomatis (*Ct*) is the most common bacterial STI and causes severe reproductive tissue damage in women. Unresolved chronic infection in mucosa epithelium and/or uncontrolled immune responses may contribute to *Ct*-induced tissue pathology. TLR2 is a pathogen-sensing molecule expressed broadly by epithelium and immune cells, yet, its role in *Ct* pathogenesis remains controversial. To this end, we compared the course of infection in $TLR2^{+/+}$ and $TLR2^{-/-}$ mice following intravaginal inoculation of *Chlamydia muridarum* (*Cm*). Compared to $TLR2^{+/+}$ mice, $TLR2^{-/-}$ mice had significantly increased vaginal bacterial shedding and markedly increased tissue pathology at day 75 post-infection although they seemingly had reduced tissue pathology at day 55. In parallel with the dynamic change in tissue pathology, $TLR2^{-/-}$ mice also displayed a time-dependent T helper 2 (T_H2) immune response, indicated by the levels of IL-13 production from splenocytes upon *Cm* antigen re-stimulation. However, $TLR2^{-/-}$ mice showed consistently reduced IL-17A production, indicating a critical role of TLR2 in developing T_H17 response. Bone marrow chimeric mice were generated to further define the contribution of TLR2-expressing immune cells and TLR2-expressing epithelium. Notably, IL-17A was only detected in mice having TLR2-expressing bone marrow-derived immune cells. Collectively, our data demonstrate a critical role of TLR2 in host defence against *Chlamydia* infection and the development of *Chlamydia*-induced tissue pathology. Our data also highlight an essential role of TLR2-expressing bone marrow-derived leukocytes in driving the development of T_H17 response, which may be required for controlling pathological T_H2 response and oviduct pathology in later stages of *Chlamydia* genital infection.

DOI: 10.1002/eji.201970400

O220

Investigating the Mycobacterium tuberculosis-specific CD4 T cell memory profiles: a comparison of immune responses between primary and established M.tb infection

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Recent *Mycobacterium tuberculosis* (M.tb) infection is associated with higher risk of progression to disease, compared to remote, established infection. Targeting preventative treatment to high-risk individuals is challenging because current immunodiagnostic tools fail to distinguish these infection states. We aimed to determine memory (CD45RA, CCR7, CD27, KLRG1), activation (HLA-DR) and functional (IFN-g, IL-2, TNF) kinetics of M.tb-specific CD4 T cells during different stages of infection. We characterized M.tb-specific CD4+ T cells from a longitudinal adolescent cohort of recent QuantiFERON (QFT) converters -with no evidence of M.tb-sensitisation at baseline- (n=28), and persistent QFT-positive (LTBI, n= 30) individuals. PBMCs were stimulated with M.tb lysate (cross-reactive with other mycobacteria sp.) or CFP10/ESAT6 (only present in M.tb) and detected by flow cytometry.
CFP10/ESAT6-specific CD4+ T cells were undetectable in QFT-negative individuals. Low but detectable mycobacteria-specific functional responses (IFN-g±TNF±IL-2±) were observed in QFT converters prior to M.tb infection. Mycobacteria-specific CD4 T cells pre-QFT-conversion possessed increased proportions of stem cell (T_{SCM}) and decreased proportions of effector (T_E) memory T cells compared to LTBI individuals. Upon QFT-conversion, we observed an increase in CD27+KLRG1-T_E mycobacteria-specific CD4 T cells, as well as a decrease in T_{SCM} mycobacteria-specific CD4 T cells compared to baseline. After M.tb infection, converters showed CFP10/ESAT6- and mycobacteria-specific functional responses similar to LTBI individuals, but decreased proportions of Mycobacteria-specific CD27-KLRG1+T_E and increased CFP10/ESAT6-specific activated T cells.
QFT conversion was associated with a shift from a quiescent to an activated and highly differentiated memory phenotype, which could distinguish individuals with primary or established M.tb infection.

O221

CagA inhibits protective gastric mucosal $CD4^+CD25^-$ effector memory T cells recruitment through CX3CL1 / CX3CR1 pathway in helicobacter pylori infected mice

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Background: Cytotoxin-associated gene A (CagA) positive Helicobacter pylori (*H. pylori*) is more likely to cause gastric disorders than CagA negative strains. The influence of CagA to the recruitment of CD4 memory T cell to gastric mucosa is unclear.
Methods: Twenty-four chemokines in gastric mucosa of CagA+ and CagA- *H. pylori* infected mice were measured. The target chemokines' effects on CD4 T cell were evaluated. Then, phenotypes of the CD4 T cells which expressed corresponding chemokine receptor in gastric mucosa were identified. At last, we valued the function of the recruited CD4 memory T cell. Also, the influence of CagA to the chemokines secreted by gastric epithelial cell were investigated.
Results: Chemokines CCL1, CCL3, CXCL2, CXCL5 and CX3CL1 concentrations were significantly

DOI: 10.1002/eji.201970400

elevated in CagA- *H. pylori* infected gastric mucosa than CagA+ *H. pylori* infected ones. Among them, CX3CL1 promoted CD4 T cell migration dramatically. CX3CR1, the only known ligand of CX3CL1, was upregulated on the surface of the gastric CD4 T cells in CagA- *H. pylori* infected stomachs. And most of CX3CR1 positive gastric CD4 T cells were demonstrated to be CD25- memory T cells. Pearson correlation analysis showed that the recruited $CX3CR1^{+}CD25^{-}$ CD4 Tem cells population was negative correlated with *H. pylori* colonization level (Pearson Correlation coefficient, r = -0.718, $p<$ 0.001) and histopathologic damage score (r = -0.804, $p<$ 0.001). Also, CagA inhibited gastric epithelial cell secreting CX3CL1.
Conclusion: CagA inhibits protective gastric mucosal $CD4^{+}CD25^{-}$ Tem cells recruitment through CX3CL1 / CX3CR1 passway in *H. pylori* infected mice.

O222

Daptomycin resistance mutations occurring in Staphylococcus aureus impede innate immune recognition by dendritic cells

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Methicillin-resistant Staphylococcus aureus (MRSA) represents an endemic public health threat. Resistance to last-line antibiotics is on the rise, correlating with increased morbidity and mortality in the hospital setting. The mutations conferring resistance to the last-line antibiotic daptomycin affect the composition of the bacterial cell membrane and wall, with recent findings showing that mutations in the cardiolipin synthesis gene, *cls2*, lead to thickening of the cell membrane and an increased ratio of cardiolipin:phosphotidylglycerol. Importantly, these changes reduce neutrophil trafficking to the site of infection, and prolong bacterial survival.
Here we investigate how daptomycin resistance affects innate sensing by dendritic cells (DCs). Using clinical isolates of MRSA we show daptomycin resistance is associated with a significant decrease in production of pro-inflammatory cytokines and chemokines by DC, as well as reduced expression of surface activation markers CD40 and CD80. These differences are almost entirely dependent on the acquisition of a point mutation in *cls2*, with recreation of the wild-type gene restoring DC activation. We show DC surface activation in response to these bacteria is dependent on MyD88 signalling. However, cytokine production is dependent on both endosomal TLR9 and cytosolic cGAS/STING sensors. Importantly, acquisition of daptomycin resistance impedes phagocytosis by DCs, suggesting that evasion of DC activation is regulated at the level of uptake, with potential implications for the presentation of antigen from these bacteria.
These findings highlight a novel aspect of antibiotic resistance- showing for the first time that point mutations associated with antibiotic resistance can concomitantly impede innate immunological recognition by DC.

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DOI: 10.1002/eji.201970400

O223

A subset of tissue resident macrophages controls cutaneous bacterial infection through recruitment of IL-17 producing T cells

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Tissue resident macrophages are a heterogeneous population of immune cells that patrol peripheral tissues. Certain tissue resident macrophages differ from monocyte-derived macrophages as they maintain their presence by homeostatic proliferation. We were able to locate a subset of Ly6C- tissue resident macrophages that express siglec-1(CD169) macrophages residing in the dermis. Thus, we aimed to investigate the role of the tissue resident macrophages in case of cutaneous infection. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common bacterial pathogens in skin infection. Infection of MRSA by subcutaneous injection induce an IL-17 mediated immune response. In mice depleted of CD169, larger area of dermonecrosis was observed when infected with MRSA. There was a smaller number of neutrophils in CD169 deficient mice compared to wild type mice. The number of dermal γδ T cells and CD4+ T cells were increased when infected. A lesser degree of recruitment for dermal γδ T cells and CD4+ T cells, but not dendritic epidermal T cells was observed in CD169+macrophage depleted mice. The frequency and number of dermal IL-17+ γδ T cells and CD4+ T cells, but not IFN-γ were smaller in mice depleted of CD169+ macrophages. Taken together, our results indicate that CD169+ tissue macrophages serve a crucial role in defeating MRSA.

O224

Opposite effects of maternal GAPDH vaccination: the critical impact of animal models to study group B Streptococcus neonatal infection

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Group B Streptococcus (GBS) is the leading cause of severe invasive neonatal infections. Our group developed an experimental vaccine against this neonatal pathogen based on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that proved to be effective in an experimental model, in which newborn mice were infected after delivery. However, this is not the natural route of infection in humans, since the bacterium is vertically transmitted. Thus, we developed a novel mouse model in which the pathogen is transmitted to the offspring from vaginally colonised pregnant females, as it occurs in humans, and assessed the efficacy of GAPDH maternal vaccination.In contrast to what was previously observed by our group, pups born from GAPDH-vaccinated mothers were not protected, presenting a mortality rate higher than controls, with higher bacterial levels. Unexpectedly, we found that 50% of GAPDH-vaccinated progenitors developed disease and died after birth. Histopathological analysis, together with the high bacterial load observed in their organs, demonstrated that they suffered from septicaemia. The potential contribution of anti-GAPDH IgG antibodies to the vaccine-induced mortality was excluded, as no death was observed when pregnant dams were passively immunized before bacterial inoculation. Interestingly, an alteration on the cellular immune response to a more inflammatory profile, with IFN-γ production, was observed in splenocyte supernatants of

DOI: 10.1002/eji.201970400

GAPDH-vaccinated compared to sham-vaccinated mice, upon stimulation with homologous antigen. In conclusion, our results highlight the danger of using an experimental model that does not mimic the human pathophysiology of disease to predict the effectiveness of treatment and/or preventive strategies in clinical trials.

LB019

The effect of malaria on the efficacy of BCG vaccination against *Mycobacterium tuberculosis* in mice

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Introduction: Bacillus Calmette Guerin (BCG) remains the only licensed vaccine against tuberculosis. It protects against severe disseminated forms of TB in infants but shows poor efficacy against pulmonary TB in adults. Coinfections have been reported as one of the factors implicated in the loss of vaccine efficacy.
Here, we compared virulent *Plasmodium berghei* and non-virulent *Plasmodium chabaudi*; their effects on the B cells, effector and memory T cells and the outcome on BCG induced efficacy against *Mycobacterium tuberculosis.*
Methods: C57Bl/6 mice were infected intraperitoneally with either *P. berghei* or *P.chabaudi* six weeks after BCG vaccination. *P. berghei* infected mice were treated with chloroquine for 10 days starting from day 6 post-P. berghei infection followed by Mtb H37Rv aerosol challenge whereas *P. chabaudi* infected mice were challenged with Mtb H37Rv on day 28 after malaria (n=10). Mice were killed 4 weeks after Mtb aerosol infection and bacterial burdens analysed in lungs and spleens. Splenocytes from BCG vaccinated or unvaccinated and Plasmodium infected mice were also stained for flow cytometry (n=6). Data was analyzed using ANOVA on graphpad prism.
Results: *P. berghei* induced a significant reduction in the central memory T and follicular B cells. Both strains induced a depletion of the marginal zone B cells but stimulated a strong effector T cell response enhanced by BCG vaccination. However, there was no loss of protection against *Mycobacterium tuberculosis* in both strains.
Conclusion: A post malaria infection does not alter the efficacy of BCG vaccination against *Mycobacterium tuberculosis* irrespective of the malarial virulence.

LB057

Contribution of neutrophils to IL-10 production during pneumococcal pneumonia and its impact on disease progression

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Introduction: Pneumococcal pneumonia is characterized by a high recruitment of neutrophils and lung damage. Recent studies have shown that neutrophils can exhibit anti-inflammatory properties; however, there are no data available regarding this fact in pneumococcal pneumonia. Our previous results showed that neutrophils produce IL-10 during a *Streptococcus pneumoniae* lung infection. Nevertheless, the relevance of these IL-10-producing neutrophils in response to pneumococcal infection remains unknown.
Objectives: To evaluate the role of IL-10-producing neutrophils during pneumococcal pneumonia and to determine whether this IL-10 production is induced by host or bacterial factors.

DOI: 10.1002/eji.201970400

Methodology: C57BL/6 wild type (WT) and IL-10$^{-/-}$ mice (highly susceptible to *S. pneumoniae* infection), were transferred with WT or IL-10$^{-/-}$ neutrophils. 24 hours later, mice were infected with *S. pneumoniae.* Infiltration of pro-inflammatory cells in lungs and bacterial loads were evaluated after 2 days post-infection and a survival curve was performed. Further, bone-marrow neutrophils were exposed to *S. pneumoniae* or mice bronchoalveolar lavage, and IL-10 was evaluated from culture supernatants by ELISA after 24 hours post-treatment.
Results: The adoptive transfer of neutrophils improved IL-10-/- mice survival rate. In contrast, WT mice transferred with WT cells showed an increased clinical score and decreased survival. Furthermore, *S. pneumoniae* molecular patterns showed to be enough to induce high levels of IL-10 production in neutrophils.
Conclusions: We provide evidence about the relevance of IL-10-producing neutrophils in pneumococcal pneumonia. Moreover, our results show that *S. pneumoniae* is able to directly affect neutrophils response, potentially modulating the immune response during the infection and the outcome of disease.

LB093

Longitudinal immunoprofiling of plasma cytokines predicts outcomes in melioidosis

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Melioidosis is a severe tropical infectious disease caused by *Burkholderia pseudomallei*. New immunotherapeutic approaches to treatment are needed to reduce mortality. To characterize the plasma cytokine response in melioidosis and identify cytokines associated with death, we conducted a prospective longitudinal study in two hospitals in Northeast Thailand. We enrolled 161 melioidosis patients, 13 uninfected healthy controls, and 11 uninfected diabetic controls. We obtained blood from all individuals at enrollment (day 0) and at days 5, 12, and 28 from surviving melioidosis patients. We assayed IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-23, and TNF-α in plasma using a bead-based assay and analyzed the association of each with outcome. Of melioidosis patients, 131/161 (81%) were bacteremic, and 93/161 (58%) died. Enrollment levels of IFN-γ, IL-6, IL-8, IL-10, IL-23 and TNF-α were higher in melioidosis patients compared with uninfected controls, and all but IFN-γ were positively associated with 28-day mortality. IL-8 demonstrated optimal discrimination of mortality (AUROCC 0.78). Cox proportional hazards analysis identified IL-10, IL-6, IL-8, and TNF-α as predictors of survival. Over time, non-survivors had persistently elevated or increasing IL-6, IL-8, IL-17A, and TNF-α concentrations. In joint modeling, temporal trajectories of IFN-γ, IL-6, IL-8, IL-10, IL-17A, and TNF-α predicted survival. In conclusion, specific pro- and anti-inflammatory and Th17 cytokines are associated with survival from melioidosis, at enrollment and over time. Persistent inflammation precedes death. These findings support further evaluation of these mediators as prognostic biomarkers and to guide targeted immunotherapeutic development in melioidosis.

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DOI: 10.1002/eji.201970400

LB094

Effect of activin A on chemotaxis and bactericidal activity of mouse neutrophil

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Activin A, a member of the transforming growth factor beta (TGF-β) superfamily, acts as a pro-inflammatory factor in acute phase response, and influences the pathological progress of neutrophil-mediated disease, for example, ARDS, rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus. Recently, we found that activin A may exert an effect on neutrophil activation in an autocrine/paracrine manner. Herein, we evaluated the role of activin A in neutrophil chemotaxis and clearance of staphylococcus aureus (S.aureus). The results revealed that activin A inhibited neutrophil chemotaxis and killing of S. aureus in vitro. In mouse model of S. aureus skin infection, high levels of activin A in serum and infected skin were observed. After treatment with activin A, neutrophils influx to the site of infection was decreased, and clearance of invading bacteria was impaired. Thus, activin A may play a role in inhibition of neutrophil chemotaxis and bactericidal activity.

LB113

The preliminary study of *Porphyromonas gingivalis* promoting malignant transformation of oral mucosa

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Objective: The role of *Porphyromonas gingivalis*(*P.g*) in the progression of oral cancer has been widely studied, but the relationship of *P.g*, malignant transformation of oral mucosa and immune response were largely unknown. This study aims to provide new insights into the potential role of *P.g* in oral carcinogenesis.
Methods: The murine oral carcinogenesis model was established by using 4-nitroquinoline-1 oxide (4NQO). At week 16, the mice were randomly divided into *P.g* treated group and control group (medium or *Streptococcus mutans*). Bacterial feeding experiments were performed for 10 weeks and the mice were sacrificed at week 26. Hematoxylin and eosin (H&E) was performed to oral lesions for histopathological analysis. Immunohistochemistry (IHC) and fluorescence in situ hybridization(FISH) were performed to detect the existence of *P.g* in oral lesions. The abundance of T cells, $CD11b^+$ cells, $CD11b^+$ $Gr\text{-}1^+$ cells (myeloid-derived suppressor cells, MDSCs) in oral lesion, draining lymph node and spleen were analyzed by flow cytometry. Immunofluorescence(IF) was performed to detect the infiltrating immune cells in the oral lesions.
Results: *P.g* group had more invasive carcinoma lesions than the control group. *P.g* invaded in the lesions of *P.g* group and the abundance of *P.g* was correlated with the pathologic degree of the lesions. Notably, $CD11b^+$ cells, MDSCs were expanded in the oral lesions of *P.g* group, but there were no differences in draining lymph nodes or spleens.
Conclusion: *P.g* infection enhanced oral carcinogenesis in 4NQO-treated mice, and the potential mechanism might be related to the expansion of MDSCs in the local lesions.

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DOI: 10.1002/eji.201970400

P0323

Tick saliva-assisted transmission protein SATP15 facilitate the infection of *Borrelia garinii*, a causative agent of Lyme disease

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Lyme disease, caused by the most common tick-borne infection in parts of the USA, Europe and Asia, can lead to clinical manifestations such as arthritis, carditis, and neurological disorders. The causative agents of Lyme disease are spirochetes belonging to the *Borrelia burgdorferi sensu lato* group and are transmitted by the bites of infected ticks. *Borrelia garinii* is the main genotype of the Lyme disease agent in China and predominantly transmitted by *Ixodes* tick vectors with the benefit of the tick saliva molecules. Here we identified a blood feeding induced saliva-assisted transmission protein (SATP15) from salivary glands of the *Ixodes persulcatus* tick. SATP15 functions as an immunosuppressive factor to facilitate the transmission of *B. garinii* to hosts by blocking the lymphotoxin beta receptor signaling pathway, which has been shown to be critical for host defense against microbial infection through activating NF-κB signaling pathway and inflammatory responses. SATP15 administration in vivo advanced *B. garinii* survival and replication, which led to abnormal inflammatory response. Furthermore, treatment with antibody to SATP15 inhibited tick-mediated *B. garinii* infection in vivo. Together, our findings indicate an essential role for SATP15 and LTβR signaling in recognizing and cleaning of the spiorchetes and may be applicable for the controlling of tick-borne diseases.

P0324

Rhesus brain transcriptomic landscape in an *ex vivo* model of the interaction of live *Borrelia burgdorferi* with frontal cortex tissue explants

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Lyme neuroborreliosis (LNB) is the most dangerous manifestation of Lyme disease caused by the spirochete *Borrelia burgdorferi* which can reach the central nervous system most commonly presenting with lymphocytic meningitis; however, the molecular basis for neuroborreliosis is still poorly understood. We incubated explants from the frontal cortex of three rhesus brains with medium alone or medium with added live *Borrelia burgdorferi* for 6 h, 12 h, and 24 h and isolated RNA from each group was used for RNA sequencing with further bioinformatic analysis. A total of 2249, 1064, and 420 genes were significantly altered, of which 80.7%, 52.9%, and 19.8% were upregulated and 19.3%, 47.1%, 80.2% were downregulated at 6 h, 12 h, and 24 h, respectively. **Gene ontology and KEGG pathway analyses revealed various pathways related to immune responses during the spirochete infection were enriched which is suggested to have a causal role in the pathogenesis of neurological Lyme disease. Moreover, we propose that the overexpressed FOLR2 which was demonstrated by the real-time PCR and western blotting could play a key role in neuroinflammation of the neuroborreliosis based on PPI analysis for the first time. Comprehensive transcriptomic signatures insights into the immune responses and other neuro-immune interactions have greatly advanced our understanding of immunity to *Borrelia burgdorferi* and identified FOLR2 as new therapeutic possibilities in Lyme neuroborreliosis.**

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DOI: 10.1002/eji.201970400

P0325

Ubiquitin binding to a Mycobacterium tuberculosis surface protein triggers host xenophagy

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Ubiquitin targeting to intracellular bacteria plays a fundamental role in selective autophagy called xenophagy, a crucial innate immune mechanism in mammalian cells against intracellular pathogens including *Mycobacterium tuberculosis* (Mtb). However, it remains unclear how the host ubiquitin system recognizes the intracellular bacteria and which bacterial proteins would be targeted for triggering this process. In our efforts to search for novel potential ubiquitin-binding proteins that located on the surface of Mtb, we identified a eukaryotic-like ubiquitin-associated (UBA) domain-containing protein Rv1468c (PE_PGRS29), a member of mycobacteria-specific PE_PGRS protein family. Interestingly, we found that host ubiquitin chains could hydrophobically bind to the UBA domain of Rv1468c which was surface-exposed on mycobacterial wall. In macrophages, the UBA-mediated direct binding of ubiquitin to Rv1468c could recruit autophagy receptor p62 to deliver mycobacteria into LC3-associated autophagosomes. Further investigations by using a mouse infection model demonstrated that disruption of Rv1468c-ubiquitin interaction led to increased bacterial loads in mice with elevated host inflammatory responses. Together, our findings reveal a previously unrecognized host antimicrobial autophagy pathway via targeting of ubiquitin that directly binds to a mycobacterial surface protein, and indicate a previously unrecognized role of ubiquitin in host innate immune defense against invading pathogens.

P0326

IL-27/IL-27R mediates protective immunity against chlamydial lung infection by suppressing Th17 responses and reducing neutrophils inflammation

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Interleukin-27, a heterodimeric cytokine of IL-12 family, has diverse influences on immune response and development of multiple inflammatory diseases. In this study, we identified the protective role of IL-27/IL-27R in host defense against Chlamydia muridarum (Cm) lung infection and further investigated the immunological mechanism. We first found that IL-27 was involved in Cm respiratory infection considering that IL-27 (EBI3 and p28) and IL-27R (WSX-1) mRNA expression were significantly induced on day 7 post-infection (p.i.). Further results found that IL-27R knockout mice (WSX-1$^{-/-}$ mice) suffered more severe diseases with greater body weight loss, higher chlamydial loads and more severe inflammatory reactions in lungs compared with WT mice. IL-17 producing CD4^{+}T cells (Th17) was dramatically enhanced in lungs on day 7 p.i. and day 14 p.i. in WSX-1$^{-/-}$ mice compared with WT mice. Neutrophils (CD11b^{high} Gr-1^{high}) and correlative cytokines and chemokines, including IL-17, TNFα, IL-6 and CXCR2 in lung were also increased on day 14 p.i. in WSX-1$^{-/-}$ mice compared to WT mice. In addition, WSX-1$^{-/-}$ mice treated with mouse IL-17/IL-17A-blocking antibody showed improved disease status and decreased neutrophils and chemokines, cytokines IL-6 and MIP-2 expression compared to those without IL-17 blocking mice. Taken together, we concluded that IL-27/IL-27R plays a protective role in host defense against Chlamydia lung infection by suppressing neutrophils inflammation mediated by Th17.

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DOI: 10.1002/eji.201970400

P0327

Molecular mechanism of pyroptosis induced by *Brucella melitensis* lipoproteins

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Brucellosis is a zoonotic disease caused by the gram-negative bacterium *Brucella* species, and most of the human brucellosis are caused by *Brucella melitensis*. Brucellosis can cause multiple organs damage and inflammation throughout all courses, severely impacting the patients´ living quality. However, the pathogenesis, immunosuppression and pathogen mechanism of brucellosis have not been elucidated yet. Lipoproteins are key components of *Brucella* invasion and replication. Our study showed that both lipoprotein 19 (OMP19) and 16 (OMP16) of *Brucella melitensis* induced pyroptosis. Inflammatory cytokines, including IL-1β, IL-18, IL-6 and TNF-α, cleaved caspase-1 and GSDMD were all detected in THP-1 cells incubated with OMP19 and 16 by western blot. OMP19 induced the up-regulation of TLR-2 and apoptosis inhibitor “XIAP” expression in THP-1 cells. However, for OMP16, the mRNA expression of pyroptosis effector “GSDME” was up-regulated and inactive IL-18 (15/16 KD) was produced, which could affect the IFN-γ synthesis of effective immune cells, thus inhibits IFN-γ mediated immunity response. Additionally, THP-1 cells incubated with OMP16 were annexin-V staining positive by flow cytometry after 4 hours, indicating that caspase-3 had been activated, which suggested that the pyroptosis induced by OMP16 may be mediated by both caspase-1 and caspase-3. These results suggest that each lipoprotein has different virulence characteristics, with multiple effects on cell death, inflammation and immune evasion caused by *Brucella melitensis*.

P0328

Molecular regulation mechanism of functional glycolipid antigens of Mycobacterium tuberculosis in innate and adaptive immunity

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Mannose-capped lipoarabinomanan (ManLAM) is a lipoglycan serving as a major cell wall component in Mycobacterium tuberculosis (M.tb). Here, we demonstrated a novel ManLAM mediated immune evasion mechanism by inhibitory effects on the polarizations of M1 macrophages and Th1/Th17 cells via CD44. ManLAM induced IL-10 production by upregulating PI3K/AKT signaling pathways in B cells mainly via TLR2, next Mannose receptor (MR). These B10 cells skew CD4+ T towards a Th2 subtype. Collectively, these findings provide a novel mechanism in which M.tb ManLAM negatively regulates host anti-TB immunity and ManLAM is potential as a novel therapeutic agent in autoimmunity. Our data demonstrate that an aptamer specifically against ManLAM with low and no toxicity, holds a potential antimycobacterial agent and tuberculosis vaccine immune adjuvant.application.

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DOI: 10.1002/eji.201970400

P0329

STAT1 contributes to host defense against intracellular bacteria downstream of NOD1/2

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Sensing intracellular pathogens is crucial for the induction of inflammatory processes and effective adaptive immune responses against pathogenic microbes. Nucleotide binding oligomerization domain 1(NOD1) and nucleotide binding oligomerization domain 2 (NOD2) are intracellular sensors for small peptides derived from the bacterial cell wall component, peptidoglycan. And they can coordinate signaling events that culminate in the induction of inflammation and anti-microbial responses. However, the signaling mechanisms involved in this process are not fully understood. Here, using Mass Spectrometric Analysis, we identify STAT1 as candidate protein interacts with NOD2. Our results reveal that activating NOD1 or NOD2 signal phosphorylate STAT1 at serine 727 directly and tyrosine 701 through JAK-STAT signaling. Phosphorylated STAT1 does not regulate classical NOD1/2 pathway but plays an important role in the expression of specific antibacterial genes downstream of NOD1/2 activation. These studies thus establish that NOD1/2 can activate STAT1 to provide better protection against intracellular bacterial infection.

P0330

A bacterial effector protein attacks host cells by acting as a eukaryotic-type E3 ubiquitin ligase, kinase and protease

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Tuberculosis (TB), which is caused by the pathogen *Mycobacterium tuberculosis* (Mtb), remains the leading cause of death from a single infectious disease. Drug-resistant TB, especially extensively drug-resistant TB is a cause of great concern, because it renders patients untreatable with available drugs. Therefore, drug discovery based on novel targets is a major focus of TB research. Mycobacterial phospho-signaling proteins have become a prime target for the development of novel therapeutics in TB treatment. Ubiquitin-binding protein 2 (Ubp2), one of the eleven eukaryotic type serine-threonine protein kinases (STPKs) in Mtb, is secreted into the cytosol of infected macrophages to promote intracellular survival of mycobacteria and has been considered as a promising therapeutic target for TB treatment. However, the molecular details of Ubp2-host intracellular interactions remain obscure. Here, **we demonstrate that Ubp2 suppresses host innate immune responses by serving as an unconventional E3 ubiquitin ligase that facilitates the proteasomal degradation of tumor necrosis factor receptor associated factor 2 (TRAF2) and TGF-β-activated kinase 1 (TAK1)**, which are two key components of host NF-κB signaling. **Ubp2 also inhibits pathogen clearance by phosphorylating AS160, the GTPase-activating protein (GAP) for Ras-related small GTPase Rab14, to inhibit phagosome maturation in infected macrophages**. Moreover, **Ubp2 promotes host cell proliferation, depending on its self-cleavage activity triggered by Ubiquitin**. Taken together, **we demonstrate that Ubp2 interferes with multiple host cellular functions by acting as a eukaryotic-type E3 ligase, kinase and protease during mycobacterial infection. Our findings provide a potential TB treatment via targeting of unconventional enzymatic activities of Mtb Ubp2.**

DOI: 10.1002/eji.201970400

P0331

Histone demethylase JMJD2D contributes to host defense against enteric bacteria through up-regulating IL-17F to induce β-defensin expression

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Histone demethylase JMJD2D can specifically demethylate H3K9me2/3 to activate its target gene expression. Our previous study has demonstrated that JMJD2D can promote colorectal cancer progression through activating Wnt signaling. However, its involvement in host defense against attaching and effacing bacterial infection remains unclear. In this study, we showed that JMJD2D was significantly up-regulated in colonic epithelial cells of wild-type mice in response to *Citrobacter rodentium* infection. In light of these results, we hypothesize that JMJM2D plays a role in enteric bacterial infection. To test it, we compared the responses of JMJD2D$^{-/-}$ and wild-type mice to oral infection of *C. rodentium*. We found that JMJD2D$^{-/-}$ mice displayed impaired clearance of *C. rodentium*, more body weight loss, and more severe colonic tissue pathology compared with wild-type mice. JMJD2D$^{-/-}$ mice exhibited an impaired expression of colonic IL-17F, which restricts *C. rodentium* infection by inducing the expression of antimicrobial peptides. Accordingly, JMJD2D$^{-/-}$ mice showed a decreased expression of β-defensin 1, β-defensin 2, β-defensin 3, and β-defensin 4 in the colons. Collectively, our study suggests that JMJD2D contributes to host defense against enteric bacteria through up-regulating IL-17F to induce β-defensin expression.

P0332

M-like protein may not contribute to the virulence in Streptococcus equi subsp. ruminatorum

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A *Streptococci* bacterial isolate, named FJ1804, was isolated from a blood sample of dead *Macaca mulatta* in China, which was classified as *Streptococcus equi subsp. ruminatorum* (*S.e. ruminatorum*) through 16S rDNA alignment. After whole genome sequencing and analyzing, a M-like protein coding gene, which was coding a SrM protein, homologous with SzP, a crucial virulence factor and protective antigen of *S.e. zooepidemicus*, was found in the genome of FJ1804. In order to evaluate the function of SrM in this bacterium, a deletion strain of *srm*, as well as complement, were constructed. Through *in vitro* studies like cell adherence, invasion and phagocytosis assay, *in vivo* study like animal challenge and histopathological analysis, were carried on among the mutant, complement and wild type. Results shows that after deletion of *srm*, the anti-phagocytosis ability the adherence rate of this strain is decreased, but the invasion rate, pathological feature and LD_{50} of both zebrafish and BALB/c mice model shows no difference with WT.
To our knowledge, this is first time reported *S.e. ruminatorum, which is a newly identified zoonotic pathogen,* infection in macaque, and our data suggest that, compared with other *S.e. zooepidemicus* stain, this SzP homologous protein may not contribute to the virulence in this bacterium.

DOI: 10.1002/eji.201970400

P0333

BRD4 promotes the autophagy dependent bactericidal activity of m1 macrophages against listeria monocytogenes infection

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Acetylation is an important means of regulating proteins. BET proteins, including BRD2, BRD3, and BRD4, are a group of regulators controlling transcription through binding acetylated histone and recruitment of transcription complexes. BET inhibitors (such as JQ1) used in research and clinical settings are usually designed to act against cancer and suppress the immune system. Recently we found that BRD4 could regulate the intracellular mechanism by which bacteria are killed. This study focuses on how BRD4 regulates autophagy and how it affects the bactericidal effect of macrophages on *L. monocytogenes*. Here we applied the small molecule inhibitor of BRD4 and constructed the models of infection by *Listeria monocytogenes* in vitro and in vivo. We found that BRD4 can promote the bactericidal effects of macrophages against *L. monocytogenes*. We also found that this effect is achieved by autophagy, which is affected by BRD4 . BRD4 can affect rapamycin-induced autophagy but has no effect on rehalose-mediated autophagy. This indicates that BRD4 regulates the formation of autophagy through the mTOR pathway. In conclusion, BRD4 is an important promoter of macrophage bactericidal action by inhibiting the mTOR pathway of autophagy. These studies have laid a good foundation for elucidating the regulatory mechanism underlying acetylation associated with autophagy in the course of intracellular infection.

P0334

TcpC promote kidney cells to produce CXCL2 via p38 MAPK pathway

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Pyelonephritis is an infection of the upper urinary tract. The most common pathogen for pyelonephritis is uropathogenic *Escherichia coli* (UPEC) bearing distinct virulence factors. Toll/interleukin-1 receptor domain containing protein encoded by *E. coli* (TcpC) is an important virulence factor in most strains of UPEC and inhibits the macrophage-mediated innate immunity, which plays crucial role in the pathogenesis of pyelonephritis. In this study, we showed that TcpC could induce kidney cells to produce CXCL2 both *in vivo* and *in vitro*. CXCL2 concentration in kidney homogenates from TcpC-secreting UPEC CFT073 ($TcpC^{wt}$) caused murine pyelonephritis model was significantly higher than that in kidney homogenates from *tcpC* knock-out CFT073 ($TcpC^{-/-}$) caused model. $TcpC^{wt}$ dose-dependently promoted CXCL2 secretion by HEK-293 cells. In the presence of anti-TcpC antibody, this enhancement effect of $TcpC^{wt}$ on CXCL2 production was completely abrogated, suggesting this enhanced production of CXCL2 was mediated by the secreted TcpC. Furthermore, $TcpC^{wt}$ treatment resulted in activation of p38 MAPK in HEK-293 cells, while $TcpC^{-/-}$ did not. Inhibition of p38 MAPK with SB203580 could significantly decrease CXCL2 concentration and neutrophil recruitment activity in the supernatants of HEK-293 cells co-cultured with $TcpC^{wt}$, indicating that TcpC promote kidney cells to produce CXCL2 via p38 MAPK pathway. Our data provided novel clues to the pathogenicity of TcpC secreting UPEC.

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DOI: 10.1002/eji.201970400

P0335

The *Brucella abortus* Cu^{2+}/Zn^{2+} superoxide dismutase in the Th17 and regulatory T cells (Treg) immune response

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Brucellosis is considered the main zoonosis worldwide, with around half a million cases annually. This infectious disease is caused by intracellular bacteria of the genus *Brucellae*. Currently there is not a vaccine for use in humans and the pharmacological treatment for this condition, although it is not expensive is very long.
It is for this that the development of vaccines for clinical use is still a very active area in the field of research.
The aim of this study consisted in assessing the protective capacity of the SOD Cu^{2+}/ Zn^{2+} from *Brucella abortus* when administered intragastrically and otherwise evaluate the immunological mechanisms involved in response to this protein.
Recombinant SOD was obtained from induction of TOP 10 pBAD-SOD plasmid transformed *E. coli* strain, the protein was purified first by affinity chromatography and subsequently by FPLC.
In a murine model, it was shown that SOD is able to induce systemic protection against *B. abortus* strain 2308 challenge.
When exploring the mechanisms that may be involved in the generation of protection, we found that immunized mice produced antibodies capable of recognizing SOD and also these mice increased the percentage of Th17 cells and decreasing the percentage of TGF-β producing regulatory T cells. These findings may explain some of the SOD protection mechanisms.

P0336

The influence of TNFRp75 on alveolar macrophage and neutrophil responses to early BCG infection

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The World Health Organization reported in 2017 that there were approximately 1.5-2 million deaths per year, with over 10 million new cases of Tuberculosis (TB) worldwide. These statistics suggest that TB remains one of the leading causes of death globally. When *Mycobacterium tuberculosis* (M.tb) enters the respiratory system it is confronted by the innate immune system. The resident alveolar macrophages (AM) and early recruited neutrophils form the first line of defence against pulmonary pathogens. These cells produce tumour necrosis factor ligands and receptors (TNFRp55 and TNFRp75), which has previously been implicated in early protection against M.tb infection. In the present study we investigated the influence of TNFRp75 on AM and neutrophil responses to BCG infection. We studied the differences between wild type and TNFRp75 deficient mice by fluorescently labelling the cells to monitor the difference in neutrophil recruitment, AM and neutrophil pathogen uptake and presentation, and differences in pro-inflammatory cytokine production. Our findings showed that in the absence of TNFRp75 CCL2, CCL3 and IL-1β were significantly upregulated in the BAL fluid as compared to the WT mice. We also found that the number of TNFRp75 deficient AMs expressing MHCII and GFP (BCG-GFP uptake) were significantly higher than in the WT. There were no differences in the neutrophil responses. This data suggests that TNFRp75 supresses the AM responses in response to pulmonary BCG infection and therefore reduces host protection. We want to

DOI: 10.1002/eji.201970400

further investigate if there would be a similar outcome if we infect with the more virulent M.tb H37Rv strain.

P0337

Actinomycetoma development and antibody response to *nocardia brasiliensis* in different strains of mice

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In the present work we study actinomycetoma development and antibody immune response in the infection with *N. brasiliensis* of the immunocompetent mice strains BALB/C and C57BL/6, as well as in the DBA/2 strain (Characterized by NK cell dysfunction), CBA/N mice (With defective B1 cells X-linked), and (CBA/N X DBA/2) F1.
Each strain of mice were infected with *N. brasiliensis* in the rear footpad ($1X10^6$ bacteria in 0.1 ml). We observed the inflammated area until 90 days post-infection. Mice sera were obtained to quantitate IgG anti-*N. brasiliensis* by ELISA.
Infected mice developed acute and chronic inflammation, which are characteristic in mycetoma, except for the DBA/2 strain, in which only 90% of mice developed acute inflammation and died before day 40 without developing mycetoma, recording the highest mortality of all groups. Similar IgG antibody titers anti *N.brasiliensis* antigens were observed in all infected mice strains, except for the CBA /N, which showed a lower concentration.
We found that *Nocardia brasiliensis* infection induces development of mycetoma in BALB/C, C57BL/6, CBA/N, and (CBA/N X DBA/2)F1 mice. IgG anti *N. brasiliensis* antibodies were demonstrated in all infected mice including DBA/2 that died before actinomycetoma development.

P0338

The intergenerational burden of bacterial sepsis - evidence for an acquired and hereditary immunodeficiency

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Sepsis represents the utmost severe consequence of infection, involving a dysregulated and self-damaging immune response of the host. Three months after sepsis onset, animal survivors exhibit sustained alterations in their hematopoietic niche, with an increase of granulocyte-monocyte precursors. Furthermore, RNA-seq on mature $CD11b^+Ly6C^+$ bone-marrow monocytes revealed persisting transcriptomic, resulting in an elevated cytokine response to ex vivo stimulation as well as an increased glycolysis rate. While this “trained immunity” phenotype might be a beneficial and protective adaption for the individual animal, we asked if this phenotype carries on to the offspring. Different environmental exposures like e.g., chronic stress or malnutrition, have been well described to reprogram the germline epigenetically and subsequently alter offspring attributes. Using *Reduced Reduction Bisulfite Sequencing* (RRBS), we were able to identify sepsis-induced changes in the DNA methylation of sperm of survivors, predominantly in the vicinity of genes involved in metabolism and development. Phenotypically, this projected into a decrease in body weight development in the F1 generation. Moreover, alveolar macrophages exclusively from male offspring exhibited a dampened cytokine response to fungal zymosan compared to offspring of healthy fathers, while the response to bacterial lipopolysaccharide (LPS) was unaltered. Contrastingly, intraperitoneal injection of LPS leads

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DOI: 10.1002/eji.201970400

to decreased systemic levels of IL-6 in male offspring. Overall, our results prove that paternal sepsis induces complex intergenerational changes in immunity, which seem to be restricted to male offspring. In sharp contrast to the adaptions of the F0 generation, these changes seem to be maladaptive and resemble an acquired and hereditable immunodeficiency.

P0339

Intrinsic antibacterial activity of nanoparticles made of beta-cyclodextrins potentiates their effect as drug nanocarriers against tuberculosis

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Multi-drug resistant tuberculosis (TB) is a major public health problem concerning about half a million cases each year. Patients hardly adhere to the current strict treatment consisting of more than 10,000 tablets over a 2-year period. There is a clear need for efficient and better formulated medications. We have previously shown that nanoparticles made of cross-linked poly-β-cyclodextrins (pβCD) are efficient vehicles for pulmonary delivery of powerful combinations of anti-TB drugs. Here, we report that in addition to be efficient drug carriers, pβCD nanoparticles are endowed with intrinsic antibacterial properties.
In mouse infected lung, we observed that pβCD nanoparticles are mainly engulfed by alveolar macrophages and modulate host cells properties impairing *M. tuberculosis* (Mtb) establishment. pβCD hamper colonisation of macrophages by Mtb by interfering with lipid rafts, without inducing toxicity. Moreover, pβCD provoke macrophage apoptosis leading to depletion of infected cells, thus creating a lung micro-environment detrimental to Mtb persistence. Taken together, our results suggest that pβCD nanoparticles loaded or not with antibiotics have an antibacterial action by their own and could be used as carrier in drug regimen formulations effective against TB. This activity could fit into the emerging and promising concept of anti-TB approaches by host-directed therapy, which aims to empower host immune properties for the elimination of mycobacteria and/or for the reduction of tissue damage induced by the infection.

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DOI: 10.1002/eji.201970400

P0340

Human macrophages derived from induced pluripotent stem cells express M1/M2 combined phenotype similar to alveolar macrophages, are phagocytic and able to restrict mycobacterial growth

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Macrophages represent a heterogeneous population of phagocytic cells and play important role in immune response, homeostasis and resolution of inflammation. Understanding mechanisms underlying macrophage generation and functioning is important and requires adequate experimental models. Currently, the most widely used models are human cell lines and monocyte-derived macrophages; they do not allow addressing cell differentiation process, population heterogeneity and do not model tissue macrophages, cells that are primarily responsible for local response to infection. To model macrophage interactions with pathogens, we have generated human macrophages from induced pluripotent stem cells (iPSC) and have characterized their phenotypic and functional properties. iPSC-derived macrophages expressed $CD45^+CD14^+CD11b^+$ phenotype typical for mononuclear phagocytes. The cells were heterogeneous with regards to the expression of CD86, CD163, CD206, and HLA-DR. Compared to blood monocyte-derived macrophages, iPSC-derived macrophages contained more $CD163^+$ and $CD86^+$ cells and less $HLA\text{-}DR^+$ cells, i.e., expressed combined M1/M2 phenotype characteristic for alveolar macrophages. The cells were large, highly vacuolated and exhibited high phagocytic activity. In *in vitro* cultures, iPSC-derived macrophages inhibited *Mycobacterium tuberculosis* (*Mtb*) growth for up to 95%. IFN-γ did not increase significantly the antimycobacterial activity of iPSC-derived macrophages, likely because they were already activated during the differentiation process. In summary, iPSC-derived macrophages express phenotype close to human lung macrophages, are phagocytic and able to partially inhibit *Mtb* growth, which allows using them to model the interactions of human macrophages with different pathogens, including *Mtb*.
The study supported by RAS program “Fundamental studies for biomedical technologies” and RSF grant #19-75-20176.

P0341

SND1 promotes type-I immunity against chlamydial lung infection through enhancing dendritic cell function

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To date, no reports have linked the multifunctional protein, staphylococcal nuclease domain-containing protein 1 (SND1), to host defense against intracellular infections. In this study, we investigated the role and mechanisms of SND1, by using SND1 knockout ($SND1^{-/-}$) mice, in host defense against the lung infection of *Chlamydia muridarum*, an obligate intracellular bacterium. Our data showed that $SND1^{-/-}$ mice exhibited significantly greater body weight loss, higher organism growth, and more severe pathological changes compared with wild-type mice following the infection. Further analysis showed significantly reduced *Chlamydia*-specific type 1 immune responses in $SND1^{-/-}$ mice after infection. **Interestingly, the dendritic cells (DCs) isolated from $SND1^{-/-}$ mice showed lower costimulatory**

DOI: 10.1002/eji.201970400

molecules expression and IL-12 production, but higher IL-10 production compared with those from wild-type control mice. In the DC-T cell co-culture system, DCs isolated from SND1$^{-/-}$ infected mice showed significantly reduced ability to promote *Chlamydia*-specific IFN-g producing Th1 cells but enhanced capacity to induce CD4^{+}T cells into Foxp3^{+} Treg cells. **Adoptive transfer of DCs isolated from SND1$^{-/-}$ mice, unlike those from wild-type control mice, failed to protect the recipients against challenge infection.** These findings provide *in vivo* evidence that SND1 plays an important role in host defense against intracellular bacterial infection, and suggest that **SND1 can promote type 1 T cell immunity and inhibit the expansion of Treg through modulation of the function of DCs.**

P0907

Immunologic functions of murine conventional and plasmacytoid dendritic cell subsets response to mycobacterium bovis BCG infection in vitro and in vivo

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Dendritic cells (DCs) are known to consist of distinct subsets, however, their immunologic function especially in pathogenic infection such as mycobacteria is poorly understood, largely because of their rarity and prepared difficulty. We used murine Flt3 ligand to generate conventional (FL-cDCs) and plasmacytoid DCs (FL-pDCs), as well as purified splenic cDCs and pDCs to evaluate their immunological response to bacillus Calmette-Guérin (BCG) infection *in vitro* and in vivo. BCG cells were observed inside both cDCs and pDCs by confocal microscopy, however, pDCs showed a significantly higher infection rate and intracellular bacterial counts compared with cDCs analyzed by FCM and CFU counting respectively, indicating greater infection susceptibility of pDCs to BCG than cDCs. FCM analysis showed significantly lower expression of CD40, CD80, CD86, and MHC-II molecules on pDCs than that on cDCs since 4 h after BCG exposure, suggesting less efficiency of maturation and activation of pDCs than cDCs. Cytokines detection results displayed that pDCs secreted hither TNF-α and IL-6 while cDCs expressed higher IFN-γ and IL-12p70 in the first 12 h post infection. Although both subsets proved capable of presenting a mycobacterial antigen, pDCs exhibited weaker activity in this respect than cDCs. In summary, cDC and pDC, can extensively participate in immunity against BCG infection, pDC showed a greater capacity to internalize bacilli and induce inflammatory response, meanwhile, cDC had important roles in activation and maturation as well as antigen presentation to trigger T cell immune response in vitro and in vivo.

P0908

New insights on the mechanisms of C5aR1 (CD88) involved in human urinary tract infection

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Uropathogenic *Escherichia coli* (UPEC) is a common pathogenic bacteria of urinary tract infections(UTIs). The type 1 fimbriae of *E. coli* is an important virulence factor of adhesion and binding with mannose glycoprotein receptors. C5aR1 participates in the pathogenesis of ascending UTIs, but the mechanism is less known. Here we show that mannose glycans mainly expression in the cortex and medullary junction of human kidney, which is the renal proximal tubule localised area. *In vitro* experiments, we culture the proximal renal tubular epithelial cells (PTECs), founding that the expression of D-mannose on PTECs can be increased by LPS or C5a stimulation. Antagonizing C5aR1 reduced adhesion/transmigration of *Escherichia coli* in renal tubule cells. In addition, C5aR1 signaling remarkably up-regulated pro-inflammatory cytokines(i.e. TNF-α) production by renal tubule

DOI: 10.1002/eji.201970400

cells under bacteria infection. The mechanism studies founed that ERK and NF-κB phosphorylation after C5a stimulation. Furthermore, clinical sample studies showed that C5a concentration was significantly higher in urine of urinary tract infection patients than healthy controls and UPEC binding to the epithelium exactly depend on the D-mannose expression. In summary, our recent studies emphasize a novel pathogenic mechanism that C5aR1 facilitating bacterial attachment/colonisation to renal epithelium. In addition, our findings also suggest a potential new therapeutic target for UTIs.

P0910

Preparation of the novel neutralized monoclonal antibodies against Staphylococcus enterotoxin B

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Staphylococcal enterotoxin B (SEB) is a potent superantigen that elicits life-threatening polyclonal T-cell proliferation and cytokine production at very low concentrations. SEB binds to both MHC-II on antigen presenting cells (APCs) and to TCRs in corporating particular Vβ chains on T-cells, which triggers the activation of many intracellular signaling processes. Because of the rapid binding to the receptors in vivo and difficulty in detection in serum, the development of therapeutic reagents and vaccines that neutralize or prevent the devastating effects of SEB remains a challenge. Here, we screened the specific antibodies against SEB in naïve full human antibodies library by phage display. Two full human monoclonal antibodies (named as YG11-1 and YG11-2) against SEB were obtained after four rounds screening. The results of ELISA showed that YG11-1 and YG11-2 had a high affinity with 16.59nM and 21.35nM separately. Furthermore, treatment with YG11-1 and YG11-2 resulted in a significant reduce the mortality of murine lethal shock models induced by SEB. Our findings present the specific human monoclonal antibody against SEB can protect mice from SEB challenge, which may constitute a better treatment strategy and provide a promising therapeutic agent for treatment of SEB intoxication.

P0911

IL-17 signaling involved in *Borrelia hermsii*-induced central nervous system diseases

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Relapsing fever (RF) neuroborreliosis (RFNB) is a central nervous system (CNS) syndrome caused by *Borrelia hermsii* infection. By establishing the RFNB mouse model, our previous findings showed that the T-cell deficient mice had no neurological symptoms, indicating that T cells play a key role in this CNS complication. Both Th1 and Th17 subtypes of CD4-positive T cells play important roles in inflammatory and infectious CNS diseases. However, Th1 cell deficiency (Tbet-/-) had no effect on this mouse model. Therefore, we proposed in this study that Th17/IL-17 signaling may play some roles in *Borrelia hermsii*-induced CNS disease. Firstly, our present studies have shown that infection of *Borrelia hermsii* can stimulate the expression of IL17 mRNA in the spinal cord of infected wild-type C57BL/6 mice. Multiplex cytokines assay showed that IL-17A became detectable on day 7 post infection. Moreover, the IL-17A-deficient mice also had high bacterial replication efficiency when infected with RF, as did the wild-type mice. This infection also induced IL-17A-deficient mice to produce *Borrelia hermsii*-specific IgM and IgG with recurrent peaks. Notably, no observable CNS syndrome was induced in IL-17A-deficient mice by *Borrelia hermsii* infection. Histologically, spinal cords and brains of IL-17A-deficient mice showed no significant inflammation post *Borrelia hermsii*

DOI: 10.1002/eji.201970400

infection. In summary, results in this study indicated that IL-17-signaling plays critical roles in induction of CNS diseases by RF *Borrelia hermsii* infection. Cellular sources for IL-17 signaling will be investigated for further pathogenesis of RF *Borrelia hermsii*-induced CNS diseases.

P0912

Biofilms assist salmonella in escaping from the immune surveillance of intestinal mucosal dendritic cells

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The intestinal tract is the primary invasion site for the *S.typhimurium*. Intestinal mucosal dendritic cells (DCs) can form transepithelial dendrites (TEDs) to sample luminal bacteria for establishing immune surveillance system. Frequently, *S.typhimurium* in the manner of biofilm formation access to intestinal tract. It remains unclear whether biofilms of *S.typhimurium* affect the immune surveillance of intestinal mucosal DCs. Here, mice ligated loop model was established and the results showed that biofilm formation strains, in comparison with planktonic strains, attenuated the recruitment of DCs to the intestinal epithelial cells (ECs), and decreased the TEDs formation for luminal bacteria uptake. Biofilms significantly suppressed the secretion of the chemokine CCL20 from the apical ECs, which drove DC recruitment and TED formation. *In vitro,* biofilms attenuated the *S.typhimurium* uptake by DCs, suppressed the expression of phenotypic markers (MHC II, CD40, CD80, and CD86 molecules), pro-inflammatory (TNF-α, IL-1β, IL-6 and IL-12p70) and anti-inflammatory (IL-10) cytokines, arrested the promoting ability of *S.typhimurium*-induced DCs for allogeneic T cell proliferation. These findings suggested that biofilms posed the ability to assist *S.typhimurium* in escaping from the immune surveillance of intestinal mucosal DCs, which could be a new pathogenesis mechanism of *S.typhimurium*.
Keywords: *S.typhimurium*; Dendritic cells; Biofilms; Immune escape

P0913

Wolbachia utilize host innate immunity to promote its symbiosis formation in dengue vector mosquito Aedes aegypti

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The host immune system plays a central role in shaping the composition of the microbiota and, in return, resident microbes influence the immune responses. Symbiotic associations of the maternally transmitted bacterium *Wolbachia* occur with a wide range of arthropods. It is, however, naturally absent from the dengue vector mosquito *Aedes aegypti*. When *Wolbachia* is forced to form symbiosis with this new mosquito host, it boosts a basal immune level and enhances this mosquito's resistance to pathogens, including dengue virus and malaria. However, the mechanisms involved in establishing a symbiotic relationship between *Wolbachia* and *A. aegypti* and the long-term outcome of this interaction are unclear. Here, we have demonstrated that both the IMD and Toll pathways are activated by the *Wolbachia* strain *w*AlbB upon its introduction into *A. aegypti.* Notably, *Wolbachia* induces PGRPLE to initiate the IMD pathway activation. Silencing Toll and IMD pathways via RNA interference reduces *w*AlbB load. Using transgenic mosquitoes with stage-specific induction of the IMD and Toll pathways, we have shown that elevated *w*AlbB infection in this background is maintained via maternal transmission. Hence, our study suggests that *Wolbachia* utilizes the host immune system to promote the establishment of a symbiotic relationship with its new host, the mosquito *A. aegypti*.

DOI: 10.1002/eji.201970400

Our findings have important implications for further development of novel strategies in the control of mosquito-borne diseases.

P0914

A study on role of improved immune function in treatment of chronic brucellosis with positive Brucella DNA in peripheral blood phagocytes

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Objective: Observing therapeutic effect on chronic brucellosis patients with DNA positive in peripheral blood phagocytes by improving immune function in case of not using antibacterial drugs.
Methods: The anti-brucella antibody in the serum of the patients was detected by tube agglutination test; The DNA of Brucella in the living blood phagocytes and in the serum were separated by a self-developed Brucella DNA extraction kit, and detected by PCR; 32 patients with chronic brucellosis negative for brucella DNA in serum but positive for it in phagocytes were observed as therapeutic objects; Lymphocyte subsets in peripheral blood were detected by flow cytometry; Serum Ig and complement components were detected by immunoluminescence technique. Treatment was carried out by improving immune function with combination of Mongolian medicine and chemical drugs.
Results: Clinical symptoms of 32 patients disappeared. The intracellular brucella DNA was negative after treatment. It showed, before treatment γδT cells increased, Treg cells decreased, Monocytes decreased; IgM level was a little low; C3 level decreased. After treatment, γδT cells were lower, Monocytes and Treg cells were both higher than that before treatment, IgM was a little higher than that before treatment, C3 returned to normal. There was no significant change in other immune function items including CD3+, CD4+, CD8+T cells, NK cells, B cells, IgG, IgA, IgE and C4 before and after treatment.
Conclusions: It difficult to remove the brucella within cells. due to the reduced innate immune response. Brucella from patients could be eliminated by improved immune function without antibacterial drugs.

P0915

Hdac3 deficiency in macrophages alters the inflammation response in pseudomonas aeruginosa-induced airway injury model

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Macrophage plays a vital role in pulmonary infection, and its inflammatory response is regulated by chromatin-modifying enzymes, including histone deacetylases (HDACs).Our research aimed to elucidate the regulatory role of Hdac3 in this process. In the LPS-induced airway injury model, the inflammatory response and lung damage of Lysm conditional Hdac3 knockout mice which specifically depletes Hdac3 expression in macrophages were significantly reduced.The ratio of macrophages and neutrophils were changed as well.Decreased levels of inflammatory response was also detected in Gram-negative pseudomonas aeruginosa-induced airway injury model,and resulted in higher mortality rates .While employing a clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9-based gene silencing technology,we found HDAC3 knockout Raw264.7 cells expressed less inflammatory mediators than that of wild type,including chemokines, cytokines, ISGs(interferon-stimulated genes) and so on. Same phenomenon was observed in bone-marrow derived

DOI: 10.1002/eji.201970400

macrophages(BMDMs).A series of inflammatory signaling pathways were involved, of which NF-κB signaling pathway played an important role.The basal and phosphorylated level of P65 significant declined in Hdac3 knockout Raw264.7 cells,BMDM and HEK 293T,as the stability of P65 protein was impaired.In summary,these findings suggests the important role of Hdac3 in the regulation of macrophages' function ,and this affect the inflammatory condition and immune system of body.

P0916

Prepration and preliminary pharmacodynamic evaluation of fully human novel anti-SEB antibody

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Staphylococcal enterotoxin B (SEB) is one of the most common and most toxic bacterial exotoxins produced by Staphylococcus aureus. SEB causes poisoning and even toxic shock syndrome, and the mortality rate can reach 50%. Till now, there are no vaccines or specific drugs for SEB in clinical application. Many anti-SEB neutralizing antibodies have been reported in lab recently. They have shown good in vivo protection effects in the mice model (for example,200 µg of antibody, GCI064, GCI075 and GCI079, was administered 1 hour after exposure to lethal dose of SEB, and all the mice survived.) Using the human phage antibody library, we successfully screened a novel anti-SEB human antibody, named as 08-1, possessed the high affinity 0.1 nM. And then, we constructed the Balb/c mice model with lethal dose of SEB, the protected activity of 08-1 was evaluated. The experimental results showed that the mice were completely survived with administration of 2.5mg/kg - 10mg/kg antibody 08-1. In addition, the empty window period is about 2 hours. It was suggested that 08-1 can effectively neutralize SEB infection and has potential clinical application value.

P0917

Effect of probiotic on gut microbiota in peritoneal dialysis patient with protein-energy wasting: 1 year follow up of a randomised controlled trial

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The purpose of this study was to evaluate the clinical efficacy of probiotics on PEW in peritoneal dialysis (PD) patients.
One hundred and twelve PD patients with PEW in Guizhou provincial people's hospital from July 2014 to July 2015 were randomly divided into intervention group (n=56) and control group (n=56). Lactobacillus bulgqricus and Streptococcus thermophilus) daily for two months, while the control group received similar table without probiotics for the same duration. Biochemical indicators, physical measurement and human body bioelectrical impedance were measured before and two months after intervention. The peritonitis and cardiovascular events were also recorded after 1 year follow up.n patients receiving probiotic, levels of CRP and triglyceride significantly decreased after two months of treatment, while levels of serum albumin, HDL and fat mass significantly increased. In contrast, there were no significant changes in levels of uremic toxin and physical measurement between groups before or after treatment. In a multiple analysis, the cumulative incidence of peritonitis was significantly affected by probiotic regime compared to the control group (OR 0.64, 95 % CI 0.39-0.89, p = 0.023). In

DOI: 10.1002/eji.201970400

addition, there was as trend towards a lower cumulative incidence of cardiovascular events in the probiotic group.

Conclusions: Probiotics could significantly reduce the cumulative incidence of peritonitis in PD patients via decreasing the serum levels of CRP and triglyceride, increasing the serum levels of serum albumin, HDL and fat mass.

P0918

Altered gut microbiome contributes to the diabetogenic effects of sirolimus in mice

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Background: Metabolic disorders are a frequent complication after the use of immunosuppressive agents. Previous studies focused on pancreatic toxicity, however, the contribution from the 'gut-pancreas' axis remains unknown. Here, we aimed to examine the alterations induced in intestinal microbiota (IM) by sirolimus (SIR) a mammalian target of rapamycin inhibitor, and its underlying mechanism of the diabetogenic effects in male C57BL/6 mice.
Results: Intraperitoneal administered SIR markedly increased the blood levels of glucose and lipids in a dose-dependent manner. In consistent with previous reports, high dose SIR (2.0 mg/kg) reduced insulin secretion and decreased the number and area of pancreatic beta-cells. Despite the clinical-mimicking dose, low dose SIR (0.5 mg/kg), caused no obvious injury in the pancreas, it induced significant insulin resistance (IR). Further, the low dose SIR mice exhibited increased intestinal permeability, reduced intestinal mucosal mucins, and elevated expression of circulating lipopolysaccharide (LPS) and pro-inflammatory cytokines compared with the control mice. In addition, altered microbiome in the low dose mice significantly correlated with glucose and IR respectively, which were characterized by the depletion of benefit-promoting Akkermansia bacteria and enrichment of LPS-producing Proteobacteria bacteria.
Conclusions: The clinical-mimicking dose of SIR altered the microbiome, disturbed the intestinal barrier function, increased the circulating LPS level, and led to system inflammation response and IR. This study firstly provided evidence that the IM might participate in SIR-induced glucose disorders via disturbing the intestinal barrier function, suggesting a novel therapeutic target for the disease.

P0919

Lypd8 suppresses the attachment of pathogenic bacteria to colonic epithelia

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Mucosal barriers serve as a segregator of commensal microbes and intestinal epithelia to maintain gut homeostasis. Ly6/Plaur domain containing 8 (Lypd8), a highly glycosylated GPI-anchored protein highly and selectively expressed on enterocytes of the colon, promotes the segregation of commensal bacteria and colonic epithelia by inhibiting bacterial invasion of the colonic epithelia. However, the

DOI: 10.1002/eji.201970400

function of Lypd8 against bacterial pathogens is totally unknown. *Citrobacter rodentium* is one of enteric bacterial pathogens causing colitis by inducing attachment and effacement (A/E) lesion on colonic epithelia. Here, we demonstrate that Lypd8 contributes to early-phase defense against *C. rodentium* by inhibiting the bacterial attachment on intestinal epithelial cells by suppressing the interaction of intimin and translocated intimin receptor (Tir). Therefore, in Lypd8-deficient mice infected with *C. rodentium*, a larger number of *C. rodentium* attached to colonic epithelia, resulting in severe colitis with the expansion of Th17 cells and neutrophils in the lamina propria. This study identifies a novel function of Lypd8 against A/E bacteria and highlights the enterocytes as a crucial innate immune player in the protection against enteric bacterial pathogens.

P0920

Irg1 induction in macrophages infected with Mycobacterium tuberculosis depends on STING/IFNAR-dependent signaling pathway

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Despite the existence of prophylactic and therapeutic strategies, TB caused by *Mycobacterium tuberculosis* (*Mtb*) remains a serious public health problem. IRG1 is an enzyme induced by inflammatory stimuli, which generates itaconate, a metabolite that plays a key role in the regulation of inflammatory responses. This property makes IRG1 a potentially important mediator in preventing excessive inflammation and tissue damage in *Mtb* infection. Here, we investigated the pattern recognition receptors and signaling pathways by which *Mtb* triggers Irg1 gene expression in control and genetically deficient murine macrophages. We observed a partial role for TLR2 (but not TLR4 or 9), MyD88 and NFkB signaling pathways in Irg1 induction by live Mtb bacilli. In addition, drug inhibition studies revealed a major requirement for phagocytosis and endosomal acidification in Irg1 gene expression triggered by *Mtb*, but not by LPS or PAM3CSK4. Importantly, the *Mtb*-induced Irg1 response is also dependent on bacterial ESAT-6 expression, as well as STING and type 1 interferon receptor (IFNAR) signaling with IFN-g playing a minor role. Based on these findings we hypothesize that *Mtb* induces Irg1 expression in macrophages via two interacting signaling pathways: 1) TLR2- and MyD88-dependent NFkB induction, presumably at the host cell plasma membrane; and 2) a critical enhancing signal stimulated by phagocytized *Mtb* products released into the cytosol by an ESAT-6-dependent mechanism, which activates the STING/IFNAR pathway.
This work was partially by the intramural research program of the NIAID and a fellowship from FAPESP to Caio Bomfim

P0921

Intragastric administration of *Lactobacillus helveticus* SBT2171 controls *Porphyromonas* gingivalis-induced inflammation and bone resorption

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Lactobacillus helveticus SBT2171 (LH2171) is a lactic acid bacterium with high protease activity and used in starter cultures in the manufacture of cheese. We assessed the potential of gastric

DOI: 10.1002/eji.201970400

administration of LH2171 for *Porphyromonas gingivalis* infection. LH2171 treatment significantly reduced alveolar bone loss and bacterial colonization by subsequent *P. gingivalis* challenge. Furthermore, the expression and secretion of TNF-α and IL-6 in gingival tissue was significantly decreased in LH2171-administered mice after bacterial infection. Conversely, β-defensin 3 in saliva was found to be produced one week after LH2171 administration, and showed a maximum amount after 3 weeks. Furthermore, LH2171 induced an increase in $CD11c^{+}CC6^{+}$dendritic cells in the gingival mucosa and submandibular gland. When the serum from LH2171-sensitized mice was intravenously injected into recipient mice, enhancement of production and expression of β-defensin 3 in saliva, tongue and submandibular gland was observed. These results suggest that gastrically administered LH2171 can enhance immunoregulation followed by periodontitis prevention in oral mucosa via the gut immune system; i.e., the possibility by homing of nucleic acid component in the blood.

P0922

Purification and molecular cloning of a cecropin-like peptide from the Japanese oak silkworm, Antheraea yamamai

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A cecropin-like antibacterial peptide gene was isolated from the immunized larvae of the japanese oak silkworm, Antheraea yamamai and designed Ay-CecA.
The complete Ay-CecA cDNA consists of 419 nucleotides with 195 bp open reading frame encoding a 64 amino acid precursor that contains a putative 22-residue signal peptide, a 4-residue propeptide and a 37-residue mature peptide with a theoretical mass of 4046.81. The deduced amino acid sequence of the peptide evidenced a significant degree of identity (62-78% identity) with other lepidopteran cecropins. Like many insect cecropin, Ay-CecA also harbored a glycine residue for C-terminal amidation at the C-end, which suggests potential amidation. To understand this peptide better, we successfully expressed bioactive recombinant Ay-CecA in Escherichia coli that are highly sensitive to the mature peptide. For this, we fused mature Ay-CecA gene with insoluble protein ketosteroid isomerase (KSI) gene to avoid the cell death during induction. The fusion CecA-KSI protein was expressed as inclusion body at high level (more than 40% of the total proteins). The expressed fusion protein was purified by Ni-NTA immobilized metal affinity chromatography (IMAC), and cleaved by cyanogen bromide (CNBr) to release recombinant Ay-CecA. The purified recombinant Ay-CecA showed considerably antibacterial activity against Gram-negative bacteria, E. cori ML 35, Klebsiella pneumonia and Pseudomonas aeruginosa. Our results proved that this peptide with a potent antibacterial activity may play a role in the immune response of japanese oak silkworm.

P0923

Production of recombinant Stx2eB protein from Shiga toxin using the baculovirus expression system

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Edema disease of swine is an infectious disease caused by enterotoxigenic E. coli stain F18ab, which is produce shiga toxin Stx2e, and its mortality rate reaches 80%. The Stx2e consist of two major subunits, an enzymatically active A subunit that joins noncovalently to a pentamer of five identical B subunits. The function of the B subunits is to bind to a specific glycolipid receptor, globotriaosylceramid, Gb3, found primarily on endothelial cell.

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DOI: 10.1002/eji.201970400

In this study, we generated recombinant Shiga toxin (Stx2eB) protein based on non-toxic B subunits via baculovirus expression system (BES). The recombinant gene encoding for Stx2eB protein was cloned and expressed in the Sf9 cell by Autographa californica multiple nucleopolyhedrovirus (AcMNPV). After expression and purification procedures, the recombinant Stx2eB was verified by SDA-PAGE and western blot analysis. As a result, recombinant Shiga toxin (Stx2eB) protein was efficiently expressed and extracted from the Sf9 cell using the BES. We expect to be able to build a recombinant vaccine production system for swine diseases.

P0924

Antigen-induced production of eight cytokines reflects pulmonary tuberculosis severity and predicts patients' responsiveness to treatment

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Pulmonary tuberculosis (TB) is a heterogeneous disease that may exhibit various clinical manifestations and severity. It is understood that the heterogeneity in TB severity and outcomes is determined by host immune reactivity to the infection. However, exact immune factors which evaluation may be used to predict disease outcomes and adjust therapy based on individual need are not known. Here we aimed to identify immunological parameters associated with patients' responsiveness to TB treatment and disease outcomes.
Thirty seven patients with active TB were included in the study and divided into test (n=19) and validation (n=18) subgroups. For each participant QuantiFERON-TB Gold Plus was performed, and the levels of 44 cytokines in patients' serum and antigen-induced plasma (AG-plasma) were determined using xMAP multiplex assay. Immunological data were analyzed in R-studio using non-parametric correlation and hierarchical clustering analysis and compared to the results of clinical evaluation of TB severity and patients' response to therapy.
In hierarchical clustering analysis, test group clustered into two subgroups (“high” and “low” producers) based on the levels of 14 cytokines. Clinically, “high-producers” had more severe TB and much slower response to TB therapy compared to “low-producers”. In validation analysis, 8 of 14 cytokines (type I, III interferons, members of IL-10 and IL-12 cytokine superfamilies) divided the patients into “high” and “low-producer” groups that again differed by TB severity and therapy response.

The results suggest that early evaluation of immune reactivity may help identifying patients with poor prognosis for whom personalized immunotherapy may be beneficial.

Supported by Grant RSF-17-75-10197

P0925

Endogenous intoxication and pathogenesis of facial flegmons

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Aim: Assess the indices of endogenous intoxication (EI) in patients with phlegmons.
Materials and methods: In investigation 82 patients with facial phlegmons and 26 healthy volunteers was included. The absolute content and composition of blood leukocytes was evaluated. To characterize the EI a complex of leukocyte indices was used. Data were processed using the unpaired version of Student´s *t*-test.
Results: In 46.4% of patients the leukocyte intoxication index (LII) did not exceed 3 (1st group), while

DOI: 10.1002/eji.201970400

in others (2nd group) LII value was higher than 3 ($p < 0.05$). Patients of the 2nd group were hospitalized statistically significantly longer. In patients of the 1st group inflammation was more often localized in the 2-3, and in the 2nd - occupied more than 3 cellular tissue spaces. In patients of the 2nd group the left leukocyte formula shift was more significant. The number of monocytes in the compared groups was not statistically significant. In the 2nd group relative lymphocytopenia was observed. These patients have a low index of body resistance, which indicates obligatoriness of detoxification therapy. EI was expressed more in comorbid pathology. So, against the background of chronic pyelonephritis, leukocytosis and leukocyte shift to the left are more pronounced, as well as a decrease in the number of monocytes and lymphocytes than in other groups.
Conclusion: In the pathogenesis of facial phlegmons EI plays a significant role, which requires medical (detoxification) measures, especially for spilled forms of the disease and against the background of comorbid pathology.

P0926

A zinc metalloproteinase of Brucella abortus regulates its resistance inside phagolysosomes of macrophages

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Macrophages eliminate bacteria within phagolysosomes which have an acidic pH and producing reactive oxygen and nitrogen species. Nevertheless, *Brucella abortus*, causative agent of brucellosis, survives in this environment and escapes to the endoplasmic reticulum where it develops its replicative niche. The open reading frame (ORF) BAB1_0270 of *B. abortus* 2308 codifies a Zinc-metalloproteinase involved in its survival and replication within macrophages and epithelial cells. Since the function of this ORF is unknown, we evaluated its contribution to the resistance to oxidative stress, low pH and to the intracellular traffic in RAW 264.7 macrophages. Results showed that the deletion of ORF BAB1_0270 significantly reduced the growth of *B. abortus* cultured in the presence of hydrogen peroxide or low pH due to a reduction in the expression of enzymes having anti-oxidative activity (*katE* and *ahrC*), of regulatory genes (*oxyR*, *phyR*, *HutC* and *vjbR*) and of virulence factors (virB1, virB2, BtpA, BceA), Thus, this deletion significantly reduced the capacity of the mutant strain to escape from phagolysosomes. Bioinformatics analyses allowed us to suggest that this metalloproteinase cleaves repressor proteins, allowing the expression of the genes required by this bacterium to survive under low pH and oxidative conditions to which it is exposed during its traffic inside of macrophages. Therefore, ORF BAB1_0270, which codifies a Zn-metalloproteinase, could regulate the gene expression of diverse factors involved in the resistance to acid and oxidative conditions during its intracellular traffic and helping *B. abortus* 2308 to escape from phagolysosomes in macrophages.
Supported by Grant FONDECYT 1180122

P0927

On the possible role of cadaverine in the survival of *Escherichia coli* in inflammatory foci

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Aim of investigation: was to evaluate the effect of cadaverine on the activity of phagocytic cells and microorganisms.
Materials and methods: Phagocytic activity of peripheral blood leukocytes of healthy donors was

DOI: 10.1002/eji.201970400

assessed (Shilov *et al*., 2003) after preincubation with cadaverine (0.01 M) for 60 minutes at 37°C. *Escherichia coli* K12 was cultured in Luria-Bertani broth and cadaverine content was determined by thin-layer chromatography.
Results: *In vitro* experiments found that the synthesis of cadaverine by *E. coli* depends on the oxygen content. When cultivated under aerobic conditions the content of cadaverine in the medium significantly decreased. In the situation when aeration was decreased concentration of cadaverine increased by more than 10 times compared with standard conditions. It was also shown that cadaverine has the ability to alter the phagocytic activity of human peripheral blood leukocytes. First of all, the proportion of neutrophils involved in phagocytosis decreases (48%). At the same time among phagocytic cells the number of those that engulfed more than 2 objects increases.
It can be assumed that in the foci of *E. coli* persistence the concentration of cadaverine increases, which inhibits the phagocytic activity. It is likely that in leukocytic phagosomes, where hypoxia is observed, the active production of cadaverine contributes to the survival of *E. coli* by neutralizing the radicals.
Conclusion: Thus, the ability of cadaverine to influence the activity of pro- and eukaryotic cells in different directions can ensure the survival of *E. coli* both in the inflammatory foci and during uptake by leukocytes.

P1514

Preparation and Immunization of ferritin nanoparticles carrying antigenic epitopes from *Enterococcus feacalis*

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For constructing efficient nano-epitope vaccines for collagen-associated adhesin(Ace) of *Enterobacter faecalis*, epitopes of collagen adhesin were deduced, and relevant gene fragments were combined respectively with that of ferritin H subunit to construct fusion expression vectors; the pET-32a-ace-H plasmid was constructed as control. Western blotting was taken to verify the immunogenicity of the fusion proteins, and transmission electron microscopy was carried out for the morphology of the target proteins. Then, the epitope/protein bearing ferritin nano-particles were vaccinated into rabbits, while conventional immunogen Ace was used as control. Specific antibody levels were determined by indirect ELISA, periodically. The results showed that six epitope-carrying fusion vectors were obtained, designated as pET-32a-ace1-H to pET-32a-ace6-H, among which pET-32a-ace1-H, pET-32a-ace3-H, pET-32a-ace5-H and pET-32a-ace6-H turned out to be soluble, and the four fusion proteins showed strong immunogenicity. TEM images indicated that five H-fusion proteins were characterized by ferritin nano-cages, four of which were hollow, with similar diameter sizes to those of natural ferritin; only pET-32a-ace-H did not form a hollow center, and its diameter was larger. The fusion proteins successfully induced specific antibody response, and the antibody dynamics of the single-epitope-carrying Ace1-H, Ace3-H, Ace5-H and Ace3-H showed good consistency with that of the multi-epitope-bearing Ace and Ace-H. Ferritin nanoparticles can effectively enhance the immunogenicity of the protein antigens, and might become a promising immune adjuvant for epitope vaccines.

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DOI: 10.1002/eji.201970400

P1515

Porphyromonas gingivalis promotes colorectal cancer development by regulating NLRP3 inflammasome signaling

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Metagenomic analyses indicate that some oral microbiota is associated with human colorectal carcinoma (CRC), however, whether this is a causal link remains unclear. We found that *Porphyromonas gingivalis* (*P. gingivalis*) from oral cavity enriched in human stool or FFPE samples from CRC patients compared to colorectal adenoma or healthy subjects. Cohorts studies demonstrated that *P. gingivalis* infection is associated with poor prognosis of CRC. Moreover, in *Apc*$^{Min/+}$ mouse model of colorectal tumorigenesis, *P. gingivalis* increases tumor counts and in orthotopic rectal MC38 model increases tumor growth. Further, tumors from orthotopic MC38 model exposed to *P. gingivalis* exhibited a tumor-infiltrating myeloid cells recruiting signature that is confirmed in human *P.gingivalis*-positive CRC. We next evaluated the role of NLRP3 in *P. gingivalis* promoting on CRC progression. When comparing with WT mice, the increased tumor growth was subtracted in *Nlrp3*$^{-/-}$ mice by the same orthotopic MC38 model exposed to *P. gingivalis*. To determine relevant cell compartment responsible for NLRP3 function, we generated *Nlrp3*$^{-/-}$ chimeric mice with adoptive bone marrow transplantation by using the same orthotopic tumor model. The results showed the effect of NLRP3 on *P. gingivalis* pathogenesis is from both hematopoietic and non-hematopoietic sources. Additionally, we performed expression profile to show that NLRP3 attenuates multiple pathways associated with *P. gingivalis* promoting CRC development. Collectively, these data suggest that, through recruitment of tumor-infiltrating immune cells by regulating NLRP3 inflammasome activity, *P. gingivalis* generates a proinflammatory microenvironment that is conductive for colorectal neoplasia progression.

P1516

TNF-α and IFN-γ productions of human peripheral T cell subsets stimulated by M. tuberculosis antigen differ among the pulmonary tubercuosis and latent infection

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Objective: To investigate the difference of production of TNF-α and IFN-γ in human peripheral blood T cell subsets that stimulated by low molecular peptide (10K) of *Mycobacterium tuberculosis* heat-resistant antigen (Mtb-HAg) between patients with pulmonary tuberculosis and latent infection.
Methods: PBMCs were isolated from healthy adults' peripheral blood, and cultured with Mtb-HAg-10k, Mtb-HAg or PHA as control, the proportions of TNF-α and IFN-γ producing cells in T cell subsets were detected by flow cytometry, the number of IFN-γ producing cells from patients with pulmonary tuberculosis and latent infection was detected by ELISPOT.
Results: The proportion of TNF-α producing γδ T cells was significantly higher than IFN-γ^{+} γδT cells in Mtb-HAg-10k antigen group($P<0.01$). The proportion of γδ T cells producing TNF-α and IFN-γ in Mtb-HAg-10k antigen group were markedly higher than that in PHA group ($P<0.01$). The proportion of αβ T cells producing TNF-α and IFN-γ were markedly lower than that in PHA group($P<0.01$). The number of IFN-γ producing cells in Mtb-HAg-10k group was dramatically lower than that in Mtb-HAg and PHA groups ($P< 0.01$).The number of IFN-γ producing cells in PBMCs in patients with PTB was

DOI: 10.1002/eji.201970400

obviously lower than that in LTBI.
Conclusion : Mtb-HAg-10k can markedly induce γδ T cells to produce TNF-α and IFN-γ, Mtb-HAg-10k-stimulated release of IFN-γ by ELISPOT assay can discriminate between pulmonary tuberculosis and latent infection.
Funding: The Innovation Team Project in Scientific Research Platform of Anhui Colleges and Universities (2016-40); Key Scientific Research Projects by Anhui Provincial Education Department (KJ2018A0227)

P1517

Epigenome-wide DNA methylation profiling of lung tissue and peripheral blood in active pulmonary tuberculosis

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Tuberculosis (TB) remains a major cause of death worldwide. However, mechanisms underlying immune response against TB are still dilemma. More and more evidence support epigenetic regulation plays important roles in both immune protection and pathology. To decipher whether epigenetic regulation is involved in immune responses on TB, we performed DNA methylation analysis in paired lung tissue samples from active pulmonary tuberculosis (PTB) patients and peripheral blood samples from active PTB patients and healthy controls. Gene ontology analysis of differentially methylated genes (DMGs) demonstrated that immune related pathways were enriched both in lung and peripheral blood. To dissect frequencies of subpopulations in lung, we obtained 9 cell-type fractions from methylation data by HEpiDISH, including B cell, NK cell, $CD4^+$T cell, $CD8^+$T cell, monocyte, neutrophil, eosinophil, epithelial and fibroblast. Results have shown that proportions of monocyte and $CD4^+$T cell are significantly increased in TB granulomas. Among the 9 fractions, 7 immune cell fractions could be analyzed in peripheral blood by EpiDISH. However, compared to healthy controls, proportions of $CD4^+$T cell and monocyte do not change but ratio of $CD8^+$T cell is dramatically decreased in active PTB patients. Next, we comprehensively analyzed DMGs, 9 genes showed significant and consistent methylation changes both in lung and peripheral blood. Among 9 genes, our further study focuses on highly conserved transcription factor RUNX1, which modulates angiogenesis, immune responses and inflammatory response. Our results may support DNA methylation plays a role in reprogramming of immune cell function against TB.

P1518

Fibronectin deficiency inhibit the differentiation and phagocytosis of mononuclear macrophages in septic mice

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The treatment of immunosuppressive phase of sepsis has always been the focus of clinical researchers. Previous studies have found that the survival of patients with sepsis could be significantly improved by adding Fibronectin (FN)-rich precipitation. However, the mechanism of FN´s participation in anti-infection process remains unknown. In this study, we constructed Fn conditioned gene knockout mice successfully, and then cecum ligation puncture (CLP) sepsis and other infection models

DOI: 10.1002/eji.201970400

were constructed in mice. The determined survival, bacterial load in the blood, remove bacteria ability of macrophage cell in abdominal cavity, macrophages differentiation direction and degree of the inflammatory cytokine secretion were assessed in these models. We found that, compared with Fn Loxp and wild-type mice, FnKO mice showed significantly greater mortality after CLP (FN KO 85.0 VS Fn loxp 55.0%, P=0.023), the mechanism was that Fn gene knockout increased the bacterial load in the blood of mice after CLP, weakened the phagocytosis and bacterial clearance ability of abdominal macrophages, and affected the expression of pro-inflammatory cytokines after CLP. Furtherly, we found that FN can promote the differentiation and phagocytic killing function of macrophages in septic mice. However, high concentration of pFN shows its anti-inflammatory function. We also find FN can stimulate and activate macrophages through integrin a5b1/ Fak-src signaling pathway. In conclusion, We demonstrated that the absence of FN inhibited the differentiation and phagocytosis of macrophages in septic mice, thus aggravated the immunosuppression and increased the mortality of mice, suggesting that FN play a protective role in the treatment of sepsis.

P1519

The transcriptional profile of macrophage in response to mycobacterium leprae sonicate stimulation

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Objectives: To investigated the immune response of THP-1 derived macrophage stimulated with *Mycobacterium leprae* sonicate (MLS) to verify the genetical associations of leprosy susceptibility genes.
Methods: We sequenced the expression of mRNA, long non-coding RNA (lncRNA) and microRNA of THP-1 derived macrophage. And compared the RNAome of MLS stimulated macrophages with unstimulated macrophages.
Results: In total, 133 mRNAs, 28 lncRNAs and 4 microRNAs were observed to be significantly differentially expressed in MLS stimulated macrophage. KEGG pathway enrichment of differentially expressed mRNA and lncRNA both demonstrated that TNF signaling, NF-κB signaling, cytokine-cytokine receptor interaction, chemokine signaling, NOD-like receptor signaling and TLR signaling pathway were involved in the immune response. The inflammation mediator miR-146 and miR-122 which can regulate interferons signaling pathway were also found to be up regulated in the MLS stimulated macrophage.
Conclusions: Our in vitro transcriptional profile further confirmed the involvement of these immune pathways that genetically associated with leprosy, providing an in vitro disease model for further leprosy pathogenesis studies.

P1520

The effects of MAPK4 deficiency on the pathology of LPS-induced murine acute lung injury

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Background: Acute lung injury (ALI) and its more serious form acute respiratory distress syndrome (ARDS) are characterized by extensive inflammations in lungs. Previous works have shown that mitogen-activated protein kinase(MAPK) subfamilies (such as ERK, p38 MAPK) played important roles in the pathology of ALI. However, the role of MAPK4 in the development of ALI remains unclear.
Objective: To detect the effects of MAPK4 deficiency on the pathology of murine ALI, and

preliminarily explore its significance.
Methods: Murine ALI model was established by intraperitoneal injection of LPS in WT or MAPK4$^{-/-}$ mice. Then, the survival ratio was observed and the pathologic injury of lung tissues was measured by HE staining. Moreover, the weight index, lung weight index and lung wet-dry ratio were recorded. Furthermore, the expressions of related cytokines in lung tissues were analyzed by Real-time PCR and ELISA. Finally, the changes of related signaling pathways were measured by western blot.
Results: Compared with WT mice, MAPK4$^{-/-}$ mice exhibited less mortality after LPS challenge. The pathological damage in lung tissues was significantly alleviated in MAPK4$^{-/-}$ mice. Real-time PCR and ELISA results showed that pro-inflammatory cytokines IL-1β and TNF-α significantly decreased, by contrast, the expression of anti-inflammatory cytokine TGF-β obviously increased. Meanwhile, western blot results showed that the expression levels of p-AKT and p-JNK decreased markedly in MAPK4$^{-/-}$ mice.
Conclusion: MAPK4 deficiency could ameliorate the pathology of murine ALI, which was closely related to the transduction of AKT and JNK pathway, indicating it played an important role in the development of ALI.

P1521

An endosomal LAPF is required for endocytosis and elimination of bacteria by macrophage

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Innate cells such as macrophages may internalize pathogens by endocytosis through caveolae and/or clathrin-dependent pathways to eliminate invading pathogens. Here, we show that LAPF (lysosome-associated and apoptosis-inducing protein containing PH and FYVE domains) is required for caveolae-mediated internalization and elimination of bacteria. LAPF localized to early endosomes and interacted with caveolin-1, the caveolae protein in macrophages. *Lapf*-deleted macrophages had impaired capacity of phagocytosis and further elimination of bacteria, and produced less proinflammatory cytokines than WT macrophages. Moreover, survival from *E.coli* infection was significantly reduced in macrophage-specific *Lapf*-deficient mice, with higher bacteria loads in liver and spleen. *Lapf* deficiency also impaired TLR4 endocytosis and TLR-triggered inflammatory cytokine production. The phosphorylation of LAPF by tyrosine kinase Src promoted the endocytosis of bacteria by inducing LAPF-Src-Caveolin complex. Our findings reveal a critical role for LAPF-mediated endocytosis in defense against bacterial infection and outlined that activating Src or LAPF is promising for antibiotic drug target.

P1522

The Chemerin-CMKLR1 axis aggravates *Staphylococcus aureus* skin infection via inhibition of IL-33-induced neutrophil recruitment

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Staphylococcus aureus (*S. aureus*) is the major cause of severe skin and soft tissue infections, and neutrophils play a vital role in bacterial clearance. Chemerin and its receptor CMKLR1 have been

DOI: 10.1002/eji.201970400

reported to exert pro- or anti-inflammatory activities depending on the context. Recent studies also demonstrated an antimicrobial activity of chemerin in vitro. However, it remains unclear about the role of Chemerin-CMKLR1 axis in *S. aureus* skin infection. We demonstrated up-regulated chemerin levels in *S. aureus* infected skin, and mice deficient in chemerin (*Rarres2*$^{-/-}$) or CMKLR1 (*Cmklr1*$^{-/-}$) had attenuated *S. aureus* skin infection as evidenced by smaller skin lesions and less bacterial growth accompanied by enhanced skin-infiltrating neutrophils. Depletion of neutrophils abrogated such effects in *S. aureus* infected *Rarres2*$^{-/-}$ mice. Notably, *S. aureus* infection caused higher IL-33 levels but similar levels of neutrophil-attracting chemokines CXCL1/2 and pro-inflammatory cytokines in the skin of *Rarres2*$^{-/-}$ or *Cmklr1*$^{-/-}$ mice compared with WT controls. Accordingly, *Il33*$^{-/-}$ mice exhibited aggravated *S. aureus* skin infection, demonstrating a protective role of IL-33. Furthermore, concomitant up-regulation of CXCR2 expression in blood neutrophils and IL-33 levels was observed in *S. aureus* infected *Rarres2*$^{-/-}$ mice. In vitro study showed that IL-33 rescued down-regulated CXCR2 expression in neutrophils treated with heat-killed *S. aureus* and their chemotactic ability toward CXCL1/2. Collectively, our study reveals an undescribed promoting effect of the Chemerin-CMKLR1 axis on *S. aureus* skin infection and the underlying mechanism, and suggests that blockade of this axis may be a potential therapeutic strategy for control of *S. aureus* skin infection.

P1523

The surface receptor VEGFR-3 coordinates phagocytosis, pyroptosis and inflammasome activation in bacteria-infected macrophages

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Macrophages play an essential role in clearing invading microorganisms. Upon infection, macrophages engulf pathogen via phagocytosis or autophagy, which is closely linked to inflammasome activation and pyroptosis. Keeping balance of these events is critical for maintaining the host homeostasis during infection. However, the underline mechanism is largely unknown about the cross-talk of phagocytosis/autophagy and inflammasome activation. In this study, we have demonstrated that the surface receptor VEGFR-3 (vascular endothelial growth factor receptor-3) could regulate caspase-1 activation, IL-1β secretion and pyroptosis during macropahges are infected with Salmonella typhimurium. Moreover, VEGFR-3 regulates LC3-associated phagocytosis (LAP), phagosome maturation and the clearance of Salmonella typhimurium. Our study suggests VEGFR-3 as a key surface receptor, which could coordinate phagocytosis, inflammasome activation, pyroptosis during bacteria infection (Submitted manuscript).

P1524

Investigating the role of circulating CXCR5-expressing CD8$^+$ T-cells as a biomarker for bacterial infection in subjects with pneumonia

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Background: Recently, lymphoid follicle-confined and circulating CD8$^+$T cells expressing CXCR5 were described, which was involved in anti-virus immune response. However, the dynamics and role of these subsets during bacterial infection is unknown. So, we asked whether CXCR5$^+$CD8$^+$T cells were generated during bacterial infections in lower respiratory tract.

DOI: 10.1002/eji.201970400

Methods: 65 pneumonia patients were divided into groups as tuberculosis, bronchiectasis and community or hospital acquired pneumonia (CAP, HAP).Procalcitonin (PCT) was used to evaluate disease severity. We characterized the number and phenotype (PD-1 and CD103) of $CXCR5^+CD8^+$T cells in the peripheral circulation and analyzed their association with the serum PCT level.
Results: Patients were mainly infected with Escherichia coli, Acinetobacter baumannii, Klebsiella pneumonia (K.p), Pseudomonas aeruginosa, and Staphylococcus aureus. PCT was weakly correlated with severity of respiratory infections. Furthermore, it was revealed an increase of CXCR5-expressing $CD8^+$T cells of uncontrolled CAP and progressive HAP compared to controlled CAP and HAP, respectively (P< 0.05). Strikingly, the subsets in K.p-infected group was higher than non-K.p-infected group (P< 0.05). Meanwhile, the ratio of $CXCR5^+CD8^+$/CD8 was positively correlated with PCT level (P< 0.05). The determination of CXCR5-expressing $CD8^+$Tcells showed better results compared to PCT and can be useful for the prediction of exacerbation of CAP or HAP. Phenotypically, $CXCR5^+CD8^+$T cell expressed comparable level of PD-1 and lower CD103 compared to $CXCR5^-$ counterparts.
Conclusion: The circulating CXCR5-expressing $CD8^+$T cell has diagnostic value for current pneumonia severity, and could act as a biomarker for identifying a bacteria-associated exacerbation. These cells may provide novel insight for the pathogenesis of pneumonia.

P1525

Airway epithelial hepcidin coordinates lung macrophages and nutritional immunity against bacterial pneumonia

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Hepcidin is the master regulator of iron metabolism via the degradation of ferroportin, the only cellular iron exporter. Accumulated evidence has shown the important roles of hepatic hepcidin in host defense and infections. Hepcidin is also expressed by airway epithelial cells. However, the function of epithelial hepcidin during bacterial pneumonia remains unknown. We investigated the role of airway epithelial hepcidin in bacterial pneumonia. Pneumonia was induced through intratracheal administration of the most common bacterial agents. Hepcidin expression levels were manipulated through intratracheal infections using recombinant adenoviruses. We found that hepcidin levels in the airway epithelium decreased while lung macrophage-derived ferroportin levels and pulmonary iron concentrations increased during bacterial infections. Hepcidin-1 knockout (*Hamp1*$^{-/-}$) mice showed increased mortality and elevated pulmonary bacterial burdens during pneumonia, caused by a lack of hepcidin in the airway epithelium. However, in mice with macrophage-specific ferroportin deletion, neither knock-down nor overexpression of hepcidin in the airway epithelium affected the outcomes of pneumonia. The increased pulmonary iron concentration not only facilitated bacterial growth but also led to the defective phagocytic function of pulmonary macrophages by disrupting actin cytoskeletal dynamics via activation of RhoA GTPase. Furthermore, both enhancing the hepcidin expression level in the airway epithelium and chelating pulmonary iron rescued mice from lethal bacterial pneumonia. In conclusion, airway epithelial hepcidin, coordinating alveolar macrophages and nutritional immunity, plays a key role in the protection against bacterial pneumonia. Therapeutic strategies targeting airway epithelial hepcidin or pulmonary iron provide novel alternative approach for combatting bacterial pneumonia.

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DOI: 10.1002/eji.201970400

P1526

Intelectin 3 is dispensable for resistance against a mycobacterial infection in zebrafish (danio rerio)

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Tuberculosis is a multifactorial bacterial disease, which can be modeled in the zebrafish (*Danio rerio*). Abdominal cavity infection with *Mycobacterium marinum*, a close relative of *Mycobacterium tuberculosis*, leads to a granulomatous disease in adult zebrafish, which replicates the different phases of human tuberculosis, including primary infection, latency and spontaneous reactivation. Here, we have carried out a transcriptional analysis of zebrafish challenged with low-dose of *M. marinum*, and identified *intelectin 3* (*itln3*) among the highly up-regulated genes. In order to clarify the *in vivo* significance of Itln3 in immunity, we created nonsense *itln3* mutant zebrafish by CRISPR/Cas9 mutagenesis and analyzed the outcome of *M. marinum* infection in both zebrafish embryos and adult fish. The lack of functional *itln3* did not affect survival or the mycobacterial burden in the zebrafish. Furthermore, embryonic survival was not affected when another mycobacterial challenge responsive *intelectin*, *itln1*, was silenced using morpholinos either in the WT or *itln3* mutant fish. In addition, *M. marinum* infection in dexamethasone-treated adult zebrafish, which have lowered lymphocyte counts, resulted in similar bacterial burden in both WT fish and homozygous *itln3* mutants. Collectively, although *itln3* expression is induced upon *M. marinum* infection in zebrafish, it is dispensable for protective mycobacterial immune response.

P1527

Reduced plasma PCSK9 response in patients with bacteremia is associated with mortality

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The proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme controls blood cholesterol levels by down-regulating the expression of the low-density lipoprotein receptor (LDLR). Since pathogenic lipids (e.g. LPS…) are removed from the bloodstream by an LDLR/PCSK9 dependent mechanism, PCSK9 inhibitors have been suggested to be beneficial also in the treatment of infections. We quantitated the plasma PCSK9 levels in 481 blood culture-positive infection patients with culture-positive bacteremia, on days 0 to 4 after admittance to the emergency department, and explored pathogen and site-of-infection dependent effects as well as correlations between patient characteristics and outcome.
The plasma PCSK9 level was elevated equally in patients with Gram-positive or Gram-negative bacterial infections; especially high levels were seen in patients with a lower respiratory tract infection and Streptococcus pneumoniae bacteremia. PCSK9 levels showed a significant positive correlation with CRP. Bacteremia patients with liver disease or a history of abusive alcohol use had significantly lower levels of plasma PCSK9. Reduced PCSK9 plasma responses in patients were significantly associated with day 7, 28 and 90 mortalities.
In conclusion, PCSK9 levels in the circulation become upregulated in blood culture-positive infections.

DOI: 10.1002/eji.201970400

Plasma PCSK9 resembles acute phase proteins; its expression is induced during an infection, reduced in liver disease and correlates positively with CRP. PCSK9 levels were lower in patients with a fatal prognosis, which is conflicting with some of the previous studies. It remains questionable, whether PCSK9 inhibitors could be a novel approach for treating septic patients.

P1528

Mechanisms of H. pylori-induced feedback regulation in human dendritic cells

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Introduction: *H. pylori* is a gastric pathogen, which colonizes 50% of the world population. It was stated as class I carcinogen by the WHO due to the fact that chronic infection is presumed to be a high risk factor for gastric cancer. Infected individuals frequently fail to eliminate the pathogen, because *H. pylori* evolved efficient strategies to dampen the host immune response. Suppressor of cytokine signaling (SOCS) proteins are an important family of feedback regulators limiting inflammatory responses in various immune cells. Therefore, we aimed to investigate whether *H. pylori* could hamper the host immune responses by modulating SOCS expression.
Results: Infection studies of human monocyte-derived DCs (moDCs) reveal that *H. pylori* rapidly induces SOCS3 expression. By blocking TLRs, we could show that the early *H. pylori*-induced SOCS3 expression appears to be independent of TLR2 and 4. In contrast, infection with a mutant strain lacking a functional type IV secretion system (T4SS) highlighted the importance of the T4SS for *H. pylori*-induced SOCS3 expression within the first hour of infection. Additionally, we monitored phosphorylation of p38 already 30 minutes post-infection and blocking experiments confirmed that p38 is as a pre-requisite for SOCS3 expression in response to *H. pylori*. Functional analyses of SOCS3 by RNA interference based silencing experiments finally indicate that *H. pylori*-induced SOCS3 may dampen the release of pro-inflammatory cytokines.
Conclusion: On the basis of our data, we propose that up-regulation of negative feedback inhibitors by *H. pylori* represents an additional mechanism to dampen host-immune responses against the pathogen.

P1529

From host transcriptomics to statins as host-directed drug therapy for tuberculosis

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Using CAGE transcriptomics we redefined the transcriptional regulatory dynamics of differentially activated classical (M1-IFNγ) or alternative (M2-IL-4/IL-13) macrophages and identified new genes and noncoding RNA species as potential targets for host-directed drug therapy for tuberculosis. We identified Batf2 as a transcriptional inducer of inflammatory responses during *M. tuberculosis* (Mtb) infection in mice and showed that BATF2 is a predictive biomarker for tuberculosis disease in humans. Additionally we demonstrate that non-coding microRNAs, miR-143 and miR-365, promote the intracellular growth of *M. tuberculosis* in alternatively IL-4/IL-13 activated macrophages by differentially targeting of c-Maf, Bach-1 and Elmo-1. We further demonstrated that Mtb exploits the host cholesterol pathway for its survival and we were able to increase host protection against tuberculosis by reducing cholesterol using statins. Mechanistically, statins increased phagosomal maturation and autophagy as host-protective functions to contain and reduce Mtb growth within macrophages. Here we present a

DOI: 10.1002/eji.201970400

proof-of-concept clinical trial to evaluate whether statins, widely used cholesterol-lowering drugs, could be employed as a repurposed drug for host-directed drug therapy in tuberculosis. In summary, targeting Batf2, miR-143 and miR-365 together with repurposed drugs such as statins offers possible adjunctive host-directed drug therapy that may reduce the burden and pathological inflammation of tuberculosis.

P1530

Chlamydia trachomatis CPAF, HSP60 and MOMP antigens elicit production of TNF-α, IL-1β and IL-6 pro-inflammatory cytokines

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Persistent inflammation caused by *Chlamydia trachomatis* in the female genital compartment represents one of the major causes of pelvic inflammatory disease (PID), ectopic pregnancy and infertility in females. Here, we examined the pro-inflammatory cytokine response following stimulation with three different types of *C. trachomatis* antigens, viz. chlamydial protease-like factor (CPAF), heat shock protein 60 (HSP60) and major outer membrane protein (MOMP). A total of 19 patients with genital *C. trachomatis* infection and 10 age-matched healthy controls were recruited for the study. Peripheral blood mononuclear cells (PBMCs) isolated from genital *C. trachomatis*-infected females were cultured in the presence of CPAF, HSP60 and MOMP antigens, and cytokines were measured by ELISA assay. We reported that pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) were robustly secreted following antigenic exposure. Notably, CPAP and MOMP were more potent in triggering IL-1β, as compared to HSP60. Our study highlights distinct ability of chlamydial antigens in triggering pro-inflammatory response in the host immune cells.

P1531

Inhibition of Notch signalling in gastric epithelial cells in response to *Helicobacter pylori* infection

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Introduction: *Helicobacter pylori* causes chronic gastritis, ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Eradication rates have fallen due to antibiotic resistance. A deeper understanding host-pathogen interaction is vital to uncover alternative therapeutic strategies. The highly conserved Notch pathway is central to numerous biological processes including cell differentiation, proliferation and survival. The role of Notch signalling in *H. pylori* pathogenesis is not fully understood.
Aim: To evaluate whether Notch signalling is modulated during *H. pylori* infection and assess the role of the virulence factors CagA and VacA in this response.
Methods: AGS gastric epithelial cells were infected with *H. pylori* 60190 or isogenic CagA and VacA mutant strains and RNA isolated over time. RNA was also isolated from stomach antral biopsies of *H. pylori* infected and uninfected patients. Expression of Notch pathway genes was measured by quantitative PCR.
Results: *H. pylori* infection led to a significant decrease in the production of several Notch pathway components in AGS cells, including the Notch1 and Notch3 receptors, the Notch ligands Jagged1,

DOI: 10.1002/eji.201970400

Jagged2 and Delta-like 1, and the target genes Hes1 and Hey1. These decreases were also observed following infection with *H. pylori* lacking functional CagA or VacA. Median levels of Jagged2, Hes1 and Hey1 were decreased in the gastric mucosa of *H. pylori*-infected patients compared to uninfected controls.
Conclusions: *H. pylori* infection inhibits Notch signalling in gastric epithelial cells, independent of the CagA and VacA virulence factors. Further experiments are required to elucidate the role of decreased Notch signalling in *H. pylori* pathogenesis.

P1532

Immunological profile in pulmonary tuberculosis patients with zinc supplementation during anti tuberculosis therapy: exploratory study

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Introduction: During pulmonary tuberculosis a strong immune response is developed by the host against the pathogen, involving the activation of T cell subpopulations and the cytokine release. Likewise, the effect of zinc as modulator of immune response is well documented. Nevertheless, they were not sufficiently studied the effects of zinc supplementation on the behaviour of immune response in pulmonary tuberculosis patients. The purpose of this study was to know the behaviour of immune system in response to zinc supplementation in pulmonary tuberculosis patients.
Methodology: An intervention study was conducted with twenty-one patients with pulmonary tuberculosis on pharmacological therapy. They were divided into two groups who were given zinc (45 mg/day as gluconate) or placebo for 3 months.
Results: The concentrations of cytokines IL-17, IL-4, IL-10, INF-gamma, TNF alpha not showed differences between patients and controls and also between times before and after intervention. Regarding the index CD4/CD8, the values of both groups were increased before and after 3 months of nutritional intervention regarding values of control group.
Conclusions: It is concluded that addition of a daily supplement of 45 mg of zinc to conventional anti-tuberculosis therapy did not have any additive effect on the behaviour of immunological profile of these patients.

P1533

Alteration of endocrine hormone profiles in different spectrum of TB disease

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Hormones often act as immunomodulators. Immune and endocrine interaction during infectious diseases may determine the failure or success of the immune response. This is particularly true for an infection like tuberculosis, in which pathogen and immune system coexist in a continuous interaction. The aim of this study was to assess selected endocrine hormones profiles in different spectrum of TB disease from repository plasma samples at the Armauer Hansen Research Institute (AHRI) laboratory. A total of 226 plasma samples, collected from pulmonary and extra pulmonary TB patients, close household contacts of PTB patients and leprosy patients, were retrieved randomly from AHRI biorepository and evaluated for DHEA, cortisol, testosterone, estradiol, growth hormone and leptin hormones concentration using ELISA. Plasma cortisol level was significantly higher in PTB, TBLN and

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leprosy patients compared to both LTBI uninfected groups and infected groups. The levels of DHEA and leptin were significantly low in PTB patients compared to LTBI uninfected groups. Similarly, levels of leptin was significantly lower in TBLN and leprosy patients compared to LTBI uninfected groups. On the other hand, plasma levels of DHEA, estradiol, testosterone and leptin significantly increased in PTB patients following treatment, whereas the concentration of cortisol and human growth hormone declined significantly after treatment. This alteration of hormones during TB disease suggests that hormones might play a role in immune response to *M. tuberculosis.*

Keywords: Hormone, Tuberculosis, immunity latency

P2103

Mycobacterial lipoprotein Z triggers effective innate immunity during *M.tb* infection

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Mycobacterial lipoproteins exhibit both virulence and immunity properties during *Mycobacterium tuberculosis* infection. Our previous work indicated that Mycobacterial Lipoprotein Z (LppZ) was of high immunogenicity and antigenicity in tuberculosis with the potential as a novel vaccine candidate. Nevertheless, the modulation of LppZ on innate immunity was still unknown. In this study, by using RAW264.7 cell line and peritoneal macrophages (PMs) from C57BL/6 mice it was revealed that RAW264.7 cells were able to secrete high amount of pro-inflammatory cytokines including TNFα and IL-6 upon *in vitro* stimulation of LppZ. In PMs, IL-12 secretion was apparently detected in addition to high levels of TNFα and IL-6 when compared to control protein. The expression of costimulatory molecules and MHC molecule on macrophages were up-regulated both with the percentages and MFI values after LppZ stimulation including CD80, CD86 and MHC-Ⅱ. What is more, when RAW264.7 cells were infected by BCG, LppZ stimulation in advance enhanced the capacity of bacterium killing partially through the generation of ROS. Taken together, mycobacterial LppZ is able to trigger macrophage activation leading to the enhancement of mycobacterium killing and the induction of pro-inflammatory cytokines and antigen presentation, which will probably in turn mediate the adaptive immunity against *Mtb* infection.

P2104

Bacterial signaling molecule c-di-AMP as adjuvant induced immune responses and its application in tuberculosis vaccine

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Cyclic di-AMP (c-di-AMP) is a bacterial second messenger which regulates various cellular processes. Studies reported that c-di-AMP activates the innate immune leading to IFN-β release, stimulates autophagy and induces IL-1β secretion through inflammasome. In addition, c-di-AMP as mucosal adjuvant could induce Th1/Th2/Th17 immune responses. Thus the vaccine potential of c-di-AMP should be investigated. In previous work, we firstly reported that c-di-AMP is produced from two ATP by diadenylate cyclase DisA (Rv3586) and degraded to AMP by phosphodiesterase CnpB (Rv2837c) in *Mycobacteria tuberculosis* (Mtb). And CnpB mutation resulted in c-di-AMP accumulation and induced the IFN-β response and showed attenuation. Another report found that overexpressing DisA

DOI: 10.1002/eji.201970400

in Mtb activated Mφ autophagy and resulted in attenuation virulence. Bacillus Calmette-Guerin (BCG) is a live attenuated vaccine against tuberculosis (TB). However, BCG has varied protective efficiency in adults and has safety concerns in immunocompromised population. We constructed a recombinant BCG (rBCG) by overexpressing DisA, the Mtb diadenylate, and named as rBCG-DisA. After Mtb infection, the splenic lymphocytes from rBCG-DisA-immunized mice produced more Th1/Th2 cytokins than BCG group and provided protection against Mtb infection. The mechanism study showed that the innate immune memory, also named as trained immunity, may contribute to the enhanced immune responses induced by rBCG-DisA. Thus our study demonstrated that elevated c-di-AMP in BCG as endogenous adjuvant enhanced immune responses induced by BCG, especially after *M. tuberculosis* infection, which suggested that c-di-AMP could act as a promising immune-modulator for better vaccine against TB.

P2106

The association between serum vitamin D levels and immune response modulation in patients with urinary tract infection

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To examine whether there is any association between serum vitamin D levels in patients with urinary tract infection (UTI) and both humoral and cellular immune responses, women (65 in the patient group and 35 in the control group), who were between 18-60 years old, were studied. The serum levels of 25(OH) vitamin D, cytokines (IFN-γ, IL-4, IL-17A and TGF-b1), and antibodies (IgG, IgM and IgA) were measured by ELISA.
A significant decrease in vitamin D levels was shown in patients compared to the control group. The results showed that both IFN-γ and IL-17A production increased in the patient group compared with *those* of the *controls* and these cytokines associated significantly with serum 25(OH) vitamin D levels. IL-4 production was reduced in patients without any significant association with serum 25(OH) vitamin D levels. Despite no significant difference in the level of TGF-β1 between the two groups, a significant positive association was observed between this cytokine and serum 25(OH) vitamin D levels. Although serum IgG levels were not significantly different between the two groups without any significant association with serum 25(OH) vitamin D levels, serum IgM and IgA levels were significantly higher in patients than in the control group. A significant association was also observed between serum levels of IgM and IgA with serum 25(OH) vitamin D levels in the two groups. Therefore, the modulation of Th1and Th17 responses might be attributed to the vitamin D levels. Antibody response may be another indicator of its effect in directing the host immune responses.

P2107

Introducing a novel porcine whole lung ex vivo infection model to investigate Klebsiella Pneumoniae host-pathogen interactions

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Klebsiella Pneumoniae (*K.p*) is a Gram-negative bacterial pathogen responsible for nosocomial and community-acquired infections including devastating necrotising pneumonia. The World Health Organisation named *K.p* as a global threat to human health due to the seemingly exponential rise in

DOI: 10.1002/eji.201970400

multi-drug resistant (MDR) isolates. Although several virulence factors have been described for *K.p*, we still know little regarding its pathogenesis. To address this issue we have developed a novel infection model using whole lungs to observe immune responses to *K.p* in real time.
Whole lungs were surgically prepared and, using the LS-1 perfusion system, returned to a functional state prior to infection with the clinical isolate *Kp52145.* Whole genome sequencing has revealed that pigs share 80% homology with the human immune system whereas mice share >10%. This, together with anatomical similarities and abundant availability, led to the selection of porcine lungs for this model.
Herein we have replicated several clinical features of *K.p* associated pneumonia in porcine lungs. This study revealed that despite significant tissue damage, *K.p* infection prevents predicted inflammation and interestingly drives macrophage polarisation toward an M2 phenotype. We confirmed that this process is STAT6 dependent by infecting porcine bone marrow-derived macrophages. Furthermore, our results reinforce the crucial role played by the capsule polysaccharide to subvert lung innate immune mechanisms. Therefore we propose that this novel infection model is a useful tool investigating host-pathogen interactions and may be used as a platform for testing novel therapies to treat infection - a highly important issue as we approach a post-antibiotic era.

P2108

Identification of immune effector mechanims controlling Brucella growth in a murine intranasal infection model

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Brucela is an alpha-proteobacterium which induces brucellosis, a worldwild zoonotic disease infecting mammals, including humans. *Brucella melitens*is is the specie the most often isolated in human brucellosis (80 % of cases). Inhalation of airborne agents constitutes one of the main transmission route. Up to now, no efficient treatment is available, leading to serious economic loss and public health problems in some endemic countries.
Despite recent progress in mouse models of brucellosis, very little is known about the first steps of *Brucella* infection *in vivo*. Following intranasal infection with mCherry-expressing *B. melitensis*, flow cytometry analysis showed that the alveolar macrophages (AMs, identified as $CD11c^{+}SIGLECF^{+}$ cells) are the main infected cells (>95 %) in the lung during the first 48 hours post-infection.
Since few years, itaconate, a TCA derivate, produced by aconitate decarboxylase1 gene (*acod1*), is emerging as a new potential immunoregulatory metabolite. This compound, produced in macrophage lineage-cells, shows an inhibitor role on the growth of different bacteria such as *Mycobacterium*and *Legionella*. We observed that itaconate also inhibits the *in vitro Brucella* growth. In addition, we observed that *acod1*$^{-/-}$C57BL/6 mice display enhanced susceptibility to *Brucella* infection, confirming its possible implication in the early control of *Brucella* in our model. To better understand how *acod1* is involved in the inhibition of *Brucella* growth, we also analyzed by transmission electronic microscopy the intracellular trafficking of *Brucella* in AMs purified from WT and *acod1*$^{-/-}$infected mice.
The understanding of how itaconate impacts the growth of *Brucella* could allow the development of therapeutic drug for brucellosis.

DOI: 10.1002/eji.201970400

P2109

LUBAC- mediated NF-κB signaling is involved in *Mycobacterium tuberculosis* effector PPE60 recombinant interacting with host cells

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Tuberculosis, caused by *Mycobacterium tuberculosis* infection, remains a global public health threat. The success of *M. tuberculosis* largely contributes to its manipulation of host cell fate. The role of *M. tuberculosis* PE/PPE family effectors in the host destiny was intensively explored. In this study, the role of PPE60 (Rv3478) was characterized by using *Rv3478* recombinant *M. smegmatis*. PPE60 can promote host cell pyroptosis via caspases/NLRP3/gasdermin. The production of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-12p40 and TNF-α was altered by PPE60. We found that LUBAC was involved in PPE60-elicited NF-κB signaling by using Linear Ubiquitin Chain Assembly Complex (LUBAC) -specific inhibitor gliotoxin. The PPE60 recombinant *M. smegmatis* survival rate within macrophages is increased, as well as elevated resistance to stresses such as low pH, surface stresses and antibiotics exposure. LUBAC-mediated NF-κB signaling was firstly reported to be involved in *M. tuberculosis* effector PPE family.
Keywords: *Mycobacterium tuberculosis, PE/PPE, Pyroptosis, LUBAC, PPARγ*
Funds: This work was supported by National Natural Science Foundation [grant numbers 81871182, 81371851]

P2110

Immunological and inflammatory local response produced by *Mycobacterium abscessus* and its glycopeptidolipid mutant in a murine model of subcutaneous infection

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Mycobacterium abscessus is an opportunistic pathogen which causes harmful infection in the subcutaneous cellular tissue, which is characterized by extensive inflammation, abscesses formation and necrosis, that in chronic stages produce fibrosis. The expression of glycopeptidolipid (GPL´s) by these bacteria is codified by the mmpL4b regulon that *in vitro* has been associated with the smooth colonies phenotype and evasion of the immune response, while the absence of these components generates rough phenotypes that show higher pathogenicity. The aim of this work was to determine the GPL´s contribution in the immunopathology produced by the infection of distinct phenotypes of *M. abscessus* using a subcutaneous infection model in BALB/c mice. Mice were infected subcutaneously in both footpads with different mycobacterial phenotypes: strains 390S (smooth colonies), 390R (rough colonies), the mutant ΔmmpL4b (lack GPL) and ΔmmpL4b-comp (complemented mutant). The kinetics of footpad inflammation, bacillary loads, cytokine production and fibrosis were determined during 150 days of infection. Regardless of the strains phenotype, there were non-cultivable bacteria in the tissue after day 28th post-infection; however, until day 150 was detected by PCR the expression of mycobacterial ribosomal subunit 16S, which indicate live bacilli which are probably in latent or dormant phase, in coexistence with extensive inflammation and fibrosis particularly induced by the rough strains. Interestingly, the infection with the mutant strain ΔmmpL4b induced significant lesser inflammation without fibrosis. These results suggest that subcutaneous infection with *M. abscessus*

DOI: 10.1002/eji.201970400

can maintain dormant or latent bacteria and their GPL´s contribute to producing extensive chronic inflammation and fibrosis

P2111

The differentially expressed miRNAs and circRNAs in the multidrug-resistant tuberculosis

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Background/Aims: MiRNAs and circRNAs are involved with various diseases in the lung. This study aimed to identify the key miRNAs and circRNAs involved in the tuberculosis.
Methods: The differentially expressed miRNAs (DEmiRNAs) and circRNAs (DEcircRNAs) in the drug susceptibility and multidrug resistant (MDR) tuberculosis were identified by non-coding RNA sequence analysis. Quantitative real-time polymerase chain reaction (qRT-PCR) was then used to validate the expression of selected DEmiRNAs and DEcircRNAs in the different groups. Based on our previous RNA sequence analysis of the differentially expressed mRNAs (DEmRNAs) in the drug susceptibility and drug resistant tuberculosis. The DEcircRNA-miRNA-DEmRNA interaction network was constructed. Functional annotation of DEmRNAs that shared the binding miRNAs or DEcircRNAs was performed using gene ontology (GO) and pathway analyses.
Results: A total of 65 and 150 significant DEcircRNAs were obtained in the drug susceptibility and MDR tuberculosis, respectively. The qRT-PCR results were generally consistent with the non-coding RNA sequence results. The DEcircRNA-miRNA-DEmRNA interaction network and pathway analysis indicated that hsa-miR-451a, has-miR-103a-3p, has-miR-125b-5p, has-miR-378a-3p, hsa_circRNA_0001386, hsa_circRNA_0002908, has_circRNA_0006337, has_circRNA_0028299, hsa_circRNA_0031446, has_circRNA_0037002 and has_circRNA_0043815 might be involved with MDR tuberculosis.
Conclusions: In conclusion, our study identified the key DEmiRNAs and DEcircRNAs in the drug susceptibility and MDR tuberculosis, which might provide new clues for further exploring the mechanism of MDR tuberculosis.

P2112

Platelet extracellular traps kill bacteria

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Platelets have significant functions in hemostasis and in regulating immune and inflammatory responses. However, the role of platelets killing bacteria in innate immunity are largely unexplored. The current study identifies an unappreciated asset of platelets and shows that platelets release azurophilic granules and polymerize fibrinogen upon activation. The fibrin is a major structural component together with granule proteins from platelet, such as neutrophil elastase and myeloperoxidase, which formed extracellular fibers bind Gram-positive and -negative bacteria. These platelet extracellular traps (PETs) degrade virulence factors and kill bacteria. PETs are abundant *in vivo* in experimental sepsis and spontaneous human appendicitis, two typical entities of acute inflammation. PETs seem to be a form of innate response, binding microorganisms, restraining them from spreading, and ensures a high local concentration of antimicrobial agents to degrade virulence

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DOI: 10.1002/eji.201970400

factors and kill bacteria. This assigns platelets a critical role in innate immune responses and as potential targets to reduce inflammatory tissue damage in severe systemic infection.

P2113

Age-related segregation of immunoreactivity to mycobacterial antigens in BCG vaccinated Chinese population

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Bovis Calmette-Guérin (BCG) is currently the only vaccine available for tuberculosis prevention with effectiveness in children but less efficacy in adults. How BCG vaccination induces long term adaptive immunity to mycobacterial antigens is not well investigated. Herein, 478 health donors (HDs) from Shanghai region were enrolled and subgrouped into ESAT6/CFP-10- and ESAT6/CFP-10+ groups by gamma-interferon release assays (IGRA). The recombinant proteins Rv0934, Rv3006 and Rv3841 were expressed prokaryotically. Among them, Rv3006 exhibited the highest immunoreactivity in active tuberculosis patients while immunoreactivity to Rv3841 was higher in HDs than in patients. In ESAT6/CFP-10- population, Rv3841 showed the strongest and maintained immunoreactivity along the aging. However, Rv3006 and Rv0934 attenuated the performance in ESAT6/CFP-10- population older than 45 years old. In addition, the latters induced predominantly Th1 responses, while Rv3841 triggered similar level of Th1 and Th17 cells. This is the first report to investigate the immunoreactivity of mycobacterial antigens at different age periods in BCG vaccinated non-infected healthy population. The different immune patterns of antigens along aging imply the existence of immunoreactivity segregation, which provided important clues for the selection of proper antigens for vaccine development in diverse infected population. (Yang and Zhang have contributed equally to this work.)

P2114

Gene expression profiling reveals a role of Pgp3 protein Chlamydia trachomatis pathogenesis

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Chlamydia trachomatis is the causative agent for the most common bacterial sexually transmitted infection around the globe. Ascending genital tract infection by *C. trachomatis* in females is related to the development of adverse long-term reproductive sequelae which encompass pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI). Most of the strains of *C. trachomatis* naturally bear a conserved plasmid of around 7.5 kb that carries eight open reading frames (ORF) designated *Pgp1-8* with uncertain biological significance. The plasmid *Pgp3* gene encodes for a ~28 kDa protein that is produced by *C. trachomatis* as a homotrimer (~84 kDa) late in its developmental cycle that is secreted into the cytosol of the infected cells. Pgp3 protein is highly immunogenic in humans and anti-Pgp3 antibodies are developed in a majority of *C. trachomatis*-seropositive patients. Furthermore, animal studies showed that *C. trachomatis* isolates transformed with shuttle vectors harboring *Pgp3* gene displayed enhanced infectivity and elicited stronger host inflammatory response in comparison with the plasmid-deficient parental isolate, suggesting Pgp3 as a virulence factor in chlamydial pathogenesis. Nonetheless, the molecular mechanisms underlying Pgp3-host interactions remain undefined. In the present study, we cloned, expressed *Pgp3* and performed stimulation of Hela cervical epithelial cells with Pgp3. Panel RNA analysis (NanoString) was used to investigate the

DOI: 10.1002/eji.201970400

effects of Pgp3 on host cells. The results provide a paradigm for greater understanding and insight into the role of Pgp3 protein in the pathogenesis of *C. trachomatis*.

P2115

Alveolar macrophages provide an early mycobacterium tuberculosis niche and initiate dissemination

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Mycobacterium tuberculosis (Mtb) infection is initiated in the distal airways, but the bacteria ultimately disseminate to the lung interstitium. Although various cell types, including alveolar macrophages (AM), neutrophils, and permissive monocytes, are known to be infected with Mtb, the initially infected cells as well as those that mediate dissemination from the alveoli to the lung interstitium are unknown. In this study, using a murine infection model, we reveal that early, productive Mtb infection occurs almost exclusively within airway-resident AM. Thereafter Mtb-infected, but not uninfected, AM localize to the lung interstitium through mechanisms requiring an intact Mtb ESX-1 secretion system. Relocalization of infected AM precedes Mtb uptake by recruited monocyte-derived macrophages and neutrophils. This dissemination process is driven by non-hematopoietic host MyD88/interleukin-1 receptor inflammasome signaling. Thus, interleukin-1-mediated crosstalk between Mtb-infected AM and non-hematopoietic cells promotes pulmonary Mtb infection by enabling infected cells to disseminate from the alveoli to the lung interstitium.

P2116

Transcriptional patterns in patients with diabetes under poor glycemic control associated to altered immune functional pathways in patients with tuberculosis-diabetes comorbidity

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Diabetes and tuberculosis are two convergent diseases of great impact globally. It has been described that diabetic patients with deficient glycemic control (HbA1c> 10%) have a relative risk of 3.78 to develop tuberculosis compared to those with normal HbA1c levels. However, the molecular mechanisms that determine the susceptibility of diabetic patients to develop tuberculosis and whether the causes and effects are bidirectional are unknown. To analyze these processes, we performed a transcriptional analysis in whole blood comparing patients with Diabetes mellitus type 2 with poor glycemic control (PDM2), with tuberculosis comorbidity (TB-DM2), subjects with only tuberculosis (TB), patients with diabetes (DM2) and healthy subjects as a control group (CTRL). The analysis of near 27,000 genes by Agilent´s Human GE 4x44K v2 Microarray, identified the overexpression of 61 genes and the downregulation of 75 genes in the PDM2 group; on the other hand, a set of 36 overexpressed genes and 48 down-regulated genes were identified in subjects with the TB-DM2 comorbidity. Bioinformatic analysis of these set genes defined some related functional pathways for dysregulated genes in the PDM2 group and the TB-DM2 patients. Then, we defined genes in the TB-DM2 group, that are strong candidates to alter regulatory cascades that explain the susceptibility of diabetic patients to develop tuberculosis. A model proposed for this phenomenon will be presented. These genes are now being evaluated functionally to define their role in the immune response in

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DOI: 10.1002/eji.201970400

models of *in vitro* infection with Mycobacterium tuberculosis with cells from diabetic patients under hyperglycemic conditions.

P2117

A rapid test for the identification of pulmonary tuberculosis in humans using serum biomarkers

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Tuberculosis (TB) has become globally the leading cause of death by a single infectious agent, surpassing even HIV / AIDS (WHO report, 2017). Thus, TB control is a priority for public health.
The diagnosis of pulmonary tuberculosis is a cornerstone for the control of the disease. Unfortunately, *Mycobacterium tuberculosis* (Mtb) culture from sputum takes up to 8 weeks to obtain a diagnostic. According to WHO, to improve the diagnosis of tuberculosis it is necessary to develop sputum-free "*point of care"* (POC) tests based on serological biomarkers.
Previously, we identified by transcriptional analysis using microarrays, qPCR, and ELISA, a protein called "BM1", whose serum levels at picogram levels allowed to discriminate patients with active tuberculosis (ATB) from patients with latent tuberculosis infection (LTBI) or control healthy subjects (CTRL). To comply with the WHO objectives, we use this protein to develop a rapid serum test based on lateral flow chromatography for the identification of pulmonary tuberculosis in humans using BM1. Using serum samples from subjects with ATB, LTBI and CTRL group without tuberculosis, a lateral flow chromatography assay was standardized according to the protocol of Zhang, et al 2009. With a specific BM1 antibody, a strip prototype was made to evaluate its utility for tuberculosis diagnosis in serum samples, defining the optimal test time to achieve the greater discrimination between ATB, LTBI and CTRL subjects, calculating the values of specificity and sensitivity. The reactive POC strip prototype obtained has results of sensitivity and specificity superior to 90%.

P2118

Neutrophils Dampen Adaptive Immunity in Brucellosis

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Brucella organisms are intracellular stealth pathogens of animals and humans. The bacteria overcome the assault of innate immunity at early stages of an infection. Removal of polymorphonuclear neutrophils (PMNs) at the onset of adaptive immunity against Brucella abortus favored bacterial elimination in mice. This was associated with higher levels of interferon gamma (IFN-γ) and a higher proportion of cells expressing interleukin 6 (IL-6) and inducible nitric oxide synthase (iNOS), compatible with M1 macrophages, in PMN-depleted B. abortus-infected (PMNd-Br) mice. At later times in the acute infection phase, the amounts of IFN-γ fell while IL-6, IL-10, and IL-12 became the predominant cytokines in PMNd-Br mice. IL-4, IL-1β, and tumor necrosis factor alpha (TNF-α) remained at background levels at all times of the infection. Depletion of PMNs at the acute stages of infection promoted the premature resolution of spleen inflammation. The efficient removal of bacteria in the PMNd-Br mice was not due to an increase of antibodies, since the immunoglobulin isotype responses to Brucella antigens were dampened. Anti-Brucella antibodies abrogated the production of

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DOI: 10.1002/eji.201970400

IL-6, IL-10, and IL-12 but did not affect the levels of IFN-γ at later stages of infection in PMNd-Br mice. These results demonstrate that PMNs have an active role in modulating the course of B. abortusinfection after the adaptive immune response has already developed.

P2119

Helicobacter pylori utilizes type IV secretion system and motility to induce NLRP3-dependent production of IL-1b in neutrophils

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Helicobacter pylori colonizes the human gastric mucosa chronically and affects more than 50% of the world's population and lasts a lifetime. *H. pylori* infection is the etiologic agent of gastrointestinal disorders that causes chronic gastritis, peptic ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. *H. pylori* expresses two virulence factors: the vacuolating cytotoxin A (VacA) and cytotoxin-associated genes pathogenicity island (cagPAI). The cagPAI encodes the components of a type Ⅳ secretion system (T4SS), which delivers oncoprotein CagA and bacterial peptidoglycan into host cells. Many studies have shown that IL-1β is a key factor for the development of gastrointestinal diseases. However, the mechanism of IL-1β production during *H. pylori* infection in mouse neutrophils is unknown. Therefore, in this study, we demonstrated that production mechanism of IL-1β in mouse neutrophils infected with *H. pylori*. We found that H. pylori T4SS and flagellin was involved in IL-1β production by H. pylori in mouse neutrophils. To determine upstream molecules involving in *H. pylori*-induced IL-1β production, we performed separated experiments using mouse peritoneal neutrophils isolated from NLRP3-inflammasome-related gene-deficient mice. The production of IL-1β in response to *H. pylori* was absolutely abolished in neutrophils from NLRP3, Caspase-1/11, and ASC-deficient mice. In addition, host TLR5 and NLRC4 in IL-1β production by bacterial flagellin were not required. Our findings suggest that H. pylori T4SS and motility of *H. pylori* can induce IL-β production in NLRP3-inflammasome dependent manner in mouse neutrophils.

P2120

Deciphering the markers of Tuberculosis latency

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Mycobacterium tuberculosis (M.tb) has surpassed HIV to become the leading cause of mortality by an infectious disease. Currently one third of the world's population is infected with TB, among which only 10% develops active disease. Majority of the infected individuals remains lifelong carrier of the bacteria constituting a vast reservoir of the disease which in turn makes the eradication of the disease a major challenge. We are exploring biomolecules that can be utilized in the diagnosis of latent TB as well as in disease progression and immune evasion. This could largely help in identifying the latent individuals and management of the disease. We are studying the role of a type of extracellular vesicle known as exosome. These membrane vesicles carries a wide variety of cargo including proteins, lipids, Nucleic acids such as mRNA as well as miRNAs which could be involved in quorum sensing as well as immune evasion. We are trying to screen biomolecules in these vesicles so as to develop novel therapeutic strategies against tuberculosis.

DOI: 10.1002/eji.201970400

P2121

Rescuing protective T cell response by blocking PD-1 facilitates bacillary clearance and modulation of the expression of Efflux pump of Mycobacterium tuberculosis

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Rational: Previously, we have shown critical involvement of regulatory T (Treg) cells and PD-1 pathway in mediating suppressed state of T cell response against *Mycobacterium tuberculosis* (*Mtb*) among Tuberculosis (TB) patient. We also reported in category II Pulmonary TB patients, that 75% of patients who did not show chemotherapy induced decline of Treg cells eventually were diagnosed as Multi Drug Resistance (MDR)-TB. Here, we attempted to understand the status of host immune response among the MDR and Drug Sensitive (DS)-TB patients. We also checked the contribution of PD-1 pathway and Tregs on poly-functional T cells (PFTs), critical for protective immunity in TB.
Results: We observed marked rescue of PFTs in TB patients by blocking PD-1 pathway, which resulted in effective clearance of *Mtb* in MDM *in vitro* model. Blocking PD-1 pathway *in Mtb* infected mice, demonstrated decrease in Tregs and restoration of PFTs with enhanced reduction of bacillary load relative to chemotherapy alone. Among MDR patients, we observed increase in the frequency of Tregs and decrease in frequency of *Mtb* specific T cell cytokine producers (IFN-γ or TNF-α or IFN-γ+TNF-α+) compared to DS TB patients, suggesting a tight correlation of Treg mediated suppression with MDR status. Furthermore, the efflux of Mtb could be substantially modulated by pro-inflammatory (IFN-γ , TNF-α) and anti-inflammatory cytokines (IL10 and TGF β) in *in vitro* MDM model.
Conclusions: Rescuing appropriate immune response may improve the efficacy of anti-tubercular therapy in TB, especially for MDR TB with possible reduction of duration of drug therapy and relapse.

P2123

Tie2/angiopoietin-mediated endothelial dysfunction during severe orientia tsutsugamushi infection

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Orientia tsutsugamushi infection can cause acute lung injury and high mortality in humans; however, the underlying mechanisms are unclear. Here, we tested a hypothesis that dysregulated pulmonary inflammation and Tie2-mediated endothelial malfunction contribute to lung damage. Using a murine model of lethal O. tsutsugamushi infection, we demonstrated pathological characteristics of lung damage: 1) a significant increase of ICAM-1, VEGFR2, and angiopoietin-2 (Ang2) proteins in inflamed tissues and lung-derived endothelial cells (EC), 2) a progressive loss of endothelial quiescent and junction proteins (Ang1, VE-cadherin/CD144, occuludin), and 3) a profound impairment of Tie2 receptor at the transcriptional and functional levels. In vitro infection of primary human EC cultures and serum Ang2 proteins in scrub typhus patients support our animal studies, implying endothelial dysfunction in severe scrub typhus. Evidence for neutrophil-mediated inflammation and tissue damage included in vitro infection and activation of neutrophils, lung accumulation of activated ($CD63^{+}CD11b^{-}$

DOI: 10.1002/eji.201970400

Ly6G$^+$) neutrophils, co-localization of lung bacteria with Ly6G$^+$ or myeloperoxidase (MPO)$^+$ neutrophils, and co-localization of MPO$^+$ neutrophils with Ang2$^+$ ECs. At the onset of disease, pulmonary macrophages (MF) were polarized toward an M1-like phenotype (CD80$^+$CD64$^+$CD11b$^+$Ly6G$^-$), with limited detection of M2-like (CD206$^+$CD64$^+$CD11b$^+$Ly6G$^-$) cells. Comparative studies of LPS- versus IL-4-primed MFs revealed extensive bacterial replication in M2-type MFs that preferentially expressed the IL-10 and SOCS1 genes. This is the first detailed investigation of lung cellular immune responses during acute O. tsutsugamushi infection. It uncovers specific biomarkers for vascular dysfunction and neutrophil- and M1-skewed inflammatory responses, highlighting future therapeutic research for the control of this neglected tropical disease.

P2873

The IL23R G149R promotes IL-23 unresponsiveness in human memory Th17 cells in leprosy

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Objectives: The SNP (c.445G > A; p. G149R) in IL-23 receptor (IL23R) was found to be association with leprosy by our group. But, the functional consequences of this variant are unclear in the disease pathogenesis.
Methods: In this study, functional investigation of the G149R variant in IL23R was performed in independent cohort of healthy individuals. The expression level of Th17 cell relative cytokines were detected by qPCR and Western Blot, and the percentage of memory Th17 cells in CD4+Tcells were analyzed by flow cytometry in G149R carriers and controls.
Results: We found that the expression level of IL-17A, IL-17F, IL-22 and IFN-γ, which are produced by Th17 cells were significantly increased in infection of Mycobacterium marine that mimic M. leprae infection in vitro. Meanwhile the percentage of circulating IL-17A, IL-17F, IL-22 and IFN-γ-producing T cells did not differ in the isolated memory CD4+T cells of G149R carriers and controls. But, reduced IL-23-induced Th17 response in memory Th cells with decreased IL-17A and IL-17F production of G149R carriers were identified.
Conclusion: Taken together, our results demonstrated that IL-23/IL-17 pathway was involved in leprosy pathogenesis. G149R attenuate IL-23 responsiveness in human memory Th 17 cells, although this variant has no biological effect on development of these cells.

P2874

Chlamydia trachomatis inclusion membrane protein CT813 interacts with host protein CREB3

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Chlamydia trachomatis, a human pathogen, is a leading cause of trachoma and sexually transmitted bacterial infection. Like other chlamydial species, *C. trachomatis* possesses a unique obligate intravacuolar biphasic life cycle. It can accomplish its entire biosynthesis, particle assembly and differentiation within the cytoplasmic vacuole called inclusion. To establish and maintain its intravacuolar growth, chlamydia must exchange both materials and signals with the host cell across the inclusion membrane. *The C. trachomatis*-specific inclusion membrane protein CT813 has the potential to interact with host cells and induce host immune responses during natural infection. To investigate host cell binding partners of CT813, we used a yeast-two hybrid screen assay to screen CT813 against a HeLa cell cDNA library, and found that CT813 interacted with the host cell protein

DOI: 10.1002/eji.201970400

cyclic adenosine monophosphate (cAMP)-responsive element (CRE)-binding protein (CREB3). The interaction of CT813 with ectopically expressed CREB3 in HeLa cells was detected by both co-immunoprecipitation and co-localization under laser confocal fluorescence microscopy. The above observations suggest that CREB3 may directly bind to the cytoplasmic domain of CT813 to mediate the interactions of chlamydial inclusions with host cell endoplasmic reticulum.

P2875

Two novel cross-protective antigens for bovine *Pasteurella multocida*

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Pasteurella multocida is an important pathogen that leads to a range of diseases that have severe economic consequences on cattle production. In order to develop an effective cross-protective component vaccine, an immunoproteomics approach was used to analyze outer membrane proteins (OMPs) of the *P. multocida* serotype A, B and F strains. Candidate antigen molecules from the whole genome were screened via linear trap quadrupole mass spectrometry and bioinformatics analysis, and the reactogenicity of the candidate antigen molecules was analyzed via cloning, expression, and ELISA or protein immunoblotting, and the vaccine efficacy of the candidate molecules was determined in infective animal models and cross-protective antigens may be obtained. rPmCQ2_2g0128, rPmCQ2_1g0327 and rPmCQ2_1g0020 proteins were selected. Their protective rates were 40/30/20% (rP mCQ2 _ 2g 0128), 50/40/0% (rP mCQ2 _1g 0327) a nd 0/40/30% (rPmCQ2_1g0020) against ten-fold median lethal dose (10LD50) of the *P. multocida* serotypes A, B and F in a mouse model, respectively. The results suggested that the two proteins rPmCQ2_2g0128 and rPmCQ2_1g0327 may be used as vaccine candidates against bovine *P. multocida* serotypes A, B. To the best of our knowledge, the present study was the first to identify cross-protective antigens, extracted OMPs from bovine *P. multocida*.

P2876

The serum lyme disease screening analysis of patients with joint symptoms in sanya

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Objective: About Sanya regional arthritis of Lyme disease infection.
Methods: People´s Hospital of Sanya city, Hainan Province, provide serum 537, For arthritis patients. Using the indirect immunofluorescence method (Indirect Fluorescent-Antibody Test, IFA) and immunoblotting (Western Blot, WB) Lyme disease antibody detection of serum samples; And nested-PCR method is used for WB antibody positive serum samples for pathogen detection.
Results: The IFA method tested serum sample 537 copies, 52 positive and 9.68% positive. WB method is used to test the above 52 IFA positive serum samples, 10 positive. A nested PCR method was used to detect 10 WB positive samples, including 6 IgG positive and 4 positive IgM.
Conclusion: This study confirms that Lyme disease exists in the population in Hainan Province. Recommendations when local doctors in the diagnosis and treatment of patients with arthritis, you might consider whether it suffered from Lyme disease.
Funding: Key research and development program (scientific and technological cooperation) project of hainan province 2DYF2017163

DOI: 10.1002/eji.201970400

P2877

PGAM1 hypomethylation drives aerobic glycolysis in CD4 T cells to facilitate the host defense against tuberculosis

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Background: DNA methylation is important epigenetic mechanism that mediates cellular development and function, but whether DNA methylation is involved in regulating CD4 T cells in the context of tuberculosis (TB) is unclear.
Results: We used oxidative bisulfite sequencing to measure global DNA methylation levels in isolated CD4 T cells from patients with tuberculous pleuritis (TP), latent TB infection (LTBI) and healthy controls (HC). Compared to peripheral blood mononuclear cells (PBMCs), CD4 T cells from pleural fluid mononuclear cells (PFMCs) showed a significant different global DNA methylation profile, with much lower 5-methylcytosine levels in PFMC. A signature of 376 differentially methylated regions could discriminate between TP PBMC and PFMC T cells, including hypermethylated LAPTM5, FAM134B and FOXP1, and hypomethylated CCL5, TNF and AIM2 in PFMCs. KEGG pathway analysis showed that numerous metabolic pathways, especially 'Carbon metabolism', were highly enriched among the differentially methylated genes with lower methylation levels in TP PFMCs. Hypomethylation of the glycolytic enzyme PGAM1 promoted gene expression and increased aerobic glycolysis in CD4 T cells to permit CD4 T-cell proliferation and effector function.
Conclusions: Diminished DNA methylation levels - especially in genes involved in metabolism - are an important CD4 T-cell signature at the site of infection in TB. DNA hypomethylation of PGAM1is critical mechanism in regulating the CD4 T-cell immune response by enhancing aerobic glycolysis. These findings provide novel insights into the epigenetic regulation of CD4 T-cell responses and identify potential targets for immunotherapy in TB.

P2878

The tst gene facilitates Staphylococcus aureus pathogenesis by promoting the secretion of inflammatory cytokines and inducing immune suppression

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Staphylococcus aureus (S. aureus) is an important pathogen causing various limited or systemic infections. Methicillin resistant S. aureus (MRSA) in particular presents a major clinical and public health problem. Toxic shock syndrome toxin-1 (TSST-1) encoded by the gene tst is an important virulence factor of tst positive S. aureus. However, the mechanism of TSST-1 in pathogenesis is only partly clear. In this study, we investigated the presence of the tst gene in clinical isolates of S. aureus. Then, animal experiments were performed to evaluate the influence of tst gene on serum cytokine concentrations and the bacterial load in different organs. In addition, macrophages were used to analyze the secretion of cytokines in vitro. Finally, pathological analysis was carried out to evaluate organ tissue impairment. The results demonstrated that the tst gene was present in 17.8% of the

DOI: 10.1002/eji.201970400

clinical strains. BALB/c mice infected with tst+ isolates exhibited a severe loss of body weight and a high bacterial load in organ tissues. Pathological analysis demonstrated that tissue impairment was more severe after infection with tst gene positive isolates. Moreover, the secretion of IL-6, IL-2 and IL17A by macrophages infected with tst+ isolates clearly increased. Notably, IL-6 secretion in mice infected with tst+ isolates was higher than that in mice infected with tst- isolates. Together, these results indicated that the tst gene may play a critical role in the pathological process of infection via a direct and persistent toxic function, and by promoting the secretion of inflammatory cytokines that indirectly induce immune suppression.

P2879

Next generation sequencing reveals changes of the γδ T cell receptor repertoires in patients with pulmonary tuberculosis

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Tuberculosis (TB) is a severe global threat to human health. The immune protection initiated by γδ T cells play an important role in mycobacterial infection. Vaccines for Mycobacterium tuberculosis (Mtb) based on γδ T cells provide a novel approach for TB control. In our previous studies, we found a preponderant complementarity-determining region 3 (CDR3) sequence of the γδ T cell receptor (TCR) in TB patients, and successfully identifed a tuberculosis antigen that can efectively activate γδ T cells with a reverse genetic strategy. However, due to the throughput limitation of the method we used, the information we obtained about the γδ TCR repertoire and preponderant CDR3 sequences was limited. In this study, we introduced next generation sequencing (NGS) to study the γδ TCR CDR3 repertoires in TB patients. We found that the CDR3δ tended to be more polyclonal and CDR3γ tended to be longer in TB patients; the γδ T cells expressing CDR3 sequences using a Vγ9-JγP rearrangement expanded signifcantly during Mtb infection. We also identifed new preponderant CDR3 sequences during Mtb infection. This study comprehensively characterized the γδ T cell receptor repertoire changes, and provides useful information for the development of new vaccines and adjuvants against TB.

P2880

Neutrophils and immunity: from bactericidal action to being conquered

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The neutrophil is the major phagocyte and the final effector cell of the innate immunity, with a primary role in the clearance of extracellular pathogens. Using the broad array of cytokines, extracellular traps, and effector molecules as the humoral arm, neutrophils play a crucial role in the host defense against pathogen infections. On the other hand, the pathogen has the capacity to overcome neutrophil-mediated host defense to establish infection causing human disease. Pathogens, such as S. aureus, have the potential to thwart neutrophil chemotaxis and phagocytosis and thereby succeed in evading killing by neutrophils. Furthermore, S. aureus surviving within neutrophils promotes neutrophil cytolysis, resulting in the release of host-derived molecules that promote local inflammation. Here, we provide a detailed overview of the mechanisms by which neutrophils kill the extracellular pathogens and how pathogens evade neutrophils degradation. This review will provide insights that might be

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DOI: 10.1002/eji.201970400

useful for the development of novel therapies against infections caused by antibiotic resistant pathogens.

P2881

Wip1 regulates mitophagic process and antibacterial response via dephosphroylating ULK1 at serine 757

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Wip1 is a serine/threonine phosphatase that plays a critical role in regulation of a wide spectrum of cellular activities including cellular proliferation, differentiation, senescence and cell cycle arrest, but its role in the regulation of innate immune response remains largely unknown. Our current study demonstrated that Wip1 deficiency exaggerated inflammatory response and impeded macrophage bactericidal ability, leading to defective bacterial clearance, exacerbated disease severity and higher mortality in mice succumbed with pulmonary staphylococcal infection. Mechanistically, we showed that Wip1 specifically interacted with ULK1, the key signaling molecule required for autophagic pathway, and de-phosphorylated it at ser757, which in turn facilitated the autophic pathway and anti-bacterial responses in macrophages. Conversely, loss of Wip1 impaired autophagic pathway and hence caused the accumulation of dysfunctional mitochondria and the failure in bacteria eradication. Collectively, we herein provide the first evidences to support a critical role for Wip1 in host defense against bacterial infection, primarily through promoting autophagic pathway and maintaining mitochondrial homeostasis.

P2882

Preliminary study on inflammatory mechanism of Chlamydia trachomatis-infected HeLa229 cells regulated by Atg5

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Objective: To study the role of autophagy-related gene Atg5 in the inflammatory reaction in HeLa229 cells infected by Chlamydia trachomatis.
Methods: Detected the expression level of Atg5 and LC3 in HeLa229-shAtg5 and HeLa229-shNC via Western blot, and analyzed the cytokine profile(IL-1β, IL-6, IL-10, IFN-γ, TNF-α, IL-17)secreted by host cells in different infectious conditions via Milliplex multi-cytokine detection technology.
Results: The basic expression level of LC3 and Atg5 in HeLa229-shAtg5 was significantly decreased than HeLa229-shNC, and the ratio of LC3-II/LC3-I was significantly decreased, the difference was statistically significant(p< 0.01); The expression of LC3 in HeLa229-shAtg5 infected by *Chlamydia trachomatis* was significantly decreased than HeLa229-shNC. The level of inflammatory cytokines IL-1β, IL-6, IFN-γ secreted by the *Chlamydia trachomatis*-infected HeLa229-shAtg5 were significantly increased than HeLa229-shNC, and the level of anti-inflammatory cytokine IL-10 in HeLa229-shAtg5 were significantly decreased than HeLa229-shNC, the difference was statistically significant; the level of TNF-α secreted by HeLa229-shAtg5 was higher than HeLa229-shNC, however, the overall level of TNF-α in the two cell-lines were low, and the IL-17 was not detected in our experiments.
Conclusion: (1) Atg5 was the important factor to regulate the level of autophagy in Hela229 cells infected by *Chlamydia trachomatis*, the level of autophagy had significantly decreased when the *Atg5* gene had been silenced; (2) The inflammatory reaction in HeLa229-shAtg5 cells were higher than HeLa229-shNC cells after infected by *Chlamydia trachomatis*, the inflammatory cytokines IL-1β, IL-6,

DOI: 10.1002/eji.201970400

IFN-γ were increased and the anti-inflammatory cytokine IL-10 was decreased than HeLa229-shNC. Our data showed that autophagy had the anti-inflammatory effect.

P2883

Transcriptomic analysis of microRNAs-mRNAs regulating innate immune response of zebrafish larvae against Vibrio parahaemolyticus infection

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In recent years, microRNAs (miRNAs) have been shown to play important roles in immunity. Analyses of the functions of miRNAs and their targets are useful in understanding the regulation of the immune response. To understand the relationship between miRNAs and their targets during infection, we used zebrafish as an infection model to characterize the miRNA and mRNA transcriptomes of zebrafish larvae infected with *Vibrio parahaemolyticus* to identify the differentially expressed miRNAs and mRNAs. Overall, 34 known zebrafish miRNAs were differentially expressed in the infection group, and the predicted targets of 26 of these miRNAs were differentially regulated in the mRNA transcriptome. These miRNAs and their targets are associated with specific Gene Ontology (GO) terms, such as peripheral nervous system neuron axonogenesis, organophosphate metabolic process, heme binding, protein binding, tetrapyrrole binding, protein dimerization activity, and aromatase activity, which regulate nerve conduction, energy metabolism, hematopoiesis, and protein synthesis, and with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways such as phototransduction, tryptophan metabolism, notch signaling, and purine metabolism. Our findings indicate that miRNAs regulate the innate immune response via complex networks and that zebrafish (dre) miR-205-3p, dre-miR-141-5p, dre-miR-200a-5p, dre-miR-92a-2-5p, dre-miR-192, and dre-miR-1788 may play important roles in the innate immune response.

P2884

Seroprevalence of tick-borne anaplasma phagocytophilum infection in healthy adult population and patients with acute undifferentiated fever from the yunnan province of China

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Anaplasma phagocytophilum—the causative agent of human granulocytic anaplasmosis (HGA)—is a tickborne pathogen transmitted by *Ixodid* ticks infecting wild and domestic mammals as well as humans. Despite the availability of evidence regarding this emerging infection among vectors, host animals, and individuals in China, there is limited knowledge on the prevalence and distribution of *A. phagocytophilum* in the Yunnan Province which is a high-incidence area for insect-borne infectious diseases because of diverse topography and climate. The aim of this study was to assess the seroprevalence of *A. phagocytophilum* in healthy adults and patients with acute undifferentiated fever from four regions in the Yunnan Province. The enzyme-linked immunosorbent assay and indirect immunofluorescence assay were used to detect IgG and IgM against *A. phagocytophilum* in sera obtained from 1185 healthy blood donors and 245 patients with acute undifferentiated fever, respectively. Demographic variables were assessed as potential risk factors using the chi-squared test. The rates of seropositivity rates were 7.59% and 4.49% in healthy donors and fever patients, respectively. Analysis of risk factors such as gender, age groups, and place of residence showed statistically significant differences. Infections with *A. phagocytophilum* occur widely among individuals residing in southwestern China. Our results indicate that there is serological evidence of HGA in this

DOI: 10.1002/eji.201970400

population and presence of acute *A. phagocytophilum* infections in patients with undifferentiated fever in the Yunnan Province.

P2885

Mycobacterium tuberculosis PE_PGRS31 enhances the intracellular survival of mycobacterium smegmatis within macrophages by inhibiting IL-1β and IL-12 p40 production

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Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains a formidable threat to global public health. The success of M. tuberculosis as a pathogen largely contributes to its ability to manipulate the host immune responses. Proline-glutamic acid (PE) and proline-valine-glutamate (PPE) are mycobacteria-specific proteins family that are widely reported to be involved in bacterial virulence, physiological activity and interaction with the host. Despite of intense research, the roles of PE_PGRS proteins in mycobacteria pathogenesis remain elusive. PE_PGRS31 is an important member of the PE/PPE family. Macrophages infected by Mycobacterium smegmatis, a non-pathogenic species intrinsically defcient of PE_PGRS, harboring PE_PGRS31 decreased the production of IL-1β and IL-12 p40. In addition, PE_PGRS31 boosted the survival of M. smegmatis within macrophage through inhibiting the cell apoptosis. Taken together, this study implicated that PE_PGRS31 is a virulent factor actively engaged in the interaction with host macrophage.
Keywords: *Mycobacterium tuberculosis, PE_PGRS31, Macrophage, IL-1β, IL-12 p40*

P2886

PE_PGRS26 reduces the survival of Mycobacterium smegmatis within macrophages via caspase-mediated apoptosis

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Tuberculosis continues to be an important infectious disease worldwide, killing millions of people every year. PE_PGRS proteins the largest subfamily of PE proteins, plays a role in interfering with the host´s natural immunity. PE_PGRS26 recombinant is sensitive under hydrogen peroxide, Zinc ions and anti-tuberculosis drugs isoniazid. The recombinant can increase the host cell production of cytokines IL-6 and TNF-α, reduce the survival of M. smegmatis and inhibit apoptosis. This process does not change intracellular reactive oxygen species levels.
Keywords: *Mycobacterium tuberculosis; PE_PGRS; apoptosis; macrophage*

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DOI: 10.1002/eji.201970400

P2887

Invariant NKT cells from patients with pulmonary tuberculosis decreased in numbers but increased expression of activation markers

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Invariant natural killer T (iNKT) cells are small subset of T cells, as innate immune cells, play regulatory role in adaptive immunity. Recent studies have shown that iNKT cells decreased in numbers in infectious diseases such as *Mycobacterium tuberculosis* infection, human T-cell lymphotropic virus infection, and human immunodeficiency virus infection. In our study we performed flow cytometry to detect the proportion and phenotypes of iNKT cells in fresh whole blood from 45 active pulmonary TB patients (29 initial treatment and 16 retreatment TB patients) and 23 healthy controls (HC). We found a significant decrease in absolute numbers of lymphocytes and iNKT cells ($TCRVa24^+$ $CD3^+$) in TB patients compared to HC group. While iNKT cells from TB patients decreased in numbers, the expressions of CD69, CD25 and HLA-DR were much higher than that in HC group. CD161 surface expression on iNKT cells of TB patients significantly decreased than that in HC group. In addition, the proportions of $CD4^+$ iNKT cells in retreatment-TB patients were obviously higher, but DN iNKT cells lower than that in HC group. We also found that the expression levels of CD45RO and CD27 on iNKT cells significantly varied in retreatment-TB group compared to initial treatment TB and HC groups. Our findings that changes of iNKT cells in TB patients were consistent with other investigations, and demonstrate that iNKT cells are involved in the immune response to infection of *Mycobacterium tuberculosis* (*M.tb).*
Funding: The Innovation Team Project in Scientific Research Platform of Anhui Colleges and Universities (2016-40)

P2888

Regulatory effect of lipoarabinomannan from Beijing genotype Mycobacterium tuberculosis on T lymphocyte differentiation

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Tuberculosis (TB) is still the leading cause of death from a single infectious agent. It is particularly significant to investigate the pathogenesis of TB for developing more efficient diagnostic and treatment approaches. Recently, more and more evidence showed that the antigen-lipid arabinomannan (LAM), which locates on the cell wall of *Mycobacterium tuberculosis* plays an important role in the immune responses against *Mtb* infection. In this work, the Beijing genotype *M. tuberculosis* clinical strains were collected and cultured. Then the LAM of the strains was purified. After that, 30 pulmonary tuberculosis patients and 12 health control were grouped, and the blood of 2 ml of each subject was collected. The PBMC cells were isolated and stimulated with purified BJ-LAM. Cytokine levels were tested after 48 h stimulation. The results showed that IL-10 and γ-interferon(IFN-γ) were significantly higher in patients than that in healthy controls. IL-10 is an anti-inflammatory factor, which plays a down-regulation of inflammatory response during bacterial infection. IFN-γ can regulate the expression levels of 30 genes and has anti-bacterial infection and other immune regulation. Also, the results showed that BJ-LAM

DOI: 10.1002/eji.201970400

had an immune-enhancing effect, and can inhibit inflammation. It was found that the release of IL-17 from the supernatant of PBMCs after LAM stimulation was higher than that of the control group, and the difference was significant, which may indicate that BJ-LAM had the ability to regulate the release of IL-17 by T cells, and enhance the immuno-protecive effect.

P2889

Study on immune response of mice to eukaryotic expression recombinant plasmid of mycoplasma ovipneumoniae Hsp70 C-terminal protein gene

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To research the possibility of Mycoplasma ovipneumoniae Hsp70 C-terminal protein as a target gene of genetic engineering vaccine, the endotoxin-free plasmid pcDNA 3.1-Hsp70 was injected into mice with 50μg, 100μg, 150μg .The growth and decline of specific antibodies and IL-2, IL-4 and IFN-γ in serum and spleen cells were analyzed by ELISA, and the proliferation of spleen cells in mice was analyzed by MTT.The results showed that the recombinant plasmid could induce mice to produce specific antibodies, of which 100 μg/single dose was the best.After injecting recombinant plasmid into mice, IL-2 and IFN-γ in serum and spleen cells were significantly higher than those in control group ($p < 0.01$),reaching the highest levels in 100μg group at 4w or 5w, which was significantly different from those in 50μg and 150μg group ($p < 0.01$), while IL-4 in serum and spleen cells was significantly lower than those in control group ($p < 0.01$),reaching the lowest level at 5w.The MTT showed that the spleen cells in the recombinant plasmid group were significantly higher than those in control groups ($p < 0.01$), of which 100μg injection group were significantly higher than those in the 50μg and 150μg groups ($p < 0.05$). The results of this study confirmed that Mo Hsp70 C-terminal protein can stimulate specific humoral and cellular immune responses in mice and the results provide basic research data for exploring the feasibility of Mo Hsp70 C-terminal protein participating in the construction of Mo gene vaccine.

P2890

Rab32 GTPase, as a direct target of miR-30b/c, controls the intracellular survival of Burkholderia pseudomallei by regulating phagosome maturation

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Burkholderia pseudomallei is a gram negative, facultative intracellular bacterium, which causes a disease known as melioidosis. Professional phagocytes represent a crucial first line of innate defense against invading pathogens. Uptake of pathogens by these cells involves the formation of a phagosome that matures by fusing with early and late endocytic vesicles, resulting in killing of ingested microbes. Host Rab GTPases are central regulators of vesicular trafficking following pathogen phagocytosis. However, it is unclear how Rab GTPases interact with B.pseudomallei to regulate the transport and maturation of bacterial-containing phagosomes. Here, we showed that the host Rab32 plays an important role in mediating antimicrobial activity by promoting phagosome maturation at an early phase of infection with B.pseudomallei. And we demonstrated that the expression level of Rab32 is increased through the downregulation of the synthesis of miR-30b/30c in B.pseudomallei infected

DOI: 10.1002/eji.201970400

macrophages. Subsequently, we showed that B.pseudomallei resides temporarily in Rab32-positive compartments with late endocytic features. And Rab32 enhances phagosome acidification and promotes the fusion of B.pseudomallei-containing phagosomes with lysosomes to activate cathepsinD, resulting in restricted intracellular growth of B.pseudomallei. Additionally, Rab32 mediates phagosome maturation depending on its guanosine triphosphate/guanosine diphosphate (GTP/GDP) binding state. Finally, we report the previously unrecognized role of miR-30b/30c in regulating B.pseudomallei-containing phagosome maturation by targeting Rab32 in macrophages. Altogether, we provide a novel insight into the host immune-regulated cellular pathway against B.pseudomallei infection is partially dependent on Rab32 trafficking pathway, which regulates phagosome maturation and enhances the killing of this bacterium in macrophages.

P2891

Detection of citrullinated histone 3-containing fabric web structure in caseous granulomas of pulmonary tuberculosis in humans

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Extracellular trap is a fabric web structure composed of DNAs, histones and serine proteases that trap and kill extracellular microbes. It is produced by myeloid cells during inflammation and tissue damage and has been implicated in triggering thrombosis. Patients with tuberculosis has thrombotic conditions. Neutrophil extracellular traps (NETs) have recently been detected from the peripheral blood and sputum of the patients. However, it remains unclear whether extracellular traps can be found within the pathological lesions at the lungs of patients with pulmonary tuberculosis. We used an antibody against citrullinated histone 3 as a marker of NETs marker. Caseous granulomas and blood vessels in lung tissues surgically removed from patients with tuberculosis were examined for NETs. Web-like structures stained positive with citrullinated histone 3 could be seen at caseous granulomas and blood vessels. Our result warrants further studies on whether the web-like structure is indeed NETs, and whether it played a role in the pathogenesis of tuberculosis

P2892

Mechanism of persistent infection and promotion of tumor progression of *Fusobacterium nucleatum* mediated by miR-31

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***Fusobacterium nucleatum* (*F. nucleatum*)** is a Gram-negative, obligate anaerobic bacterium located in human oral cavity. Recent studies have shown that *F. nucleatum* enriched in **colorectal cancer (CRC)** tissues, affecting multiple stages of cancer progression, including tumor cell proliferation promotion, tumor immune escape, recurrence, and chemo resistance. There are various types and large numbers of microorganisms in the human intestinal tract, forming a complex environment. Therefore, as an invasive bacteria, it is difficult for *F. nucleatum* to colonize in human intestinal tract. In this study, we found that *F. nucleatum* can upregulate the expression of **miR-31**, mediating its **persistent infection** in colorectal epithelial cells to **escape immune elimination.** Autophagy can maintain cell homeostasis and plays a vital role in the body´s elimination of infectious pathogens. miR-

31 inhibits **autophagic flow** by inhibiting the fusion of autophagosomes and lysosomes by **SNAREs protein STX12** to colonized in cells. At the same time, miR-31 can activate **RAS signaling pathway** by inhibiting the expression of RASA1 to **promote tumor progression.** In this study, we focused on miR-31 to explore the interaction pattern of "*F. nucleatum* infection, autophagy and tumor". It demonstrated that *F. nucleatum* infection can inhibit autophagic flow and promote cancer progression via miR-31. This study provides a theoretical basis for the clinical diagnosis, prognosis evaluation and precise prevention of colorectal cancer, and also a new idea for the study of other intracellular pathogenic microorganisms.

P2893

L-serine lowers lung inflammatory responses in mice during Pasteurella multocida infection

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Pasteurella multocida causes a variety of infectious diseases in a wide range of mammals and birds, resulting in enormous economic loss to the modern livestock and poultry industry. However, the mechanism of interactions between host and *P. multocida* are still unclear. Previous studies had found that some amino acids played important roles associated with *P. multocida* infection. In present work, we found that L-serine concentrations were significantly decreased in murine lungs infected with *P. multocida*. Intriguingly, exogenous supplementation with L-serine significantly ameliorated the pulmonary inflammatory responses and increased the survival rate of *P. multocida*-infected mice. Notably, L-serine decreased the macrophage-mediated inflammatory responses in *P. multocida* infection mice. Mechanistically, L-serine inhibited the production of inflammatory cytokines in macrophages by inhibiting the activation of cellular inflammasome, and inhibiting macrophage polarization though reprogramming the cellular transcription and metabolism. This work initially revealed that the L-serine metabolic cross talk between *P. multocida* and host, and provided a theoretical basis for the treatment and prevention of diseases caused by this bacterium.
Keyword: Inflammatory responses; Inflammasome; L-serine; metabolic reprogramming; *Pasteurella multocida*
Support Fund: China Agriculture Research System (Beef/Yak Cattle, CARS-37)

P2894

The mechanisms of lipophagy in persistent intracellular infection of *Burkholderi pseudomallei*

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Burkholderia pseudomallei (*B. pseudomallei*) is the causative agent of melioidosis, which is a potential threat to public health and national security. As a intracellular pathogen, *B. pseudomallei* can escape from the vacuole and replicate within the host-cell cytosol which causing persistent infection and complicating treatment. The interaction between host lipid metabolism and pathogen infection plays an important role in the pathogenesis of pathogenic microorganisms. Previous study showed that NR1D2 was up-regulated in response to *B. pseudomallei* infection by microarray assay and the lipids accumulated, the autophagy-mediated anti-*B. pseudomallei* response was inhibited. However, the mechanism is not clear. In this paper, as a potential mechanistic explanation for this observation, we demonstrate down-regulate NR1D2 by eukaryotic transfection in *B. pseudomallei* infected cells and animal models, and use WB, LSCM, TEM, FLOW and EMSA techniques to study the function in

DOI: 10.1002/eji.201970400

lipophagy in persistent intracellular infection of *B. pseudomallei* . Furthermore, we aim to clarify the mechanism of lipophagy inhibited by *B.pseudomallei* in vivo and in vitro, and search for new approaches to the treatment of *B. pseudomallei* infection to provide theoretical guidance.

P2895

Evaluation of the neutrophil volume conductivity scatter (VCS) parameter during the period of stable and acute exacerbation of chronic obstructive pulmonary disease patients

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Objectives: We aimed to investigate the importance of neutrophil volume conductivity scatter (VCS) parameter in patients with chronic obstructive pulmonary disease (COPD) for identifying the severity of inflammation and recognition of acute exacerbation.
Methods: All the patients in this prospective study were enrolled from October 2017 to April 2018, including 118 male AECOPD patients and 60 male stable COPD patients from the department of respiratory diseases, and 95 healthy non-smoking male controls from the department of medical examination. The mean neutrophil volume (MNV) with its distribution width (NDW) and the mean neutrophil scatter (MNS) as well as the neutrophil-lymphocyte ratio were studied.The neutrophil-lymphocyte ratio was calculated from complete blood count (CBC). The Receiver operating characteristic curve (ROC) analysis was used to highlight the clinical implication.
Results: MNV、NDW、MNS and NLR were found to be significantly elevated in exacerbated COPD compared to stable COPD and control participants. For an NDW cutoff of 22.205, sensitivity for detecting exacerbation of COPD was 84.7% and specificity was 75.0% (AUC 0.852, $P< 0.05$). Combined NDW with NLR resulting in a increase sensitivity of 88.1% and specificity of 76.7% to detect AECOPD(AUC 0.890,$P< 0.05$).
Conclusions: Elevated NDW can be used as a marker in the determination of increased inflammation in acutely exacerbated COPD. Combining NDW with NLR may improve this.

P2896

Leukadherin-1-mediated activation of CD11b inhibits LPS-induced pro-inflammatory response in macrophages and protects mice against endotoxic shock by blocking LPS-TLR4 interaction

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Dysregulation of macrophage has been demonstrated to contribute to aberrant immune responses and inflammatory diseases. CD11b, expressed on macrophages, plays a critical role in regulating pathogen recognition, phagocytosis, and cell survival. In the present study, we explored the effect of leukadherin-1 (LA1), an agonist of CD11b, on regulating LPS-induced pro-inflammatory response in macrophages and endotoxic shock. Intriguingly, we found that LA1 could significantly reduce mortalities of mice and alleviated pathological injury of liver and lung in endotoxic shock. *In vivo* studies showed that LA1-induced activation of CD11b significantly inhibited the LPS-induced pro-inflammatory response in macrophages of mice. Moreover, LA1-induced activation of CD11b significantly inhibited LPS/IFN-γ-induced pro-inflammatory response in macrophages by inhibiting MAPKs and NF-κB signaling pathways *in vitro*. Furthermore, the mice injected with LA1-treated BMDMs showed fewer pathological lesions than those injected with vehicle-treated BMDMs in endotoxic shock. In addition, we found that activation of TLR4 by LPS could endocytose CD11b and

activation of CD11b by LA1 could endocytose TLR4 *in vitro* and *in vivo*, subsequently blocking the binding of LPS with TLR4. Based on these findings, we concluded that LA1-induced activation of CD11b negatively regulates LPS-induced pro-inflammatory response in macrophages and subsequently protects mice from endotoxin shock by partially blocking LPS-TLR4 interaction. Our study provides a new insight into the role of CD11b in the pathogenesis of inflammatory diseases.

P2897

Antibody production is concomitant with altered NK cell and CD8$^+$ T cell frequencies in peripheral blood of patients with active tuberculosis

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The effect and potential mechanism of antibodies (Abs) in immunity against tuberculosis (TB) is not completely clear. In this study, we evaluated Ab responses by detecting serum IgG titers against a panel of *Mycobacterium tuberculosis* (Mtb) specific antigens in patients with active TB. Meanwhile, we determined the peripheral lymphocyte profile of these subjects and analyzed whether Ab production affects immune cell distribution. We found that a combination of 38KD-MPT32-MPT64, CFP10-Mtb81-EspC and LAM antigens were superior for detection of TB Abs, with a sensitivity of 74.0%, and a specificity of 88.2%. We detected decreased percentages of natural killer (NK) cells, increased CD4$^+$ T cells and NK T-like (NKT-like) cells, and no significant change in CD8$^+$ T cells and B cells in patients with active TB compared with healthy donors. Importantly, we found that TB-specific IgG Ab production was accompanied with altered cytotoxic cell frequencies, characterized by increased NK cells and decreased CD8$^+$ T cells in patients with detectable TB Abs compared with the patients without detectable TB Abs during active TB. These results suggest that NK cells and CD8$^+$ T cells may mediate a potential role of Abs in immunity against active TB. In summary, our study provides preliminary clues for further investigation of the effect of Abs against TB and offers a better understanding of the immune state of patients with active TB.

P2898

Alpha-hemolysin of uropathogenic Escherichia coli induces GM-CSF-mediated acute kidney injury

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Uropathogenic *Escherichia coli* (UPEC) is the leading cause of urinary tract infections (UTIs). Acute pyelonephritis caused by UPEC is the most severe form of UTIs, which may result in permanent renal scarring and failure. Alpha-hemolysin (HlyA) is a key virulence factor of UPEC, which causes serious tissue damage. More than 70% of pyelonephritis isolates are *hlyA*-positive. The mechanism by which HlyA triggers kidney tissue damage during acute pyelonephritis remains unknown. Recently, we found that HlyA induced GM-CSF secretion from human renal epithelial cells and in an acute pyelonephritis mouse model, which triggered M1 macrophage accumulation in kidney and caused sever tissue damage. Macrophage elimination or GM-CSF neutralization markedly reduced kidney injury by HlyA. Anti-GM-CSF antibody, which protects against HlyA-induced kidney injury, has the potential to treat acute pyelonephritis. Invasion of bacteria into epithelial cells is beneficial for their survival by escaping

DOI: 10.1002/eji.201970400

immune cell mediated clearance. We found HlyA promoted UPEC invasion into renal epithelial cells and Nectin-2 was identified to be a renal epithelial receptor for HlyA, which is involved in HlyA-induced UPEC invasion. The interaction between HlyA and Nectin-2 (including ectopic, endogenous and purified Nectin-2) was confirmed. Nectin-2 has the potential to be used as a target to treat kidney infections. Antibiotics are frequently used for UTIs treatment, and the multidrug-resistant strains are rising. Therefore, alternative therapeutics are necessary to be developed. Identification of host factors responsible for HlyA-induced kidney injury could lead to novel strategies to treat acute pyelonephritis.

P2899

The expression of MicroRNA-146a is related with antibody titers and clinical symptoms in brucellosis patients

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Brucellosis is one of the most common zoonosis worldwide. Human brucellosis is characterized by untypical symptoms, including fever, sweating, arthralgia/arthritis, constitutional symptoms, hepatomegaly, splenomegaly and host immune inhibition. MicroRNAs (miRNA) are endogenous 22-nucleotide RNAs that play important regulatory roles in gene regulation. MiRNAs also modulate the innate and adaptive immune responses to pathogens. The potential applications of miRNAs as diagnostic or prognostic biomarkers have been demonstrated in various types of diseases. MicroRNA-146a (miR-146a) is involved in immune response and related with many infectious diseases. In this study, the expression of microRNA-146a in sera samples from 20 brucellosis patients and 20 healthy volunteers were analyzed by real time PCR assay. The mean expression level of miR-146a in brucellosis patients was 0.71μM and ranged from 0.04 to 1.58μM. While that was 6.75μM and ranged from 0.18 to 22.53μM in healthy volunteers. The relation of expression level of miRNA-146a and antibody titer was analyzed by standard agglutination test (SAT), a confirmative serological assay for brucellosis. The 1:50 group showed an average of 4.6 ng/ul, and 1:100 showed 3.31, while 1:200 showed 1.54 ng/ul. The expression levels of the three groups differed significantly ($p< 0.01$). We also analyzed the possible correlation between miR-146a level and common clinical symptoms. Patients with symptom of fever and/or sweating showed lower level of miR-146a. The results indicate that the expression levels of miR-146a are significantly inhibited in brucellosis patients and negatively relate with serum antibody titers and clinical symptoms of fever and sweating.

P2900

Lactobacillus protects intestinal barrier against *S.* Typhimurium infection through regulation of epithelial proliferation by Wnt/β-catenin pathway

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The influence of the intestinal microbiota, such as *Lactobacillus*, on the intestinal mucosa, particularly intestinal stem cells, remains incompletely understood. This study demonstrated that *S.* Typhimurium infection caused intestinal epithelial damage and affected the growth of intestinal organoids through significantly increased secretion of TNF-α. *S.* Typhimurium also colonized the intestine and invaded mesenteric lymph nodes (MLN) in mice and then caused pathological changes to the intestinal epithelium, intestinal inflammation, and even death. However, *L. acidophilus* alleviated damage to intestinal organoids, increased the survival ratio of mice infected with *S.* Typhimurium, and reduced

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DOI: 10.1002/eji.201970400

TNF-α secretion and *S.* Typhimurium colonization in mice feces and MLNs. Moreover, *L. acidophilus* affected the differentiation of epithelial cells through inhibition of the excessive expansion of Paneth cells induced by *S.* Typhimurium to avoid over-exhaustion. Finally, we also demonstrated that *L. acidophilus* ameliorated overactivation of TLRs and the Wnt/β-catenin pathway to affect the proliferation of the intestinal epithelium. This study demonstrated that *L. acidophilus* protects the intestinal mucosa against *S.* Typhimurium infection through not only the inhibition of pathogen invasion but also determination of the fate of the intestinal epithelium.

P2901

CP-PGN protects against MRSA infection by MyD88-mediated moderate inflammatory reaction

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In our previous studies, it has been verified that a novel peptidoglycan of *Corynebacterium pyruvate* (CP-PGN) could resist the infection caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) through TLR2 signaling pathway. Here, we show that not all ligands of TLR2 could be used as anti-infective agents. Exactly, peptidoglycan derived from MRSA(M-PGN) aggravated serious MRSA infection. In addition, *CP-PGN as an agonist of TLR2 activated the macrophages and reversed MyD88 and TNF-αexpression induced by infection,* which was very helpful for the body to maintain moderate inflammatory response and avoid excessive damage. But, *this dual effect of TLR2 ligands is first reported on macrophages,* which seems to be challenging the well-known knowledge of Toll-like receptors (TLRs) network. Furthermore, these data clearly demonstrate that *such down-regulation of MyD88 is mainly dependent on the basal level of MyD88 at the CP-PGN time-dependently exposure, which is vital to balance the immune disorders caused by infection.* Overall, MyD88-mediated moderate inflammatory reaction may be a novel mechanism in CP-PGN against MRSA infection.

P2902

Borrelia burgdorferi basic membrane protein A initiates Proinflammatory Chemokine Storm via the Receptors TLR1 and TLR2

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Lyme arthritis, the most common, serious and harmful manifestation during the late stages of Lyme disease, is closely associated with the *Borrelia burgdorferi* basic membrane protein A (BmpA). Chemokines are also reported to have an important role in Lyme arthritis. Toll-like receptors (TLRs) recognize and bind to pathogen-associated molecules which are structurally conserved among microbes, to activate transcriptional events, including cytokine production, inflammation, and tissue damage. We speculated that BmpA could induce a storm of proinflammatory chemokines via TLRs and downstream moleculars, and that TLR1, TLR2, TLR5, TLR6 and the adaptor protein, MyD88, may be involved in this process. We explored this hypothesis using the human monocytic leukemia cell line, THP-1, and recombinant BmpA (rBmpA). Cell surface TLR1 and TLR2 were neutralized using specific antibodies before stimulation with rBmpA and analysis of chemokine secretion using a chemokine chip. Further, the expressions level of the four TLRs and MyD88 were analyzed following

stimulation with rBmpA. Stimulation with rBmpA resulted in elevated levels of seven cytokines. Further, TLR1 and TLR2 antibody treated cells exhibited an overall reduction in rBmpA-induced chemokine expression. TLR1, TLR2, and MyD88 expression levels (both mRNA and protein) increased after stimulation with rBmpA. Our data confirm that TLR1, TLR2, and MyD88 are involved in BmpA-induced proinflammatory chemokines, which may be closely involved in Lyme arthritis pathogenesis.
Funding: This study was supported by grants from the National Natural Science Foundation of China (No.81560596, 81860644 and 31560051), the Yunnan Applied Basic Research Projects (No. 2017FE467-001 and 2014FA011).

P2903

Caspase-11 plays a protective role in pulmonary Acinetobacter baumannii infection

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Objective: As a conditional pathogenic bacterium, *Acinetobacter baumannii (A. baumannii*) can infect skin, respiratory tract and urinary system. In this study, we established *A. baumannii* lung infection model, to investigate the role of caspase-11 in *A. baumannii* pulmonary infection.
Methods: Wild-type C57BL/6 mice (*caspase-11*$^{+/+}$) and *caspase-11*$^{-/-}$ mice, and bone marrow-derived macrophages (BMDM) from these mice were studied in this study. *A. baumannii* was used to infect *in vivo*, and *A. baumannii* lysate was used to infect *in vitro*.
Results: *A. baumannii* could activate noncanonical inflammasome signaling mediated by caspase-11. Caspase-11 deficiency impaired neutrophil recruitment and bacterial clearance in the early stage of *A. baumannii* infection; caspase-11 deficiency inhibited IL-1α secretion by *A. baumannii* infection mice, and IL-1α neutralizing antibody suppressed neutrophil recruitment and bacterial clearance in the early stage of *A. baumannii* infection on wild-type mice; caspase-11 deficiency aggravated pulmonary pathological change in the persistant stage of *A. baumannii* infection.
Conclusion: Caspase-11/ IL-1α is involved in neutrophil recruitment and bacterial clearance in the early stage of *A. baumannii* pulmonary infection, and caspase-11 deficiency increases susceptibility to *A. baumannii*, leading to more severe pathological changes of lung. These data indicate that caspase-11 plays a protective role in pulmonary *A. baumannii* infection.
Keywords: *A. baumannii*; caspase-11, IL-1α

Immunity to Fungi and Parasites

O089

IL-1α promotes liver inflammation and necrosis during blood-stage Plasmodium chabaudi malaria

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DOI: 10.1002/eji.201970400

Malaria causes hepatic inflammation and damage, which contribute to disease severity. The pro-inflammatory cytokine interleukin (IL)-1α can be released by non-hematopoietic or hematopoietic cells during liver injury. This study established the role and the source of IL-1α during liver pathology caused by blood-stage *P. chabaudi* malaria. During acute infection, hepatic inflammation and necrosis were accompanied by NLRP3 inflammasome-independent IL-1α production. Systemically, IL-1α deficiency attenuated weight loss and hypothermia but had minor effects on parasitemia control. The levels of transaminases in the sera of *Il1a*$^{-/-}$ mice were lower in comparison to C57BL/6 mice. In the liver, the absence of IL-1α reduced the number of TUNEL^{+} cells and necrotic lesions. This finding was associated with a lower inflammatory response, concerning both cell infiltration and cytokine production, including TNF-α. The main source of IL-1α in the liver of infected mice was inflammatory cells, particularly neutrophils and the number of IL-1α-producing neutrophils were found to increase substantially in response to infection. The implication of IL-1α in liver inflammation and necrosis caused by *P. chabaudi* infection, as well as in weight loss and hypothermia, opens up new perspectives for improving malaria outcomes by inhibiting IL-1 signaling.

O090

Combinatorial proteomics and cellular immunology identify a protective *Leishmania* antigen, dihydrolipoyl dehydrogenase and its responding CD4^{+} T cells at clonal level

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There is currently no clinically effective vaccine against leishmaniasis due to poor understanding of the antigens that elicit protective CD4^{+} T cell immunity. Using proteomics and cellular immunology, we identified a naturally processed peptide (DLD$_{63-79}$) that is derived from *Leishmania* dihydrolipoyl dehydrogenase (DLD) protein. The gene encoding DLD is conserved in all species of pathogenic *Leishmania* and the protein is expressed by both the promastigote and amastigote stages of the parasite. DLD elicits strong CD4^{+} T cell responses in mice infected with *L. major*. We generated I-A^{b}-DLD$_{63-79}$ tetramer and used it to identify DLD-specific CD4^{+} T cells at clonal level. Following *L. major* infection, DLD$_{63-79}$-specific CD4^{+} T cells produce effector cytokines (IFN-γ and TNF) and undergo expansion, contraction and stable maintenance following lesion resolution. In addition, DLD$_{63-79}$-specific CD4^{+} T cells in healed mice displayed strong memory response (rapid proliferation and strong IFN-γ production) following secondary *L. major* challenge. Vaccination with DNA expressing full length DLD or recombinant DLD protein induced strong protection in mice. The remarkable protection observed in vaccinated mice suggests that DLD is an immunoprotective antigen and offers a possibility for development of an effective vaccine against cutaneous leishmaniasis by targeting DLD. Identification of the antigens that elicit protective immunity and their corresponding antigen-specific T cells are critical steps necessary for developing effective vaccines and vaccination strategies against infectious agents, including protozoan parasites.

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DOI: 10.1002/eji.201970400

O091

Transglutaminase 2 controls NLRP3 inflammasome activation during *Schistosoma japonicum* infection

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The mechanisms that promote hepatic inflammation induced by *Schistosoma japonicum(Sj)* eggs's deposition are not well defined. We showed that classical NLRP3, NLRC4 and AIM2 inflammasomes and non-canonical inflammasome were activated in mice liver during *Sj* infection. NLRP3 deletion strikingly decreased inflammasome activation and numbers of infiltrating immune cells around *Sj* eggs in mice liver during *Sj* infection, but without affecting Sj eggs load. Soluble egg antigen (SEA) induced activation of NLRP3 inflammasome in macrophages with the priming of TLR2 & TLR4 ligands, which indicated that SEA only acts as the signal 2 of this activation. The activity of NLRP3 inflammasome was decreased in tissue transglutaminase 2 (TG2) knockout mice liver during *Sj* infection, which was consistent with the results in vitro macrophages. With interfere of TG2 expression, level of phosphorylated JNK and p65 were down regulated, which suggested that TG2 operated signal 1 of NLRP3 inflammasome activation through JNK & NF-kB pathway. Transfecting TG2 plasmid into HEK 293T cells together with caspase1, IL-1B and NLRP3 vectors increase level of IL-1β and cleavage of caspase 1 in the supernatant of transfected cells, which indicated TG2 controls signal 2 of NLRP3 inflammasome activation. In conclusion, TG2 operates NLRP3 inflammasome activation during *Schistosoma japonicum* infection through regulating both signal 1 and signal 2. The component inside SEA of *Sj* as the signal 2 inducer needs further study.

O092

Whole exome sequencing of Chagas disease cardiomyopathy families reveals accumulation of rare variants in mitochondrial and inflammation-associated genes

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DOI: 10.1002/eji.201970400

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Background: Chagas disease is caused by lifelong infection with the protozoan *Trypanosoma cruzi*. Thirty percent of infected patients develop Chagas cardiomyopathy (CCC), a Th1 T cell-rich inflammatory dilated cardiomyopathy that is a leading cause of heart failure in Latin America. The remaining infected individuals are free from heart disease (asymptomatic, ASY). Myocarditis and mitochondrial dysfunction play a major pathogenetic role in CCC. Host genetic factors may underlie the differential progression of chagasic patients towards CCC, as indicated by familial aggregation of cases and association of common gene polymorphisms with CCC. Whole exome sequencing (WES) has been used to identify rare nonsynonymous pathogenic mutations in genetic cardiomyopathies. Here we used WES to search for genetic determinants of CCC development in families containing multiple cases of Chagas disease.
Methods: WES was performed in 26 patients belonging to 6 families. In each family, we searched for rare pathogenic variants that were shared only by family members with CCC, but absent in all infected siblings with the ASY form of disease.
Results: We identified CCC-associated heterozygous pathogenic variants in inflammation-related and mitochondrial genes in all studied families. Identified variants include truncation and complete loss-of-function. Variants and genes were different in each family.
Conclusion: Different heterozygous rare variants in mitochondrial or inflammatory genes may play an important role in progression to CCC. Mechanistically, carriers of such variants could display increased mitochondrial dysfunction and myocarditis after lifelong exposure to the high IFN-γ levels that are typical of Chagas disease, leading to myocardial energy starvation and cardiomyocyte death.

O093

Analysis of IgG autoantibody repertoire during asymptomatic malaria in children of Abobo endemic area (Cote d'Ivoire)

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Despite a considerable number of studies, the mechanisms of natural acquired immunity that contribute to protection in asymptomatic malaria are only partially understood. Among them the role played by the self-reactive antibody response induced during infection has not been clarified yet. This study aims to analyze the contribution of self-reactive antibody repertoires and also to determine the impact of parasite transmission levels in asymptomatic malaria in Ivory coast. Blood samples, n = 94 of patients to symptomatic malaria attacks (MM), n=47 asymptomatic carriers (AM) of parasites and n= 38 endemic control (EC) from different settings were enrolled in the study. IgG antibodies against a whole parasite (3D7), brain extract and Anopheles salivary gland peptide gSG6-P1 were quantified by the enzyme-linked immunosorbent assay (ELISA). Human self-reactive antibody repertoires were analyzed by quantitative immunoblotting. The association of self- and parasite specific- antibody repertoires was evaluated using Kruskal-wallis test and sperman's rank correlation. The level of total IgG response against 3D7 in AM were lower than those of MM. Moreover, children with AM exhibited a high level of autoantibodies recognizing brain antigen. In addition, a significant correlation between the exposure marker (IgG against gSG6-P1) and the self-reactive antibody response was found in all groups ($p< 0.01$). These results suggest that in addition to IgG to parasite 3D7, the intense production of autoantibodies observed in asymptomatic malaria may contribute to protective immune mechanisms. However the identification of the set of antigens that may contribute to the protective response in asymptomatic carriers needs further investigation.

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DOI: 10.1002/eji.201970400

O094

The discovery of new insertions in antibodies against malaria

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We previously isolated antibodies from malaria-exposed individuals that carried a large DNA segment inserted in between the V_H and DJ_H regions or in the switch regions of the immunoglobulin locus. The insert originates from chromosome 19 and encodes for the extracellular Ig-like domain of an inhibitory receptor named LAIR1. We found that the LAIR1 domain was sufficient to bind to various *Plasmodium falciparum* strains by acquiring somatic mutations along with antibody maturation. We, therefore, hypothesized that malaria surface antigens target host inhibitory receptors to evade immune response, and the host may develop inhibitory receptor-based antibodies to compete against such evasion mechanisms. Here, by screening malaria-exposed individuals, we discovered natural antibodies containing a new inhibitory receptor domains (LILRB1) that can cross-react with various malaria isolates. The new LILRB1 insert was found in the switch regions and after RNA splicing, the insert fused precisely after the variable region of the antibody. Moreover, we identified that the isolated LILRB1-containing antibodies recognize members of RIFIN family on *P.falciparum*-infected erythrocytes which maybe potential candidates for the development of a malaria vaccine.

O095

Antibodies elicited during natural infection in a predominantly P. falciparum transmission area cross-react with sexual stage-specific antigen in P. vivax

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Exposure to malaria parasite elicits immune responses during infection and it is generally believed that the immunity is stage specific and species specific. However, partial genomic similarity for various antigens in different *Plasmodium* spp. raises the possibility of immunological cross-reactivity at the level of specific antigens. The availability of recombinant antigens allowed us to investigate the immune cross reactivity against *P. falciparum* and *P. vivax* antigens using human sera from a *P. falciparum* endemic region in Zimbabwe. Serum samples were screened for antibody reactivity against Pfs48/45, a *P. falciparum* gametocyte antigen and Pvs48/45, a *P. vivax* homolog of Pfs48/45 using, ELISA and Western blotting. PCR was used to confirm *P. falciparum* infection and rule out *P. vivax*. Twenty-seven percent of the participants were found to be sero-positive for Pfs48/45 and 77 % of these Pfs 48/45 positive participants also showed reactivity with Pvs48/45. Western blotting confirmed cross reactivity in 23 Pfs48/45 and Pvs48/45 positive samples. Molecular diagnosis confirmed infection with *P. falciparum* and generalized the absence of *P. vivax* except for a single case which was positive for both pathogens. While it was not possible to rule out *P. vivax* transmission, it was reasonable to conclude that the higher sero-prevalence of Pvs48/45 positivity was likely a reflection of cross-reactivity of antibodies elicited against the predominant *P. falciparum* infection. This raises the possibility of partial cross-protective immunity and a beneficial development in the search for a malaria vaccine that could induce simultaneous protection against both *P. falciparum* and *P. vivax.*

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DOI: 10.1002/eji.201970400

O096

Role of treg cells in pathogenesis of post kala azar dermal leishmaniasis

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Background: The persistence of post kala-azar dermal leishmaniasis in some patients has been difficult to explain.There are many gaps in knowledge concerning the pathogenesis of PKDL
Design: This is a prospective,longitudinal study that was carried out between March 2010-April 2012
Setting: Patients from eastern Sudan were recruited for the present study
Objective: 1.to study the role of Treg cells in the pathogenesis of Post Kala-azar Dermal Leishmaniasis.2.To define the cell phenotypes and cytokines profile in tissues and peripheral blood of patients suffering from persistent PKDL before and after treatment.3.To correlate the pathology of PKDL lesions with the prognosis of PKDL(healing and non-healing).
Results: The reaction in the skin usually consisted of a mixed inflammatory cellular infiltrate composed of lymphocytes,macrophages and epithelioid granulomas.Patients with TypeII reaction (epitheloid granuloma)had a significantly longer duration of the disease compared with those of mixed inflammatory TypeI reaction (p= 0.001).In peripheral blood samples of a subset of patients,the number of CD4 cells before treatment was high followed byCD8 cells , followed by Treg cells.The number of cells decreased in the same manner upon treatment.Both CD8 and Treg cells showed a significant difference in count before and after treatment (p=0.005)
Conclusion: Treg cells in PKDL have a major impact on our understanding of the pathogenesis and treatment of this disease. This study has provided evidence that Treg cells can influence the disease outcome and that UVB light plays an important role in the pathogenesis of PKDL by inducing Treg cells which suppress CD 8+ cells that are needed to damage the leishmania antigen-containing macrophages and epithelioid cells.

LB020

Unraveling the role of BLIMP-1 induction during *Leishmania donovani* infection as a novel escape mechanism

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Inflammasome activation is a tightly regulated process in providing defense against pathogenic insults. Plethora of reports has been highlighted on how *Leishmania donovani* parasites hijack the immune system of host and tailor it to their convenience with few reports on altered regulation of inflammasome during infection. In this regard, the role of BLIMP-1 and its involvement in suppressing immune responses in various infectious diseases have been reported earlier for malaria. Here we report for the first time the role of BLIMP-1 in suppression of macrophage pyroptosis during *L. donovani* infection and thereby tweaking the tight regulation of NFκβ - NLRP3 signaling pathway by inhibiting caspase 1 maturation. We hypothesize that dependency of NFκβ for NLRP3 signaling pathway is cross-regulated by BLIMP-1, finally leading to increased parasitic burden upon infection. The inverse relationship between BLIMP-1 and NFκβ expression pattern in infected cells has been observed and validated by its reversal in siRNA mediated *blimp*-1 knock down study. Interestingly, we found resumption of pyroptosis in infected *blimp-1* deficient cells as compared to wild type infected cells with increased caspase 1 activity, higher percentage of LDH release and increased maturation of

DOI: 10.1002/eji.201970400

pro-IL-1β and pro-IL-18 into its active counterparts. In a nutshell, we conjecture that regulation of NFκβ activation followed by NLRP3 inflammasome formation and activation is primarily controlled and orchestrated by higher BLIMP-1 production, disrupting pyroptosis in infected cells. The current findings depict how BLIMP-1 is responsible for cross regulating a tightly regulated pathway crucial for host defense system during infection.

LB021

Emergomyces africanus, a new endemic emerging fungal pathogen of AIDS patients

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Background: *Emergomyces africanus*, is the first new dimorphic fungal pathogen to be identified in over 50 years and was recently identified in AIDS patients in South Africa. This disseminated disease is associated with high fatality rates in infected patients not receiving anti-fungal treatment. The recent implementation of improved diagnostics has resulted in a dramatic increase in the number of reported cases, although the true burden of disease is still unknown. Since discovery, an additional 4 species have been described in the genus Emergomyces and have been clinically reported over 4 different continents including *Emergomyces pasteurianus* (Europe, Asia, Africa and South Africa), *Emergomyces canadensis* (Canada and United States of America), *Emergomyces orientalis* (China) and *Emergomyces europaeus* (Germany). However, very little is known about the pathogenesis and host immune response to *E. africanus.*
Methods: To determine the host immune response to infection, we established a mouse model of disease using a clinical isolate of *E. africanus,* and determined the fungal pathogenesis in both immune competent and immunocompromised (RAG1-deficient) mice. Disseminated fungal burden was determined in homogenised organs isolated and cultured from mice after intra-tracheal infection on fungal growth media. Measured disease parameters included survival, inflammatory cytokine production by ELISA and cellular recruitment using flow cytometry and immunohistochemistry.
Results: Our preliminary data shows that we are able to infect mice with this organism and cause disseminated disease. We have established a murine model that demonstrates the susceptibility of immunocompromised mice with preliminary data suggesting that Pattern Recognition Receptors are required for fungal recognition.

LB022

Identification of antigenity for excretory-secretory products of *Clonorchis sinensis by* western blot

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Clonorchis sinensis (CS) is the parasite to live in common bile duct (CBD) and causes the persistent inflammation on CBD for several decades. The parasite is regarded to produce excretory-secretory

DOI: 10.1002/eji.201970400

products (ESP) absorbed by intestine or by CBD to raise the immune response of antibody production. Although the antigenicity of CS-ESP was reported in a study using indirect ELISA and worm culture supernatants, any antigen protein characterization was not available. Any antigen or protein band was not described neither on SDS-PAGE nor on Western blot. The antigens carried by CS-ESP were investigated using Tyrod's solution as culture media for CS worm culture. CS worms were harvested at 3~8 week post infection from rat and cultured at incubator with the daily change of culture solution, which used as CS-ESP. The protein bands visualized on stained gel for SDS-PAGE were ranged from 20 to over 170 kDa. Strong ladder like antigen bands of CS-ESP were observed at 25~62 kDa with some bands at 88 and 115K on the nitrocellose membrane prepared for Western blot. The membrane was incubated with rat serum pool from rats in clonorchiasis and peroxidase conjugated anti rat IgG. By comparison with worm crude extracts, the antigens bands of CS-ESP were much stronger and thicker. And the antigenity of CS-ESP was higher than that of worm crude extracts by 4 times when analyzed by indirect ELISA. These results indicated that CS-ESP were the good proteins to analyze the antigen profile of *Clonorchis sinensis*.

LB058

Manipulating CD4$^+$T cells response to enhance anti-parasitic immunity during visceral Leishmaniasis

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CD4$^+$ T cells are major effector cells during host immune responses against protozoan parasites such as *Leishmania donovani* causing visceral leishmaniasis (VL). The ability of CD4$^+$ T cells to produce pro-inflammatory cytokines is crucial for mediating protection. However, these cells can become functionally impaired during chronic disease. To identify molecules associated with CD4$^+$ T cell dysfunction that can be targeted to restore immune function, we performed RNASeq on CD4$^+$ T cells isolated from the blood of VL patients with active disease and identified type I interferons (IFNs) as major upstream regulators of CD4+ T cells. Furthermore, we report that mice deficient in type I IFN signalling had significantly improved control of parasites, associated with enhanced IFNγ production by parasite-specific CD4+ T cells. Dendritic cells were an important target for type I IFNs following parasite infection. Importantly, we identified a small molecule inhibitor that could be used to block type I IFN signaling during established infection and acted synergistically with conventional anti-parasitic drug to improve parasite clearance and enhance anti-parasitic CD4+ T cell responses in mice and humans. Additionally, we also identified LAG-3 as an important immune checkpoint molecules expressed on CD4$^+$ T cells from human VL and critically, we also showed, using a whole blood assay, that blockade of LAG-3 signalling enhanced antigen specific IFN-γ production. Thus, we identified both type-1 IFN and LAG-3 as potential therapeutic target to treat VL by stimulating anti- parasitic CD4$^+$ T cell responses.

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DOI: 10.1002/eji.201970400

LB059

Anti-GM-CSF autoantibodies are decreased in cerebrospinal fluid of patients with cryptococcal meningitis during the recovery phase

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Cryptococcal meningitis (CM) is a global disease with significant morbidity and mortality. The increased levels of granulocyte-macrophage-colony-stimulating-factor (GM-CSF) autoantibodies were found increased in the cerebrospinal fluid of patients with CM, which was followed in our study during disease recovery. A total of eleven patients from SPHCC were included in the study between December 2014 and June 2018. Decreased autoantibody levels and slightly increased GM-CSF were observed during recovery of CM patients. The anti-GM-CSF autoantibodies were correlated with increased CSF lactate dehydrogenase (LDH) (P=0.0085), protein (P=0.0472), and adenosine dehydrogenase (ADA) (P=0.0123) and decreased chloride (P=0.0462) and glucose (P=0.0173). The TNF-α were lower in THP1 cells upon LPS with the CSF of patient than LPS activation alone. Taken together, decreased anti-GM-CSF autoantibody levels were observed during recovery from CM, which may be of utility when monitoring CM disease activity.

LB095

Relationship of Th17/Tregs with TLR2 in chronic rhinosinusitis with nasal polyps (CRSwNP) patients after in vitro exposure to Aspergillus flavus

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Background: The pathogenesis of nasal polyposis has not been clearly understood. Presence of *Aspergillus sp*.is a source of continuous stimulation causing hypersensitivity. TLRs are engaged in modulating Th17/Treg cell functions.TLR2 may decrease the suppressive function of Tregs by regulating the balance among Treg and Th17 function.
Aim: To study the TLR2 expression and Th17/Tregs cell markers in peripheral blood mononuclear cells (PBMCs) of CRSwNP patients induced by *Aspergillus flavus* antigens
Methods: Subjects enrolled in the study included 50 cases and 50 healthy controls. Postoperatively tissue biopsy samples were subjected to histopathological and mycological investigations. Preoperatively, a blood sample (6ml) were collected for PBMCs isolation. PBMCs ($1x10^6$ cells/ml) were treated with *A. flavus* antigen and PHAM and incubated at 37°C in humidified air containing 5% CO_2 for 18 hrs. The cells were stained for Th17 (CD161+IL23-R+), and Treg (CD25+FoxP3+) cells markers for flow cytometry analysis. mRNA expression of TLR 2 was analyzed using RT-PCR.
Results: The results showed significantly increased ($p< 0.05$) percentage of CD161+IL-23R+ (Th17) cells, whereas CD25FoxP3 (Treg cells) was significantly low in cases when treated to *A. flavus* antigen as compared to controls. TLR2 expression was four-fold high in cases. A significant ($p< 0.05$) positive correlation (r=0.6308) was found between TLR 2 and CD161+IL23R+ cells.
Conclusion: Increased TLR2 and CD161+IL-23R+ levels in CRSwNP, when stimulated with *A. flavus* antigen, indicated a skewed Th17 response and probable Treg imbalance as indicated by low CD25FoxP3+ levels. Overexpression of Th17 cells response leads to persistence and chronicity of the disease.

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DOI: 10.1002/eji.201970400

LB096

Lung endothelial cell antigen cross-presentation to CD8$^+$ T cells drives malaria-associated lung injury

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Malaria-associated acute lung injury (MA-ALI) is a clinical complication of severe malaria. MA-ALI is characterized by edematous lung, along with marked infiltration of inflammatory cells and damaged alveolar-capillary barrier. However in MA-ALI, the pathogenic mechanisms remain largely unclear. Murine models mimicking MA-ALI in humans are powerful tools to gain insights into the pathogenesis. Here, we show that C57BL/6 mice infected with *Plasmodium berghei ANKA* (PbA), a murine MA-ALI model, had increased transmigration of total and malaria-specific CD8$^+$ T cells in the lung and that antibody depletion of CD8$^+$ T cells prevented lung vascular leakage. When we transfer malaria-specific CD8$^+$ T cells to PbA-infected TCRβ$^{-/-}$ mice (devoid of T cells population), lung vascular leakage was recapitulated. Lastly, we further demonstrated that accumulation of parasitized erythrocytes led to lung endothelium cross-presentation of parasite antigen to malaria-specific CD8$^+$ T cells. In summary, lung vascular injury in MA-ALI is a consequence of the chronological occurrence of parasitized erythrocytes in the lung microvasculature, followed by antigen capture and processing by the activated endothelium, which cross-presents to antigen-specific CD8$^+$ T cells infiltrated in the lung during PbA infection.

P0092

Seroprevalence of chagas disease among blood donors in the state of Bahia, Brazil

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Chagas disease affects 5.7 to 7 million individuals in the world. In the state of Bahia, Brazil, its prevalence has reached 25.1%. There is an association between CD and the social profile of the population and a risk of re-emergence of the disease also due to non-vectorial transmission, such as blood transfusion.
Objective: Describe the seroprevalence of *T. cruzi* infection among blood donors in the state of Bahia, Northeastern Brazil, and their epidemiological profile in the last 10 years.
Methods: Descriptive, cross-sectional study, conducted through database review. Data was gathered from patients who had a non-negative result for *T. cruzi* infection over the last 10 years.
Findings: 3,084 (0.62%) non-negative samples were identified in a first sample, and 810 (0.16%) were again defined as non-negative for *T. cruzi* infection in a second sample. Correlation between infection and age (30 years and older) and infection and lower educational level (12 years or less) in both samples were statistically significant. Males presented a higher risk for T. cruzi infection only in the first sample. 99,52% of the municipalities of Bahia were identified as cities of residence among all blood donors. Livramento de Nossa Senhora was the most prevalent city in both samples and the capital, Salvador, presented the greatest recurrence of cases.
Main conclusions: The seroprevalence found in these populations is lower compared to other similar

DOI: 10.1002/eji.201970400

studies conducted in Brazil, but some condensed areas with higher prevalence were found. In general, the epidemiological profile was in agreement with previous studies.

P0342

Inflammasome activation negatively regulates MyD88-IRF7 type I IFN signaling and anti-malaria immunity

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The inflammasome plays a critical role in inflammation and immune responses against pathogens. However, whether or how inflammasome activation regulates type I interferon (IFN-I) signaling in the context of malaria infection remain unknown. Here we show mice deficient in inflammasome sensors AIM2, NLRP3 or adaptor Caspase-1 produce high levels of IFN-I cytokines and are resistant to lethal Plasmodium yoelii YM infection. Inactivation of inflammasome signaling reduces interleukin (IL)-1beta production, but increases IFN-I production. Mechanistically, we show inflammsome activation enhances IL-1beta-mediated MyD88-TRAF3-IRF3 signaling and SOCS1 upregulation. However, SOCS1 inhibits MyD88-IRF7-mediated-IFN-I signaling and cytokine production in plasmacytoid dendritic cells. By contrast, ablation of inflammsome components reduces SOCS1 induction, and relieves its inhibition on MyD88-IRF7-dependent-IFN-I signaling, leading to high levels of IFN-alpha/beta production and host survival. Our study identifies a previously unrecognized role of inflammasome activation in the negative regulation of IFN-I signaling pathways and provides potential targets for developing effective malaria vaccines.

P0343

SIV infection aggravates malaria in a Chinese rhesus monkey coinfection model

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Background: The co-occurrence of human immunodeficiency virus (HIV) infection and malaria in humans in endemic areas raises the question of whether one of these infections affects the course of the other. Although epidemiological studies have shown the impact of HIV infection on malaria, the underlying mechanism(s) are not yet fully understood.
Methods: We randomly assigned ten Chinese rhesus monkeys to two groups based on body weight and age. The coinfection animals (S+P group) were inoculated intravenously with SIVmac251 eight weeks prior to with *Plasmodium* cynomolgi (Pc) infected red blood cells (iRBCs), and the control animals (P group) were inoculated intravenously with only Pc-iRBCs. After malaria was cured with chloroquine phosphate, we also initiated a secondary malaria infection that lasted 4 weeks in both groups.
Results: The animals in S+P group exhibited more severe malaria symptoms characterized by longer

DOI: 10.1002/eji.201970400

fever episodes, higher parasitemia, more severe anemia, greater body weight loss and higher body temperature than the animals infected with Pc alone. Concurrent SIV infection also impaired immune protection against the secondary Pc challenge infection. The coinfected animals showed a reduced B cell response to Pc malaria and produced lower levels of Pc-specific antibodies. In addition, the coinfected animals also had reduced total $CD4^+$ T cells, including $CD4^+CD28^{high}CD95^{high}$ central memory and $CD4^+CD28^{low}CD95^-$ naïve T cells, which may result from the imbalanced immune activation and faster $CD4^+$ T cell turnover caused by SIV infection.
Conclusions: SIV infection impairs the immunity against *Plasmodium* parasites and aggravates malaria physiologically and immunologically in Chinese rhesus monkeys.

P0344

NF-κB subunit p50 binds to TGF-β promoter and up-regulates its expression in macrophages to promote Treg induction

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Long-lived helminth parasites are remarkable for their unique ability to modulate host immunity. $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) remain most functionally prominent among immunoregulatory cell populations during helminth infections. TGF-β is an essential factor for the generation of Tregs from $CD4^+CD25^-$ T-cell precursors by initiating the master transcription factor Foxp3 expression. Helminth infections are commonly accompanied by a high level of TGF-β production. We recently reported the discovery of *Schistosoma japonicum* (*S. japonicum*)-derived stress protein HSP60 (SjHSP60) that stimulates TGF-β expression in macrophages (Mφ) via TLR4 (Toll-like receptor 4)-Mal (MyD88 adaptor-like protein) pathway. However, the exact mechanism underlying the transcriptional regulation of TGF-β generation after schistosome infection remains uncharacterized. Here we further found that activation of TLR4-Mal signaling by SjHSP60 increased expression of NF-κB subunit p50 (NF-κB1), that bound to TGF-β promoter and subsequently up-regulated TGF-β expression in Mφ, which was required for Treg induction and liver immunopathology restriction in *S. japonicum*-infected mice. Thus, our data identify that p50 is a critical regulator in Mφ, mediating TGF-β expression for Treg induction, and suggest that this novel mechanism could be targeted for potential therapeutics.

P0345

Circumsporozoite protein suppresses the IFN-γ-mediated killing of *Plasmodium* liver stage through enhanced autophagy related proteins ubiquitination

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Malaria is still one of the most devastating diseases worldwide, which is caused by infection of the genus *Plasmodium*. Liver stage is an essential early step of malaria parasite infection, and

DOI: 10.1002/eji.201970400

plasmodium replicates in parasitophorous vacuole (PV) of hepatocytes at this stage. Although encountering with hepatocyte autonomous immunity, exoerythrocytic forms (EEFs) in PV can still survive and successfully complete infection of hepatocytes. The underlying mechanism is largely unknown. Here, we show that sporozoite circumsporozoite protein (CSP) translocates from the parasitophorous vacuole into the hepatocyte cytoplasm to significantly inhibit the killing of exo-erythrocytic forms (EEFs) by interferon-gamma (IFN-γ). Nitric oxide were found to be indispensable for the resistance to IFN-γ-mediated killing of EEFs by CSP. Attenuation of the IFN-γ-mediated killing of EEFs by CSP is dependent on its ability to reduce the levels of ATGs in hepatocytes. The ATGs downregulation occurs through its enhanced ubiquitination mediated by E3 ligase NEDD4, an enzyme that is upregulated by CSP when it translocates from the cytoplasm into the nucleus of hepatocytes via its NLS domain. Thus, we have revealed an unrecognized role of CSP in suppressing host autonomous immunity, and shed new light for a prophylaxis strategy against liver-stage infection.

P0346

Characterization of a novel transmission blocking vaccine candidate PbGPR180 in Plasmodium berghei

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Transmission-blocking vaccine (TBV) would be a promising advance for malaria control and elimination. However, limited candidate TBV antigens hindered the TBV vaccine development, which highlighting the urgency of identifying new antigens. In this article, we detailed analysis a GPCR protein in *Plasmodium berghei*, PbGPR180, and evaluated its transmission blocking potential as a vaccine target. PbGPR180 was detected in western blot as highly expressed in gametocyte stage. IFA revealed localizations of PbGPR180 on the surface of infected red blood cells (iRBCs), gametocytes, zygotes, retorts and mature ookinetes. Deletion of the *pbgpr180* gene (Δ*pbgpr180*) did not affect asexual growth, suggesting this gene is not essential for asexual stage. However, the ookinetes differentiation and oocyst formation in mosquito midguts were significantly reduced in Δ*pbgpr180* parasites, indicating that PbGPR180 protein is required for parasite sexual development. In order to assess the transmission-blocking potential of PbGPR180, specific anti-sera against recombinant PbGPR180-his fuse protein was developed. The anti-PbGPR180 sera significantly inhibited male gametocytes exflagellation and ookinetes differentiation in a dose-dependent manner. Furthermore, mosquito feeding experiments confirmed that mosquitoes fed on mice passive transferred with immunized anti-PbGPR180 sera had 49.2 % reduction in the prevalence of infection and almost 54.4 % reduction in oocyst density, indicating blocked transmission to mosquitoes. Together, these results highlight PbGPR180 as a potential TBV candidate that is worth future investigations in human malaria parasites.

P0347

Extracellular Vesicles from a carcinogenic fluke-Clonorchis sinensis promotes TNF-α production in macrophage by a TLR2-dependent manner

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DOI: 10.1002/eji.201970400

Little is known about the roles of extracellular vesicles (EVs) from a carcinogenic fluke *Clonorchis sinensis* in the regulation of immune responses during infection which may ultimately lead to cholangiocarcinoma. In the present study, we isolated and purified EVs derived from *C. sinensis* via differential centrifugation and identified them by transmission electron microscopy. Further study showed that extracellular vesicles of *C. sinensis* (CsEV) can be internalized by RAW 246.7 cells. We also found that CsEV potently promotes macrophage to express various proteins including CD68, CD86 and CD206, and cytokines like TNF-α, IL-6, MCP-1 and IL-10 in bone marrow-derived macrophages (BMDM). However, CsEV failed to TNF-α induce in BMDM from *Tlr2-/-* mice, suggesting that TNF-α induced by CsEV in BMDM is in a TLR2-dependent manner. Furthermore, we found that Rapamycin-pharmaceutical inhibitor of mTORC1 promotes the production of TNF-α in CsEV-stimulated BMDM, but the elevated TNF-α production was blunted in CsEV-stimulated BMDM from *Tlr2-/-* mice when the cells were pretreated by Rapamycin. These data demonstrated that TLR2 might negatively regulate mTORC1 to promote TNF-α production in CsEV-stimulated macrophage.

P0348

Naturally acquired antibody responses to *Plasmodium vivax* and *Plasmodium falciparum* $MSP1_{19}$ in an area of unstable malaria transmission in Southeast Asia

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Understanding naturally acquired immunity to *Plasmodium* merozoite surface antigens in different malaria endemicity settings is needed for better vaccine designs and for exploring the antibody responses as a proxy marker of malaria transmission intensity. This study investigated the sero-epidemiology of malaria focusing the key candidate antigen of $MSP1_{19}$ expressed on the surface of parasite along the international border between China and Myanmar, where malaria elimination action plans are in place. Antibody responses to respective $MSP1_{19}$ were detected in 50.9% and 78.2% patients with acute *P. vivax* and *P. falciparum* infections, respectively. There were cross-reacting antibodies in *Plasmodium* patients against these two recombinant proteins. IgG1, IgG3 and IgG4 were the major subclasses. Interestingly, 43.2% of the healthy endemic population also had antibodies against $PfMSP1_{19}$, whereas only 3.9% of this population had antibodies against $PvMSP1_{19}$. Higher antibody levels were correlated with age and parasite density, but not with season, gender or malaria history. Both total IgG and individual IgG subclasses underwent substantial declines during the convalescent period in three months. This study demonstrated that individuals in a hypoendemic area with coexistence of *P. vivax* and *P. falciparum* can mount rapid antibody responses against both $PfMSP1_{19}$ and $PvMSP1_{19}$. The significantly higher proportion of responders to $PfMSP1_{19}$ in the healthy endemic population indicates higher prevalence of *P. falciparum* in the recent past. Specific antibodies against $PvMSP1_{19}$ could serve as a marker of recent exposure to *P. vivax* in epidemiological studies.

P0349

Molecular cloning of enolase from Trichinella spiralis and the protective immunity in mice

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Trichinella spiralis, the main pathogen of trichinosis, infects a wide range of mammalian hosts and is one of the most widespread parasites worldwide. For parasites, glycolysis is the most important way to generate energy. Previous studies showed that some enzymes involved in the glycolytic pathway play roles in regulation the host immunity. In this paper, enolase from *T. spiralis* was cloned and the protective potentials were studied. One hundred and sixty ICR mice were divided into four groups and

DOI: 10.1002/eji.201970400

vaccinated with recombinant enolase (pET-ENO), eukaryotic recombinant plasmid encoding enolase (pVAX1-ENO) and negative controls (pVAX1 and PBS), respectively. Two weeks after the second immunization, each mouse was challenged orally with 200 muscle larvae (MLs) of *T. spiralis*. Results showed that mice vaccinated with pET-ENO and pVAX1-ENO induced specific antibodies of IgG, IgA, IgM, but no IgE. Subclasses of IgG antibodies showed that mice immunized with recombinant protein and recombinant plasmids induced a Th1/Th2 immune response. Concentrations of serum cytokines were detected and showed significant increase of IFN-γ, IL-4 and TGFβ1, while IL-17 in each group was not significantly different. Flow cytometric analysis showed significant increase of CD4+ and CD8+ T lymphocytes in the groups immunized with recombinant protein and recombinant plasmids. Challenge infection demonstrated that immunized groups had a reduced number of worm burdens. The reductions of larvae per gram muscle (LPG) in pET-ENO and pVAX1-ENO group were 17.7% and 15.8% when compared with PBS control.

P0350

T cells are essential for the natural resistance to Schistosoma japonicum infection in non-permissive rat

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Schistosoma japonicum infects numerous mammals which cause chronic inflammatory response of the hosts that affect many different organs. However very little is known on the immunogenetic basis of resistance or susceptibility to this life-threatening disease. In mice, we compared wildtype B6 and B6 mice deficient in T cells or myeloid cells, and found that the immune deficiency did not alter worm recovery results indicating that the survival of worm in B6 mice was not affected by immune deficiency. Indeed, we found that the adult worms were significantly smaller in width when development occurred in CD3e deficient mice, however such phenomenon was not observed in Gfi1 mutant mice which had severe deficiency in neutrophils and natural killer cells. Based on the results in susceptible models, we were intrigued to investigate how T cell could impact *Schistosoma japonicum* infection in the non-permissive host SD rat, an outbred strain of *Rattus norvegicus*. By CRISPR/Cas9 genome editing in SD rat, we obtained animals in which Lck was completely loss of function and absent in peripheral T cells. Surprisingly, our results found that the natural resistance of SD rats to *Schistosoma japonicum* infection was completely abolished by T cell deficiency. We further found that SD rats had significantly increased of master transcription factors for T cell differentiation including T-bet and Bcl6, but not the regulatory T cell driving Foxp3. Our results determined that the natural resistance of SD rats to *Schistosoma japonicum* infection was completely T cell dependent involving Th1 and Tfh differentiation.

DOI: 10.1002/eji.201970400

P0351

The novel potential cytokine LYG1 has protective effects in Candida albicans infection

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Fungal infection is the main reason for the high mortality of immunocompromised patients in clinical practice. Cytokines such as IFN-γ, IL-17A and IL-22, which secreted by Th1 and Th17 cells, play an important role in antifungal infection. LYG1 is a novel potential cytokine identified by our laboratory. It can promote the differentiations of Th1 cells and increase IFN-γ secretion. GEO database suggests that the expression level of *LYG1* is positively related with the virulence of the pathogen. We found that *Lyg1* was up-regulated in fungi-stimulated BMDM and BMDC cells. In order to detect the expression level of LYG1, we prepared and identified anti-LYG1 monoclonal antibodies which could be used for Western blot and IP. Functional studies showed that recombinant human LYG1 can enhance the killing ability of THP-1 cells against *C.albicans.* To elucidate its physiological function, we constructed *Lyg1* knockout mice. LYG1 deficiency has no obvious influence on the development of main organs and immune cells. The phagocytic ability of BMDM and BMDC from *Lyg1* knockout mice decreased obviously compared with cells from WT mice. *Lyg1* knockout mice were more sensitive to *C.albicans* infection than WT mice. Our results indicated that LYG1 was up-regulated upon *C.albicans* stimulation and might play important roles in fungal infection.

P0353

Performance of recombinant chimeric proteins in the serological diagnosis of Chagas disease in dogs

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Canine *T. cruzi* infection precedes human infection; therefore, they are considered sentinel species, acting as an alert for human transmission. ELISA is the most used method for Chagas disease (CD) serodiagnosis. However, its performance depends on the adopted antigenic matrix and there are no commercially available veterinary tests. Previously, our group developed and evaluated four *T. cruzi* chimeric antigens (IBMP-8.1, 8.2, 8.3 and 8.4) for human diagnostic, which outperformed several commercially available tests. Hence, this study aimed to evaluate the diagnostic potential of these antigenic matrixes for canine *Trypanosoma cruzi* infection. *T. cruzi* chimeric constructs were optimized and synthesized for recombinant production in *E. coli*, purified, and assessed by SDS-PAGE. The assays were optimized, their diagnostic potential validated and performance parameters were determined employing serum samples from naturally and experimentally infected dogs, with Y, Berenice and Colombiana strains. Cross-reactivity was evaluated for babesiosis, ehrlichiosis, dirofilariasis, anaplasmosis, and leishmaniasis. All antigens demonstrated high diagnostic performance, especially IBMP-8.3 and -8.4. IBMP-8.3 antigen demonstrated a 100% sensitivity, followed by -8.4 (96.7% - 100%), -8.2 (73.3% - 87.5%) and -8.1 (50% - 100%). The highest specificity was for IBMP-8.2 (100%) and -8.4 (100%). No cross-reaction was observed using IBMP-8.2 and -8.4. IBMP-8.3 rendered one false-positive result for a *Leishmania* spp.-positive sample. Conversely, IBMP-

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DOI: 10.1002/eji.201970400

8.1 produced 13.5% of false-positive results. The use of chimeric antigens efficiently addressed hurdles seen in CD serodiagnosis, especially regarding efficiency parameters variation according to parasite's strain, cross-reactivity with other infectious diseases and is a promising tool to diagnose CD.

P0354

Cellular dynamics of immune evasion during *Leishmania major* infection

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Concomitant immunity against intracellular parasites is dictated by the strength of the immune response and parasite-driven escape mechanisms that prevent complete clearance. Infection by *Leishmania major* causes severe skin lesions, and, while strong T cell responses are generated, parasite clearance is incomplete and often leaves a small pool of persistently-infected cells. What remains unclear is the cellular mechanisms that allow for persistent infections to be established. We utilized in vitro and in vivo microscopy approaches to visualize the dynamics of anti-*Leishmania* CD4 T cell responses and to characterize mechanisms that restrain their effector functions. Using a novel TCR transgenic mouse model, where T cells recognize the immunodominant *Leishmania*- glycosomal phosphoenolpyruvate carboxykinase (PEPCK) peptide, we show that monocyte-derived macrophage:T cell interaction dynamics were transient at steady-state, but prolonged in the presence of PEPCK peptide. PEPCK-specific T cells produced high levels of IFNg in response to peptide or *Leishmania* infection. We also show that in vitro expanded Treg cells can suppress effector T cell functions, and that greater suppression was observed by PEPCK-specific Treg cells. Ongoing studies that address the cellular and molecular mechanisms of Treg-mediated blunting of T cell responses will be presented. Intravital microscopy studies characterizing PEPCK-specific T cell migration dynamics and tissue localization within skin lesions directly in live mice will also be presented. The goal of our study is to identify barriers that need to be overcome in order to achieve complete parasite clearance in infected individuals and to better describe the dynamic aspects of concomitant immunity generation.

P0355

Critical role of candidalysin in immune response and pathogenesis of *Candida albicans*

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Candida albicans is the most common opportunistic fungal pathogen that causes life-threatening disseminated candidiasis in immunocompromised patients. Understanding the molecular basis of host-pathogen interaction may enable development of novel treatment options. Phagocytes such as macrophages, serve as a critical line of host defense. Upon infection, macrophages recognize a major component of the fungal cell wall, β-1,3-glucan, by a pattern recognition receptor, Dectin-1 and directly engulf *C. albicans* yeast cells via phagocytosis. Here, we show that the hypha-associated protein candidalysin is critical for the fungus to escape from macrophages. After phagocytosis, the engulfed *C. albicans* yeast were able to produce hyphae inside macrophages, allowing them escape from the phagocytes in a candidalysin-dependent manner. In contrast, phagocytosed *C. albicans* lacking candidalysin were trapped in macrophages, unable to escape apparently due to inability to penetrate the membrane. During the escape, candidalysin disrupted plasma membrane integrity and induced potassium (K^+) efflux in macrophages. The K^+ efflux mediated by candidalysin caused NLRP3 inflammasome activation and subsequent IL-1b and LDH secretion *in vitro*. This was significantly inhibited by high concentration of KCL and an NLRP3 inhibitor. Importantly, candidalysin deficient *C. albicans* induced less pro-inflammatory cytokine. Taken together, our study provides new insights into

DOI: 10.1002/eji.201970400

host-fungal interactions, and candidalysin might be a potential therapeutic target for disseminated candidiasis.

P0356

The role of innate immune receptors in Candida albicans skin infections

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Intradermal infections with *C. albicans* are eliminated by neutrophils recruited at the site of infection with an unknown mechanism.
To dissect how neutrophils are recruited at the site of infection, we analyzed the involvement of PRR that recognize *C. albicans* (TLR4, TLR2, Dectin1 and Dectin2) as well as CARD9, activated downstream Dectins, and MyD88 that transduces the signal derived from TLRs.
WT, PRR-deficient and CARD9-deficient mice did not show any defect in neutrophil recruitment after *C. albicans* intradermal injection. Diversely, MyD88-deficient mice do not recruit neutrophils after *C. albicans* skin infection.
Since MyD88 is involved in IL-1 signaling, we tested the role of IL-1β and IL-1α in the initiation of inflammation and neutrophil recruitment. In vitro and in vivo studies revealed that both IL-1β and IL-1α were involved in this process. IL-1α is constitutively expressed in epithelial, endothelial and stromal cells and can be released through proteolytic cleavage or cell death, enhancing the production of CXCL1, a chemokine with neutrophil chemoattractant activity. We confirmed that CXCL1 production in vivo depends on IL-1α release.
We concluded that PRRs were not involved in the initiation of the inflammatory process during primary *Candida* skin exposure. Diversely, the initiation of the inflammatory process during primary infection can be due to the unspecific release of alarmins (like IL-1α) by distressed cells stimulating neutrophils recruitment at the site of infection.

P0357

Successful treatment of human visceral leishmaniasis restores antigen-specific IFN- γ, but not IL-10 production

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One of the key immunological characteristics of active visceral leishmaniasis (VL) is a profound immunosuppression and impaired production of Interferon-γ (IFN-γ). However, recent studies from Bihar in India showed using a whole blood assay, that whole blood cells have maintained the capacity to produce IFN-γ. Here we tested the hypothesis that a population of low-density granulocytes (LDG) might contribute to T cell responses hyporesponsiveness via the release of arginase. Our results show that this population is affected by the anticoagulant used to collect blood: the frequency of LDGs is significantly lower when the blood is collected with heparin as compared to EDTA; however, the anticoagulant does not impact on the levels of arginase released. Next, we assessed the capacity of

DOI: 10.1002/eji.201970400

whole blood cells from patients with active VL to produce IFN-γ and IL-10 in response to antigen-specific and polyclonal activation. Our results show that whole blood cells produce low or levels below detection limit of IFN-γ and IL-10, however, after successful treatment of VL patients, these cells gradually regain their capacity to produce IFN-γ, but not IL-10, in response to activation. These results suggest that in contrast to VL patients from Bihar, India, whole blood cells from VL patients from Gondar, Ethiopia, have lost their ability to produce IFN-γ during active VL and that active disease is not associated with sustained levels of IL-10 production following stimulation.

P0929

Dynamics of Th9 cells in mice infected with Trichinella Spiralis

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Backgroud: Th9 cells play critical roles in the immunomodulation and immunopathology of helminths infection by secreting the characteristic cytokine IL-9, but little in vivo data are available on the dynamics of Th9 cells after *Trichinella Spiralis* infection. We aimed to analyze the dynamic characteristics of Th9 cells in mice infected with *T. spiralis*.
Methods: Forty-eight Balb/c mice infected with *T. spiralis* were used as the infected group, and six normal mice were applied to the control group. The proportion of Th9 cells in spleens were analyzed by flow cytometry.
Results: Compared with the normal controls, the proportion of Th9 in $CD4^+$ T cells of spleens from the infected group significantly elevated. The percentage of Th9 cells maintained at a stable level from 2 days to 7 days post infection(dpi), and then gradually increased, peaked at 21 dpi, and decreased dramatically at 28 dpi. The proportion of Th9 cells increased again at 42dpi to 56dpi.
Conclusion: The proportion of Th9 cells in spleens from mice infected with *T. spiralis* showed a significant fluctuations as the infection developed, suggesting that Th9 cells play an important role in the progression of trichinosis.
Keywords: *Trichinella spiralis*, Th9 cells, IL-9, flow cytometry

P0930

The IL-22-secreting cells of mice infected with Trichinella spiralis in early encapsulated stage

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Objective: To investigate the main source of IL-22-secreting cells of mice infected with *Trichinella spiralis* in early encapsulated stage.
Methods: Twelve Balb/c mice were randomly divided into the infected group and the control group. The infected mice were intragastrically administrated with 300 muscle larvae of *T. spiralis*, and the control mouses were given the same amount of normal saline. The IL-22-secreting cell subsets in mouse splenic lymphocytes were detected by flow cytometry at the fourth week after infected.
Results: The proportion of IL-22-secreting cells in splenic lymphocytes of mice infected with *T. spiralis* increased when compared with normal mice($t = -4.899$, $P < 0.05$). There was no significant difference between the proportion of $CD3^+$ lymphocytes and $CD3^-$ lymphocytes producing IL-22 in the splenic lymphocytes of the infected group ($t = -2.195$, $P > 0.05$), and the percentage of IL-22-secreting $CD3^-$ lymphocytes were similar between the infected group and the normal group($t = -0.600$, $P > 0.05$). The proportion of IL-22-producing $CD3^+$ lymphocytes in the infected group was significantly higher than that in the normal group ($t = -3.068$, $P < 0.05$), and the percentage of IL-22-secreting $CD4^+$T cell and γδT cell subsets obviously increased in $CD3^+$ lymphocytes.

Conclusion: The proportion of IL-22-secreting splenic lymphocytes increased in mice infected with *T. spiralis* at early encapsulated stage. The rise was caused by increased IL-22-secreting $CD3^+$ lymphocytes, especially $CD4^+$ T cells and γδT cells.
Keywords: *Trichinella spiralis*; IL-22; $CD4^+$T cell; γδT cell

P0931

Immune exhaustion and reversal by blocking checkpoint receptor TIGIT in patients and experimental mice with alveolar echinococcosis

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The cestode *Echinococcus multilocularis* (*E. multilocularis*) infection is a serious health problem worldwide, which causes alveolar echinococcosis (AE), a tumor-like disease predominantly located in the liver, and able to spread to any organs. Until now, there have been few studies to explore how T cell exhaustion contributes to parasite's escape from immune attack and how it might be reversed. In this study, we found that liver TIGIT expression was significantly enhanced, and positively correlated with lesion activity in AE patients. High TIGIT expression on both liver-infiltrating and blood T cells was associated with their functional exhaustion, and its ligand CD155 was highly expressed by hepatocytes surrounding the infiltrating lymphocytes. In co-culture experiments using human peripheral blood T cells and normal hepatic cell line HL-7702, CD155 induced functional impairment of $TIGIT^+$ T cells, and *in vitro* blockade with TIGIT antibody restored the function of AE patients' T cells. Similar TIGIT-related functional exhaustion of hepatic T cells, and an abundant CD155 expression on hepatocytes were observed in *E. multilocularis*-infected mice. Importantly, *in vivo* blocking TIGIT prevented T cells exhaustion and inhibited disease progression in *E. multilocularis* mice. Mechanistically, $CD4^+$ T cells were totally and $CD8^+$ T cells partially required for anti-TIGIT-induced regression of parasite growth in mice.
Conclusion: This study demonstrates that *E. multilocularis* can induce T cell exhaustion *via* inhibitory receptor TIGIT, and that blocking this checkpoint may reverse the functional impairment of T cells, and represent a possible approach to *E. multilocularis* immunotherapy.

P0932

Polyclonal antibody against proflilin inhibited the proliferation of *Toxoplasma gondii* in vitro

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Toxoplasmosis is one of the most common zoonotic diseases in the world. It can parasitize almost all nucleated cells and cause serious illness in humans. The gliding movement, invasion or egress of *Toxoplasma* depend on the normal aggregation and decomposition of actin. As an actin regulatory protein, *Toxoplasma* profilin (TgPRF) plays a key role in regulating directional migration and cell invasion of *T. gondii*. Therefore, we evaluated the inhibition of its antibodies on the proliferation of tachyzoites (RH) in mouse embryonic fibroblasts. Unexpectedly, anti-TgPRF antibody not only had no cytotoxicity, but also significantly inhibited the cell death after they grew to plateau in vitro, which in turn promoted apoptosis. InCuyte ZOOM™ assay showed that anti-TgPRF antibody had a good inhibition on the proliferation of tachyzoites, and could confine the parasite to intracellular, while less extracellular. In addition, anti-TgPRF antibody could promote the apoptosis of cells after *T. gondii* challenge, which was also concentrated in the tachyzoites proliferation area. These results suggest that anti-TgPRF antibody had obvious restriction on parasite egress, and ultimately induce the

DOI: 10.1002/eji.201970400

apoptosis of infected cells to eliminate tachyzoites. Further study of TgPRF and its antibody will contribute to the treatment of toxoplasmosis and even other diseases.
Keywords: *Toxoplasma gondii*, proflin, apoptosis, anti-TgPRF antibody

P0933

The local immune response during *Echinococcus granulosus* growth in a quantitative hepatic experimental model

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The local immune mechanisms responsible for the establishment of *Echinococcus granulosus sensu stricto* infection and its development in the liver, its major location, have been little explored. We developed a suitable experimental model that mimics naturally infected livers using portal injection of increasing numbers of protoscoleces, comparable to the similar *Echinococcus multilocularis*(*E. multilocularis*) model. We showed that, opposite to what happens with *E. multilocularis* infection in which infection is dose-dependent, fully mature hydatid cysts can be established in the liver whatever the injection dose; although most of the infection sites were seen at the establishment phase as inflammatory granulomas associated with fibrosis but never matured into cysts, no fibrous scars were observed after healing. At the establishment phase, a strong immune response composed by T and B cells, with the involvement of T2-type cells and cytokines as well as IL-10-secreting $CD8^+$ T cells surrounded the injected protoscoleces in liver. At the established phase, results suggest a local production of antibodies by B cells, and an involvement of NK and NKT cells. Infection outcome and local immune response, at the whole liver level, were different in the mice models of *Echinococcus granulosus sensu stricto* and *E. multilocularis* respectively; however, only specificities at the microenvironment level might explain the differences actually found between the lesions induced by the 2 species, both quantitatively and qualitatively. Our quantitative experimental model appears fully appropriate to further study this microenvironment and its relationship with the respective biochemical nature of the laminated layer of each cestode species.

P0934

The pathological difference between *Echinococcus multilocularis* strains is likely due to the different exosomic profiles

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Echinococcus multilocularis （*E. multilocularis*）causes alveolar echinococcosis (AE), which is a lethal parasitic disease. One remarkable feature of the disease is that the interface between host and parasite contains a large amount of fibrosis and granuloma. In contrast, cystic echinococcosis (CE) caused by *Echinococcus granulosus*（*E. granulosus*）, a very close pathogen to *E. multilocularis* induced a weak cellular immune responses in the host-parasite interface. We collected two strains of *E. multilocularis* and found one caused more severe pathological response than another strain. Mitochondrial sequence analysis showed 1% of difference between the two strains of *E. multilocularis*, which had 4% difference to *E. granulosus* mitochondrial sequence. Infection animal model by hepatic portal vein injection of *E. multilocularis* protoscoleces revealed that the mice infected with EM-1 showed more granuloma responses than that of pathology by EM-4. Masson staining showed that EM-1 induced more fibrosis in the livers. Immunohistochemical staining showed EM-1 produced more α-

DOI: 10.1002/eji.201970400

SMA and ERK than EM-4. Exosomic analysis showed the different protein profiles between *E. multilocularis* strains and *E. granulosus*, which may induce different pathological responses.

P0935

Nosema bombycis serpin 6 inhibits host hemolymph melanization

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Nosema bombycis (*N. bombycis*, Nb) is the pathogen of silkworm. Previous studies found that *N. bombycis* infection will inhibit silkworm hemolymph melanization. It is known that the hemolymph of invertebrates function as both hemoid and lymphoid tissue, and hemolymph melanization is a major part of the innate immune responses against pathogens. However, the molecular mechanism has not been elucidated. Hemolymph melanization relies on serine protease mediated prophenoloxidase (PO) cascade activation, thus tightly regulated by serine protease inhibitor proteins (serpin). Previous study indicated that Nb can secret serpins (NbSPNs), thus we hypothesize that Nb utilizes NbSPNs to inhibit host hemolymph melanization in order to evade host innate immunity. In current study, we identified Nb serpin 6 (NbSPN6) expression in the hemolymph of infected silkworm, and recombinant NbSPN6 can inhibit PO cascade activation *in vitro*. In addition, *in vivo* analysis by RNA interference technology showed that when NbSPN6 expression is interfered in the infected silkworm, the decreased PO activity will be reverted. Furthermore, we identified the serine protease PPAE (prophenoloxidase-activating enzyme) in the PO activation cascade is the targeted protease of NbSPN6 in the host. Our novel study is the first to elucidate the molecular mechanism of pathogen-derived serpin inhibiting host innate immune responses thus facilitate propagation within host. In addition, it will also help to provide novel target for microsporidiosis prevention.

P0936

Effect of antifungal and antiflammatory therapy on the granulomatous response in experimental paracoccidioidomycosis

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Paracoccidioidomycosis is a very severe granulomatous mycosis caused by the fungus *Paracoccidioides brasiliensis (Pb)*. Previously, we characterized the striking differences in granulomatous lesions associated with susceptible or resistant mice. In the former, we found multiple non-encapsulated granulomas with numerous budding *Pb* yeasts, suggesting viability. In the later, we observed massive destruction of *Pb* contained in few granulomas, delimited by thick collagen type I fibers.
With these parameters established, we could monitor whether a novel, experimental combined therapy could elicit alterations in granulomas patterns from susceptible to resistant pattern.
Swiss mice were intraperitoneally infected with *Pb* and three days later treatment was initiated with daily doses of either itraconazole (3mg/mL), celecoxib (6mg/mL) or both itraconazole (3mg/mL)/celecoxib (6mg/mL) for 15 days, when they were sacrificed and the spleen, lungs, liver and

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DOI: 10.1002/eji.201970400

epiploon were collected, processed, and stained by conventional methods for light microscopy and analyzed for cellular composition, presence of *Pb* and granulomas architecture.
Inflammatory cells and *Pb* numbers decreased in all organs in all treated groups, as did the total numbers of granulomas in the liver, lung and epiploon.
The numbers of viable *Pb* and the concentration of nitric oxide were determined and showed that both these parameters were reduced in the itraconazole/celecoxib-treated group, being CFU in all organs and NO in the liver and epiploon.
Our results allow to conclude that both itraconazole and celecoxib are effective against *Pb* at the lesions and that the combination of these drugs resulted in an improvement the control of the granulomatous response.
E.B. Grants: CNPq 305216/2016-3 and FAPEMIG APQ 012941-16.

P0937

Critical role for Dectin-2-mediated signaling in the engulfment of *Cryptococcus neoformans* by dendritic cells

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Cryptococcus neoformans causes fatal meningoencephalitis under a condition with immunodeficiency including AIDS. *C. neoformans* grows within macrophages, which makes Th1-mediated immune response required for host defense against this fungal pathogen. Dendritic cells (DC) engulf *C. neoformans* and present its antigen to naïve T cells for differentiation of Th1 cells. Our previous report revealed that Dectin-2 was involved in the cytokine production by DC infected with this fungus. In this study, we addressed the role of Dectin-2 in the DC-mediated engulfment of *C. neoformans*. Bone marrow-derived DC were prepared from Dectin-2-deficient (KO), MincleKO, CARD9KO and wild type (WT) C57BL/6 mice. The engulfment of *C. neoformans* by DC from Dectin-2KO or CARD9KO mice was significantly decreased compared to those from WT mice. By contrast, this observation was not found in DC from MincleKO mice and from mice treated with Ab against mannose receptor or SIGN-R1. In the experiments to examine the effect of inhibitors of signaling molecules, PI3K inhibitor totally abrogated this engulfment, whereas Syk inhibitor affected only on the Dcetin-2-based response. Addition of cytochalasin D led to total abrogation of the DC engulfment, suggesting the indispensable role of actin polymerization. In line with this finding, actin-polymerization in DC caused by *C. neoformans* was completely dependent on Dectin-2 and CARD9, but not on Mincle. These results indicated that Dectin-2-mediated signaling play an important role in the engulfment of *C. neoformans* by DC and suggested that Dectin-2 may act as a scavenger receptor at the stage of innate immune response.

DOI: 10.1002/eji.201970400

P0938

Gut mycobiota and colon carcinogenesis

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The importance of the intestinal microbiome for tissue homeostasis, as well as the pathogenesis and treatment of a wide range of inflammatory, immune, and malignant disease, has become increasingly evident. However, most studies have focused on the role of bacteria. Our recent study has proved fungi enter the stage of colon carcinogenesis. Several mammalian C-type lectin receptors (CLRs), including Dectin-3, function as pattern recognition receptors sensing fungal infections and inducing multiple signaling cascades, such as CARD9, which lead to expression of various pro-inflammatory cytokines and antimicrobial proteins. Using a colitis-associated colon (CAC) mice model, we show Dectin-3-deficient mice and CARD9 -deficient mice developed more colon tumors than WT mice. The impaired fungicidal functions of *Card9*$^{-/-}$ macrophages led to increased fungal loads and variation in the overall composition of the intestinal mycobiota, with a notable increase in *C. tropicalis*. Bone marrow cells incubated with *C. tropicalis* exhibited MDSCs features and suppressive functions. Fluconazole treatment suppressed CAC in Dectin-3 and CARD9 deficient mice, and was associated with decreased MDSCs accumulation. The frequency of MDSCs in tumor tissues of colon cancer patients correlated positively with fungal burden, pointing to the relevance of this regulatory axis in human disease. Taken together, we demonstrate the important function of Dectin-3 and CARD9 and its functional interaction with commensal fungi in intestinal immune responses and regulation of colonic homeostasis.

P0939

Inflammatory and immunologic responses in mice after intranasal instillation of spores of some aspergillus and penicillium spp monitored in Southwest Nigeria

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Fungi are an increasing public health problem worldwide because they have a great impact on human health. A mouse model was devised to compare the adverse effects provoked by four most abundant fungi isolated from southwest Nigeria. Fifty-four Balb/c mice were grouped into eight treatments of six per cage with a control group. The animals were exposed intranasally to the spores of *A. flavus*, *A. penicilloides*, *Penicillium citrinum* and *Penicillium chrysogenum* at 2.3 x 10 7 and 3.2 x 10 5 m/L for 24hours and 48 hrs. Both dose-response, time-course and toxic responses were investigated after inhalation of the microbes. Inflammatory and histopathological analysis was also done. Biochemical and histopathology results revealed that all the four different fungi instilled in the mice provoked inflammation but the magnitude and its characteristic features were different. The spores of *A. flavus*, and *A. penicilloides* provoked a very intense acute inflammation indicated by production of increased malondialdehyde, myeloperoxidase, protein in the lungs. All the fungi inoculated resulted in significant ($p< 0.05$) increase in the monocyte and basophils cell. P. citrinum significantly increased the eosinophils as *A. terreus* increased the lymphocytes. The neutrophils content was higher in the *A. penicillioides*, *P. chrysogenum* and *P. citrinum* incoculated treatments. Both haemoglobin and red blood cell count were significantly increased in the mice inoculated with *A. funiculosum*, *P. chrysogenum* and *A. flavus* compared to control. The results show that the selected microbes have potential to cause inflammatory and toxic responses in mice.

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DOI: 10.1002/eji.201970400

P0940

Host transcriptomic signatures as alternative test-of-cure in visceral leishmaniasis patients co-infected with HIV

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Human Immunodeficiency virus (HIV) infection has been identified as a significant challenge facing visceral leishmaniasis (VL) control. VL treatment in HIV patients very often fails and results in extended treatments and/or high case-fatality rates. Diagnosis and test-of-cure are based on invasive spleen or bone marrow aspiration for microscopical parasite detection. RNA sequencing offers a mean to investigate the mechanisms behind these treatment outcomes, which remain largely unknown, on a transcriptome-wide scale. Moreover, such studies provide a basis for the identification of clinically relevant biomarker signatures. Twenty-eight VL-HIV patients were sampled during a 29-day treatment regime of Ambisome or Ambisome/miltefosine in Northwest Ethiopia. Whole blood transcriptome sequencing was performed at baseline (D0) and end of treatment (D29). Only 12 patients (43%) reached parasitological cure after 29 days. Distinct signatures of differentially expressed genes between D0 and D29 were identified for treatment failure (194 genes) and success (397 genes) cases. Pathway analyses highlighted changes related to mechanisms of action of the drugs used and patterns of a symbiotic interaction, with a clear downregulation of immune-mediated responses and cell cycle factors at cure. In addition, a minimum set of gene transcripts for accurately discriminating treatment success and failure was calculated with different methods and showed an average 81% sensitivity and 100% specificity. Although more validation of these transcriptomic signatures is required across different populations, a restricted blood transcriptome signature could be a promising proxy outcome for research and drug development in VL-HIV patients, and should also be investigated in non-HIV VL patients.

P0941

Prognostic value of IgG-anti-MSPs levels of Plasmodium falciparum: variability following the recruitment period

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Introduction: Malaria is one of the leading causes of death among the African population infected with Plasmodium falciparum. However early diagnosis and prognosis of severe form is still very difficult.
Methodology: Thus we studied the naturally acquired antibody levels (OD ratio) against the conserved and variable regions of the merozoite surface proteins MSP1-19, MSP3, MSP4-20 and MSP5 by ELISA in plasma samples of 251 patients admitted to the Principal Hospital of Dakar, over

DOI: 10.1002/eji.201970400

two periods 10-years separated with 177 patients suffering from severe malaria and 74 patients with mild malaria.
Results: Persons suffering from severe malaria and survived, showed very high levels of IgG against the antigens studied. Conversely, those with severe malaria who died had significantly lower levels of IgG against the same antigens examined (for MSP1, MSP4-20 and MSP3 $p < 0.005$). Similarly, patients enrolled in period 1 had higher antibody levels than those recruited in period 2 (for MSP1, MSP4-20, and MSP5, $p < 0.001$).
Conclusion: These results demonstrate the prognostic value of some of these anti-MSPs antibodies in severe malaria. In addition, their level of expression would vary according to age, the level of exposure to P. falciparum, and / or the implementation of a malaria eradication policy implemented by Senegal, affecting the responses Immunity.

P0942

Development of plasmodium specific liver resident-memory CD8$^+$T cells after heat-killed sporozoites immunization

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It is of major interest to develop an efficient vaccine to prevent malaria. Living sporozoites attenuated by radiation (RAS) have been shown to confer sterile immunity by enhancing the development of specific memory CD8$^+$ T cells. We have previously described a T cell receptor (TCR) transgenic mouse, termed PbT-I, in which all CD8$^+$ T cells specifically recognize a *Plasmodium* antigen. Using these TCR transgenic mice and RAS vaccination, we have shown that resident-memory T cells (Trm cells) develop in the liver and are essential for protection against pre-erytrocytic stages of Plasmodium development. Here we examined whether non-living sporozoites could trigger the development of *Plasmodium*-specific memory CD8+T cells and, specifically, liver-Trm. We used heat-killed sporozoites to immunize mice transferred with PbT-I cells and then assessed the differentiation of PbT-I cells into memory cells. We found that after vaccination with heat-killed sporozoites, memory PbT-I cells developed in the spleen and liver, but the number of these cells was significantly reduced relative to RAS immunised mice. Importantly, we were able to detect liver-Trm generation by heat-killed immunization, but again, the number of *Plasmodium*-specific liver-Trm recovered after heat-killed sporozoite immunization was less than one tenth that induced by RAS. However, we showed that the development of *Plasmodium*-specific liver-Trm after heat-killed immunization can be significantly improved by combining this form of antigen with α-galactosylceramide as an adjuvant. In the future, this work could lead to development of an anti-malaria vaccination strategy that does not require live sporozoites, providing greater utility.

DOI: 10.1002/eji.201970400

P0943

Inflammatory CD11c$^+$ monocytes constitute a main reservoir for high proliferation and cell-to-cell transmission of Leishmania major

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The virulence of intracellular pathogens such as *Leishmania major* (*L. major*) relies largely on their ability to undergo cycles of replication within phagocytes, release, and uptake into new host cells. While all these steps are critical for successful establishment of infection, neither the cellular niche of efficient proliferation, nor the spread to new host cells have been characterized in vivo. Using biosensors for measuring pathogen physiology in the living tissue, we found that monocyte-derived Ly6C$^+$CCR2$^+$ phagocytes expressing CD11c constituted the main cell type harboring rapidly proliferating *L. major* in the ongoing infection. Intravital 2-photon imaging and adoptive monocyte transfers showed that these high proliferating parasites preferentially underwent direct cell-to-cell spread without any long-lasting extracellular state. These newly infected cells might mainly fuel the cycle of intracellular proliferation and cell-to-cell transfer during the acute infection.
Thus, besides the well-described function for priming and activating T cell effector functions against *L. major*, CD11c-expressing monocytes recruited to the infected skin provide a reservoir for rapidly proliferating parasites that disseminate at the site of infection.

P0944

The absence of cysLTR1 ameliorates liver granulomatous inflammation during acute schistosomiasis in mice

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Schistosomiasis is a debilitating disease responsible for significant morbidity and mortality world-wide. The disease currently affects over 240 million people worldwide, 80% of these are in the sub-Saharan Africa. The disease is driven by the parasite eggs that get trapped in the host's tissues such as the liver and intestines; and drive chronic Th2 driven fibrogranulomatous pathology. Remarkably, however no vaccines are available for human use. Praziquantel remains the first and only efficient drug used to treat schistosomiasis. However, it does not prevent re-infection, a common occurrence in endemic areas. Hence, there is a new for alternative therapeutic drugs and development of vaccine to treat fibro-proliferative pathology driven by infection. In this study, we hypothesized that cysteinyl leukotrienes (cysLTs) play an important role in mediating fibro-proliferative pathology. In order to address this, we percutaneously infected cysteinyl leukotriene receptor-1 deficient mice (cysLTR1-/-) with 100 live S. mansoni cercariae and killed 8 weeks post-infection for evaluation of pathology and immune responses. We found that cysLTR1-/- mice had a significant reduction of granuloma area in the liver as compared with wildtype mice. Moreover, there was a low production of IL-4 and IL-13 protein in liver homogenates as well as stimulated of mesenteric lymph node cells with α-CD3 and

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DOI: 10.1002/eji.201970400

schistosome egg antigen (SEA) of the cysLTR1-/- mice as compared to wild-type mice. Therefore, these data demonstrated that signalling via cysLTR1 drives fibrogranulomatous liver pathology during acute schistosomiasis in mice.

P0945

Expression of α4β7 and PD1 on human T lymphocytes surface stimulated by schistosoma antigens

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α4β7 and PD1 markers are cell adhesion molecules which expressed on human T lymphocytes surface and sharply involved in those cell immune activity above all in case of infections pathology. Schistosomes have parasitical endemics which chronocity is linked to an immune tolerance phenomenon probably linked to an intense immunomodulation activity.
So we have made blood,, urine and stools samples carried out among population living in endemic zones in the area around lac de GUIERS in the North of SENEGAL. Lymphocytes cells have been isolated before freezing then in culture with antigen from two sorts of schistosomic species (*S.haematobium, S.mansoni)*. The T-lymphocytes and their surface markers analysis has been done by cytometry flux with Becton Dickinson LSR2 device.
We have obtained results which globaly showed us the highest level of expression of a α4β7 on TCD8+ lymphocytes surface simultaneously exposed to antigens from the two types of schistosomes compared to those obtained on regulating and T helper CD4+ lymphocytes surface. The α4β7 expression was the highest on the cells surface which were simultaneously exposed to the two schistosomes types antigens. The membranous marker PD1 expression is the highest on T helper CD4+ lymphocytes and T simultaneously exposed to the types of schistosomes antigens. Moreover the study beetween the α4β7 expression on T regulating lymphocytes surfaces and PD1 levels at helper CD4+ lymphocytes surface has revealed a positive link of the two variables in the case of simultaneous stimulation with the two types of schistosomes antigens

P1534

In vivo roles of the immune response and cardiomyocytes in the heart response to Trypanosoma cruzi parasites

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Chagas disease, an illness due to infection with *Trypanosoma cruzi* parasites, affects around 6 million people, mostly in Latin-America and results in ten thousand deaths per year. Around 30% of the patients develop chronic chagasic cardiomyopathy (CCC), a complication thought to result from parasite persistence at the heart. In a CCC model (C3H/HePAS mice chronically-infected by Sylvio X10/4 *T. cruzi* parasites) we evaluated the effects of boosting the anti-*T. cruzi* response by associating anti-PD1/PDL1 antibodies treatment with inoculation of irradiated parasites; while this association

DOI: 10.1002/eji.201970400

diminished subpatent parasitaemia levels, no parasite reduction was observed at the heart, in spite that CD4+ and CD8+ heart-infiltrating cells did increase. More important, this therapeutic association resulted in increased heart damage, evidenced by deeper bradicardia. These results indicate that chronic heart pathology is not the result of an insufficient immune response, but highlights the double-edge effect of immune reactivity.
We then analyzed if cardiomyocytes play an active immune role in CCC by using a conditional Cre-Lox model in which MyD88 can be selectively eliminated in cardiomyocytes following tamoxifen treatment. After validating this model, we observed that MyD88 elimination in cardiomyocytes results in decreased heart transcription of IFNg and TNFa, as well as of CCL5, demonstrating that cardiomyocites directly or indirectly signal parasite presence *in vivo*. Yet, we did not find any difference between control and tamoxifen-treated mice in the intensity of cardiac inflammatory infiltrates or levels of heart parasitism.

P1535

CsHscB as a novel TLR2 agonist from a carcinogenic liver fluke *Clonorchis sinensis* modulates host immune response

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Clonorchis sinensis-a fluke dwelling on the intrahepatic bile ducts causes clonorchiasis, affecting about 15 million people wide-distributed in eastern Asia. During C. sinensis infection, worm-host interaction results in activation of PPRs such as TLRs and further triggers immune responses which determine the outcome of infection. However, the mechanisms by which pathogen-associated molecules patterns from C. sinensis interacted with TLRs were poorly understood. In the present study, we identified a ~34 kDa lipoprotein CsHscB from C. sinensis which physically bound with TLR2 as demonstrated by molecular docking and pull-down assay. We also found that recombinant CsHscB (rCsHscB) potently activated macrophage to express various proteins including TLR2, CD80, MHCII, and cytokines like IL-6, TNF-α, and IL-10 in a TLR2-dependent manner but rCsHscB failed to induce IL-10 in macrophages from Tlr2-/- mice. Moreover, ERK1/2 activation was required for rCsHscB-induced IL-10 production in macrophages. In vivo study revealed that rCsHscB triggered a high induction of IL-10 in the wild-type (WT) but not Tlr2-/- mice. Consistently, compared to the WT mice, rCsHscB-triggered ERK1/2 phosphorylation was also attenuated in Tlr2-/- mice. Our data thus demonstrate that CsHscB from C. sinensis is an unidentified TLR2 agonist with immune regulatory activities, and may have some therapeutic implications in future beyond parasitology.

P1536

Helminth-induced immunomodulation signatures on gut microbiota and metabolic pathways in mice

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According to “hygiene hypothesis”, the decreased incidence of infections with parasites and bacteria in developed countries may be the underlying cause for immune system deregulation and consequential

DOI: 10.1002/eji.201970400

development of allergic and autoimmune diseases. Dissecting the complex mechanisms that determine the cross-talk between GI helminths, gut microbiota and the host immune system is a challenging work. In this study, we performed 16SrRNA gene sequence and untargeted LC/MS-based metabolomic analyses of the fecal and serum sample from the different development stages of *Trichinella spiralis*-mouse model, respectively. Gut microbiota alterations and metabolic activity accompanied with parasite-induced immunomodulation were detected. Duodenum inflammation parameters(villus/crypt ratio, the number and size of goblet cells, and histological score) were shown to be involved in active inflammation and oxidative metabolite profiles, which were increased penylalanine, tyrosine and tryptophan biosynthesis; decreased cholesterol metabolism, primary and secondary bile acid biosynthesis. These disrupted metabolism were adapted to infection stress in enteral and parenteral phase, following by return to homeostasis in convalescent phase. There was a switch from elevated *Bacteroides* in parenteral phase to probiotic *Lactobacillus* and Treg associated-*Clostridia* in convalescent phase.The corresponding changes of Th2 immune response(IL-4/IL-5/IL-13), lamina propria Treg and immune hyporesponsiveness metabolic pathways(decreased tropane, piperidine and pyridine alkaloid biosynthesis and biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid) were observed. **These findings enhance our understanding of gut microbiota and metabolic profiles of helminth-infected mice, which could be driving force in parasite shaping immune system maintenance.**

P1537

Dog vaccination with EgM proteins against Echinococcus granulosus

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Background: Dogs play a pivotal role in the transmission of cystic echinococcosis (CE), a zoonosis caused by the tapeworm Echinococcus granulosus. We showed previously that dogs vaccinated with two E. granulosus adult-worm specific proteins, EgM9 and EgM123, emulsified with Freund's adjuvants induced significant protective efficacy in terms of reduction in worm burden and egg production after 45 days post-infection. It was not known whether this protection can be sustained using adjuvants suitable for use in dogs.
Methods: Recombinant EgM9 and EgM123 were mixed with Quil A or ISCOMs for vaccinating dogs. After three vaccine injections, all the dogs were orally challenge-infected with 200 000 protoscoleces of E. granulosus. After 45 days of infection, all the dogs were euthanized and necropsied for collecting and counting E. granulosus worms. Immunoglobins, including the IgG subclasses IgG1 and IgG2, were detected in the sera of vaccinated dogs by ELISA. To determine whether the protection efficacy could be maintained after 45 days post-infection, we implemented a longevity trial to count eggs in dog faeces for 170 days after infection.
Results: The dogs vaccinated with EgM9 and EgM123 mixed with Quil A and ISCOMs showed similar protective efficacy as the proteins emulsified with Freund's adjuvants in terms of reduction of worms and eggs at 45 days post-infection. The longevity trial showed that EgM9 protein-vaccinated group released lower number of eggs per gram compared with the egg counts in the control dogs.
Conclusion: EgM9 and EgM123 are thus suitable vaccine candidates against E. granulosus infection in dogs.

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DOI: 10.1002/eji.201970400

P1538

Visceral leishmaniasis patients display altered composition and maturity of neutrophils as well as impaired neutrophil effector functions

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Introduction: Active visceral leishmaniasis (VL) is characterised by profound immunosuppression, severe systemic inflammatory responses and an impaired capacity to control parasite replication. Neutrophils play a crucial role in the induction as well as the resolution of inflammation, the control of pathogen replication and the regulation of immune responses. Neutrophil functions have been investigated in human cutaneous leishmaniasis; however, their role in human visceral leishmaniasis is poorly understood.
Methods: In the present study we evaluated the effector functions of neutrophils in patients with active VL and after successful treatment. Neutrophils were isolated using double layer density gradient technique and were used immediately. *E.coli* particle and GFP-labeled *Leishmania donovani* were used to evaluate the phagocytic capacity of neutrophils. Pyocyanin and *Leishmania donovani* were used to evaluate reactive oxygen species production capacity of neutrophils. Purified neutrophils were induced to release neutrophil extra cellular traps by phorbol myristate acetate (PMA). The effector functions of neutrophils were measured by Flowcytometry using FACS calibur
Results: Our results show that neutrophils in VL patients have an impaired capacity to release neutrophil extracellular traps, produce reactive oxygen species and phagocytose bacterial particles, but not *Leishmania* parasites.
Conclusion: Our results suggest that impaired effector functions of neutrophils play a key role in the pathogenesis of VL.

P1539

Association of modulatory cytokine levels and gene polymorphisms in polyparasitism infection indicating survival trait in Zimbabwe

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The global distribution of neglected tropical diseases shows polyparasitism as a common phenomenon in sub-Saharan Africa that could impact on systemic cytokines. Blood samples from 492 children provided DNA for genotyping using ARMS-PCR for TNF-α (-308 G/A), IFN-γ (+874 A/T), TGF-β (C/T codon 10; G/C codon 25); IL-10 (-1082 G/A and -819/-592 C/T). Systemic cytokines were measured in plasma using ELISA. The distribution of individuals with SNPs of TNF-α (-308 GG) was 76.1%, while 23.8% were predicted high producers. For IL-10 (-819/-592) the distribution of high, medium and low was 59.6%, 22.9% and 17.5%, respectively. While for IL-10 on position -1082 revealed that most of the samples were high producers. For IFN-γ (+874 A/T), 70.5% were associated with high cytokine secretion. Analysis on the sample population also revealed that at the TGF-β locus (C/T codon 10) 88.5% were homozygous (TT) which predicts high production. Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% predicted moderate TGF-b production. Equal distribution of IL-10 (-819/-592 C/T) and the rare occurrence of allele associated with low IL-10 (-1082 AA) production suggest moderate to high IL-10 responses in the population where polyparasitism is prevalent. The high prevalence of TGF-β codon 10 genotype (TT) and IL-10 (AA, high producers) might suggest the dominance of an anti-inflammatory environment in the population

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DOI: 10.1002/eji.201970400

who are faced with diverse polyparasitism infection. These observations suggest a complex interaction between various cytokine SNPs, infections and inherent genetic make-up playing an important role to downplay severity of infection.

P1540

The increased inflammatory monocytes are the hallmark of human visceral leishmaniasis caused by *Leishmania donovani*: the activation of signature chemokines

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The protozoan parasite *Leishmania* causes leishmaniasis, a devastating human disease which threatens 350 million people worldwide. At present visceral leishmaniasis is extremely endemic in India and East Africa; claiming maximum lives if left untreated. Intermediate monocytes contribute to pathologic immune responses in different forms of inflammatory diseases. The involvements of intermediate and non- classical monocytes have been studied in the individuals (healthy control, kala-azar patients and post-treated) by the flow cytometric analysis of the peripheral blood in the endemic regions of West Bengal, India. We found that, the frequency of viable $CD16^+$ [both intermediate ($CD14^+CD16^+$) and non-classical ($CD14^-CD16^+$)] monocytes was increased in the peripheral blood of patients (as diagnosed by rk39 antigen positivity), infected with *L. donovani*, compared with endemic controls; whereas in most of the post-treated individuals both the inflammatory monocytes restored back to the normal condition with respect to the uninfected populations. These results suggest that $CD16^+$ monocytes might contribute to the development of disease. Our study depicted a substantial increase in the expression of CCR7 and CX3CR1 in both $CD14^+CD16^+$ and $CD14^-CD16^+$ monocyte subsets of VL patients, when compared to the endemic uninfected populations, suggesting that CCR7 and CX3CR1 may guide the patrolling of these monocytes to the infected site. Our proposition was further strengthened by the fact that the expressions of certain chemokine ligands (CCL5 and IP10) were increased in the plasma samples of VL patients that might be responsible for the infiltration of monocytes and T cells for the subsequent resolution of infection.

P1541

Distinct activation of Dectin-2-mediated signaling by capsular polysaccharides from Cryptococcus neoformans and C. gattii

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There are Cryptococcus neoformans and C. gattii as causative fungi of cryptococcosis: the former causes meningitis when cellular immunity is impaired, and the latter develops meningitis even in the absence of immunodeficiency. It has been suggested that the Th1 immune response is decreased in C. gattii infection. We previously reported that Dectin-2 is involved in dendritic cell (DC) activation by C. neoformans. In this study, capsular polysaccharides were prepared from C. neoformans (H99) and C. gattii (R265), and the CTAB-unbound fraction was applied to a ConA-affinity column. Among the capsular polysaccharides from both strains, binding to Dectin-2-Fc fusion protein, activation of Dectin-

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DOI: 10.1002/eji.201970400

2-NFAT GFP reporter cells and production of TNF-α and IL-12p40 by dendritic cells were detected in the CTAB-unbound/ConA-bound fraction. These activities were higher in the fraction from H99 than that from R265. Although galactoxylomannan (GalXM) was detected in this fraction, neither Dectin-2-binding nor activation of Dectin-2-mediated signaling was found when purified GalXM was used. From these results, it was demonstrated that the CTAB-unbound/ConA-bound fraction from C. neoformans and C. gattii was recognized by Dectin-2 and suggested that the distinct recognition of the capsular polysaccharides by Dectin-2 may be involved in their different pathogenicity. Mannoprotein may also be thought as a candidate for its responsible polysaccharide, distinct from GalXM, among the CTAB unbound/ConA-bound fraction. It is necessary to further conduct the structural analysis of the responsible polysaccharide components and to examine their influence on the Th1-immune response during infection with these fungal pathogens.

P1542

Efficacy of combined itraconazole and low level LASER therapy in the initial phase of experimental paracoccidioidomycosis

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Paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America, often has severe clinical manifestations, requiring complicated treatment, justifying studies to amplify the therapeutic options. Itraconazole (itra), an effective drug against the fungus *Paracoccidioides brasiliensis* (*Pb*), causative agent of paracoccidioidomycosis, is widely used. Previously we demonstrated activation of neutrophils by Low Level LASER Therapy (LLLT).
Here we inoculated *Pb* in mice subcutaneous air pouch and evaluated the effect of itra and/or LLLT on their size, cellular composition, tissue architecture, protein metabolism, mitochondrial activity, production of reactive oxygen species (ROS) and number of recovered viable *Pb*.
Neutrophils constituted about 80% of incoming cells. None of the treatments had adverse effects on cellular viability. Mitochondrial activity and cell counts were significantly lower in LLLT-treated than in infected controls and also in itra-treated mice, in a dose/response way, with or without LLLT. ROS production was significantly increased in itra-treated cells, mainly with 10mg/mL itra. Air pouch volume, weight and diameter significantly decreased in parallel with the increment in itra dosage and also when this treatment was associated with LLLT. The intensity of the inflammatory process and the number of vessels and neovases decreased when the concentration of itra in combination with LLLT increased. *Pb* numbers decreased significantly as a result of itra treatment in any concentration, and this effect was enhanced when itra was used in combination with LLLT.
Our results strongly suggest that itra efficiency is enhanced by LLLT and that this combined therapy provides control of the initial stages of experimental PCM.
E.B. Grants: CNPq 305216/2016-3, FAPEMIG APQ012941-16.

DOI: 10.1002/eji.201970400

P1544

IL-4 receptor alpha on Foxp3+ regulatory T cells is required to control hypersusceptibility to Leishmania major infection in mice

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Immunity to the protozoan parasite *Leishmania major* is dependent on the successful generation of a T helper (Th) 1 response while interleukin 4-receptor alpha (IL-4Rα)-driven Th2 responses drives susceptibility to this parasite in BALB/c mice. T regulatory cells (Tregs) have been implicated in suppressing excessive Th2-mediated disease progression but it is unclear if IL-4Rα-responsive Tregs play a significant role in this process.
To investigate this further, a novel BALB/c mouse lacking IL-4Rα expression specifically on T regulatory cells (Foxp3^{cre}IL-4Rα$^{-/lox}$) was generated by gene targeting and site-specific recombination using the cre/*lox*P system under control of the *foxp3* locus. Following infection with *L. major* LV39 parasites, host immune parameters were analysed at 8 weeks post-infection. Following infection with *L. major* LV39, protein characterisation demonstrated abrogated IL-4Rα expression specifically on Tregs. As a result, Foxp3^{cre}IL-4Rα$^{-/lox}$ BALB/c mice became hypersusceptible to disease as shown by increased footpad swelling, necrosis and a striking increase in parasite burdens in the footpad and draining lymph node. Hypersusceptibility to infection was further associated with an increase in IL-4 and IL-13 secretion by CD4^{+} Th2 and B effector 2 (Be2) B cells. Upregulated Th2/Be2-mediated responses correlated with an augmented IgE antibody response, altogether leading to a hypersusceptible type-2 immune response.
Together, these data demonstrate that abrogation of IL-4Rα signaling on Tregs is severely detrimental to the host revealing a protective role for IL-4Rα-responsive Tregs in suppressing excessive type-2 immune responses during *L. major* LV39 infection in BALB/c mice.

P1545

Role of cytosolic innate immune sensing pathways in *Leismania major* infection

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Introduction: Leishmaniasis is a parasitic disease caused by several different species of Leishmania protozoa. Depending on the infecting Leishmania species, the disease manifests as recurrent cutaneous, mucocutaneous or visceral leishmaniasis. The lack of a potent licensed vaccine, the toxicity of the drugs used in treatment of Leishmaniasis and emergence of drug resistant/LRV positive parasites, necessitates a thorough understanding of parasite/virus detection/immune evasion mechanisms to develop better anti-*Leismanial* therapeutics. In this work, we focused on the role of cGAS/STING signaling pathway, NLRP3 inflammasome, and RIG-I/MAVS pathway on infection of monocyte/macrophage cell lines by *Leismania Major* parasites that are LRV negative or positive.
Materials & Methods: PMA differentiated THP1-Dual wild-type, cGAS KO, STING KO, STING GOF, NLRP3-deficient, ASC-deficient and TREX1-KO cells were infected with the abovementioned Leishmania strains. 24-48-72 h following infection, parasite load was determined using flow cytometry.
Results: Infection of THP1-Dual STING-KO and THP1-Dual cGAS-KO cells were reduced by 65% compared to their wild-type counterparts.
Conclusions: Data presented herein suggests that *Leismania Major* parasites utilize the cGAS-STING-IRF3 pathway to their advantage. Furthermore, the presence of RNA virus may synergize with the cGAS/STING pathway in IRF3-activation, that somehow contributes to parasite survival/proliferation. Inhibitory drugs that target cGAS/STING and or IRF3 activation may be pivotal for the success of anti-Leishmanial immunotherapy.

DOI: 10.1002/eji.201970400

P1546

Integrative analysis of the human macrophage microRNA-mRNA response to *Leishmania major* infection

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Micro-RNAs are a class of small non-coding RNAs of 20 to 24 nucleotides in length known to be involved in almost all biological processes. Knowing the crucial potential of miRNAs to regulate concomitantly several messenger RNAs, they have become the focus of studies revolving around the uncovered complexity of pathogen survival strategies inside their hosts. The case of *Leishmania* (*L.*) parasites is edifying in this regard. But despite huge community efforts, only a small number of host and parasite molecules have been identified to date for their role during the infectious process. In this study, we conducted an integrated analysis of miRNA and mRNA expression profiles of human macrophages infected, through a time-dependent follow-up, with *Leishmania major* parasites in order to identify parasitic escape routes to the immune response.Our results revealed that host predicted and experimentally validated target genes, that are under the control of up- or down-regulated miRNAs in response to *Leishmania major* infection, are influencing during the infectious process key pathways such as MAPK signaling pathways, PI3K-Akt signaling, as well as endocytosis, among others. This comprehensive approach is likely to allow the identification of functional pathways that could be targeted to fight this parasitic infection.

P1547

Serum amyloid P component as an essential component of resistance against *Aspergillus fumigatus*

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Serum amyloid P component (SAP), also known as Pentraxin 2 (PTX2), is a component of the humoral arm of innate immunity involved in resistance to infection and regulation of tissue remodeling. The present study was designed to investigate the role of SAP/PTX2 in antifungal resistance. SAP-deficient mice showed a dramatic increase in susceptibility to *A. fumigatus*infection. The defective anti-fungal resistance of gene targeted mice was rescued by *in vitro*opsonization of *A. fumigatus* conidia and *in vivo*administration of SAP. Mouse and human SAP bound conidia and increased phagocytosis by mouse and human neutrophils. Defective resistance of SAP-deficient mice was associated with defective *in vivo*phagocytosis by neutrophils in lungs. The opsonic activity of SAP was dependent on the classical pathway of complement activation. In immunosuppressed mice, SAP administration protected hosts against *A. fumigatus*infection and death. Thus, SAP is a fluid phase pattern recognition molecule essential for resistance against *A. fumigatus*. The results reported here also suggest that SAP has therapeutic potential against an infectious agent which represents a formidable clinical challenge.

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DOI: 10.1002/eji.201970400

P1548

Endothelial protein C receptor contributes to experimental malaria-associated acute respiratory distress syndrome

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The severity of *Plasmodium falciparum* malaria is associated with parasite cytoadherence, but there is limited knowledge about the effect of parasite cytoadherence in malaria-associated acute respiratory distress syndrome (ARDS). Our objective was to evaluate the cytoadherence of infected red blood cells (iRBCs) in a murine model of ARDS and to appraise the role of endothelial protein C receptor (EPCR) in ARDS pathogenesis. DBA/2 mice infected with *P. berghei* ANKA were classified as ARDS- or hiperparasitemia (HP)-developing mice according to respiratory parameters and parasitemia. Lungs, blood and bronchoalveolar lavage were collected for gene expression or protein analyses. Primary cultures of microvascular lung endothelial cells from DBA/2 mice were analyzed for iRBC interactions. Lungs from ARDS-developing mice showed evidence of iRBC accumulation along with an increase in EPCR and TNF concentrations. Furthermore, TNF increased iRBC adherence *in vitro*. Dexamethasone-treated infected mice showed low levels of TNF and EPCR mRNA expression and, finally, decreased vascular permeability, thus protecting mice from ARDS. In conclusion, we identified that increased iRBC cytoadherence in the lungs underlies malaria-associated ARDS in DBA/2-infected mice and that inflammation increased cytoadherence capacity through EPCR expression, suggesting a potential target for drug development.

P1549

Impact of human *Loa loa* transmission on immune response in endemic area of Gabon

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Loa loa is a filarial worm restricted to central and some West African countries with many imported cases reported all over the world. We compared the level of specific IgG subclasses (plasma), proliferation and cytokines (PBMC) from villagers exposed to two different annual transmission potentials (ATP) of 9000 infective larvae per person per year (V1) and 1000larvae/person/year, using extract of all development stages of *L.loa* (adult, microfilaria, L3) and nonspecific antigen (concanavalin A, PPD).The results show that the level of L3- and microfilaria-specific IgG1 were significantly higher in amicrofilaremic individuals compared with microfilaria carriers (p=0.04 and p=0.03,respectively). In contrast, microfilaria-specific IgG2 was significantly higher in a high-transmission village (V1) than in a low-transmission village (V2) (p=0.0009). In addition, the levels of L3-specific IgG3 were significantly higher in the village with low transmission (V2) (p=0.006), and both microfilaria- and adult-specific IgG4 were significantly increased in microfilaria carriers compared with amicrofilaremic subjects (p=0.0015 and p=0.003,respectively), while PBMC from individuals in the low-transmission village (V2) responded better to stimulation with adult antigen and to PPD than did PBMC from individuals in the high-transmission village (p=0.0031 and p=0.0012, respectively). **These results suggest that the expression of antigen-specific IgG3 and IgG2 are more likely to vary with transmission intensity, whereas antigen-specific IgG4 and IgG1 vary with adult worm and microfilaria burden.Furthermore, high levels of *L. loa* transmission depress both specific and nonspecific T-cell proliferative responses.**

DOI: 10.1002/eji.201970400

P2124

Cellular profiles in the interface of host and echinococcus

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Alveolar echinococcosis (AE) and cystic echinococcosis (CE) caused by Echinococcus multilocularis and E. granulosus, respectively, are lethal parasitic diseases of world wide distribution. The treatment of these diseases are very difficult. An effective drug is urgently needed. A big barrier for finding effective drugs is that we knew less on the interface of host and parasite. Pathogenesis of AE is characterized by a continuous, granulomatous, periparasitic infiltration of immune cells the metacestode of E.multilocularisin the affected liver. In the present study, we probed cells in the lesions of AE, we compared with those cells in CE lesions, which cause less pathological responses. In AE lesions, infiltrated cells mainly composed of plasms cells and lymphocytes, also observed small amounts of neutrophils, fibroblasts and vascular endothelial cells, while in the CE lesions, infiltrated cells predominantly as vascular endothelial cells with a few of plasms cells, lymphocytes and fibroblasts. Immunohistochemistry staining further confirmed the results above, and also discovered that macrophage markers were positive in both AE and CE lesions. We also showed pathological responses in different time points after infection in mice. In the early stage of AE, parasitic lesions contained a large amount of cellular responses showed mixed Th1/Th2 cells. Early infectious lesions included mostly infiltrated lymphocytes. In late stage 8 to 12 weeks, AE lesions contained numerous lymphocytes, NK cells,fibroblasts, and monocytes. In the late stage, the periparasitic area was composed of inflammatory fibrous tissue and necrotic areas, that formed small granulomatous nodules.

P2125

Immunolocalization of antigen gene CsSQ1 and CsSQ2 of clonorchis sinensis

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Clonorchiasis caused by *Clonorchis sinensis* is an important fish-borne zoonotic parasitic disease and predominantly leads to hepatobiliary diseases. The most severe complication is associated with cholangiocarcinoma (CCA). So *C.sinensis* was reclassified as group Ⅰ bio-carcinogen by the International Agency of Cancer Research. To study the localization of the Antigen gene of Clonorchis sinensis. Two Amino acids highly repetitive sequences were obtained by Construction and screening of a cDNA expresssion library of Clonorchis sinensis etacercaria and adult worm. Then we synthesize peptides with highly repetitive gene sequences and coupling with the carrier protein. Animals were immunized and serum was collected. Then Antibody purification by Ammonium sulfate precipitation and affinity chromatography, At last antigenic gene were located by Immunofluorescence technique. Antigen gene CsSQ1 and CsSQ2 mainly distributed in oral sucker and ventral sucker respectively. The localization of the Antigen gene of *Clonorchis sinensis* was confirmed. We speculate that the recombinant antigen may be related to the immune adhesion of *Clonorchis sinensis.* It provided the reference for the Applied research in diagnosis and immunological characteristics of antigen of *Clonorchis sinensis* in the future.

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DOI: 10.1002/eji.201970400

P2126

Altered expression of microRNAs in infections with cutaneous and visceral strains of Leishmania

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Leishmaniases are caused by protozoa of the genera *Leishmania*. *Leishmania* modulates several mechanisms within the host in its favor including through the expression of microRNAs (miRNAs). Their expression profile may differ depending on the *Leishmania* species thus miRNA expression in THP-1 human macrophages infected with *Leishmania (Viannia) braziliensis* that causes tegumentary disease or *Leishmania (Leishmania) infantum* that causes visceral disease was evaluated. Further miRNAs present in plasma from patients with tegumentary leishmaniasis caused by *L. (V.) braziliensis* were evaluated. cDNAs synthesized from total RNA both from *in vitro L. (V.) braziliensis*- or *L. (L.) infantum*-infected THP-1 cells incubated for 6 h or 24 h and from patients´ plasma were evaluated for inflammatory response specific-miRNAs through qPCR. Significant results were selected for computational target prediction. Significantly altered miRNA expressions were identified in both *L. (V.) braziliensis* and *L. (L.) infantum* infections. The former altered the expression of 20 miRNAs of which 6 were up regulated in 6h and 8 were overexpressed in 24h. In *L. (L.) infantum* infection, 11 differentially expressed miRNAs were identified when compared with non-infected cells at 6 and 24 hours. Significantly altered miRNA expression was observed in plasma from patients presenting different clinical manifestations. Some miRNAs were similarly altered in in vitro infection and in patient plasma. The predicted targets of those significantly altered miRNAs included cytokine coding genes, TGF-beta pathway and MHC II proteins suggesting participation in evasion mechanisms. Validation of certain miRNAs altered both in vitro and in patients is in progress.

P2127

The role of laccase in modulating immune response in Cryptococcus gattii infection

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Cryptococcosis is an infectious disease caused by two fungal species; *C. neoformans* and *C. gattii*. While *C. neoformans* affects mainly immunocompromised patients, *C. gattii* infects both immunocompetent and immunocompromised individuals. Laccase is an important virulence factor that contributes to the virulence of *C. neoformans* by promoting pulmonary growth and brain dissemination. The presence of laccase in *C. neoformans* is able to shift host immune response towards non-protective Th2-type immunity. However, the role of laccase in immunity to *C. gattii* remains unclear. In this study, we characterized laccase activity in *C. neoformans* and *C. gattii* isolates from Thailand and investigated whether *C. gattii* deficient in laccase could modulate immune responses against *C. gattii* infection. *C. gattii* was found to have higher laccase activity than *C. neoformans*, indicating the importance of laccase in pathogenesis of *C. gattii* infection. Mice infected with *LAC1* mutant strain of *C. gattii* had reduced lung burdens at the early but not late stage of infection. *LAC1* mutant strain of *C.*

DOI: 10.1002/eji.201970400

gattii did not alter Th1 and Th2 cytokines. Indeed, we found elevated IL-17 cytokine in mice infected with mutant compared with wild type strain. Interestingly, infection with *LAC1* mutant induced more neutrophil infiltration and enhanced expression of neutrophil-recruiting chemokines G-CSF and CXCL1. Similar to parental strain, infection with mutant complemented with *LAC1* gene significantly impaired neutrophil recruitment. Thus, our data suggest that laccase is an important virulence factor of *C. gattii* that plays role to modulate Th17-type immunity and neutrophil function during the early stage of infection.

P2128

Vesicular Stomatitis Virus -based vaccines elicit strong immune response against Plasmodium falciparum

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In the life history of *Plasmodium*, only the erythrocytic stage of malaria can cause illness or even death. Therefore, the invasion of red blood cells by *Plasmodium* is considered to be a key node for the development and reproduction of *Plasmodium*. The apical membrane protein 1 (AMA1) and the reticulocyte-binding prote in homolog (RH5) are key proteins in the process of *Plasmodium* invasion of red blood cells and are potential targets for vaccine research. The vesicular stomatitis virus VSV is a single-stranded negative-strand RNA virus of the Rhabdoviridae and moreand more used in the preparation of vaccines. Therefore, in this project, we constructed the recombinant VSV vaccines that expressed *Plasmodium falciparum* AMA1345 , RON2sp and RH5ΔNL proteins and proved that the VSV-based viral vaccines can effectively induce strong immune responses and suppress *Plasmodium* invasion. In a mouse model of *P.yoelli* infection, the rVSV prime - protein-in-adjuvant boost regime induces a better immune response and protects against *P.yoelli* infection. Our study will provide a new idea for the research of *Plasmodium falciparum* vaccines.

P2129

Infection with echinococcus granulosus alleviated IBD response through AgB which impacting M1/M2 polarization

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Background: IBD is a chronic idiopathic disease. It showed helminth infection can reduce IBD symptom. However the underling machines are not clear.
Methods: 1)We established an *E.granulosus* (*E.g*) infection model with mice challenged with dextran sodium sulphate (DSS). 2) Purified *E.g* antigen B (AgB, a major secreted *E.g* protein) was injected into mice peritoneal cavity, and then the mice were challenged with DSS. 3) AgB blocking assay was done by cultivation of RAW264.7 cells with AgB and stimulated by LPS.
Results: *E.g* infection and AgB significantly reduced gut inflammation by DSS in terms of increasing weight and colon length, and lower DAI and histological scores. AgB intervene reduced iNOs level and increased Fizzl expression in colon quantitated by RT-PCR and flow cytometry. AgB inhibited NO and M1 cytokines expressed in RAW264.7 induced by LPS.
Conclusion: *E.g* infection reduces IBD responses through AgB impacting M1/M2 polarization.

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DOI: 10.1002/eji.201970400

P2130

Alteration of lrNK/cNK balance and their phenotype in mice of long term infection with Schistosoma japonicum

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Bulk NK were reported to be involved and impacted in *Schistosoma* infection and are composed of two major subsets in liver, conventional(cNK) and liver resident NK(lrNK). To investigate the impacts of schistosomiasis on lrNK and cNK respectively, we observed their number changes and phenotype alteration in *S.japonicum* infected mice liver using multicolor FCM, at a series time points (3wks, 4wks, 6wks, 8wks and 12wks)post infection(p.i.). Comparing to uninfected mice, infected group had more lrNK and less cNK at 8 and 12 wks p.i., which was not observed at 3wks, 4wks or 6wks p.i..Similar Ki67 level indicated no changes in their proliferation ability upon infection. CD69, CD27, NKG2D, CXCR3, CXCR6 expression decreased on lrNK cells in mice of 8 weeks post-infection. Meanwhile, the CD69 and CD27 expression increased and the KLRG1 and NKG2D expression decreased on their liver cNK cells. Our data indicated differential effect of schistosomiasis on lrNK and cNK and suggested a developmental or/and a migration impact, instead of a proliferation impact, on murine lrNK and cNK from long term *S.japonicum* infection.Further observations and related mechanisms investigation are planed in our ongoing projects.
*This study was supported by the National Natural Science Foundation of China (No. 81772221,81672047), Fundamental Research Funds for the Central Universities (HUST: 2015QN147, 2017KFYXJJ056), Natural Science Foundation of Hubei Province, China (No. 2016CFB419), Foundation of Health and Family Planning Commission of Hubei Province, China (No. WJ2017X006) The National Undergraduate Training Programs for Innovation and Entrepreneurship (HUST: 16A171, 17A212)

P2131

Repurposed drugs acting on host mechanisms of *T. cruzi* invasion synergize with Benznidazole: New therapies for Chagas disease

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Chagas disease affects about 7 million people worldwide, up to 30% of chronically infected people develop cardiac alterations. Benznidazole and Nifurtimox are the only available trypanocidal drugs are effective only at the acute phase of infection. Our hypothesis is that treatment with repurposed drugs known to act on host cell mechanisms to block *T. cruzi* escape, combined with reduced doses of benznidazole, may show higher efficacy and less toxicity than high-dose benznidazole alone. *In vitro* and *in vivo* response of the colchicine and chloroquine, selected because of their known blocking effects on important steps of the host cell invasion/replication by *T. cruzi*.
Experiments measuring *T. cruzi* Y strain (partially BZN-resistant) trypomastigote release to the supernatant of infected phagocytic and non-phagocytic mammalian cells indicated Chloroquine was as potent as BZN in suppressing *T. cruzi* parasitism, and that the combination of Chloroquine and Colchicine with BZN potentiated its effect several-folds.
Furthermore, Female BALB/c were infected intraperitoneally with blood trypomastigote forms of the

DOI: 10.1002/eji.201970400

Benznidazole-resistant Colombian strain of *T. cruzi*, followed by gavage treatment after 10 days of infection. The levels of parasitemia were significantly lower after 30 days of infection in the group of mice treated with the combination of Benznidazole and Chloroquine in comparison with either BZN alone or saline. These results indicate that the repurposed drugs could boost the anti-*T. cruzi* effect of the standard but suboptimal anti-*T. cruzi* drug Benznidazole (BZN), optimizing its therapeutic efficacy for Chagas disease.

P2132

The drug-resistant and drug-sensitive parasites elicit different immune responses

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The protozoan parasite *Leishmania donovani* causes visceral leishmaniasis or kala-azar and is responsible for significant mortality and morbidity, if untreated. Four drugs are used for the treatment of visceral leishmaniasis. These are antimony, miltefosine, amphotericin B and paromomycin. Now, the parasite is emerging as resistant against each of these drugs causing a concern for its treatment options. It was previously reported that the drug-resistant parasites altered the cellular responsiveness and functions. As a result, the host-immune responses were suppressed. However, the relationship between infection with a drug-resistant parasite and the ensuing immunosuppression remains unknown till date. Here, we have performed the growth kinetics of the promastigotes in cell-free cultures and amastigotes in macrophages. The drug-resistant parasites survived longer than the drug-sensitive parasites within macrophages, although the cell-free growth of promastigotes between the strains was not significantly different. The cytokine productions also were significantly different. We performed the immune responses against these parasites. It was observed that the drug-resistant parasites elicit more of a disease-promoting response than host-protective response in a susceptible host. Therefore, these results suggest that the success in anti-leishmanial therapy against drug-resistant parasites becomes compounded by the additional immuno-suppression reinforced by these parasites.

P2133

A 'carbohydrate-fraction' from wild mushroom Astraeus hygrometricus protects the host against Leishmania donovani infection by reinstating the pro-inflammatory responses: searching the sweet solution

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Although several formulations are in use widely as anti-leishmanial drugs, severe side effects along with the emergence of resistant strains are a continuous nuisance in treatment efficacy. In this study, we report a 'carbohydrate-fraction', isolated from an edible mushroom *Astraeus hygrometricus,* capable of tweaking the host machinery towards pro-inflammatory pathways of immune responses of the host. It was found capable of promoting the expression of iNOS2 and pro-inflammatory cytokines like TNF-α and IL-12, with subsequent down-regulation of the anti-inflammatory cytokines as TGF-β

DOI: 10.1002/eji.201970400

and IL-10, *in vitro* and *in vivo* and a remarkable increase in the expressions of IL-6, IL-1β, IFN-γ, IRF-7 and IRF-8 *in vivo*. Activation of TLR-2, TLR-7, and TLR-9 was also detected, *in vitro*. Interestingly, it also reduced the parasite burden in spleen and liver dose-dependently, *in vivo*. The proliferation of $Ly6C^+$ cells in bone marrow of *Leishmania*-infected experimental animals with an increase in monocyte population along with a dose-dependent increase in the expression of PU.1 by the 'carbohydrate-fraction' presumably signify the expansion of protective macrophages. Thus, this fraction might be a resourceful impact in the development of new adjuvant as an efficient anti-leishmanial lead with comparatively least toxicity but high promises.

The first third authors equal contribution.

P2135

Characterization of the Neutrophil Extracellular Trap (NET) process induced by the human parasite E. histolytica

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NETosis is a new mechanism of innate immunity related to defense against different pathogens. It involves sequential steps in neutrophils that culminate in the extrusión of DNA associated with histones, granule enzymes and antimicrobial peptides. Amoebiasis, the third leading cause of human death among parasitic infections, is associated with the induction of inflammation with a large number of infiltrating neutrophils. These neutrophils have been implicated in the defense against this parasite by mechanisms not fully understood. Here we show that E.histolytica trophozoites trigger NETosis rapidly (less tan 15 mins), depend on contact with viable amoebas and involve both nuclear and mitochondrial DNA. The process is independent of ROS from neutrophils but seems to be dependent on ROS from the parasite. Translocation of nuclease elastase to the nucleus and serine-protease activity were necessary, but surprisingly, while calcium chelation reduced NET release induced by amoebas, PAD4 inhibition failed to block extrusion of DNA, suggesting that citrullination is not involved. The effect of several pharmacological inhibitors on amoeba-induced NETs formation was assessed showing that the parasite activates a signaling pathway involving Raf/MEK/ERK, but it is independent of PKC and TAK1. Finally, we showed by video microscopy that NET were important for trapping and killing the pathogenic amoebas but in contrast, the non-pathogenic E. dispar did not induce NET. In conclusion, we showed that amoeba triggers NETosis by a rapid non-classical mechanism, which involves a signalling pathway different from that of PMA or FcγRIIIb, and is associated with the pathogenicty of the parasite.

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DOI: 10.1002/eji.201970400

P2136

IgM antibody naturally acquired to Plasmodium vivax blood stage antigen ($MSP1_{19}$) in individuals living in a low malaria transmission area in Amazon region, Brazil

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The acquired antibody immune response has been reported in human malaria, including antibodies against antigens from asexual stage. However, few studies have evaluated the acquisition of IgM antibody in *Plasmodium vivax* malaria. Among the best characterized antigens of this stage is the Merozoite Surface Protein 1 (MSP1). This study evaluated the naturally acquired IgM antibody in infected and residents (exposed) individuals living in low malaria transmission area, in Pará state, Brazil, where *P. vivax* is the most prevalent species. The IgM against the C-portion of MSP1 ($MSP1_{19}$) was detected by ELISA in sera from 84 malaria patients (infected) and 164 non-infected individuals (exposed). In infected, the median age was 32 years, 73% male and the median parasitaemia was 1,500 parasites/µl. The sera were collected on the day of parasite detection by thick blood smear. At the moment of diagnosis all patients received treatment. In non-infected group, the median age was 33.5 years and 70% were male. The percentage of positive sera that recognized the recombinant protein used as antigen ($PvHis_6MSP1_{19}$) was 31.0% (26/84) and 6.1% (10/164) in infected and non-infected groups, respectively. Among these 26 infected and IgM positive individuals, 22 had had previous malaria episodes, and the parasitaemia in 20 of them ranged from 10 to 5,000 parasites/µL. In non-infected, the IgM was not maintained for long time although they were living in malaria transmission area. It showed that the infection can induce an IgM short lived primary immune response in *P. vivax* malaria.

P2138

The control of a protozoa-induced lesions by Blimp-1

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B lymphocyte-induced maturation protein 1 (Blimp-1) is a transcription factor that plays a crucial role in regulation B-lymphocyte function. Its deletion in T cells increased the differentiation of naive $CD4^+$ T cells into Th9 cells. T cell-specific Blimp-1-deficient mice exibits a worsened allergic airway disease, that is inhibited by IL-9 neutralization. Here, we investigated the role of the Blimp-1 in the *Trypanosoma cruzi*-induced Th1 response. The infection induces high Blimp-1 expression in spleen and liver, 14 days post-infection (dpi) and 100% of T cell conditional Blimp-1-deficient mice (Blimp-$^{1fl/f}CD4^{Cre}$; CKO) succumbed within 15 dpi, whereas WT mice survived. The deletion of Blimp-1 in T cells resulted in an excessive hepatic inflammation with high levels of IFN-γ, CXCL10 and TNF, and higher parasite burden in heart and liver, compared with WT mice. The CKO mice showed a less regulatory T cells e low IL-10 expression in myeloid cells compared to WT mice. In contrast, *T. cruzi*-infected Blimp-1-deficient BM-derived dendritic cells (BM-DCs) showed a high IL-10 and reduced IL-12 production compared to WT BM-DCs. The lack of Blimp-1 in myeloid cells leads the resistance to the infection, with no mortality, increased Th1 response, high IL-10 levels, and control of parasite burden. In conclusion, deletion of Blimp-1 in T cells favors IFN-γ signaling, drives TNF production and contributes to hepatic and splenic pathology. In contrast, the deletion of Blimp-1 in myeloid cells contributes to IL-10 signaling and control the IFN-γ and TNF mediated destruction, promoting the

DOI: 10.1002/eji.201970400

resistance to infection.
Financed by FAPESP

P2904

Aberrantly expressed long non-coding RNA and mRNAs in the liver of experimental mice infected with *Echinococcus multilocularis*: TLRs and NLRs signaling pathways were involved in

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Alveolar echinococcosis (AE) is one of the most severe helminthic disease caused by the intrahepatic tumor-like growth of the metacestode of *E. multilocularis* (Em). Although long non-coding RNAs (lncRNA) play important roles in regulating occurrence and progression of many diseases, lncRNA has never been analyzed on mice model infected by echinococcosis. In this study, lncRNA and mRNA expression profiling were comprehensively assessed by microarray analysis on the Balb/c mice livers, which were collected at 2, 4, 8, 15, 30, 60, 90 and 150 days after intraperitoneally injected with *Em* protoscoleces. Over 10,000 common differentially expressed mRNA (DEM) and lncRNA were identified at 2, 4, 8, 15 dpi, respectively (FC>2, $p< 0.05$). And only over 1000 common differentially expressed mRNA and lncRNA were identified at 30, 60, 90 and 150dpi, respectively (FC>2, $p< 0.05$). The KEGG analysis of DEM at 30 dpi showed that Toll-like and RIG-I like receptor signaling pathways were the up-regulated pathways. Co-expression network of dysregulated lncRNA-mRNA analysis revealed that lncRNA NONMMUT081543.1, NONMMUT137613.1 and AK014756 had high connectivity with DEM ($r>0.99$, $p< 0.05$) at initial stage of the infection (including 2, 4, 8, 15, 30dpi), while LncRNA NONMMUT102390.1, AK017047 and AK044084 had high connectivity with DEM ($r>0.9$, $p< 0.05$) at middle stage of the infection (including 60, 90, 150 dpi). These findings provide new insights into the response of mice to *Em* during the course of infection, which may be useful in determining pathways that can modulate host-parasite interaction and thus potentially important for clearance of the parasite.

P2905

Effection of the ILC2 differentiation mediated by IL-33/ST2 signaling pathway on liver fibrosis of mice infected with Schistosoma japonicum

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Objectives: To study the regulation function of ILC2 differentiation mediated by IL-33/ST2 signaling in the development of hepatic fibrosis in mice infected with Schistosoma japonicum.
Methods: After the experimental mice were molded for 10 weeks, the morphological changes of granuloma and liver fibrosis were observed by HE staining. The levels of serum TNF-α, IL-1β, IL-4, IL-5, IL-13 and IL-33 were detected by ELISA. The expression of ST2 mRNA in liver tissue and of T-bet, GATA3 and RORγt mRNA in spleen cells were detected by RT-PCR. Flow cytometry was used to detect the proportion of ILCs cells in spleen.
Results: HE staining showed that IL-33 could significantly accelerate liver fibrosis of Schistosomiasis japonica, leading to obvious inflammation of liver tissue, continuous increase of fibrous tissue, and obvious increase of granuloma area of schistosomiasis. The serum levels of cytokines TNF-α, IL-1β, IL-4, IL-5, IL-13 and IL-33 in IL-33 intervention group and model control group were significantly higher than those of normal controls. The expression of GATA-3 gene was significantly increased, but the expression of T-bet and RORγt was not significantly changed. Compared with the normal control

group, the proportion of ILC2 cells in the IL-33 intervention group was significantly higher than that in the normal control group, while there was no significant change in ILC1 and ILC3 cells.
Conclusions: Schistosomiasis infection leads to the release of IL-33 in the liver microenvironment, which mediates the proliferation and differentiation of ILC2 through IL-33/ST2 signaling, amplifies the inflammatory response, and promotes the development of liver fibrosis.

P2906

Inflammation and T cells response in interleukin-33 defect mice with acute *Toxoplasma gondii* infection

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Toxoplasma gondii is an intracellular parasite, and the T cell immune responses play an important role against *Toxoplasma gondii* infection. IL-33 is a new member of the IL-1 family which induces Th2 responses. In this study, a model of acute *Toxoplasma gondii* infection in IL-33$^{-/-}$ mice was established to investigate the role of IL-33 in inflammation and T cells immune responses. Our results show that the survival rate of IL-33$^{-/-}$ mice was higher than wild-type mice; the levels of inflammatory factors in liver, spleen and peripheral blood were decreased while these were increased in brain; the mRNA level of SAG1 in spleen was decreased, while the burden of toxoplasma tzchyzoits in the peritoneal cavity was increased; the expression of PD-1 was increased in CD4^{+}T and CD8^{+}T cells with the increase of the ratio of CD44^{high} CD62^{low} CD4^{+}T and CD44^{high} CD62^{low} CD8^{+}T, while the proportion of TNF^{+} CD8^{+} T cells was increased with the decrease of the ratio of CD4^{+} T-IFN-γ/CD4^{+} T after acute *Toxoplasma gondii* infection. Our results suggest that IL-33 plays different roles in different tissues after acute *Toxoplasma gondii* infection; compared with wild-type mice, IL-33$^{-/-}$ could enhance T cell immune responses and CD8^{+}T cell function, but inhibit CD4^{+}T to polarize toward Th1 while promote macrophage to polarize toward M1.
Key Words: interleukin-33; acute *Toxoplasma gondii* infection; T lymphocyte immune response.

P2907

Comparative proteomic analysis of differentially expressed proteins in the *Bombyx mori* hemolymph during infection by the Microsporidia *Nosema bombycis*

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Host-pathogen interactions are complicated processes, which have been studied extensively in recent years. Insects possess a highly efficient immune system in which hemolymph plays an important role in the innate immune process. To investigate the effects of *N. bombycis* infection from a global perspective, third instar molted silkworm larvae were challenged with *N. bombycis* isolate CQ1, and two-dimensional gel electrophoresis (2DE) analysis was performed to analyze the differentially expressed proteins in infected and uninfected silkworm hemolymph 2, 4, 6 and 8 days post-infection (dpi). **The results showed that there were 18 differentially expressed spots in hemolymph at the 4 time points. Five proteins, namely antichymotrypsin-1, apolipophorin-III precursor, hemolymph protein, promoting protein and chymotrypsin inhibitor CI-8A, and four unannotated proteins were indentified using matrix assisted laser desorption/ ionization time-of -flight mass spectrometry (MALDI-TOF-MS).** Furthermore, we found that these proteins were mainly involved in

DOI: 10.1002/eji.201970400

energy metabolism and innate immunity. Overall, the putative functions and participation in biological reactions of the identified proteins reveal a typical host-parasite response between *B. mori* and *N. bombycis*, and those findings may provide important insights into the mechanisms of the silkworm response to *N. bombycis* infection.
Keywords: *Nosema bombycis*, *Bombyx mori,* Proteome, Innate immune

P2908

Regulation of mouse type 2 immune response induced by the thioredoxin peroxidase-2 (TPX2) from Trichinella spiralis

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Trichinella spiralis is an intracellular parasitic nematode that can infect many hosts including humans and causes trichinellosis. The enteric phase of *T. spiralis* infection generally induces a Th1/Th2 mixed response, which becomes type 2-biased during the systemic phase. Thioredoxin peroxidases from trematode can induce type 2 immune responses, but the function of thioredoxin peroxidases from *T. spiralis* in regulating type 2 immune response has not been identified. In our previous study, three members of the TPX family were cloned from *T. spiralis* muscle larvae (ML). In this study, we further confirmed the function of TsTPX2 in regulating type 2 immune response. Immunolocalization with anti-TsTPX2 mouse sera confirmed that TsTPX2 protein was strongly expressed on the surface and within the body of *T. spiralis* day 3 adults (Ad3). Immunization with recombinant TsTPX2 produced higher levers of Th2 (IL-4) and lower Th1 (IFN-γ, IL-6) cytokines; the percentage of $CD4^+$T cells increased and the percentage of $CD8^+$T cells declined at the same time. Moreover, the expression of characteristic molecular of AAMs (Arg-1, Mrc-1) was up-regulated and the expression of characteristic molecular of CAMs (iNOS) was de-regulated when RAW264.7 cells and peritoneal macrophages were stimulated respectively with recombinant TsTPX2. Taken together, results from our study suggested that the thioredoxin peroxidase-2 from *T. spiralis* can activate macrophages by the alternative pathways *in vitro* and induce a type 2-biased immune response *in vivo*.

P2909

Inhibition of GLUTs by WZB117 mediates apoptosis in blood-stage Plasmodium parasites by breaking redox balance

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Like tumour cells, during intraerythrocytic stage, Plasmodium-infected erythrocytes rely completely on glucose absorption from the blood circulation for energy metabolism. Glucose is taken up by glucose transporter 1 (GLUT1) on human red blood cells (RBCs) and glucose transporter 4 (GLUT4) on rodent RBCs. Blood-stage parasites grow rapidly; therefore, infected red blood cells (iRBCs) need much

DOI: 10.1002/eji.201970400

more glucose for energy. In previous study, WZB117 (2-fluoro-6-(m-hydroxybenzoyloxy) phenyl m-hydroxybenzoate) inhibits GLUT1 by binding the exofacial sugar-binding site and inhibits the insulin-sensitive GLUT4 with greater potency than its inhibition of either GLUT1 or GLUT3. In our study, WZB117 effectively inhibit the growth of blood-stage parasites. Mechanistically, WZB117 inhibited the activity of GLUTs and perturbed the glycolysis. Therefore, decreasing the glucose level increased the redox oxidative species (ROS) level and induced oxidative stress and apoptosis. The spleen can more easily clear apoptopic iRBCs than nonapoptotic iRBCs, effectively relieving hepatosplenomegaly. These findings provide important insights into the crucial role of glucose transporters (GLUTs) in Plasmodium glucose uptake during intra-erythrocytic stage, as the inhibition of GLUTs block Plasmodium infection during the erythrocytic stage.

P2910

Immunization of mice with rBCG-EgG1Y162 provides protection against Echinococcus granulosus infection in BALB/c mice

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Background: Echinococcosis is a zoonosis caused by cestodes of the genus Echinococcus. This study is to detect the immune protection of recombinant BCG-EgG1Y162 vaccines in Echinococcus granulosus infection, and to evaluate their immunogenicity in mice.
Methods: Experimental group mice were immunized with rBCG-Eg1Y162, BCG group and NS group were injected with BCG and saline respectively. The serum and spleen cells were isolated. The isolated spleen cells were stimulated with recombinant BCG-EgG1Y162 in vitro and the culture supernatant was collected. The levels of IgG in serum and levels of IL-4 and IFN-γ in the culture supernatant were measured by ELISA. The weight and number of the fresh hydatid cysts were evaluated. The serum ptotoscolicidal activity was measured by the complement dependent cytotoxicity assay.
Results: Serum IgG levels of mice immunized with BCG-egG1Y162 increased significantly on 2nd to 8th weeks, and the difference was significant with NS group and BCG group($P < 0.05$). The levels of IFN-γ, IL-4 and the ratio of IFN-γ/IL-4 after rBCG-Eg1Y162 immunization were significantly higher than the normal group and BCG group ($P < 0.05$). Weight and number of the fresh hydatid cysts in rBCG-EgG1Y162 immunized mice was significantly decreased with a protective efficacy of 87.3%. And, the serum protoscolicidal activity after rBCG-Eg1Y162 immunization was enhanced.
Conclusions: The rBCG-EgG1Y162 induces production of serum IgG levels and Th1 cell immune response, which enhances the protective immunity in Echinococcus granulosus challenged mice and thus may be used as a potential vaccine candidate.

This research is supported by NSFC: 81660343, 81460307

DOI: 10.1002/eji.201970400

P2911

The role of Plasmodium berghei ER-shaping protein YOP1 in cerebral malaria

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The pathogen of malaria is Plasmodium. Cerebral malaria is a serious complication of malaria with a high rate of morbidity. The proteins secreted by plasmodium could insert into the host cell membrane, leading remodeling of membrane structure. The infected erythrocytes can adhere with the vascular endothelial cells and sequestrate in the organs, which is one of the mechanisms of cerebral malaria pathogenesis. It is reported that YOP1 protein plays a role in tubular ER morphogenesis by mediating membrane high-curvature, and Atlastin plays a role in formation of the tubular ER network by mediating homotypic fusion of ER membranes and participates in COPII vesicle formation. These results indicate that ER-shaping proteins are closely related with the process of protein secretion. Here, we identified the YOP1 homologous protein in the plasmodium genome by sequence comparison, and obtained the YOP1 knockout mutant in Plasmodium berghei. We demonstrate that PbYOP1 is expressed in erythrocytic stages and sporozoites and Pbyop1Δ-infected erythrocytes have decreased cytoplasmic density compared to WT-infected erythrocytes. Digestive vacuoles in Pbyop1Δ trophozoites are significantly larger than in WT parasites. Pbyop1Δ parasites are highly attenuated in the asexual cycle, cause less mortality in infected mice and display fewer symptoms of experimental cerebral malaria. Pbyop1Δ parasites sequestration and accumulation of $CD8^+$ T cell in brain is significantly decreased compared to WT parasites-infected mice. These results indicate that ER function may be disturbed in Pbyop1Δ parasites, resulting in abnormal protein secretion and immune response, and finally inducing attenuated virulence.

P2912

Circumsporozoite protein suppresses mosquito innate immune response to promote malaria parasite development in mosquitoes

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It is well known that circumsporozoite protein (CSP) is essential for the development of malaria parasites in mosquitoes, but the mechanism behind this is still largely unknown. Here, we found the development of parasite was blocked at oocyst stage, and no sporozoite was found in either hemolymph or salivary gland of mosquitoes infected with parasite, whose CSP pexel domain was mutated. Although the maturation of mutant parasite oocyst were unaffected, the melanization of oocysts was only observed in mosquitoes infected with mutant parasite but not wild type (WT) parasite. RNAseq showed that several mosquitoes immune related genes, includingThioester containing protein (TEP)2, were greatly upregulated after infected with the mutant parasite.Strikingly, the knock-down of TEP2 partially recovered the development of mutant parasite in mosquitoes, with the disappearance of melanized oocysts and appearance of sporozoite in hemolymph. Further study showed TEP2 mainly expressed by haemocytes and much more TEP2 bound to the mutant oocysts as compared to WT oocysts. There are 3 kinds of haemocytes in anopheles, including granulocytes, oenocytoids and prohemocytes, next study also showed granulocytes are mainly responsible for expression of TEP2. Thus, our data strongly suggested that the inhibition of mosquito defense responses by naïve CSP was greatly contributed to the development of malaria parasite in mosquitoes, and firstly revealed a novel role of CSP to suppress mosquitoes innate immune responses, and which also provided us with a promising target to design the malaria blocking transmission vaccine.

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DOI: 10.1002/eji.201970400

P2913

Malaria blood stage infection induces CD4$^+$CD25$^+$ Foxp3$^+$ regulatory T cells to release sFGL2 and suppresses host innate immune responses

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The induction of Treg (CD4+CD25+ Foxp3+ regulatory T cells) is one of the major immune escaping strategies of malaria parasite, but the underlying mechanism by which malaria parasite-induced Treg dampens host immune responses remains elusive. Here, we found that malaria parasite infection induced Tregs to release soluble fibrinogen-like protein 2 (sFGL2) and significantly promoted the growth of malaria blood stage. This effect was attributed to the capacity of sFGL2 to inhibit macrophages from releasing MCP-1 and to sequentially reduce the recruitment of NK/NKT cells to the spleen and the production of IFN-γ. Further study showed that sFGL2 inhibited JNK phosphorylation in the TLR2 signaling pathway, mediating MCP-1 release dependent on FcγIIB receptors of macrophages. Strikingly, a high level of sFGL2 was also observed in the serum of malaria patients, and recombinant FGL2 significantly suppressed *Plasmodium falciparum* from inducing macrophages to release MCP-1. Therefore, we reveal an unrecognized role of sFGL2 released by Tregs in manipulating host innate immune responses and a novel immune suppression strategy of malaria parasites.

P2914

Membrane-bound IgE on B cells is increased during Clonorchis sinensis infection

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A high level of serum IgE is a hallmark of helminthic disease. Secretory IgE can bind FcεRI or FcεRII/CD23. The combination of IgE and FcεRI, a high-affinity interaction, has long received attention and is believed to facilitate helminth control, while the properties of CD23-bound IgE have long been unexplored. Here, we established a *Clonorchis sinensis* (*C. sinensis*) infection model with different mouse strains and investigated membrane-bound IgE on B cells during infection. We show that after infection, the increase in CD23 expression on B cells was obvious, even in relatively resistant C57BL/6 mice, as well as in susceptible BALB/c and FVB mice. Although the serum IgE amount was lower in C57BL/6 mice than in BALB/c and FVB mice, the level of IgE binding to peripheral B cells was also elevated. Additionally, the IgE on B cells was soon undetectable *in vitro* due to dissociable binding. The results of the present study demonstrate the dramatic increase in CD23-bound IgE on B cells after *C. sinensis* infection. The significance of CD23-bound IgE in Ag transport and presentation has gained consideration in allergy development for its potential ability to promote the Th2 response. Therefore, even though the association of IgE and CD23 is not as substantial as that of IgE and FcεRI, membrane-bound IgE on B cells may be worth further study regarding clonorchiasis and other parasitic infections.

DOI: 10.1002/eji.201970400

P2915

Neddylation contributes to CD4+ T cell-mediated protective immunity against blood-stage Plasmodium infection

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CD4+ T cells play predominant roles in protective immunity against blood-stage Plasmodium infection, both for IFN-γ-dependent effector mechanisms and providing B cell helper signals. Neddylation, an ubiquitination-like process triggered by covalent conjugation of NEDD8 to specific targets, has emerged as a potential regulator of T cell activities to TCR engagement. However, its contribution to T cell-mediated immunity to blood-stage malaria remains unclear. Here using an experimental model induced by Plasmodium yoelii 17XNL, and conditional knockout mice with T cell-specific deficiency of crucial components of neddylation pathway, we demonstrate activation of neddylation in T cells during blood-stage Plasmodium infection is essential for parasite control and host survival. Mechanistically, we show that apart from promoting CD4+ T cell activation, proliferation, and development of protective T helper 1 (Th1) cell response as suggested previously, neddylation is also required for supporting CD4+ T cell survival, mainly through B-cell lymphoma-2 (Bcl-2) mediated suppression of the mitochondria-dependent apoptosis. Furthermore, we provide evidence that neddylation contributes to follicular helper T (Tfh) cell differentiation, probably via augmenting the ubiquitin ligase Itch activity and proteasomal degradation of FoxO1, thereby facilitating germinal center (GC) formation and parasite-specific antibody production. This study identifies neddylation as a positive regulator of anti-Plasmodium immunity and provides insight into an involvement of such pathway in host resistance to infectious diseases.

P2916

Live recombinant Lactococcus lactis vaccine expressing immobilization antigen (i-Ag) for protection against Ichthyophthirius multifiliis in goldfish

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The present study was conducted to evaluate a live recombinant *Lactococcus lactis* vaccine expressing immobilization antigen (IAG-52X) in protection against *I. multifiliis*. A 1266 bp gene fragment containing a potential antigenic epitope of the 48 kDa immobilization antigen of *I. multifiliis* was assembled from six synthetic ohgonucleotides and cloned into pSIP409 and electrotransformed into *Lactobacillus plantarum* NC8. The recombinant vaccine candidate was then orally fed into goldfish. The expression of immune-related genes: complement component 3 (C3), MHC I, IgM gene in blood from goldfish at different time points after immunization were evaluated. Immunized fish were than challenged with a lethal dose of infectious *I. multifiliis*. The cumulative mortality and relative percentage survival (RPS) were also determined. Our results showed that the antibody level in the blood and skin of the immunized fish was statistically significant ($P < 0.05$) in relation to the control groups. Goldfish orally immunized with NC8-pSIP409- IAG-52X had high serum antibody titers that ranged from 32 to 256 after 28d post immunization, while fish fed with NC8-pSIP409 or PBS had no detectable immobilizing antibody response. Expression of IgM, C3, MHC I genes in the group immunized with IAG-52X were significantly ($P < 0.05$) up regulated as compared with control group, indicating that different immune cells were actively involved in cellular immune response. The results showed that the average survival rate of fish orally immunized with 10^8 and 10^6NC8-pSIP409-IAG-52X

DOI: 10.1002/eji.201970400

was 60% and 50% respectively. Therefore, NC8-pSIP409-IAG-52X could become a promising oral vaccine candidate against *I. multifiliis*.

Immunodeficiency

O097

Impaired extracellular NADPH-oxidase-mediated ROS in two ISG15 defect patients with unique clinic phenotype of recurrent bacterial infection

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ISG15 deficiency is a rare primary immunodeficiency disease classically manifested as tuberculosis infection or type I interferon disease, this study explored the new clinical phenotype and potential immunological mechanism of patients with ISG15 defect in a consanguineous Chinese family. Patient One, a nearly 2-years-old male, presented with recurrent respiratory infection. Patient Two, a 3-months-old female, showed recurrent systemic skin suppurative infection. The lymphocyte subsets frequency, absolute number, TCR repertoire and proliferation function were normal. IFN -γ- IL12 axis function was normal. Homozygous mutations c.420C>A (p.Y140X) on Exon 2 from their heterozygous parents result in absence of ISG15 protein expression in neutrophils. Productions of extracellular NADPH-oxidase-mediated reactive oxygen species (ROS) induced by PMA, fMLP and WKYMVM in neutrophils were significantly impaired. While the productions of intracellular NADPH-oxidase-mediated ROS in neutrophil was normal. The clinical symptoms of the two patients were significantly improved with antibiotic treatment. Patient Two died from accidental foreign body inhalation at the age of 1 year and 6 months.

In conclusion, We firstly described two patients with novel ISG15 mutation causing ubiquitin-like domain truncation. The patients manifested new clinical phenotype of recurrent pulmonary and cutaneous bacterial infection, which indicated the clinical heterogeneity of ISG15 defect. Truncated ubiquitin-like motif of ISG15 may be related to the disturbance of extracellular NADPH-oxidase-mediated ROS produced by neutrophils resulting in recurrent bacterial infection.

O098

Deficiency in human adenosine deaminase 2 alters cellular responses to extracellular adenosine

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Adenosine accumulates at the sites of inflammation and tumor growth. It is suggested that binding of adenosine to adenosine receptors (ADORs) induces suppression of inflammatory responses triggered by activating signals. In humans, the level of extracellular adenosine is regulated by two adenosine deaminases, ADA1 and ADA2. Decrease in the concentration of ADAs due to genetic defects in the ADA genes leads to serious perturbation in the functioning of the immune system, while an increase in

DOI: 10.1002/eji.201970400

the ADA activity is associated with numerous immune diseases and cancers. Here, it was demonstrated that both blocking of adenosine receptors with ADORs antagonists and activation with ADORs agonists or adenosine results in a dramatic decrease in the cytokines secretion by lipopolysaccharide (LPS) activated monocytes. When the concentration of adenosine is high, the activation of A_{2A} receptors reduces cytokine secretion and the addition of ADAs restores the level of the cytokines by decreasing the concentration of extracellular adenosine. In contrast, when the concentration of adenosine is low, the activation of cells is controlled by high affinity A_1 and A_3 receptors, which bind adenosine even in the presence of ADAs. A comparison of monocytes from ADA2 deficient patients with PAN and LGLL phenotypes suggests that ADA2 and ADORs play a crucial role in the regulation of cytokines secretion by activated immune cells in response to the extracellular adenosine. These results explain why the deficiency of ADA2 results in excessive TNF-α secretion and suggests new lines of therapy for this disease.

O099

Multiple myeloma patients have impaired neutrophil chemotaxis and extracellular traps production, improved by Simvastatin

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Introduction: Neutrophils migrate towards infections (chemotaxis) and kill pathogens by phagocytosis, neutrophil-extracellular-traps (NETs) and reactive-oxygen-species (ROS) release. These functions are impaired in elders compared to younger individuals. Multiple myeloma (MM) is a plasma cell malignancy, affecting mostly elders, characterised by immune dysregulation. Infections are a major cause of the high early and later death rates in MM. Statins reduce mortality in MM and have a beneficial effect on neutrophil function of elders. This study investigated neutrophil function and the effect of statins in MM.
Materials and methods: Ten newly-diagnosed MM patients (aged 50-85) and age/sex-matched controls were recruited. Neutrophils, *in vitro*, were exposed to 10µM Simvastatin and stimulated with 50nM phorbol-myristate-acetate or 15µM N-Formylmethionyl-leucyl-phenylalanine. NETs were measured as cell-free DNA, ROS by chemiluminescent reaction and Chemotaxis was reproduced using the Insall chamber.
Results: While no differences were identified in ROS, NETs production in MM patients was 14% lower than controls. Simvastatin increased NET production in 9 elders and in half of MM patients. Upon simvastatin treatment, NETs was 50% lower in MM patients compared to controls. Chemotaxis was impaired in MM patients: directionality and velocity were reduced 40%, while simvastatin treatment increased them respectively of 8% and 43%.
Conclusion: We show that MM aggravates the impairment of senescent neutrophil functions. This could have a role in the high incidence of infections afflicting MM patients. Statins treatment was able to improve chemotaxis in patients, suggesting a potential utility as an intervention to reduce infections in MM.

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DOI: 10.1002/eji.201970400

LB060

The percentage of Th1 lymphocytes in peripheral blood of patients with autosomal recessive hyper IgE syndrome

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Background: Hyper IgE syndrome is a primary immunodeficiency disease, characterized by increased susceptibility to a limited range of fungal and bacterial infections, especially Candida Albicans and Staphylococcus Aureus. The study of different subtypes of lymphocytes would be helpful in understanding of the disease pathogenesis. In this study, the percentage of Th1 lymphocytes in peripheral blood of patients with autosomal recessive hyper-IgE syndrome was investigated.
Methods: In this case-control study, six patients with autosomal recessive hyper IgE syndrome and seven age-sex matched healthy controls were studied. Peripheral blood mononuclear cells were isolated from venous blood, by ficoll gradient centrifugation. After cell stimulation and culture for 12 hours, the percentage of Th1 cells was evaluated by flow cytometry.
Results: The results of this study showed that the percentage of Th1 cells was significantly decreased in patients (9.48±1.61) compared to the control group (17.83± 6.16) ($P< 0.005$).
Conclusion: The reduction in Th1 lymphocytes may play an important role in the pathogenesis of autosomal recessive hyper-IgE syndrome and their increased susceptibility to bacterial and fungal infections.

LB097

LPS-responsive beige-like anchor (LRBA) deficiency: a systematic review of genetic, immunologic, and clinical features

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Bacground / Objective: LPS-responsive beige-like anchor protein (LRBA) deficiency is a rare monogenic primary immunodeficiency caused by biallelic mutations in the LRBA gene. These mutations usually abrogate the protein expression of LRBA, leading to a broad spectrum of clinical phenotypes including autoimmunity, chronic diarrhea, hypogammaglobulinemia, and recurrent infections.
In this study, we aimed to systematically review the existing evidence of clinical manifestations, molecular and laboratory findings, and management of patients with LRBA deficiency.
Methods: A total of 109 patients from 45 eligible articles were identified through searching in PubMed, Web of Science, and Scopus databases. For all patients, demographic, clinical, immunologic, and molecular data were collected.
Results: Of the patients with LRBA deficiency, 93 had homozygous and 16 had compound heterozygous mutations in LRBA. The most common clinical manifestations were autoimmunity (82%), enteropathy (63%), splenomegaly (57%), and pneumonia (49%). Reduction in numbers of $CD4^+$ T cells and regulatory T cells as well as IgG levels was recorded for 21.6%, 65.6%, and 54.2% of evaluated patients, respectively. B-cell subpopulation analysis revealed low numbers of switched memory and increased numbers of $CD21^{low}$ B cells in 73.5% and 77.8% of patients, respectively. Eighteen (16%) patients underwent hematopoietic stem cell transplantation due to the severity of complications and the outcomes improved in 13 of them.

DOI: 10.1002/eji.201970400

Conclusions: Autoimmune disorders are the main clinical manifestations of LRBA deficiency. Therefore, LRBA deficiency should be included in the list of monogenic autoimmune diseases, and screening for LRBA mutations should be routinely performed for patients with these conditions.

P0358

Alteration of phenotype and effector functions on NK cells contributes to immunosuppression during sepsis

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Sepsis results in life-threatening organ failure due to dysregulated host response to infection. It is the leading cause of mortality in most intensive care units and its global incidence is gradually increasing. Most information about the immune status of natural killer (NK) cells during sepsis has been obtained from animal models, while data from clinical septic patients is limited. In this study, we aimed to decipher NK cell immunity of septic patients in a more comprehensive way. We found that cytotoxicity of NK cells dramatically decreased during sepsis, likely due to the reduction of cluster of differentiation (CD)3$^-$CD56$^+$ NK cells in septic patients. Phenotypic changes of natural-killer group 2 member (NKG2) receptors, natural cytotoxicity receptors (NCR)s and killer immunoglobulin-like receptors (KIR)s demonstrated a shift towards inhibitory receptors on CD3$^-$CD56$^+$NK cells in septic patients. Cytokine production of interferon gamma (IFN)-g and tumor necrosis factor (TNF)-α on CD3$^-$CD56$^+$ NK cells in septic patients was also impaired after stimulation by phorbol-myristate acetate (PMA) and ionomycin. We found that the proportion of NK cells in lymphocytes was an independent risk factor that was associated with patient 28-day death in septic patients. Phenotypic changes of a shift towards inhibitory receptors and impairment of effector functions of NK cells might be an important mechanism of immunosuppression during sepsis

P0359

An allosteric MALT1 inhibitor as a molecular corrector for protein function rescuing in an immunodeficient patient

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MALT1 paracaspase plays a central role in lymphocyte antigen-dependent responses involving NF-κB activation. Previously defined MALT1 deficient patient with a homozygous MALT1 mutation at tryptophan (W) 580 to serine (S) presented a new class of combined immunodeficiency. The loss of tryptophan weakened interactions between the paracaspase and C-terminal immunoglobulin MALT1 domains leading to protein instability, which in turn reduced the protein levels and functions of MALT1.

DOI: 10.1002/eji.201970400

This had a direct impact on lymphocyte receptor-mediated NF-κB activation to mount immune responses of T and B cells. In searching for new potent MALT1 inhibitors, we discovered nanomolar, selective allosteric inhibitors that can bind to MALT1 by displacing the side chain of Trp580, locking the protease in an inactive conformation. Very interestingly, we also found that binding of allosteric inhibitors to MALT1 could increase the stabilization of mutated MALT1-W580S to reach that of wild-type MALT1 in proportion to their inhibitory potency. In addition, with restored levels of stable MALT1 protein, the allosteric inhibitors rescued NF-κB and JNK signaling in patient lymphocytes. After washing out these compounds, MALT1 paracaspase activity on cleaving the substrates HOIL1 and RelB was partly recovered. Altogether, this study presents a molecular corrector rescuing an enzyme deficiency by substituting for the mutated residue; this work also inspires new potential precision therapies to restore mutant enzyme activity in other deficiencies.

P0360

Establishment of an in vitro culture system for the analysis of B cell intrinsic defects in common variable immunodeficiencies

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Common variable immune deficiency (CVID) is the most common symptomatic primary immunodeficiencies (PIDs). Approximately 90% of all CVID cases display normal B-cell numbers in their peripheral blood but reduced levels of serum immunoglobulins, implicating impaired B cell function during adaptive immunity. To better understand the involvement of B cells in the etiology of CVID, we established a culture system that can be used to analyze the abnormalities in B cell activation and differentiation. B cells from CVID patients and healthy donors were cultured under T-independent (anti-IgM+CpG2006+IL-2) or T-dependent (SA-CD40L+IL-4) conditions for different time and analyzed for their survival, activation, Ig gene class switch recombination (CSR) and differentiation into antibody secreting plasma cells. Our results showed that naïve B cells from three CVID patients were largely defective in differentiating into $CD27^{+}IgD^{-}$ cells and IgM secreting plasmablasts. B cells from one of the patients exhibited reduced survival and impaired CSR. B cells from all these patients also showed elevated expression of HLA-DR molecules, which might cause abnormalities in B cell antigen presentation, and impaired responses to toll like receptor (TLR) 9 stimulation compared with healthy controls. These results demonstrate that our culture system is useful in elucidating the B cell intrinsic defects in CVID patients and should greatly facilitate the identification of the causal genes of CVID.

P0361

Prevalence of HIV/AIDS and economic development in Nigeria

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HIV/AIDS has emerged in the last decades as a major threat to health and the socio- economic conditions of developing countries in particular including Nigeria. The growing trend of HIV/AIDS is becoming worrisome to planners and the likes. The study used secondary data sourced from National Bureau of Statistics-NBS and Central Bank of Nigeria-CBN statistical Bulletin in its analysis. The analysis validated that the incidence of HIV/AIDS exerts serious negative influence on economic growth in Nigeria. This is due to the fact that HIV/AIDS reduces a great proportion of the working population and with huge cost implications, which reduces economic resources in the country. The

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DOI: 10.1002/eji.201970400

policy interventions recommended by the study for stemming the scourge of HIV/AIDS include: prevention of new infections, cost reduction of treatments, adjustments to employment, and development of activities like pycho-educational prog ramme to motivate and foster AIDS prevention behaviours among the Nigeria populace especially the youths.

P0362

Immune functional profiling for the confirmation of mendelian susceptibility to mycobacterial disease in South Africa

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In countries with high TB prevalence, individuals with Mendelian Susceptibility to Mycobacterial Disease (MSMD) are prone to severe, persistent, unusual and/or recurrent TB. Several mutations that disrupt the IL-12-IFN-γ cytokine pathways (essential for control of mycobacterial infections) have been described. These mutations result in distinct disorders, which vary in clinical presentation. The aim of this study was to implement a set of immune profiling assays to assess the IL-12-IFN-γ cytokine pathways in patients with suspected MSMD.
DNA and PBMCs were isolated from the blood of 17 participants with suspected MSMD. Whole exome sequencing was performed, and the data was processed using an in-house bioinformatics pipeline, TAPER™. IFN-γR1 and IL-12Rβ1 expression was assessed by standard flow cytometry, and IFN-γ and IL-12 signalling was assessed by the detection of pSTAT1 and pSTAT4 through intracellular phospho-specific flow cytometry. IFN-γ-induced IL-12 production and IL-12-induced IFN-γ production was assessed by ELISA. The functional and genetic data were reconciled to determine the extent of functional impairment associated with identified variants.
Plausible disease-causing variants were identified in 71% of participants, of which 47% were in known MSMD-associated genes. All but one of the identified variants were novel. Through these functional assays, we identified functional immune deficits in all participants.
The functional results were variable, which emphasises the importance of *in vitro* functional confirmation of MSMD. These assays could be routinely performed for patients with suspected MSMD. A molecular diagnosis with confirmed functional impairment paves the way for targeted treatment and improved disease management options for these patients.

P0363

Reactive dermatitis and non-tuberculous mycobacterial (NTM) infections are distinguishing features of interferon-gamma (IFNg) autoantibody syndrome in Asia

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Background: Interferon-gamma (IFNg) autoantibodies are associated with adult onset immunodeficiency in Southeast Asia; patients have exquisite susceptibility to non-tuberculous mycobacterial and other intracellular infections. We reviewed Singapore's national database and analysed clinical features of these patients, and tested functional responsiveness of their peripheral blood mononuclear cells (PMBCs).
Method: Using the national database of patients for whom the assay for IFNg autoantibodies was

DOI: 10.1002/eji.201970400

performed, we compared the clinical features of patients who were suspected and tested for IFNg autoantibodies, seeking to identify the most representative features.
Of the patients who were positive IFNg autoantibody syndrome and with the disease, we interrogated the functional immune responsiveness of their PMBCs using cytokine stimulation assays.
Results and conclusions: Between 2013 and 2017, of the 40 patients suspected of having the syndrome clinically, 10 patients tested positive. Among these, non-tuberculous mycobacterial infections (NTM) were present in 9 (90%) of the patients, reactive dermatitis in 7 (70%) and salmonellosis in 3 (30%). Co-existing non-tuberculous mycobacterial infections and reactive dermatitis were found in 6 (60%) of the patients who tested positive and none of those who tested negative. The co-existence of NTM infections and reactive dermatitis is thus extremely suggestive of IFNg autoantibody syndrome in our setting.
Cytokine stimulation assays revealed that PBMCs of patients with IFNg autoantibody syndrome were able to mount a robust response when their immune cells were challenged with *M abscessus*, lipopolysaccharide and Pam3Cys. PMBCs in the absence of IFNg-autoantibody containing plasma had normal functional responsiveness, suggesting pathogenicity of these autoantibodies.

P0364

Haploinsufficiency of murine NFκB1 confers a defective immune response and diverse late-onset complications

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Common variable immunodeficiency (CVID) is a disease characterised by enhanced susceptibility to recurrent infections, and the frequent development of non-infectious complications such as autoimmunity and cancer. The aetiology of CVID can depend on monogenic mutations, of which the most common in Europeans is the haploinsufficiency of *NFKB1*. This gene encodes a precursor protein, p105, that is cleaved into a shorter form, p50. Both p105 and p50 have undergone extensive characterisation, partly through use of the NFκB1-deficient mouse model (*Nfkb1*$^{-/-}$). We have examined young and ageing *Nfkb1*-haploinsufficient mice (*Nfkb1*$^{+/-}$) to elucidate the impact of *Nfkb1* haploinsufficiency. Young *Nfkb1*$^{+/-}$ mice developed normally, and showed no overt signs of disease. However, when challenged with lymphocytic choriomeningitis virus, *Nfkb1*$^{+/-}$ mice displayed an impaired T cell-dependent B cell response. Additionally, *Nfkb1*$^{+/-}$ mice developed late-onset complications, including splenomegaly, and a multi-organ immune cell infiltrate in the liver, lung, and salivary gland. This pathology coincided with multiple aberrant immunological manifestations, including the excessive differentiation of CD4^{+} T follicular helper cells and the marked expansion of an atypical B cell population characterised by low levels of cell surface CD21 and high expression of the key transcriptional regulator T-bet. Collectively, these results indicate that the loss of one allele of *Nfkb1* leads to early signs of immunodeficiency, followed by late-onset lymphoproliferative disease.

DOI: 10.1002/eji.201970400

P0365

CVID-associated tumors: Czech nationwide study focused on epidemiology, immunology and genetic background in a cohort of patients with CVID

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Background: Common variable immunodeficiency (CVID) is one of the most frequent primary immunodeficiencies (PIDs) manifesting with increased susceptibility to infections and immune dysregulation. There is also higher risk of malignancies including lymphomas and gastric cancers. Here, we present results of the Czech national multicentric study focused on epidemiology, immunology and genetics in patients with CVID, who developed a malignancy.
Methods: A cohort of 295 CVID patients was followed up for 3070 patient-years. SIR (Standardized Incidence Ratio) and RR (Risk Ratio) were calculated to determine risk of cancer development, to assess predictive value of comorbidities respectively. Furthermore, we performed immunophenotyping of lymphocyte subpopulations and Whole exome sequencing (WES) in all patients with lymphoma.
Results: Twenty-five malignancies were found in 22 CVID patients. The risk for malignancy was 6 times higher in CVID patients compared to the general population. Lymphomas and gastric cancers were the most common malignancies. Immune thrombocytopenic purpura (ITP) was significant negative prognostic factor with 3 times higher risk for tumor development. Post-treatment T and B cell lymphopenia associated with worse prognosis was found in the majority of patients in contrast to NK cells, which were not affected. WES revealed CVID and well as tumor susceptibility associated genes such as CTLA4, PIK3CD, PMS2; BRCA1, RABEP1, EP300, KDM5A respectively.
Conclusions: There is significantly higher incidence of malignancies in CVID patients, particularly lymphomas and gastric cancers. The history of ITP was identified as negative prognostic factor. WES confirmed wide genetic heterogeneity including causal and modifying variants of CVID as well as tumor susceptibility associated genes.

P0946

Screening of DUOX2 mutations in children with congenital hypothyroidism

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Objective: Aims to analyze the mutation spectrum and the prevalence of DUOX2 gene in 86 CH children, and analyzes the genotype-phenotype correlations of various DUOX2 gene variants.
Methods: 86 children diagnosed as CH by neonatal screening and 200 healthy children were selected. All exons and exon-introns of DUOX2 gene were sequenced by next-generation sequencing. Then the function and effect of potential pathogenic variants were analyzed by means of computational biology. The relationship between CH patients with different numbers of DUOX2 gene variants and the thyroid function test results were analyzed.
Results: The results showed that 20 different DUOX2 gene variants were found, including 10 non-synonymous variants, 4 nonsense mutations, 3 whole-code variants, and 3 frame-shift variants. Of these 20 variants, 16 are known to be pathogenic or likely pathogenic, and 4 are suspected to be uncertain significance, and their frequencies in healthy controls less than 1% . The prevalence of DUOX2 variants in 86 CH children was 30.2% (26/86), and three mutations(p.K530X, p.L1343F and p.R1110Q) had the highest prevalence in our study. Using protein homology modeling method, the analysis of its three-dimensional structure suggested that the mutations p.336_337del and p.T1107fs caused change of the protein.

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DOI: 10.1002/eji.201970400

Conclusions: 30.2% of the 86 CH children had at least one suspected disease-causing gene variants. Variants of p.K530X had the highest prevalence in our study. Children with DUOX2 single allele heterozygous mutation or compound heterozygous mutation have different thyroid gland.
Keywords: congenital hypothyroidism; DUOX2; next-generation sequencing; genotype-phenotype

P0947

Clinical characteristics and genetic profiles of severe combined immunodeficiency: a single center experience in China from 2004 to 2017

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Rationale: Severe combined immunodeficiency (SCID), a rare PID, is poorly characterized in China. In this study, we retrospectively reviewed patients with SCID referred to our hospital from 2004 to 2017, and summarized their clinical manifestations and genetic features.
Methods: Clinical features were summarized and outcome of each patient was traced from history records. Targeted gene capture combined with next-generation sequencing technology and Sanger sequencing were used to find out related gene mutation.
Results: Among 725 patients with PID, 95 (13.1%) were diagnosed as SCID. The mean time of delay in the diagnosis was 2.76 months (range, 0.4-22 months). Fifty-six of the 95 patients (58.95%) died by the end of 2016 with the mean age being 8.55 months. Respiratory tract infections were the most common (83 cases, 87.37%) including pneumonia and upper respiratory tract infection. Gastrointestinal infection and oral candidiasis was found in 70 (73.68%) and 42 (44.21 %) patients, respectively. BCG complications occurred in 30 of the 66 patients who received BCG vaccination. Transfusion-induced GVHD occurred in 8 patients. Eight patients received HSCT, one survived, who was transplanted twice. Total 30 mutations in IL-2RG were identified in 42 patients, including 18 novel mutations.
Conclusions: Diagnosis of SCID has improved over the last decade in China. Complications of BCG vaccine are an important warning sign for the presence of SCID and account for significant morbidity during disease progression. Establishing more diagnostic centers dedicated to the care of PID will facilitate early, correct diagnosis and better care of SCID in China.

P0948

How to identify Wiskott-Aldrich syndrome from immune thrombocytopenic purpura before genetic diagnosis: seeking clues from 43 patients with *WAS* gene mutation in China (2004-2016)

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Wiskott-Aldrich syndrome (WAS) is a rare and severe X-linked disorder with variable clinical phenotypes correlating with the type of mutations in the *WAS* gene. We retrospectively reviewed patients suspected WAS in our hospital from 2004 to 2016, and compared the clinical feature and laboratory examination between genetic confirmed WAS/XLT patients and patients diagnosed as immune thrombocytopenic purpura (ITP). In our study, Forty-three patients were finally diagnosed as WAS/XLT with gene confirmed. The median onset age of these patients was 1 month. The median diagnosis lag was 4 months. More than half patients had diarrhea. Twenty-three had pneumonia with 7 with severe symptom. Major bleeding events included skin spot or petechiae, per-rectal bleeding, epistaxis and intracranial bleeding. Twenty-nine patients had eczema and one patient had drug

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allergy. Three patients had autoimmune diseases among which 2 with autoimmune hemolytic anemia while one with autoimmune hemolytic anemia and IgA nephropathy. Total 43 mutations in *WASP* were identified, including 19 novel mutations. Nine patients received HSCT, all survived. Compared with 29 patients diagnoses as ITP, WAS patients had lower numbers of $CD8^+$T lymphocyte, increased NK cell number, reduced IgM level, but higher EOS, IgE and IgA level. In conclusion, the diagnosis of WAS should be considered in all males with ITP-like features, especially, if patients with far earlier onset age, decreased MPV (< 6.5 fl), elevated EOS/IgE and IgA level, reduced IgM level, increased NK cell number, diminished $CD8^+$T lymphocyte count, reversed CD4/CD8 ratio and poor response to IVIG.

P0949

Seroprevalence of hepatitis B antigen (HBs Ag) carriage HIV-infected people at the General Hospital of Grand-Yoff (HOGGY)

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Objectives: This study aimed to assess the seroprevalence of hepatitis B antigen (HBs Ag) carriage HIV-infected people at the General Hospital of Grand-Yoff (HOGGY).
Materials and methods: This is a prospective, descriptive and analytical study conducted from July to December 2017 at HOGGY and included patients from the HIV cohort through a non-exhaustive sampling. All enrolled patients gave informed consent to provide samples for HBV testing. The markers of chronic hepatitis B and hepatitis C were investigated in HIV and HBV co-infected patients using a microparticulate immunologic technique.
Results: This study included 147 HIV-infected patients and including 95 women (64.63%). Most of the HIV-infected patients - 80.95% - had medical care as their gateway and 89.8% among them were infected with HIV-1. At diagnosis, the median age at diagnosis was 46 years and the most represented age group was 30-39. 12.25% of patients were co-infected with HIV and hepatitis B virus. Women were the most affected by co-infection (61.11%) and 44.44% of them were married.
Investigation of hepatitis B chronicity markers indicated that all patient, except one, underwent anti-HBe seroconversion; no case of co-infection with HCV had been noted.
During follow-up, all patients were under antiretroviral therapy. Regarding treatment against hepatitis B virus, Tenofovir (TDF) remains the most used molecule with 83.33%, followed by Lamivudine (3TC) 16.67%.
Conclusions: HIV/HBV co-infection remains a real public health problem. Investigation of hepatitis B markers as well as liver damage should be systematic during HIV infection.

Key words: HIV/HBV co-infection - Seroprevalence - Senegal

P0950

A novel mutation in FAT4 caused a mild form of Hennekam syndrome with warty formations

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Background: Hennekam syndrome (HS) is a genetically heterogeneous disorder, with more than 50 cases diagnosed with HS worldwide. The aim of this study was to investigate the potential pathogenic

DOI: 10.1002/eji.201970400

mutations for a child with mild manifestation of HS by whole genome sequencing (WGS) analysis.
Material/Methods: The patient, a five-year-old girl, is mainly characterized by an asymmetric lymphangiectasia-lymphedema. The patient also has symptoms of warty formations on the skin of the palm, forefinger, and thumb of the right hand, which are similar to manifestations of human papillomavirus infection. The warty formations disappeared after several months of everolimus treatment. She currently has hirsutism defined on upper limbs and no any cognitive disorders. We performed a variant calling of the WGS data of the patient using GATK4 software followed by ANNOVAR. HS-related candidate mutations were defined by variants with MAF < 0.01 and the "deleterious" nomination, and they were further confirmed by Sanger sequencing.
Results: WGS identified a novel homozygous mutation (c.G11624A, rs12650153) in FAT4 as a cause of HS type II, and no previously reported mutation was detected.
Conclusions: Our findings could extend and enrich the diagnosis, management, and treatment of HS.

P0951

The role of NOD2 ligands muramyl peptides in chemotherapy induced cytopenia

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Cytopenia is a serious side effect of the treatment of oncological diseases, which prevents from carrying out an adequate and complete course of chemotherapy and radiation therapy. Neutropenia is the most common hematological complication in cancer patients, manifested by a decrease in the number of granulocyte cells in bone marrow. The presence of neutropenia is associated with a high risk of bacterial and fungal infections, and represents a threat to the lives of patients, since it can lead to septic shock and death. Muramyl peptides from bacterial cell walls, which normally form during the breakdown of commensal microflora support the immune homeostasis from the moment of birth, also affect hematopoiesis, adjusting cytopenia of various etiologies. Muramyl peptides which act via NOD2 pattern-recognition receptors to induce a cascade of reactions trigger the synthesis of cytokines, including G-CSF. GMDP provides protection against neutropenia comparable to G-CSF in an experimental model. Researchers from Canada, Russia, France and Japan demonstrated the effectiveness of muramyl peptides in the correction of cytopenia and led to the creation of a number of anticancer drugs. An innovative drug - a derivative of muramyl dipeptide (GMDP-A) has successfully passed pre-clinical trials in the implementation of the State program of the Russian Federation "Development of the pharmaceutical and medical industry" for 2013-2020 and is currently in the first phase of clinical studies.
˟The publication has been prepared with the support of the "RUDN University Program 5-100"

DOI: 10.1002/eji.201970400

P0952

Primary immunodeficiency with autoimmune polyendocrine and hemorrhagic syndromes. Surgical dental tactics

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The clinical picture of autoimmune polyendocrine syndrome varied depending on the form of PID. 12 years old patient S. with the indicated syndrome (the manifestations of which were autoimmune thyroiditis, migraine, epilepsy, ulcerative colitis, thrombocytopathy with hemorrhagic syndrome in the form of periodic petechial eruptions, ecchymosis and intestinal bleeding) was under our observation. In the oral cavity observed chronic generalized gingivitis and periodic aphthous rashes on oral mucosa. The terms of eruption were violated and the persistence of the primary tooth caused the formation of a sharp root edge and gingival trauma, which caused the exacerbation of chronic gingivitis. Required an operative intervention to remove the primary tooth. Before the planned surgery, the dose of intravenous immunoglobulin G used for anti-inflammatory purposes was increased from 0.4 g / kg to 0.8 g / kg, which ensured a reduction in the duration of bleeding in the postoperative period to 1 day. The operation was carried out on an outpatient basis of a dental clinic under local anesthesia, with the subsequent application of the hemostasis procedure and the application of a polymer film on alveolus for 1 day. Further healing of the wound occurred by secondary intention.

P1550

Expression of CD39 is correlated with HIV DNA levels in naïve Tregs in chronically infected ART naïve patients

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Background: Treg cells represent important viral reservoirs during chronic HIV infection. CD39 is closely involved in Treg-mediated immunosuppressive effects. However, CD39 expression on nTregs and mTregs and a relationship with HIV DNA levels during HIV infection is still unclear. In this study, we analyzed the distribution of HIV DNA in Treg subsets and the association between HIV DNA and CD39 expression on Treg subsets.
Methods: Sixty-two HIV-infected patients with different HIV stages and 14 uninfected individuals were enrolled. nTregs and mTregs were isolated by magnetic selection and flow cytometric sorting. HIV DNA was quantified by real-time polymerase chain reaction (PCR). CD39 expression on nTregs and mTregs was analyzed by flow cytometry.
Results: Higher levels of HIV DNA were detected in mTregs than those in nTregs during chronic HIV infection. The frequency of $CD39^+$ nTregs and HIV DNA levels in nTregs were increased in patients with advanced HIV infection. Furthermore, HIV DNA levels in nTregs correlated positively with $CD39^+$ nTreg frequency. $CD39^+$ nTreg frequency was also increased in immune non-responders.
Conclusions: mTregs and nTregs are both important reservoirs of virus during chronic HIV infection and HIV DNA levels increase in nTregs in patients with advanced HIV infection.

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DOI: 10.1002/eji.201970400

P1551

CD161 expression identifies a subset of CD4$^+$ T cells that promote clonal expansion of latent HIV-1 in ART-suppressed individuals

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The presence of an extremely stable latent reservoir for HIV-1 in memory CD4$^+$ T cells is the main obstacle to eradication despite effective antiretroviral therapy (ART). Recent studies have shown that latently infected cells undergoing clonal expansion without viral reactivation is a key phenomenon to maintain the long-term stability of the reservoir, yet its underlying mechanism remains unclear. Here we report that a subset of memory CD4$^+$ T cells, characterized by CD161 expression on the surface, are highly permissive for HIV-1 infection. These cells possess significantly higher survival and proliferative capacity comparing to their CD161-negative counterparts. More importantly, we found that these cells harbor significantly higher frequency of HIV-1 DNA and replication-competent latent viruses. By using massive single-genome proviral sequencing from ART-suppressed individuals, we confirm that CD161$^+$ CD4$^+$ T cells contain remarkably more identical proviral sequences, indicating clonal expansion of the viral genome in these cells. Together, our study identifies infected CD161$^+$ CD4$^+$ T cells as a critical driving force for the clonal expansion of HIV-1 latent reservoir, providing a novel mechanism to understand the long-term stability of HIV-1 latency, with implications to targeting the latently infected cells for clearance.

P1552

Scarless correction of Wiskott Aldrich Syndrome patient-specific iPSCs using CRISPR/Cas9 and piggyBac

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Wiskott-Aldrich Syndrome, one of the most severe immunodeficiency diseases, is caused by mutations in the WAS gene. Generation of integration-free patient-specific induced pluripotent stem cells (iPSCs) provide a safe approach to cure this disorder. Gene editing using CRISPR/Cas9 technology together with the piggyBac transposon system enables scar-less correction of the mutant gene as well as the restoration of cell function. In this study, we corrected the mutation in an integration-free WAS patient-specific iPS (WAS-iPS) without leaving any exogenous sequences in genome DNA. The corrected WAS-iPSCs (cWAS-iPSCs) can be differentiated towards myeloid-monocytic and megakaryocytic lineages in vitro. When differentiated into monocytes, the gene corrected cells reconstituted the expression of WASp as well as the IgG-regulated phagocytosis function. The macrophages we generated from corrected cells also obtained the formation of podosomes. We also restored the function in gene corrected megakaryocytes and gained normal-sized platelets compared with the parental WAS-iPSCs. Our study provides a promising safe solution towards future clinical usage of patient-specific iPSC-based cell therapy.

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DOI: 10.1002/eji.201970400

P1553

Novel genetic defect in autoimmune lymphoproliferative syndrome-like disease

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Background: Autoimmune lymphoproliferative syndrome (ALPS) is a genetic disorder of lymphocyte homeostasis due to impaired apoptosis. It was initially regarded as a very rare disease, but recent studies show that it may be more common than previously thought. Defects in a couple of genes have been identified in a proportion of patients with ALPS, but around one-third of such patients remain undefined genetically.
Objective: We describe 2 siblings presenting with ALPS-like disease. This study aimed to identify the genetic cause responsible for this phenotype.
Methods: Whole-exome sequencing and molecular and functional studies were performed to identify and characterize the genetic defect. Clinical and immunological analysis was also done and reported.
Results: The 2 patients presented with chronic lymphadenopathy, hepatosplenomegaly, autoimmune hemolytic anemia, immune thrombocytopenia, and the presence of antinuclear autoantibody and other autoantibodies, but normal double-negative T cells. They also suffered from recurrent infections. Novel compound heterozygous mutations of RASGRP1 encoding Ras guanyl nucleotide releasing protein 1 were identified in the 2 siblings. The mutations impaired T-cell receptor signaling, leading to defective T-cell activation and proliferation, as well as impaired activation-induced cell death of T cells.
Conclusions: This study shows for the first time that RASGRP1 mutation should be considered in patients with ALPS-like disease. We also propose to investigate the intracellular proteins involved in the T-cell receptor signaling pathway in similar patients but with unknown genetic cause.

P1554

Functional analysis of inflammation associated parameters in a case of NEMO deficiency before and after HSCT

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NEMO (NF-κB essential modulator) is the regulatory subunit of the IκB kinase (IKK) complex and a vital molecule in the NF-κB activation pathway. NEMO deficiency is a rare type of primary immunodeficiency that has been shown to display autoinflammatory manifestations in certain cases due to unknown mechanisms. This study used functional assays before and after hematopoietic stem cell transplantation (HSCT) to determine immunological parameters that could be responsible for autoinflammatory symptoms in a case of NEMO deficiency. Peripheral blood mononuclear cells (PBMC's) were isolated from patient and healthy donors using gradient centrifugation and treated with various TLR ligands and anti-CD3/anti-CD28. Immune responses were analyzed at the cytokine level, via protein expression, and through gene expression using the NanoString inflammation panel. Our results demonstrate that diminished IL-1β and IFN-γ cytokine responses of the patient to TLR4, TLR7/8 ligands and anti-CD3/anti-CD28 respectively, returned to healthy control levels following HSCT. Interestingly, a subset of neutrophils termed low density granulocytes (LDG) that were discovered in the PBMC fraction of SLE patients indicating an autoinflammatory state, was found at high amounts among the patients blood cells but decreased to a near-healthy amount after HSCT.

DOI: 10.1002/eji.201970400

Additionally; high ROS levels in neutrophils of the patient indicating susceptibility to activation were alleviated following treatment. To sum up, our study shows that HSCT restores deficient immune functions and immunological parameters suggested to be responsible for autoinflammatory manifestations in a case of NEMO deficiency.

P1555

Defined different etiology of adult-onset anti-GM-CSF autoantibodies in different diseases

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Over the years, anti-cytokine autoantibodies are unknows that how they act and why they appear in some diseases. Recently, it is reported that anti-GMCSF autoantibodies are found not only existing in pulmonary alveolar proteinosis (PAP) patients, but also in a few patients suffering from cryptococcal infection, nocardia infection and inflammatory bowel disease (IBD). However, the reason of manifestation these distinct different clinical phenotypes among patients with anti-GM-CSF autoantibodies are still unclear.
We hypothesis that the anti-GM-CSF autoantibodies might manifest different mechanism of action, which lead to have different biological effect and further to cause the autoimmune or infectious problem. In our previously studies' publication on *Journal of Clinical Immunology (JoCI)*, we already recruited some cryptococcal infection or PAP patients and have some preliminary findings. We also apply multiplex assay to recruit various infectious patients with anti-GM-CSF autoantibodies and identify the anti-GM-CSF autoantibodies mechanisms in different kinds of patients through clinical diagnoses or molecular biology.
In our current work, we aim to characterize the property of anti-GM-CSF autoantibodies from cryptococcosis and PAP patients through serology analysis and B cell cloning technology. We expect our study could provide a better understand the pathogenesis between cryptococcosis and PAP, and even other patients with anti-GM-CSF autoantibodies.

P1556

Vascular inflammation biomarkers levels are increased in HIV treatment-naïve subjects and decreases after one year of treatment with antiretroviral therapy

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HIV infection is associated to a greater prevalence of high-risk to develop Cardiovascular disease (CVD), even though the current antiretroviral therapies (ART) have less cardiometabolic effects, inflammation and immune activation remains, promoting a vascular activation and inflammation, which is a classic hallmark in the development of CVD.
Objective: measure soluble factors associated to vascular inflammation in treatment-naïve HIV subjects and after one year of treatment with ART.
Methods. Vascular inflammation and coagulation biomarkers were quantified by a bead-based immunoassay and ELISA; respectively. Manufacturer´s instructions were followed. Results were analyzed with no parametric ANOVA and Kruskal-Wallis test.

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DOI: 10.1002/eji.201970400

Results: In HIV naïve-treatment subjects: ICAM-1, VCAM-1, MPO, NGAL, OPN and Dimer-D concentrations were significant higher when comparing to control subjects. After a year of ART most of the biomarkers decrease to baseline levels, importantly VCAM-1, MPO, NGAL and Dimer-D reduce significantly ($p < 0.05$). ICAM-1 and Cystatin levels, present a discreet and no significant decrease after one year of ART. OPN remain higher regardless treatment. No changes were detected in myogolobin levels.
Conclusion: HIV infection promotes inflammation in the vascular endothelium, increasing the levels of biomarkers related endothelial damage and inflammatory mechanisms linked to CVD development. ART decrease the levels of these biomarkers however molecules like ICAM-1, Cystatin and OPN remain, contributing to the vascular endothelial inflammation that has been describe in HIV infection even in presence of ART. We identified an appropriate set of biomarkers for HIV positive subjects, treated or not-treated with ART, that improve the monitoring of development of CVD.

P1557

sCD163, SAA and Calprotectine contribute to the low grade chronic inflammation in HIV-infected subjects even after one year of antiretroviral therapy

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The introduction of Antiretroviral Therapy (ART) to HIV+ patients have increased the survival; however, these people have a greater risk for diseases as cardiovascular disease (CVD). The specific mechanism that leads to an increased risk of CVD in HIV+ patients is not clear; however, it is believed that involves the virus itself, effects of ART, burden of traditional and nontraditional risk factors. Additionally, the HIV-related low-grade chronic Inflammation plays a role in accelerating the development of CVD.
Objective: To measure biomarkers of inflammation in naive HIV+ patients and after one year under ART.
Methods: Cytokines and sCD163 were quantified by a bead-based immunoassay and ELISA; respectively. The manufacturer´s instructions were followed. The results were analyzed and are expressed in pg/mL or ng/mL.
Results: In response to HIV infection the concentrations of SAA, sCD163, MCP1, IL-8, IL-18, Calprotectin and IL-10 were higher. After a year of ART all but SAA tend to significantly ($p < 0.05$) decrease almost to baseline levels. sCD163, SAA and Calprotectin remain higher regardless of treatment (* $p < 0.05$ for sCD163).
Conclusion: HIV infection increase inflammatory proteins which decrease after ART; however, sCD163, SAA and calprotectin remain high contributing to the persistent low-grade inflammation state. ART reduces but it does not normalize completely the level of inflammation and immune activation. It is important to identify the main inflammatory biomarkers that remain elevated in HIV infection, even after ART, since these induce activation and damage in other tissues, increasing the risk of non-AIDS comorbidities such as CVD.

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DOI: 10.1002/eji.201970400

P1558

Emerging strains of HIV-1 subtype-C acquire multiple NFkB-binding sites in the LTR in co-infected individuals.

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Introduction: Tuberculosis remains one of the most prevalent opportunistic infections in HIV-infected persons. Higher replication and disease severity is also significant in HIV individuals co-infected with HCV and HBV infections. The molecular mechanisms responsible for this enhanced replication are obscure though. NF-κB plays an important role in regulating the gene expression from the viral promoter and controlling viral latency. HIV-1 subtype C acquires extra NFkB sites achieving higher degree of transcription. We hypothesize that the new viral strains in co-infected individuals gain a potential selective advantage as a consequence of the acquired transcription factor-binding sites in the co-infection making it more replication competent.
Material and methods: The number of NF-kB binding sites in Long Terminal Repeats region of HIV genome was analysed in HIV mono-infected as well as HIV-TB, HCV or HBV co-infected ART Naive Individuals. The genomic DNA was isolated and LTR region was amplified. Nucleotide sequence of the amplified products was obtained and multiple sequence alignment was performed. NFkB binding sites were identified in the LTR sequences.
Results: HIV-1 subtype C isolates from HIV-TB, HCV or HBV co-infected individuals had higher number of NFkB sites as compared to isolates from HIV-mono-infected individuals. The number of NFkB sites correlated positively with viral load and negatively with CD4 cell count in these individuals.
Conclusion: HIV-1 Subtype C achieved a higher degree of transcription and in turn enhanced replicative fitness with increased NFkB site in co-infected individuals indicating the virus adopting a novel strategy of strengthening its promoter to gain fitness.

P2139

Functions of ZBTB24 in human and murine B cells

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ZBTB24 has been identified as the causative gene in patients with Immunodeficiency, Centromeric Instability and Facial Anomalies syndrome type 2 (ICF2), a rare autosomal recessive disease. Most ICF2 patients harbor *ZBTB24* nonsense mutations, and suffer from recurrent respiratory and gastrointestinal infections due to hypogammaglobulinemia, most likely due to the lack of germinal center (GC) structure and circulating $CD19^{+}CD27^{+}$ memory B cells (Bmem). ZBTB24 belongs to the large ZBTB family of transcriptional repressors with members like BCL6 (ZBTB27) & ZBTB7A (LRF) playing essential roles in B-cell development/functions. ZBTB24 is highly expressed in human B-cell compartment, and knockdown of its endogenous expressions hampers the cell-cycle progression in human GC-derived B lymphoma Raji cells *via* upregulating, in a BCL6-independent fashion, the expressions of IRF4 & PRDM1, two essential transcriptional factors involved in GC-reactions. Collectively, ZBTB24 appears to control the *in vivo* Bmem development *via* regulating the proliferation and/or terminal differentiation of human GC-B cells. Intriguingly, despite the low baseline antibody levels in B-cell specific zbtb24-knockout mice, no significant differences in *in-vivo* antibody responses were observed in these mice after immunizations with T-cell-dependent or -independent antigens. Thus, functions of ZBTB24 in B cells seem to differ in mice and humans.
Funding acknowledgements: This study was financially supported by the National Key Research

DOI: 10.1002/eji.201970400

and Development Program of China (2017YFB0403805); National Natural Science Foundation of China (31670888/31370871/81470564); Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and Ministry of Education (IRT1075).

P2140

The stage-specific impairment of granulopoiesis in HIV-infected patients with neutropenia

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Objectives: To assess stage-specific impairment of granulopoiesis in bone marrow of HAART-naïve and HAART-experienced patients with neutropenia.
Methods: A total of 48 HAART-naïve and 49 HAART-experienced patients from 2016 to 2018 were recruited and divided into non, mild, and moderate-to-severe neutropenia groups according to their neutrophil counts. The bone marrow aspirates and peripheral blood were collected and analyzed by multicolor flow cytometry for granulocyte subsets, hematopoietic stem/progenitor cells (HSPC), apoptosis, and emigration and retention of different subsets.
Results: Compared with healthy donors, the percentages of circulating segmented neutrophils were significantly decreased along with an increase of immature neutrophils in both groups. In bone marrow, the proportion of segmented neutrophils among total granulocytes were decreased in HAART-naïve patients with moderate-severe neutropenia, but not in HAART-experienced patients. Apoptosis of relatively differentiated neutrophils was significantly accelerated in HAART-naïve patients with neutropenia. In HAART-experienced patients with neutropenia, the proportion of granulocyte macrophage progenitors was decreased and the expression of CXCR4 of segmented neutrophils was increased compared to patients without neutropenia.
Conclusions: HAART-naïve patients with neutropenia exhibited increased apoptosis of relatively differentiated neutrophils at committed granulopoiesis, while impaired lineage determination and enhanced retention of segmented neutrophils were observed in HAART-experienced patients.
Keywords: HIV/AIDS, Neutropenia, Multicolor flow cytometry, Granulopoiesis, Bone marrow.

P2141

114 cases of chronic granulomatous disease in mainland China

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Chronic granulomatous disease (CGD) is a rare inherited disorder. Mutations in the gene encoding CYBB are responsible for the majority. During 2003 to 2018, we enrolled 114 cases diagnosed with CGD in Children's Hospital of Chongqing Medical University. Clinical data were collected, neutrophil function was detected, and the genotype of patients and fetal cells in carrier mother´s amniotic fluid were detected.114 CGD patients (109 male, 5 female), from 20 provinces. The mean onset age was 4 months, and the mean diagnosis age was 23.1 months. To date, 33 patients have died, 27 patients have transplantation, and 12 patients were lost. For all patients, the types of genetic mutations include CYBB (95), CYBA (8), NCF1 (3) and NCF2 (2), and 6 cases have not been found yet. The type of infection symptoms in this cohort included recurrent fever (100%), pneumonia (92.1%), tuberculosis (58.8%), BCG disease (55. 1%), repeated diarrhea (45.6%) and others. In addition, we prenatally diagnosed 7 XL-CGD, 5 healthy fetuses and 3 carriers (a XL-CGD female infant patient with X-chromosome inactivated). Children with recurrent infection not easy to cure, with or without BCG disease and family history, should be alert to CGD. BCG disease and tuberculosis is still a very severe problem in CGD patients in China. Early diagnosis and prophylaxis treatment can prolong the survival

DOI: 10.1002/eji.201970400

time of CGD patients. DHR-1,2,3 test and NBT test can quickly aid in the diagnosis of CGD. Molecular analysis is an important tool for identifying diagnosis, prenatal diagnosis of CGD.

P2142

Clinical features and genetic analysis of 47 Chinese patients with x-linked hyper-igm syndrome

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Rationale: X-linked hyper-IgM syndrome (XHIGM) is one type of primary immunodeficiency diseases, resulting from defects in the CD40 ligand/CD40 signaling pathways. Here, we retrospectively reviewed clinical, laboratory and genetic characteristic of XHIGM in Chinese population, thus further improving diagnosis and treatment for XHIGM.
Methods: We collected and analyzed 47 Chinese patients, who were diagnosed and followed up in hospitals affiliated to Shanghai Jiao Tong University School of Medicine from 1999 to 2017. Targeted gene capture combined with next-generation sequencing technology and Sanger sequencing were used to find out related gene mutation.
Results: The median onset age of these patients was 7 months (range: 20 days-66 months). Thirty-six percent of them had positive family histories, with a shorter diagnosis lag. The most common symptoms were recurrent sinopulmonary infections (40 patients, 85%), neutropenia (22 patients, 47%), protracted diarrhea (21 patients, 45%), and oral ulcer (19 patients, 40%). Ten patients had BCGitis. Six patients received hematopoietic stem cell transplantations and four of them had immune reconstructions and clinical remissions. Twenty-seven unique mutations in *CD40L* gene were identified in these 47 patients, with 18 novel mutations.
Conclusion: To our knowledge, this report provides the largest cohort of patients with XHIGM in China. Mutation analysis is an important tool for XHIGM diagnosis.

P2143

Defective glycosylation leads to impairment of gp130 expression and gp130-dependent STAT3 signaling in PGM3-deficient patients

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Congenital disorders of glycosylation are a rare group of genetic disorders due to defects in protein glycosylation. Phosphoglucomutase 3 (PGM3) is an enzyme necessary for the synthesis of Uridine Diphosphate N-Acetylglucosamine, an important precursor for protein glycosylation. Patients with autosomal recessive PGM3 deficiency have a multi-systemic disorder characterized by a neurologic impairment and clinical features classically observed in autosomal dominant hyper-IgE syndrome due to STAT3 mutations; including recurrent pneumonias, skin abscesses, elevated levels of IgE, and abnormalities in connective tissues and bones.
We hypothesized that gp130, a highly glycosylated protein and co-receptor of the cytokine IL-6, would be weakly expressed on PGM3-deficient cells, due to impaired glycosylation.
We studied 6 PGM3-deficient patients from 3 kindreds and showed that IL-6-driven STAT3 phosphorylation was impaired in their PBMCs and EBV-transformed B cells. Accordingly, the induction of SOCS3 target gene was significantly decreased. In contrast, the patients had normal STAT3 phosphorylation and SOCS3 induction downstream of IL-10, a cytokine whose signaling is independent of gp130. Flow cytometry and immunoblotting showed significantly lower gp130

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DOI: 10.1002/eji.201970400

expression in peripheral T-cells and EBV-transformed B cells from PGM3-deficient patients compared to healthy donors. We did also show that *in vitro* inhibition of N-glycosylation, using tunicamycin in EBV-transformed B cell line from healthy donor, alters gp130-mediated signaling.
Collectively, our findings demonstrate that defective glycosylation in PGM3-deficient patients results in reduced expression of gp130 and consequently, impaired gp130 dependent STAT3 phosphorylation and defective IL-6 signaling. This may account for the overlapping clinical features shared by PGM3 and STAT3 deficient patients.

P2146

Lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency in a patient with hypogammaglobulinemia and elevated TCRab$^+$CD4$^-$CD8$^-$ double negative T cells

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Background: Lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency (OMIM #614700) was initially liked to early-onset hypogammaglobulinemia, inflammatory bowel disease, autoimmune disease, and respiratory infections. However, further reports expanded this phenotype and described, among others, patients with autoimmune lymphoproliferative syndrome (ALPS)-like phenotype.
Methods: We report the case of a girl with homozygous mutation in *LRBA,* presenting with unusual association of hypogammaglobulinemia and elevated TCRab$^+$CD4$^-$CD8$^-$ double negative T (DNT) cells. Next-generation sequencing was used for genetic analysis.
Case report: The patient is a 15-year-old offspring of second-degree consanguineous Algerian parents with no family history of primary immunodeficiency. At the age of 2 years, she presented with autoimmune haemolytic anemia, immune thrombocytopenic purpura (Evans syndrome), splenomegaly, lymphadenopathy, arthralgia and abdominal pain. At the age of the 13 years she developed granulomatous and lymphocytic interstitial lung disease and autoimmune hepatitis. Immunologic evaluation at the age of 15 years was notable for hypogammaglobulinemia (low IgG: 3,57 g/L, and low IgM: 0,42 g/L), nearly absent switched memory B cells, high percentage of CD21^{low}CD38^{low} B cells (22% in CD20+ B cells) and elevated DNT cells comprising 4% of CD3$^+$ T cells. Genetic analysis revealed homozygous nonsense mutation in *LRBA* (p.Gln2470Ter), with no pathogenic variants in known ALPS-causing genes (*TNFRSF6, TNFSF6, CASP10, CASP8, FADD*).
Conclusion: Our observation broadens the spectrum of clinical phenotypes associated with LRBA deficiency. It emphasizes the importance of considering mutation in *LRBA* in patient with hypogammaglobulinemia and ALPS-like phenotype.

DOI: 10.1002/eji.201970400

LB127

Outcome of 6 patients with Good's syndrome: rare coincidence combined immunodeficiency and secondary cancer from the single centre perspective

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Most of patients are diagnosed after surgical thymectomy, therefore Good´s syndrome is not recognized in the first decades of life. Contrary to CVID and humoral immunodeficiency bacterial infection with encapsulated pathogen and secondary lymphoproliferative disease are observed to a lesser extent.
We observe outcome of 6 patients with Good's syndrome, natural history and clinical data
Good´s syndrome was not associated with autoimmune processes (e.g. PRCA, poliendocrinopathy, myasthenia gravis) and rare infection with encapsulated bacteria, but in contrast to CVID a shorter survival period (average 5.5 year after thymectomy) due to secondary cancers.
Large cohort of 1470 thymoma patients show tumor recurrence in 136 patients, whereas only 64 died due to tumor. Our patients presented predominantly lymphocyte rich thymoma and secondary malignancy. In 3 of 6 patients, the main cause of death was squamous cell carcinoma of the nasopharynx, one person -breast cancer (dissemination despite radical resection), 2-cervical cancer .
As described previously by Granato F. et al. among thymomas, the cortical subtype was associated with a higher risk of additional malignancy and mortality. Interestingly, maean survival was 69.3 months i.e. similar time period for our patients: five-year survival was observed in 5 patients.
In conclusion Good's syndrome is combined immunodeficiency with unique host-pathogen and cancer constelation. Compared to immuncompetent patients with thymomas, the main complication was infection and secondary cancer rather than autoimmunity or tumor recurrence. Thus svere combined immunodeficiency in Good' syndrome may be source of both: low immune surveillance of tumor development and weak clearance of oncogenic infection.

P2917

Role of plasma exosomes on CD8$^+$T cell and its relationship with CD4/CD8 ratio in HIV-infected cART-treated complete responder

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Objective: To investigate the characteristics of plasma exosomes in HIV-infected patients received long-term ART, and to analyze the relationship between dynamic changes of plasma exosomes and CD4/CD8 ratio, in addition to explore the potential role of plasma exosomes on CD8$^+$ T cells.
Methods: Nineteen complete responders (CR) received ART were enrolled and divided into abnormal group (CD4/CD8＜0.8, n=10) and normalized group (CD4/CD8＞0.8, n=9) by the CD4/CD8 ratio. Five healthy controls (HC) were enrolled. Compared the concentrations of plasma exosomes from three groups (n=5 per group), and to investigate the effects of plasma exosomes on the function of CD8$^+$ T cells.
Results: The concentration of exosomes in abnormal group was significantly higher than that in normalized group and the HC group, and negatively correlated with the CD4/CD8 ratio (P＜0.05). Plasma exosomes from abnormal group reduced the frequency of CD38 and HLA-DR co-expression on CD8$^+$ T cells, decreased the secretion of IFN-γ and IL-2 in CD8$^+$ T cells, inhibited the proliferation

DOI: 10.1002/eji.201970400

of CD8⁺ T cells. And the inhibitory effect was correlated with donors' CD4/CD8 ratio ($P<0.05$, $P<0.001$, $P<0.001$).
Conclusions: Plasma exosomes from CD4/CD8 abnormal group inhibited the activation, secretion and proliferation of CD8⁺ T cells, suggesting that elevated exosomes in CRs with abnormal CD4/CD8 ratio have an inhibitory effect on over-activation and excessive inflammation in HIV patients.

P2918

A novel bruton tyrosine kinase gene mutation was discovered in an adult with X-linked agammaglobulinemia by ABO blood typing discrepancy

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Objective: ABO typing discrepancy could be frequently seen in the patients due to variable reasons. In this study, ABO typing discrepancy was found in a 24-year-old young man with arthralgia, whose forward ABO group was O and reverse ABO group was AB. Primary immunologic deficiency (PID) was speculated in this patient, especially X-linked agammaglobulinemia (XLA). We aim to investigate the underlying molecular mechanism of the patient's ABO typing discrepancy.
Methods: All immunoglobulin isotypes were detected by specific protein analyzer. Lymphocyte subgroups of the patient were measured by flow cytometry. All of 19 exons and boundaries of BTK gene were amplified by PCR and all PCR products were purified then sequenced by 3730 DNA analyzer. Btk protein in the leukocytes of the patient was detected by Western blot.
Results: All immunoglobulin isotypes were extremely low, with IgG< 0.26 g/L (normal, 7.0-16.0 g/L), IgA< 0.065g/L (normal, 0.7-4.0 g/L), IgM< 0.046 g/L (normal, 0.4-2.3 g/L). The absolute count of CD19+ B lymphocytes was 3/μL (normal, 80-616/μL) and accounted for only 0.03% (normal, 5-22%) of all lymphocytes. A novel nonsense mutation, c.817G>T (p.Glu272Ter) in exon 9 of BTK gene was discovered by DNA sequencing. No Btk protein expression could be detected in the leukocytes of the patient by Western blot.
Conclusion: Humoral immunodeficiency, such as XLA is one of the causes of ABO typing discrepancy. A novel nonsense mutation, c.817G>T (p.Glu272Ter) found in BTK gene, which caused XLA, was the reason for the lack of antibody.

P2919

De novo mutation in the NADPH binding domain of CYBB in a patient with severe infections

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DOI: 10.1002/eji.201970400

Chronic granulomatous disease (CGD; OMIM no. 306400) is a rare genetic disorder of the innate immune system, approximately 1 case in 200,000-250,000 newborns. Patients with CGD have frequent life-threatening infections of the lung, skin, lymph nodes, and liver accompanied by the characteristic granulomas formation in response to chronic inflammation. CGD is caused by a defect in the NADPH oxidase complex, which is essential for bactericidal function of phagocytes. CYBB gene is located on the X chromosome, encodes p91-phox beta polypeptide, which is an essential component of phagocytic NADPH-oxidase. Mutations in CYBB gene are found in about 70% of CGD patients. Most of the patients are male diagnosed in childhood, usually before age 5. Here, we described a 15 years old boy, who suffered fever, cough and chest pain for more than one month. Enhanced computed tomography chest scan showed bilateral multiple nodular and patchy consolidation. The infectious agent was Burkholderia multivorans decided by sputum, bronchoalveolar lavage fluid and lung tissue culture. Genetic analysis revealed a de novo missense mutation c.1241C>T/p.T414I in the CYBB gene, which is the critical region of the oxidase activity. The Ca2+ concentration in monocytes of the patient was lower than healthy controls detected by Calbryte-520AM. The relationship between samples was confirmed using short tandem repeats genotyping. However, the patient's mother did not carry the same mutation in CYBB gene, indicating a de novo mutation. Our findings expand the genetic spectrum of X-linked CGD and a genetic de novo mutation should be considered.

P2920

Identification of the differentially expressed genes involved in the synergistic neurotoxicity of a HIV protease inhibitor and methamphetamine

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Human immunodeficiency virus (HIV)-infected people have been shown to abuse psychostimulants such as methamphetamine (METH). Acquired immunodeficiency syndrome (AIDS) patients taking METH and antiretroviral drugs could suffer severe neurologic damage and cognitive impairment. To reveal the underlying neuropathologic mechanisms of a HIV protease inhibitor (PI) combined with METH, growth-inhibition tests of dopaminergic cells andRNA sequencing wereundertaken. A combination of METH and a PI caused more growth inhibition of dopaminergic cells than METH alone or a PI alone. Furthermore, we identified differentially expressed gene (DEG)patterns in the METH *vs*.untreated cells(1161 genes), PI *vs*. untreated cells(16 genes), METH-PI *vs*. PI (3959 genes), and METH-PI *vs*. METH groups (14 genes). The DEGs in the METH-PI group were also verified in the brains of a mouse model using quantitative polymerase chain reaction and were involved mostly in the regulatory functions of cell proliferation and inflammation. Such identification of key regulatory genes could facilitate study of their neuroprotective potential in the users of METH and PIs.

P2921

The potential binding target on HIV gp41 cytoplasmic tail region for HIV protease inhibitor

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HIV protease inhibitors were initially designed to bind HIV protease and block the maturation of viral gag-pol polyprotein. However, due to the off-target effect, HIV protease inhibitors not only block the activity of HIV protease, but also unexpectedly hinder the HIV fusion with host cells, which indicates that there are other target sites for HIV protease inhibitors. This study demonstrated that HIV protease inhibitors (atazanavir and darunavir) can bind to gp41 cytoplasmic tail region, and tentatively identified the binding site of HIV protease inhibitors at the gp41 cytoplasmic tail by molecular docking. We also

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DOI: 10.1002/eji.201970400

proved that inhibitor binding prevented the function of gp41 in membrane fusion. The mechanism of gp41 inhibition was explored by studying the conformational change of gp41 trimer using the technique of split green fluorescent protein. The finding of this project could benefit the design of specific HIV gp41 inhibitors.

P2922

A rare case of leukocyte adhesion deficiency Type 1 with glucose-6-phosphate dehydrogenase deficiency

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Leukocyte Adhesion Deficiency (LAD) is a category of Primary Immunodificiency Disease (PIDD) that inherited autosomal recessively and characterized as defective leukocyte adhesion to the vascular wall, hence causing failures of PMN migration to infection sites. The major form of LAD, LAD-1, is caused by mutations in ITGB2 gene on chromosome 21q22.3. Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency is an X-recessive genetic disorder caused by mutations on G6PD gene on chromosome Xq28 leading to congenital dysfunction of metabolic system. In this article, we reported a very rare case of LAD-1 patient accompanied with G6PD Deficiency hospitalized in Kunming Children's Hospital, China. We summarized results of the patient from clinical diagnosis and routine laboratory examination, investigated the LAD-1 diagnosis marker CD18 via flow cytometry, and identified a novel pathogenic mutation on ITGB2 by WES and a pathogenic mutation on G6PD by Sanger Sequencing. We discussed the genetic and environmental factors related to pathology of LAD-1 and G6PD Deficiency in Southwest region of China. We also proposed potential molecular mechanisms of ITGB2 deficiency in LAD-1 pathogenesis. Reporting and analyzing existing LAD-1 cases can facilitate further studies of LAD-1 diagnosis and treatment, and also provide genetics bases for the prenatal diagnosis of LAD-1.

Immune Supression, Tolerance and Exhaustion

O321

Persistent stimulation with Mycobacterium tuberculosis antigen impaired the proliferation and transcriptional program of hematopoietic cells in bone marrow

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To investigate whether *Mycobacterium tuberculosis* (*M. tuberculosis*) persistent antigen stimulation impairs the proliferation and differentiation of hematopoietic cells characterized as lineage$^-$ c-Kit$^+$ (LK cells), C57BL/6 mice were persistently stimulated with *M. tuberculosis* antigens containing ESAT6, CFP10 and MH along with adjuvant weekly for 12 or 22 weeks. The cytokine production of spleen T cells, proliferation of LK cells and transcriptional differentiation among bone marrow (BM) c-Kit$^+$ cells were investigated. Meanwhile, the mice were treated with IL-2 and the therapeutic effects were analyzed. We found that antigen specific IFN-γ production by spleen CD4$^+$ T cells increased following the antigen stimulation for 12 weeks, but it declined after continuous stimulation for 22 weeks. A long-term exposure of mice to *M. tuberculosis* antigen compromised LK cell proliferation. The expression of *IRF8* and *Batf2* involved in myeloid differentiation up-regulated, while that of *NOTCH1* and *GATA2* participated in lymphoid commitment down-regulated. The concentrations of IFN-γ in BM of antigen

DOI: 10.1002/eji.201970400

persistent group were higher than that of sham control at 12th week, while the concentrations of IL-2 were lower compared with antigen transient stimulation control. Following IL-2 treatment, the concentrations of IL-2 in BM increased while IFN-γ got declined. IL-2 treatment could restore the expression levels of those transcription factors and the proliferation of LK cells. Our results indicated that *M. tuberculosis* antigen persistent stimulation decreased the proliferating activity of LK cells, promoted myelopoietic differentiation, and repressed lymphopoietic differentiation as a consequence of elevated IFN-γ production. IL-2 complementation contributed to maintaining the homeostasis of hemopoiesis.

O322

Understanding the tumor immune escape and the immune modulatory function of cancer stem cells

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Cancer therapy is experiencing a paradigm shift due to the clinical success of immune checkpoint inhibitors (ICI), yet efficacy remains limited to a few tumor types and a minority of patients. In an increasing number of cancers, tumor populations called cancer stem cells (CSCs) or tumor-initiating cells (TIC) have been defined. Strong evidence is supporting the immunoresistance feature of CSCs. CSCs can also assist immunosuppression in the Tumor microenvironment (TME) through interaction with other tumor stroma cells. Understanding the mechanisms leading to the immunotolerance of CSCs will be essential for treatment of immune-resistant and metastatic tumors. The identification of such mechanisms faces challenges when applying the widely used transplanted tumor models since they fail to recapitulate the tumor microenvironment as it develops during the progression of human tumors. We previously generated a transgenic model of autochthonous melanoma, named TiRP, which express murine MAGE-type antigen P1A, and can be used as a more faithful preclinical model for immunotherapy. In this model, the CSCs and CTCs are immune resistant. They are devoid of surface MHC I expression and are resistant to both antigen-specific T-cells- and NK-cells-mediated killing. In contrast to non-CSC tumor clones from the TiRP tumor (T429.11), CSCs/CTCs, when transplanted into recipient mice, generate a highly immunosuppressive TME with high numbers of PMN- and Monocytic-MDSCs, and are resistant to immune therapy.

O323

Myeloid Response Score (MRS): a simple immune signature that reflects intratumoral myeloid contexture and predicts prognosis of hepatocellular carcinoma

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Myeloid cells, including monocytes/macrophages (Mo/Mφ), neutrophils, dendritic cells, and myeloid-derived suppressor cells (MDSCs), can marshal divergent immune responses according to different pathological conditions. In cancer, the myeloid immune components play important roles in disease progression as well as in therapy, suggesting that appraising the overall and exact intratumoral myeloid response can provide valuable information that may help to predict cancer prognosis and tailor more precise therapeutic strategies. In this study, we constructed Myeloid Response Score

DOI: 10.1002/eji.201970400

(MRS), a simple immune signature based on 2 immunohistochemistry features, in hepatocellular carcinoma (HCC) and validated its prognostic relevance in a large, multi-center cohort of patients (n = 1177). Using multiplex immunofluorescence staining, we observed a shift of predominant myeloid population from $CD169^+$, $CD169^-CD11b^-CD14^+$, and $CD169^-CD11b^-CD163^+$ Mo/Mφs in MRS-low HCC to neutrophils/granulocytic MDSCs and $CD11b^+CD169^-$ Mo/Mφs in MRS-high HCC. Transcriptomic and flow cytometric analyses showed that MRS-high HCC exhibited patterns of $CD8^+$ T cell exhaustion including impaired cytotoxicity and increased PD-1 expression, suggesting highly suppressive immune microenvironment. Using the RNA-seq data from TCGA-LIHC project, we depicted the dynamic "climb trajectory" of MRS values between HCC tumors, and confirmed its association with $CD8^+$ T cell dysfunction and poorer survival of patients. Clinically, MRS-based nomograms were shown highly discriminative, accurate, and useful in predicting HCC recurrence and patient survival. Moreover, we provide evidence that MRS may associate with the efficacies of post-recurrence sorafenib and transcatheter arterial chemoembolization (TACE) treatments. Together, we constructed and validated a new myeloid signature with valuable immunological and clinical potential.

O324

Hypoxia shapes immune intratumoural heterogeneity in hepatocellular carcinoma

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Recent success of checkpoint inhibitor in hepatocellular carcinoma (HCC) has revolutionized the treatment for HCC. Despite being a promising strategy, response rate of patients is highly unpredictable and the mechanistic basis for variation in response patterns remains poorly unexplained. Through a multiregional study of pretreatment resected HCC tumours using single-cell mass cytometry and multiplexed immunohistochemistry, our recent findings revealed a significant degree of immune intratumoural heterogeneity (ITH) associated with hypoxic areas within a tumour. We found that the proportion of regulatory T cells over CD4+ T cells was higher while density of CD4+ and CD8+ T cells were lower in hypoxic area. Subjecting peripheral blood from HCC patients to hypoxic condition *in-vitro* resulted in enhanced expression of genes involved in metabolic switch, antiproliferation, apoptosis and immunosuppression. Genes expression analysis in various immune subsets showed their differential phenotypes and functions under hypoxia, demonstrating that hypoxia could be one of the factors contributing to immune-ITH. Our study informs on how intratumoural variation in hypoxia could shape the localized selective pressure of immune microenvironment, potentially resulting in immunologically privileged sites within a tumour which would encumber the success of immunotherapy.

DOI: 10.1002/eji.201970400

O325

Targeting monocyte-intrinsic enhancer reprogramming improves immunotherapy efficacy in hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC), mostly developed in fibrotic/cirrhotic liver, exhibits relatively low responsiveness to immune-checkpoint blockade (ICB) therapy. With an aim to developing mechanism-based combination immunotherapy, we have recently reported enhancement of ICB efficacy by disrupting the HCC cell-myeloid-derived suppressor cell (MDSC) crosstalk (Gut 2018; Nature Communications 2018). Here we hypothesize that direct targeting of MDSCs can efficiently re-shape the immunosuppressive microenvironment to improve HCC immunotherapy.
Methods and Results: Flow cytometry showed that accumulation of monocytic MDSCs (M-MDSCs), but not polymorphonuclear MDSCs, in fibrotic livers significantly correlated with reduced tumor-infiltrating lymphocytes (TILs) and increased tumorigenicity in two orthotopic HCC models in fibrotic liver setting via carbon tetrachloride or high-fat high-carbohydrate diet. In human HCCs, the tumor-surrounding fibrotic livers were markedly enriched with M-MDSC, with its surrogate marker CD33 significantly associated with aggressive tumor phenotypes and poor survival rates. Mechanistically, activated hepatic stellate cells (HSCs) induced monocyte-intrinsic p38 MAPK signaling to trigger enhancer reprogramming for M-MDSC development and immunosuppression. Treatment with p38 MAPK inhibitor abrogated HSC-M-MDSC crosstalk to prevent HCC growth. Concomitant with patient-derived M-MDSC suppression by a clinically-trialed BET bromodomain inhibitor i-BET762, combined treatment with anti-PD-L1 synergistically enhanced TILs, resulting in tumor eradication and prolonged survival in the fibrotic-HCC mouse model.
Conclusions: Our results signify how non-tumor intrinsic properties in the desmoplastic microenvironment can be exploited to reinstate immunosurveillance, providing readily translatable combination strategies to empower cancer immunotherapy.
Acknowledgement: This project is supported by Collaborative Research Fund C4045-18W, Theme-based Research Scheme T11-706/18-N, HMRF 16170451, and the Terry Fox Foundation.

O326

Antibody targeting Na, K ATPase β3 subunit on monocytes effectively impairs T helper cell responses

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DOI: 10.1002/eji.201970400

T helper (Th) cells play a crucial role in facilitating the activity of other immune cells by releasing Th cytokines. Uncontrolled Th cell responses can elicit chronic inflammation and autoimmune disease. The new wave of treatment for autoimmunity has been focusing on T cell suppression. We have generated a monoclonal antibody (mAb) recognizing Na, K ATPase β3 subunit, termed P-3E10. We previously reported that mAb P-3E10 could reduce cell proliferation in anti-CD3-activated PBMCs with no interfering of Na, K ATPase activity. In the present study, the insight mechanism of mAb P-3E10 in impairment of Th cell responses has been elucidated. mAb P-3E10 could attenuate T cell proliferation and Th1, Th2 and Th17 cytokine production. Monocytes play a key role in inhibiting Th cell activity via mAb P-3E10 ligation. This inhibitory effect required cell to cell contact between monocytes and T cells. Only soluble factors produced by monocytes were insufficient to impair T cell activation. The mAb P-3E10 down-regulated MHC class II and CD86 levels and increased IL-6, IL-10 and TNF-α production of monocytes eventually leading to impaired Th cell functions. By sequential immunoprecipitation, we proposed that β3 subunit expressed separately from the α subunit of Na, K ATPase might exist on monocytes and implicate in T cell regulation. Altogether, our study demonstrated that the targeting of Na, K ATPase β3 subunit on monocytes by mAb P-3E10 effectively impairs Th cell responses. mAb P-3E10 might be a promising novel antibody drug for T cell-mediated autoimmunity.

O327

Syngeneic mouse red blood cells express self-tolerogenic potential after intravenous injection in a high dose

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Background: We recently observed that intravenous administration of haptenated syngeneic mouse red blood cells (sMRBC) prior to sensitization with the same hapten leads in mice to hapten-specific suppression of contact hypersensitivity (CHS) reaction, which is mediated by miRNA-150-carrying and light chain-coated extracellular vesicles (EVs). Subsequent studies revealed that mice intravenously administered with sMRBC alone generate EVs that could down-regulate antigen-presenting cell function. While erythrocytes were proposed good carriers for self-antigens to induce tolerance, little is known on the possible tolerogenic action of self-erythrocytes themselves. Thus, current studies investigated the immune effects induced in mice by mere intravenous administration of a high dose of sMRBC.
Methods: Self-tolerogenic potential of sMRBC occurred to be mediated by CD9+ EVs, and was determined in newly developed mouse model of delayed-type hypersensitivity (DTH) to sMRBC, where indicated after blocking with anti-miRNA-150. The mechanism of EV's action was tested by evaluating T cell activation and IL-2-dependent viability.
Results: We found that intravenous administration of sMRBC leads to generation of EVs that suppress DTH to sMRBC in a miRNA-150-dependent manner, and could be coated with antibody light chains of particular specificity. Furthermore, our initial results imply that sMRBC-induced EVs inhibit DTH reaction by influencing effector T cell activation and viability.
Conclusions: Current study described newly discovered mechanism of self-tolerance induced by intravenous delivery of a high dose of sMRBC and mediated by EV-carried miRNA-150. This implies the concept of naturally-occurring immune tolerance that is presumably activated in a response to overloading of the organism with altered self-antigens.

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DOI: 10.1002/eji.201970400

O328

Deciphering the aberrant immune microenvironment in acute myeloid leukemia development and its role in chemotherapy response

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Comprehensive illustration of phenotypic and functional signatures of abnormal immune microenvironment in acute myeloid leukemia (AML) is lacking. We used multiparameter flow cytometry to characterize various lymphocytes in peripheral blood and bone marrow in 67 patients with AML (before or/and after induction chemotherapy) and 24 healthy controls (HCs). The proportions of CD3+ T, NK, Th, Tc and γδ T cells were lower in newly-diagnosed AML (ND-AML) patients versus HCs. As for memory subsets, we found a significantly reduced percentage of naïve subset (CD45RA+CCR7+, relative to CD4+ or CD8+ T cells). Further analysis showed a significantly lower expression of CD28 in CD4+, CD8+ T and NKT cells, but a higher expression of CD57 for AML patients. The percentage of CD4+ T, CD8+ T, Vδ1 T and Vδ2 T cells expressing inhibitory receptor PD-1 was significantly higher in AML compared with HCs. Following induction chemotherapy, the immune landscape of AML patients was altered by chemotherapy with therapeutic response as immune reconstitution occurred. Several phenotypic and functional markers diverged between good responders (GR) and refractory or relapsed (RR) patients. Good response to induction chemotherapy significantly correlated with increased CD8+ Tnaïve cells, upregulation of NKG2D in NK and Vδ2 cells, and downregulation of PD-1 and CD57 in CD8+ T cells. Our findings suggest immunological dysfunction in AML at diagnosis, including exhaustion and senescence. Chemotherapy response and relapse significantly correlates with suppressed immune microenvironment, indicating that immunological markers may be novel prognostic risk factor in acute myeloid leukemia.

LB023

PLGA-MPs-mediated codelivery of myelin antigen/MHC complex and tolerogenic molecules maintain specific long-term tolerance in EAE

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Background: Current treatments to control pathological or unwanted immune responses in multiple sclerosis (MS) often use broadly immunosuppressive drugs or low doses allergen without immunomodulating agents. In previous work, we described the use of **synthetic, biodegradable polylactic-co-glycolic acid microparticles (PLGA-MPs)** carrying **MOG peptide/MHC complexs** and tolerogenic immunomodulators (**anti-Fas, PD-L1-Ig and TGF-β1**) to directly and selectively eliminate or inhibit auto-reactive T cells in mouse EAE model. In addition, its maintenance of immunosuppression and tolerance needs further verification.
Objective: To develop a new approach mediated by biodegradable MPs to induce **antigen-specific and long-term immunological tolerance** in EAE.
Methods: Here, the PLGA-MPs as previously described were administered *i.v.* into the MOG_{35-55}-induced EAE mice, two months after four time infusions, followed by the investigation of long-term therapeutic effects, specific immunosuppression, tolerance maintenance and histopathological recovery.
Results: Treatment with this tolerogenic MP, **in a long time**, results in lower clinical score and reduction of infiltration in CNS tissues in EAE mice. Moreover, **the inhibition of MOG-specific T cells proliferation**, **decrease in MOG-specific Th1/Th17 cells and increase in regulatory T cells** are

DOI: 10.1002/eji.201970400

presented in spleen or lymph nodes in EAE mice.
Conclusions: Together these data highlight the potential for using such specific microparticles to regulate pathogenic T cells by inducing long-term immune tolerance and targeting natural apoptotic clearance pathways in the treatment of autoimmune disorders.
Keywords: EAE; specific immunotherapy; immune tolerance; PLGA-MPs.

P0366

Biology of immune privilege

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Immune Privilege is a special immune phenomenon in immunology. There are several immune privilege sites which have been found in the body. Such as brain, liver, testis, eye, pregnent uterus, tumor, etc. The nature of immune privilege is antigen specific immune tolerance at the special sites. Suppressive microenvironment and regulatory T cells (Treg cells) have been believed to be responsible for the mechanism of immune privilege. If we furtherly investigate the details of immune privilege mechanism, we may find a noval way of understanding immunology, and may find new methods to deal with various immunological disorders.

P0367

Tim-3 signaling in peripheral NK cells promotes maternal-fetal immune tolerance and alleviates pregnancy loss

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Pregnancy loss occurs in about 15% of clinically recognized pregnancies, and defective maternal-fetal immune tolerance contributes to more than 50% of these events. We found that signaling by the type I membrane protein T cell immunoglobulin and mucin-containing protein 3 (Tim-3) in natural killer (NK) cells had an essential protective role during early pregnancy. Tim-3 on peripheral NK (pNK) cells was transiently increased in
abundance during the first trimester of pregnancy, which depended on interleukin-4 (IL-4)-signal transducer and activator of transcription 6 (STAT6) and progesterone signaling. Tim-3+pNK cells displayed immunosuppressive activities, including the production of anti-inflammatory cytokines and the induction of regulatory T cells (Tregs) in a transforming growth factor-b1 (TGF-b1)-dependent manner. Tim-3 on pNK cells was stimulated by its ligand galectin-9 (Gal-9), leading to signaling by the kinases c-Jun N-terminal kinase (JNK) and AKT. In recurrent miscarriage (RM) patients, Tim-3 abundance on pNK cells was reduced and the immunosuppressive activity of Tim-3+
pNK cells was impaired. Compared to Tim-3+pNK cells from donors with normal pregnancies, RM patient Tim-3+pNK cells exhibited changes in DNA accessibility in certain genetic loci, which were reversed by inhibiting accessible chromatin reader proteins. Furthermore, Tim-3+pNK cells, but not Tim-3−pNK cells, reduced fetal loss in abortion-prone and NK cell-deficient mice. Together, our findings reveal a critical role for Tim-3-Gal-9 signaling-mediated immunoregulation by pNK cells in maternal-fetal immune tolerance and suggest that Tim-3 abundance on pNK cells is a potential biomarker for RM diagnosis.

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DOI: 10.1002/eji.201970400

P0368

Expression pattern of TIGIT+ γδ T cell subsets in AML patients with different clinical status

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γδ T cells based adoptive immunotherapy is gaining momentum to fight acute myeloid leukemia (AML), however γδ T cells show diverse structural and functional heterogeneity which may related to the alteration of immune checkpoint. One of immune checkpoint TIGIT shares similar ligands as the co-stimulatory receptor CD226 and can suppress T cell response by inhibiting CD226 activation. However, little is known the expression profiles of these receptors in γδ T cells from AML patients. To this light, we defined subpopulations of γδ T cells in peripheral blood from different clinical status AML patients and healthy individuals (HIs) by flow cytometry. We found decreased occurrence of CD226-expressing but increased expression of TIGIT-expressing γδ T cells in de novo AML patients. The proportions of Foxp3+ γδ T cells, Foxp3+ CD226- γδ T cells, TIGIT+ Foxp3+ γδ T cells, TIGIT+ γδ T cells and TIGIT+ CD226- γδ T cells were significantly higher either in de novo or the recurrent AML groups, and showed the pattern as the recurrent AML > the de novo AML > the AML-CR > HIs. Further analysis of γδ T cells revealed the subset that positive-expressed CD226 but displayed negative TIGIT or Foxp3 were associated with clinical status. Phenotypic and functional abnormalities of TIGIT+ γδ T cells and TIGIT+ CD226- γδ T cells were partially restored in 4 de novo AML patients following achieving CR. **Altogether, we showed characteristics of TIGIT+ γδ T cell subsets which may affect clinical outcome in AML patients.**

P0369

miR-520c-3p suppresses lung adenocarcinoma progression by modulating the tumor immunosuppressive environment as well as inhibiting cancer cell migration and invasion

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Lung adenocarcinoma (LUAD), the most common subtype of lung cancer, is often diagnosed at advanced stages involving disseminated metastatic tumors with a precipitous drop in the survival rate of patients. Therefore, uncovering the mechanism of LUAD tumorigenesis to identify neoplasia-associated markers is of full significance. Accordingly, microRNAs (miRNAs) have been implicated in modulating the progression of cancer. Here, we first focused on thymic stromal lymphopoietin (TSLP), which plays a critical role in immunity. We verified that miR-520c-3p, a remarkably decreased miRNA in LUAD, directly targeted TSLP, thus inhibiting the formation of an immunosuppressive microenvironment and suppressing the progression of LUAD. In addition, we found that miR-520c-3p was correlated with good clinical outcomes in LUAD clinical stage I patients. Furthermore, overexpressed miR-520c-3p suppressed the migratory and invasive properties of LUAD cell lines by directly targeting Ras-related protein 22a (Rab22a). The experimental techniques used in this study include immunohistochemistry staining, ELISA, real-time PCR, transient transfection, plasmid construction, Transwell assays, wound-healing assays, western blot assays and dual-luciferase assays. Taken together, our findings indicate that miR-520c-3p functions as a tumor-suppressive gene by modulating the tumor immunosuppressive microenvironment via TSLP and directly regulating cell migration and invasion by Rab22a. The current study supports a key role of miR-520c-3p in modulating not only the tumor immunosuppressive microenvironment but also cell motility in LUAD, presenting a potential therapeutic target for LUAD treatment.

DOI: 10.1002/eji.201970400

P0370

Distribution features and functions of γδT cells infiltrated in ovarian cancer microenvironment

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To investigate the distribution patterns of γδT cells and main subsets in peripheral blood, and tumor tissues among ovarian cancer (OC) patients, benign ovarian tumor (BOT) patients, age-matched healthy controls (HC), as well as the expression levels of IFN-γ and IL-17A secreted from γδT cells. Moreover, immunohistochemical staining was utilized to detect the numbers of γδT cells and main subsets in different types of ovarian tumor tissues. Additionally, we also investigated the chemotaxis effects, along with cytotoxicity activity and proliferative assay of γδT cells.We found that the percentages of γδT cells and Vδ1 T cells were significantly higher in OC tissues than BOT tissues and normal ovarian tissues. Meanwhile, higher numbers of γδT cells and Vδ1 T cells were described in OC tissues by immunohistochemistry, which were positively related to advanced clinicopathological features of OC patients. Further, the levels of IFN-γ secreted by γδT cells were relatively lower, while IL-17A was in a high level both in peripheral blood and tissues of OC patients. Chemotaxis assay revealed that supernatants derived from OC tissues, possessed stronger capacity of attracting and recruiting γδT cells. However,γδT cells sorted from OC tissues showed the weakened cytotoxic activity against ovarian cancer cells, and γδT cells cocultured with OC tissues supernatants could effectively inhibit the proliferative activity of Naïve $CD4^+$T cells.These data suggested thatγδT cells might be key players in OC progression and have the potential for treatment or prognosis prediction.

P0371

Tumor-derived exosomal ORM1 messenger RNA, acting as a non-coding RNA, promotes the development of regulatory T cells in hepatocellular carcinoma

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Tumor derived exosomes, as a novel carrier of bioactive molecules, were able to educate host immune defense. However, the roles of exosomal messenger RNAs in immune system have not been fully identified. Here, we demonstrated that tumor-derived exosomal ORM1 mRNA functions as a non-coding RNA to promote regulatory T cells induction. By re-analyzing exosome sequencing dataset from patients with colorectal, pancreatic or liver cancers, we found that the levels of serum exosomal ORM1 mRNA were specifically increased only in patients with hepatocellular carcinoma (HCC). Removal of tumor dramatically decreased the levels of exsomal ORM1 mRNA, indicating that exosomal ORM1 mRNAs were derived from HCC tumor cells. The levels of ORM1 mRNA in tumor were negatively correlated with prognosis of patients with HCC and number of tumor infiltrated lymphocytes (TILs) by using TCGA. Flow cytometric analysis showed that the levels of exsomal ORM1 mRNA were negatively correlated with the frequency of CD4+T cells and Treg number in serum. Further, we constructed a vector that only expressed ORM1 mRNA by truncating translation initiation site (△ATG). Co-culturing tumor cells with immune cells showed that tumor cells or exosomes, which overexpressed ORM1 mRNA, promoted the development of thymic Treg *in vitro*. Finally, by using RNA pull-down, we found that ORM1 mRNA interacted with Foxp3. In conclusions, our study reported that

DOI: 10.1002/eji.201970400

tumor cells promoted regulatory T cells formation by secreting specific exosomal with the abundant of ORM1 mRNA.

P0372

Hepatitis B virus-specific CD8+ T cells maintain functional exhaustion after antigen reexposure in an acute activation immune environment

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Chronic hepatitis B virus (HBV) infection is characterized by the presence of functionally exhausted HBV-specific CD8+ T cells. To characterize the possible residual effector ability of these cells, we reexposed CD8+ T cells from chronically HBV replicating mice to HBV antigens in an acute activation immune environment. We found that after transfer into naive mice, exhausted CD8+ T cells reexpanded in a comparable magnitude as naive CD8+ T cells in response to acute HBV infection; however, their proliferation intensity was significantly lower than that of CD8+ T cells from acute-resolving HBV replicating mice (AR mice). The differentiation phenotypes driven by acute HBV replication of donor exhausted and naive CD8+ T cells were similar, but were different from those of their counterparts from AR mice. Nevertheless, exhausted CD8+ T cells maintained less activated phenotype, an absence of effector cytokine production and poor antiviral function after HBV reexposure in an acute activation immune environment. We thus conclude that exhausted CD8+ T cells undergo a stable form of dysfunctional differentiation during chronic HBV replication and switching immune environment alone is not sufficient for the antiviral functional reconstitution of these cells.

P0373

Remold tumor immune microenvironment with small molecule drugs

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Successfully induced competent anti-tumor immune response is essential for eradicating neoplastic cells and for providing long-term memory protection against relapse. The tumor immune microenvironment (TIME) is largely regulated by immune suppressors, therefore subverting the suppressive TIME is the hinge of an effective anti-cancer therapy. Emerging evidence suggests that genetic aberrations in cancer cells dictate the immune contexture of tumors, small molecule drugs targeting tumor cells' aberrations may reshape the TIME, which will also contribute to its anti-cancer effect, so understanding the effect of small molecule drugs on TIME is critical for the development of combination therapy. To date, many novel anti-cancer drugs have been discovered, including CDK4/6 inhibitors, epigenetic regulators and molecules targeting metabolic pathways. Though their efficacies were tempting, they were mostly evaluated in immune deficient models, and their long-term effects and resistant outcomes are still unclear. In our study, we will examine the influence of these small

DOI: 10.1002/eji.201970400

molecule anti-cancer drugs on tumor cell immunogenicity, tumor microenvironment and immune effector cells both *in vitro* and *in vivo* of immune competent models. Our research goal is to clarify effects of small molecule anti-cancer drugs on TIME, based on which we can design small molecule drug combinations as powerful tool to remold the TIME toward anti-tumor status and approaching the final goal of cancer cure.

P0374

The production of IL-10 by B cells is regulated by cytokines, but is not dependent on GATA3 and Foxp3 expression

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The possibility to modulate immune response represents a great aim for clinical applications. The regulatory effects of T cells have been well described. Apart from production of antibodies, B cells can regulate immune response in the antibody-independent manner. In this study we determined modulatory effects of IFN-γ on the activation of suppressive action of B cells stimulated with LPS. Highly purified mouse B cells stimulated with LPS and IFN-γ produced increased concentrations of IL-10 and expressed enhanced levels of the genes for FasL and PD-L1. Macrophages, which were cocultivated with B cells stimulated with LPS and IFN-γ, showed decreased expression of costimulatory molecule CD86 and reduced production of IL-6. These macrophages displayed also decreased ability to stimulate a proliferation of activated CD8+ T cells. Furthermore, we studied the role of transcription factors GATA3 and Foxp3 in IL-10 production by LPS-stimulated B cells. It was found that IL-10 production by B cells is regulated by cytokines, but is independent of GATA3 and Foxp3 expression. These findings are in a sharp contrast with the observation in T cells, where IL-10 production correlates with GATA3 or Foxp3 expression. The results have shown that IFN-γ enhances activation of suppressive functions of B cells which have the ability to inhibit immune response through their effect on macrophages. Nevertheless, the production of IL-10 by B cells is independent of the presence of GATA3 or Foxp3, while IL-10 production by T cells requires GATA3 expression, and Foxp3 is indispensable for Treg functions.

P0375

Enriched HLA-E and CD94/NKG2a interaction limits anti-tumor CD8$^+$ tumor-infiltrating T lymphocyte responses

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DOI: 10.1002/eji.201970400

Past immunotherapy treatments with anti-PD-1 demonstrated failure to boost recovery in significant number of cancer patients, indicating the complexity of the tumor microenvironment. While human leukocyte antigen-E (HLA-E) has been linked to worsen clinical outcomes in mice and human patients, the evasive immunological contributions of HLA-E and its inhibitory CD94/NKG2a ligand in cancer compared to adjacent tissue and PBMC remains unclear. In this study, we reported that not only epithelial-derived cancer cells, but importantly tumor macrophages and CD141^{+} conventional dendritic cells (cDC) contributed towards HLA-E enrichment in carcinoma, with similar pattern of enrichment exhibited across different cancer types. This correlated to CD94/NKG2a upregulation on CD8^{+} tumor-infiltrating T lymphocytes (TILs) but surprisingly not on CD4^{+} TILs. Interestingly, CD94/NKG2a is exclusively expressed on PD-1$^{high\text{-}expressing}$ TILs whilst lacking the beneficial intratumoral CD103 expression. Most importantly, we also found that the presence of enriched CD94/NKG2a on human tumor-specific T cells highly impairs IL-2/IL-2 receptor-dependent proliferation, which affected its IFN-γ-mediated responses and anti-tumor cytotoxicity, but these functionalities recovers following antibody-mediated blockade shown *in vitro* and *ex vivo*. Taken altogether, this suggest that enriched HLA-E:CD94/NKG2a inhibitory interaction can critically impairs and exhaust particularly the survivability of PD-1$^{high\text{-}expressing}$ TILs in the tumor microenvironment.

P0953

Dysfunctional CD4^{+}T cell driven by Foxp3^{+}CD4^{+}Treg cells contributes to Lewis lung cancer growth and metastasis

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Successful immunotherapy by checkpoint blockade proved that strategic manipulation of immune system be of effective measurement for the treatment of lung cancer. Nevertheless, prognosis of the majority lung cancer patients remains unsatisfactory. It has been shown that CD4^{+}T cell plays critical role in the orchestration of the anti-tumor immune responses and inhibit the progression of various cancer. However, emerging studies suggested that activated CD4^{+}T cells are susceptible for dysfunction that comprised anti-tumor immunity against tumor development. The cellular and molecular mechanisms that drive CD4^{+}T cells dysfunction are still unclear. In this study, we showed that the dysfunctional CD4^{+}T cells, which characterized by the enhanced expression of inhibitory receptors, including CTLA-4, PD-1, and TIM-3 molecules, reduced production of cytokine IL-2 and IFN-g and decreased proliferation potential, were closely related to the increased levels of Foxp3^{+}CD4^{+}Treg cells in the tumor tissue and spleen of LLC mice. Depletion of Foxp3^{+}CD4^{+}Treg cells in LLC mice resulted in functional rescue of dysfunctional CD4^{+}T cells, leading to retarded tumor growth and metastasis. These results suggest that dysfunctional CD4^{+}T cells could be driven by Foxp3^{+}CD4^{+}Treg cellscel that contribute to the LLC tumor progression. We, thus, propose that targeting Foxp3^{+}CD4^{+}Treg cells could be of great importance to functional rescue dysfunctional CD4^{+}T cell and have potential implications for lung cancer therapy.

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DOI: 10.1002/eji.201970400

P0954

LPS induced macrophage IL-10 autocrine signaling is essential for induction of endotoxin tolerance

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Prior exposure of innate immune cells like monocytes/macrophages to minute amounts of endotoxin cause them to become refractory to subsequent endotoxin challenge, a phenomenon called "endotoxin tolerance". Although it is well known that IL-10 could inhibit macrophage activity, IL-10 functions as an autocrine regulator of macrophage pro-inflammatory cytokines production remaining exclusively. By using IL-10Mdel, IL-10RαMdel and IL-10Mdel-IL-10RαMdel mice, in which macrophages could not produce IL-10 and/or response to IL-10, we systematically studied how macrophage's IL-10 signaling pathway influences the immune tolerance. We found both IL-10Mdel and IL-10RαMdel mice are susceptible for LPS induced liver injury with increased serum levels of IL-6, TNF-α and IL-1β. Macrophages lacking the IL-10 response are highly activated with upregulated expression level of CD80, CD86 and CD14. Moreover, bone marrow-derived macrophages from IL-10RαMdel mice do not display endotoxin tolerance. *In vivo* studies with IL-10Mdel, IL-10RαMdel and IL-10Mdel-IL-10RαMdel mice confirm these *in vitro* findings. Western blot analysis showed TLR4-MyD88-NFκB signaling pathway related proteins expression level are increased in macrophages losing their IL-10Rα. Together, autocrine IL-10 suppressing macrophages activation and their proinflammatory cytokines production plays an indispensable and dominant role for endotoxin induced tolerance model.

P0955

Tissue resident memory T cells is the predominant subset of exhausted $CD8^+$ T cells in human lung cancer microenvironment

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Lung cancer is the leading cause of cancer deaths worldwide. The initiation, progression and recurrence of lung cancer are closely linked to local immune disturbance. Tissue resident memory T cells (T_{RMs}) were well demonstrated to play a crucial role in sensing local perturbations and participating in protection from infection and cancer. However, the role and underling regulatory mechanism of T_{RMs} in human lung cancer microenvironment is still unclear. Our recent study found that $CD8^+$ T_{RMs} were significantly increased in human lung cancer tissue compared with matched normal tissues. We also found that $CD8^+$ T_{RMs} was the predominant subset of human lung cancer-infiltrating T_{RMs}. Furthermore, we demonstrated that human lung cancer infiltrating CD8+TRMs cells highly express immune exhaustion marker PD-1 and exhibited an immune exhaustion related transcriptome characteristics. Moreover, we had demonstrated that $CD8^+T_{RMs}$ cells is the dominant subset of exhausted $CD8^+$ T cells in human lung cancer microenvironment. It is interesting that human lung cancer infiltrating $CD8^+T_{RMs}$ shown obvious advantage in cytotoxic activity, survival and proliferation when compared with normal matched tissues infiltrating $CD8^+T_{RMs}$ or other lung cancer infiltrating $CD8^+$ T cell subsets. Our finding suggested that the immune exhaustion of human lung cancer infiltrating $CD8^+$ T_{RMs} is one of the key factors for the imbalance of immune microenvironment in lung cancer. Reversing the immune exhaustion of $CD8^+$ T_{RMs} may be the key to the success of lung cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P0956

Immunotherapy of intrahepatic cholangiocarcinoma combined with Siomycin A and PDL1 inhibitor

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Background: Forkhead box M1 (FOXM1) acts as a transcription factor plays an important role in multiple cellular processes, such as apoptosis, DNA damage repair and angiogenesis etc. Indoleamine 2,3-dioxygenase (IDO), IDO1 and IDO2, are the essential amino-acid tryptophan, which leads to immune tolerance in organ transplantation. However, the relationship between FOXM1 and IDO is still unknown.
Methods: We used FOXM1 inhibitor, Siomycin A, to suppress the expression of FOXM1. Through the RT-PCR and western-blotting to detect the expression of IDO2 under Siomycin A treatment in intrahepatic cholangiocarcinoma (ICC) cells. Besides, we also measured the level of kynurenine (degradation of tryptophan) through Elisa assay. Moreover, we also take application of Siomycin A and PDL1 inhibitor into mouse ICC model to ensure the influence of combination therapy to the survival rate of ICC. Then we used Chromatin immunoprecipitation (CHIP) to understand the relationship between FOXM1 and IDO2.
Results: We observed that the expression of IDO2 was downregulated under the treatment of Siomycin A in ICC cells. Furthermore, Siomycin A treated LO2 cells presented decreased ability of tryptophan degradation. CHIP assay indicated direct interaction of FOXM1 with IDO2 promoter in liver cells. Besides, we found that Siomycin A-mediated increase in CD8+ T cells, and the combination therapy could increase the curative rate of ICC.

P0957

SENEX gene mediate apoptosis resistance of leukemia blasts by stress-induced premature senescence in patients with acute myeloid leukemia

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In acute myeloid leukemia (AML), the underlying cause of refractory and relapse is usually related with apoptosis resistance to anticancer drugs. In endothelial cells (ECs), a novel senescence gene named SENEX resistant to TNF-α induced apoptosis and limit the inflammatory response by regulating stress induced premature senescence (SIPS). However, it remains unknown whether SENEX role in the apoptosis resistance of leukemia blasts. In this study, we found that SENEX gene expression was elevated compared with controls (4.06±2.72 Vs. 0.92±0.51, $p< 0.01$) and was positive correlated with the percentage of bone marrow leukemia blasts ($R=0.557$, $p=0.000$) in AML patients. Next, we analyzed the SENEX gene expression and relationship between senescence and apoptosis in AML NB4 cell lines under the pressure of low-dose cytarabine. Finally, we investigated that the SENEX natural expressing cells had a high proportion of senescence in 12 hours (39.6%±3.6%) and a low proportion of apoptosis in 48 hours (33.3%±3.9%), while the SENEX silencing-down cells had a low proportion of senescence in 12 hours (30.0%±2.4%, $p< 0.01$) and a high proportion of apoptosis in 48 hours (52.5%±5.7%, $p< 0.01$). Furthermore, SENEX natural expressing cells survived and regained the ability to proliferate, while SENEX silencing-down cells almost all died cultured until to 96 hours. In conclusion, our study indicated that SENEX gene was elevated in AML patients and mediated

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DOI: 10.1002/eji.201970400

apoptosis resistance of leukemia blasts by SIPS, suggesting that higher SENEX gene expression level might be related with chemotherapy resistance, even as an important biomarker in predicting response to chemotherapy.

P0958

Chronic psychological stress induces the accumulation of myeloid-derived suppressor cells in mice

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Chronic psychological stress has been shown to adversely impact immune system functions and compromise host defenses against various infections. However, the underlying mechanisms remain elusive. Recent studies have demonstrated that myeloid-derived suppressor cells (MDSCs) play an important role in regulating immunity. It is of interest to explore whether or not chronic psychological stress plays immunosuppressive functions partially by inducing MDSCs accumulation. In this work, we report that chronic psychological stress led to the accumulation of CD11b+Gr1+ cells in the bone marrow of BALB/c mice. Repeated β-agonist infusion showed no such effect. However, β-adrenergic blockade, but not glucocorticoids blockade, partially reversed the accumulation of CD11b +Gr1+ cells under the condition of chronic psychological stress, suggesting catecholamines collaborate with other factors to induce the accumulation. Further exploration indicates that cyclooxygenase 2 (COX-2)-prostaglandin E2 (PGE2) loop might act downstream to induce the accumulation. A majority of the accumulated CD11b+Gr1+ cells were Ly6G+Ly6Clow immature neutrophils, which inhibited cytokine release of macrophages as well as T cell responsiveness. Moreover, the accumulated CD11b+Gr1+ cells under the condition of chronic psychological stress expressed multiple inhibitory molecules. Taken together, our data demonstrate for the first time that chronic psychological stress induces MDSCs accumulation in mice, which can contribute to immunosuppression.

P0959

Immune state change in BALB/c mice exposed to PFOS after breast cancer cell challenge

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A total of 36 healthy female BALB/c mice were randomly divided into three groups according to body weight： the control group was administrated with Tween-80, the exposure groups were administrated with 0.1 g/L, 0.45 g/L PFOS respectively, and the treatment were conducted through drinking water for 56 consecutive days. Once the exposure finished, the experimental murine model of breast cancer was prepared by subcutaneous injecting 4T1 cell line in the right back of the mouse. Compared with the control group, the levels of IFN-γ mRNA increased significantly after 4T1 breast cancer cell challenge in all PFOS groups and the levels of IL-4 mRNA increased in the 0.45 g/L PFOS group ($P< 0.05$). Compared with control group and the 0.1 g/L PFOS group, the ratio of MDSC increased obviously in the spleen cells in the 0.45 g/L PFOS group after 4T1 breast cancer cell challenge ($P< 0.05$), while the ratio of Treg did not change obviously in all groups. The volumes of tumor in control, 0.1 g/L PFOS and 0.45 g/L PFOS group were (697.33 ±183.06), (861.5±38.35） and (946.67± 135.03) mm^3 respectively ($P>0.05$). The size of tumor mass had an increasing tendence with the increase of PFOS concentration. Compared with the control group, 0.45 g/L PFOS group showed a higher degree of malignancy with denser tumor cells, more mitosis and hypervascularization. In

DOI: 10.1002/eji.201970400

Conclusion, PFOS-exposed mice tends towards a Th2 dominant immune state type, the ratio of MDSC increases after 4T1 breast cancer cell challenge and the cancer cells showed a higher degree of malignancy.

P0960

Analysis of plasma EBV DNA and soluble checkpoint proteins in nasopharyngeal carcinoma patients after definitive intensity-modulated radiotherapy

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Background: Tumor immunotherapy and immunological checkpoint-related proteins are research hotspots. Intensity-modulated radiotherapy (IMRT) is the main treatment for nasopharyngeal carcinoma (NPC). Hence, the evaluation of its effect is very important.
The aim of this study was to assess the relationship between the concentrations of soluble checkpoint proteins, plasma EBV-DNA and cytokines in NPC patients treated with IMRT.
Methods: In this study, the plasma samples of 37 NPC patients and 40 healthy controls were collected. Luminex MAGPIX was used to detect the concentrations of 32 plasma targets. RT-qPCR was used to measure EBV-DNA.
Results: The concentrations of 33 plasma targets were detected in NPC patients before and after IMRT to explore the changes after IMRT. The results showed that IMRT could increase the expression of sPD-1 and significantly reduce the level of EBV-DNA in the plasma of NPC patients. Besides, the concentrations of 12 other targets were significantly different after IMRT, including LAG-3, PD-L1, TIM-3, IFN-γ, IL-12p70, IL-1β, IL-5, IL-6, TNF-α, IL-10, IL-17A and IL-22. High sPD-1 patients had longer survival than those with low sPD-1. Also, patients with lower EBV-DNA, TNM grade I and II/III had longer survival than those with higher EBV-DNA or TNM IV.
Conclusions: This study demonstrated that the concentration of sPD-1 was significantly increased and EBV-DNA was significantly reduced in the NPC patients after IMRT. Plasma EBV-DNA level was a highly specific and sensitive biomarker for NPC diagnosis. Both sPD-1 expression and EBV-DNA concentration in plasma were related to the survival of patients.

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P0961

The effect of IL-10-differentiated dendritic cell therapy on B cell tolerance in a mouse model of asthma

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Cell therapy with IL-10-differentiated dendritic cells (DC10) reverses the disease phenotype in mouse models of asthma, particularly OVA-specific IgG1 and IgE response. But how they induce B cell tolerance is unclear. We have reported that DC10 increased the loss of IgG1 B cells in the bone marrow and spleen of asthmatic mice, but not in the lung, compared to saline-treated group. DC10 that carried intact OVA on their cell surface, but not DC10 that had fully processed their allergen, were able to directly suppress IgG1 production by asthmatic lung cells *in vitro* or *in vivo*. These intact

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DOI: 10.1002/eji.201970400

allergen-presenting DC10 inhibited CD19- IgG1 B cells in a contact-dependent and antigen-specific manner that was not dependent on IL-10, TGF-β or indoleamine-2,3-dioxygenase. We have also reported that passive transfer of DC10-induced regulatory T cells (DC10-iTreg) significantly suppressed OVA-IgG1 and IgE antibody response and that CD4+CD25+ Treg from the lungs of DC10-treated asthmatic mice can suppress B cell IgG1 responses *in vitro*. Herein we also asked whether regulatory B cells (Breg) contribute to DC10-induced B cell tolerance. Coculturing DC10-OVA and asthmatic lung CD19+ B cells led to the outgrowth of IL-10-producing CD19+ and CD19-CD1dhiCD5+ cells *in vitro*. These CD5+ B cells could suppress T cell responses in an IL-10/TGF-β and antigen-independent manner, but we observed no differences in the numbers of IL-10-producing Breg in asthmatic mice treated with specific versus irrelevant allergen-presenting DC10. In summary, while DC10 can drive B cell tolerance *in vivo*, they do not do so via direct induction of Breg.

P0962

Tolerogenic dendritic cells transfected with MHC epitopes prolong allograft survival and reduce GVHD

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Currently, transplantation is the treatment of choice in the late stages of organ damage/failure. Although immunosuppressive therapy significantly increases the chance of successful allograft survival, it is often accompanied by severe side effects such as opportunistic infections and cancers. In addition, immunosuppressive therapy does not prevent the development of chronic rejection reactions. Therefore, the development of biological approaches to induce immunological tolerance to transplanted organs and tissues might be promising.
In this study, we induced antigen-specific tolerance in C57Bl/6 mice to histocompatibility antigens of CBA mice. For this purpose, we co-cultured C57Bl/6 splenocytes with tolerogenic dendritic cells transfected with DNA constructs encoding distinct homologous determinants from the H2 locus of CBA mice. In addition, a DNA construct encoding mouse IL-10 was used for transfection, which had previously been shown to be effective for *in vitro* induction of CD4+CD25^{hi}FoxP3+ Treg cells.
Our findings demonstrated that the used approach may be effective both in induction of nonspecific tolerance and in induction of antigen-specific suppression of the immune response to alloantigens. We demonstrated the effectiveness and antigen specificity of a tolerogenic immune response induced by tol-DCs in the mixed lymphocyte culture (MLC) assay *in vitro* as well as in acute GVHD models and skin graft transplantation *in vivo*.

This study was supported by the Russian Science Foundation (Agreement No. 16-15-00086)

P0963

Analysis of progesterone effect on pregnant humoral immunity using humanized mice

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DOI: 10.1002/eji.201970400

While immune system recognizes and attacks non-self-antigens, maternal immunity establishes a tolerance to the fetus during pregnancy. Progesterone (P4), steroid hormone which is enhanced during pregnancy, is suggested to be involved as the main factor. However, experimental animal model is insufficient for mimicking human pregnant immune system to analyze P4 effect because pregnant system is highly species-specific. Additionally, signal cascades and related gene expression profiles of steroid hormones on immune cells are highly complicated, and the outcome has not been strictly identified. Therefore, we tried to analyze the steroid hormone effects on differentiations of human immune cell subsets
Human PBMCs were stimulated by TSST-1 in the presence of P4 or cortisol for 3 days and the cellularity was analyzed by flow cytometry. Pregnant NOG mice were transplanted with hPBMCs. The lymphoid tissues and placenta were obtained and the cellularity was analyzed after 2 weeks. The plasma IgG level was measured by LC/MS.
Expression of CD25 and PD-1 on T cells and Tfh fraction were decreased at high P4 concentration, while these phenomena were not observed in the presence of cortisol *in vitro*. Plasma IgG level was significantly decreased in pregnant humanized mice, which is also observed in pregnant woman. However, Tfh cells were maintained and plasmablast-like cells were developed in the lymphoid tissues except placenta.
These results suggest that antibody production is decreased in pregnant humanized mice because of Tfh cell decrease and B cell malfunction by abundant P4 environment in the placenta, which suppress the antibody producibility of plasmablasts.

P0964

Exhausted memory T cells (T_{MEX}) maintain T cell responses in chronic infections and cancer

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Defective cytokine production, expression of inhibitory receptors such as PD-1 and metabolic dysfunction are hallmarks of exhausted CD8 T cells that are exposed to high loads of antigen for prolonged periods in situations such as chronic infections or tumours. Recent research by us and others has discovered a small population of cells that possess self-renewal capacity, high proliferative potential and concurrently display phenotypic features of exhaustion and memory. During steady state, these exhausted memory cells (T_{MEX}) constantly replenish the terminally differentiated exhausted effector T cell pool required for viral control. Importantly, the proliferative burst and rejuvenation of the T cell response observed upon checkpoint blockade is exclusively mediated by T_{MEX}.
Our research dissects the early developmental pathways of T_{MEX} and their exhausted effector counterparts, their differential metabolic requirements and transcriptional signatures. Better understanding of the molecular mechanisms that control formation and maintenance of T_{MEX} will allow current checkpoint blockade therapies to be fine-tuned to meet the requirements of the subset of cells responsible for the proliferative burst, thereby improving the efficacy of these treatments in chronic infections and tumours.

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DOI: 10.1002/eji.201970400

P1559

The research of CD47-SIRPα interaction functional epitope

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The 3-D theoretical complex of CD47 and SIRPα was constructed by computer-guided homology modeling, molecular docking and dynamics optimization based on their crystal structures. CD47 and SIRPαinteraction model was predicted by the theoretical modeling complex structure. The major and minor interaction domain of CD47 identified by SIRPα were determined. Furthermore, three mutants of CD47 (i.e. CD47-M1 denoted the major binding domain of CD47, CD47-M2 was the minor binding domain of CD47 and CD47-M3 was the reference mutant) were designed and replaced with alanine residues. Three mutant protein (i.e. CD47-M1, CD47-M2 and CD47-M3) were prepared by the above-mentioned theoretical results. ELISA and SPR results showed the binding activity to SIRPα differed from CD47 and the mutants. CD47-M3 kept the similar affinity to parent CD47. The mutants CD47-M1 and CD47-M2 possessed weaker binding activity to SIRPαthan CD47. In addition, the mutant CD47-M1 was the weakest binder. Besides, the CD47 molecule was knocked out and hominine CD47 and relevant mutants were knocked-in (named MC38-H, MC38-M1, MC38-M2, MC38-M3) in murine MC38 cell line. Compared with the other mutants, the MC38-M1 could be devoured more easily by macrophage. In conclusion, the functional domain in CD47 was predicted and determined. It highlighted the novel CD47 antagonist design and was benefited to the research about the mechanism of interaction between CD47 and its receptor.

P1560

Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation

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Myeloid-derived suppressor cells (MDSCs) are pathologically activated and relatively immature myeloid cells that have been implicated in the immunological regulation of many pathologic conditions. Phenotypically and morphologically, MDSCs are similar to neutrophils (PMN-MDSCs) and monocytes (M-MDSCs). However, they have potent suppressive activity and distinct gene expression profiles and biochemical characteristics. No or very few MDSCs are observed in steadystate physiological conditions. Therefore, until recently, accumulation of MDSCs was considered a consequence of pathological processes or pregnancy. Here, we report that MDSCs with a potent ability to suppress T cells are present during the first weeks of life in mice and humans. MDSC suppressive activity was triggered by lactoferrin and mediated by nitric oxide, PGE2, and S100A9 and S100A8 proteins. MDSCs from newborns had a transcriptome similar to that of tumor MDSCs, but with strong upregulation of an antimicrobial gene network, and had potent antibacterial activity. MDSCs played a critical role in control of experimental necrotizing enterocolitis (NEC) in newborn mice. MDSCs in infants with very low weight, who are prone to NEC, had lower MDSC levels and suppressive activity than did infants with normal weight. Thus, the transitory presence of MDSCs may be critical for regulation of inflammation in newborns.

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DOI: 10.1002/eji.201970400

P1561

B7-H4 expression in tumor cells impairs CD8 T cell responses and tumor immunity

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Background: B7-H4 has been reported to be a negative regulator of anti-tumor immune response. However, the actual immunoregulatory role of B7-H4 expressing in tumor cells is not yet clear. Here, we assessed membrane-bound B7-H4 expression in human breast cancer tissues and its correlation with CD8 T cell infiltration. B7H4 overexpressing tumor cell lines were used to address the potential mechanisms of tumor-expressed B7-H4 on T cell immune response.
Methods: The percentage of B7-H4 or CD8 positive cell population in tumor, para-tumor, adjacent normal tissues and PBMC derived from breast patients was analyzed by flow cytometry and further confirmed by immunohistochemistry (IHC). B7-H4 overexpressing murine tumor cell lines were implanted in immunocompetent mice and received adoptive transfer of antigen specific T cell.
Results: B7-H4 was highly expressed in 26 (86.6%) out of 30 breast ductal invasive carcinomas. B7-H4 surface expression on tumor cells was inversely correlated with the number of tumor infiltrating CD8 T lymphocytes ($P < 0.001$). In murine models, tumor-expressed B7-H4 enhanced tumor growth in immunocompetent mice but not in immunodeficient mice. Further investigation showed that CD8 T cells activation and expansion within the lymph node were suppressed in B7H4 overexpessing tumor-bearing mice after adoptive transfer. Additionally, B7-H4 overexpressing tumor cells impaired cytotoxicity of antigen specific CD8 T cells.
Conclusions: B7H4 on the surface of tumor cells was a negative regulator of CD8 T activation, expansion and cytotoxicity, implicating tumor cell-associated B7H4 might be a target for T cell-based cancer immunotherapy.

P1562

A systematic review update of tolerogenic dendritic cells reinfusion therapy prolong the survival of liver allograft

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Given the pivotal role of tolerogenic dendritic cells (Tol-DCs) in immune tolerance, we updated the evidence on mechanisms of the tolerogenic dendritic cells (Tol-DCs) by different modified treatments on inducing immune tolerance of liver allografts since the first systematic review published in 2014. Related literatures are obtained from different databases after December 2013. Three additional literatures were included for investigating the effects of Tol-DCs infusion on liver transplantation model. The effect of immature DCs (imDCs) without any modification on prolonging the survival time of liver recipients was rely strongly on immunosuppressor or regulatory T cells (Treg). The gene modified imDCs had outstanding performance on inducing alloantigen tolerance and prolonging liver allograft survival (35.8±25.8 days, 4.9 fold to control). Two studies reported the survival of liver recipients infused with imDCs coexpressing TGF-β1/FasL or TGF-β1/IL-10 was significant extend ($P< 0.01$). Gene modification contributed to maintain the immature state of imDCs and furtherly reduce the use of immunosuppressor. Further studies are needed to identify the optimal modification of imDCs to gain a longterm survival of liver recipients.

DOI: 10.1002/eji.201970400

P1563

Cancer-derived sialylated IgG promotes tumor immune escape by binding to Siglecs on effector T cells

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To date, IgG in the tumor microenvironment (TME) has been considered a product of B cells, serving as an anti-tumor antibody. However, in this study, using a monoclonal antibody against cancer-derived IgG (Cancer-IgG), we showed that the majority of IgG in TME is secreted by cancer cells. Furthermore, Cancer-IgG that carried an abnormal sialic acid modification of Fab domain directly inhibited effector T cell proliferation and significantly promoted tumor growth by reducing CD4+ and CD8+ T cells in tumor tissues. Mechanistic studies show that the immunosuppression effect of Cancer-sia-IgG is dependent on its sialylation by binding to sialic acid-binding immunoglobulin-type lectin (Siglec) on T cells in both human and mice. In addition, we found that T cells of the tumor bearing mice can express Siglec3, SiglecE , SiglecG in vivo , and we show an interaction between overexpressed Siglec10/G and Cancer-sia-IgG in vitro. Importantly, we show that Siglecs are overexpressed on T cells of cancer patients but not healthy donors. The findings suggest that Cancer-IgG may be a ligand for sialic acid receptors, and Siglecs may serve as potential checkpoint proteins and mediate tumor immune evasion.

P1564

Activation of de novo NAD biosynthesis sustains immune tolerance by fueling nuclear NAD pool and NAD-SIRT1-dependent epigenetics during acute inflammatory response

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A typical inflammatory response sequentially progresses from pro-inflammatory, immune suppressive to inflammatory resolution phases. While physiological inflammatory response timely resolves, severe acute inflammation usually sustains immune tolerance and causes high mortality, yet the underlying mechanism is not completely understood. We have recently reported SIRT4 as a physiological regulator of tolerance reversal, here, using THP-1 human monocytes, healthy and septic human peripheral blood mononuclear cells (PBMC), we report that endotoxin dose-dependent activation of NAD biosynthesis pathways determines inflammatory phenotypes. Low dose endotoxin triggers Nampt-dependent NAD salvage activity to adapt pro-inflammation, in contrast, high dose endotoxin drives a shift of NAD synthesis pathway from early Nampt-dependent NAD salvage to late IDO-dependent NAD de novo biosynthesis, leading to persistent immune suppression. This is resulted from the IDO-dependent expansion of nuclear NAD pool and nuclear NAD-dependent prolongation of SIR1-directed epigenetics of immune tolerance. Inhibition of IDO activity predominantly decreases nuclear NAD level, which sequentially dissociates immunosuppressive SIRT1 and RelB from the promoter of pro-inflammatory TNF-α gene, thereby, breaking endotoxin tolerance. Thus, Nampt-NAD-SIRT1 axis adapts pro-inflammation, but IDO-NAD-SIRT1 axis sustains endotoxin tolerance during acute inflammatory response. Further study on mechanism-based therapy identifies that the combination of the IDO inhibitor 1-methyltryptophan with tryptophan supplementation, instead 1-methyltryptophan alone, significantly improves outcomes of polymicrobial sepsis animals, endowing our findings translational impacts on effective therapies of immune tolerance in severe inflammatory diseases.

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DOI: 10.1002/eji.201970400

P1565

The deubiquitinating enzyme UCHL1 negatively regulates the immunosuppressive capacity and survival of multipotent mesenchymal stromal cells

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It is known that proinflammatory cytokines empower multipotent mesenchymal stromal cells (MSCs) the immunosuppressive capacity to treat various inflammatory diseases. Nevertheless, how the proinflammatory cytokines modulate the immunosuppressive capacity of MSCs is poorly understood. In the present study, we identified that the deubiquitinating enzyme ubiquitin C-terminal hydrolase 1 (UCHL1) was upregulated in MSCs upon stimulation of proinflammatory cytokines IFN-γ plus TNF-α. Interestingly, through intervening UCHL1 by shRNA knockdown or its inhibitor LDN57444 or overexpression, we found that UCHL1 played a critical role in restraining capacity of MSCs to suppress T cell proliferation. Mechanism analysis revealed that UCHL1 suppressed expression of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO), which are essential for the immunosuppressive effects of murine MSCs and human MSCs, respectively. This effect of UCHL1 was attributed to the negative role in regulating NF-κB and STAT1 signaling, as exhibited by promoting NF-κB and STAT1 activation upon inhibition of UCHL1. Besides, inhibition of UCHL1 suppressed cytokines-induced MSC apoptosis via upregulation of Bcl-2. As a consequence, UCHL1-inhibited MSCs effectively alleviated concanavalin A-induced inflammatory liver injury, with less CD4+ and CD8+ T cells infiltrated in liver. Therefore, our study demonstrates a novel role of UCHL1 in regulating the immunosuppressive capacity and survival of MSCs, which further affects their immunotherapy for inflammatory diseases.

P1566

Transient antibody targeting of CD45RC^{high} T cells to prevent the development of graft-versus-host disease in rats and NSG mice

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GVHD is a frequent complication of bone marrow transplantation and there is great need for new treatments. Transient anti-CD45RC MAb treatment induces tolerance in organ transplantation models through depletion of CD45RC^{high} cells and activation of CD45RC$^{-/low}$ Treg.
BN-LEW/F1 rats infused with LEW T cells showed aGVHD as shown by weight loss and clinical features. Depletion of CD45RC^{high} cells from the T cell inoculum before infusion resulted in prevention of aGVHD (n=4). In vivo treatment after T cell infusion with anti- CD45RC MAb alone (0 to 30d, n=3) or a suboptimal dose of rapamycine (0 to 10d, n=3) did not prolong survival while combination of both anti-CD45RC and rapamycine were synergic (65d vs 33, n=8) and 50% of the recipients showed indefinite survival. Long-term survivors rejected all third-party SPD but not BN or LEW skin grafts demonstrating active mechanisms of tolerance.
Infusion of human PBMCs in immunodeficient NSG mice resulted in aGVHD. Treatment with an anti-CD45RC MAb alone (0 to 20d) or with a suboptimal dose or rapamycine alone (0 to 10d) significantly prolonged survival (34d vs 14d, n=9 and 30d, n=10, respectively) but all recipients developed lethal aGVHD. Combination of both anti-CD45RC and rapamycine were synergic (84d, n=10) and 80% had indefinite survival. Human tumor cells implanted in NSG mice were rejected by hPBMCs infused 7d later. Treatment with anti-CD45RC MAb alone or in combination with rapamycine preserved elimination of tumor cells in all animals (n=4).

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DOI: 10.1002/eji.201970400

Thus, anti-CD45RC treatment in combination with rapamycine has a strong potential to prevent aGVHD.

P1567

Harnessing immunity by controlling the stability of antigen-specific regulatory T cells via manipulation of the functional avidity of self-dominant peptide

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The immune system elicits selective response against non-self while avoiding any harmful response towards self. Self-antigencan cause autoimmunity and induce tumour development, but manifestation of such abnormal immune responses varies with each antigen and the precise mechanism is not clear. Thus antigen-specific therapy is not generally applicable yet.
Here, we investigate how different encephalitogenic peptides itself produce distinct clinical characteristics of experimental autoimmune encephalomyelitis (EAE), irrespective of their genetic background. Particularly, unlike the peptide PLP139-151 and PLP178-191, which caused relapsing EAE with progressive capacity in SJL/J mice, PLP136-150 induced monophasic EAE without progressive capacity through efficient expansion and maintenance of a $CD69^{+}CD103^{+}$(DP) subset of $CD4^{+}CD25^{+}$regulatory T-cells (Treg). This DP-subset of Treg (DP-Treg) produced stable hybrid signature that co-expressed Foxp3 and another transcriptional factors of pathogenic effector T-cells, such as RORγt, Tbet and Eomes. The high antigen specificity elicited within the hybrid signature was correlated with the disease course and facilitated with cognate antigen. Notably, the flanking residues outside of the MHC/TCR contact sites in PLP136-150 affected the functional avidity, which were closely correlated with the kinetics of DP-Treg. Furthermore, the sensitization of PLP136-150 suppressed the chronic reactivity to other encephalitogenic peptides without affecting the foreign antigen by inducing DP-Treg, unlike peptide tolerance induction.
These results augment our understanding of the state of autoimmune diseases, which may help to develop alternative therapies, such as inverse vaccination and in the design of efficient peptide-based anti-tumor immunotherapy, through manipulation of antigen-specific Treg capable to response to disease related antigens.

P1568

Suppression of protective immunity against bystander infectious agents upon murine malaria

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In malaria endemic areas, course of viral and bacterial infection, such as EB virus and *Mycobacterium* spp., become severer than that in malaria non-endemic area. By using non-malaria parasite antigen-specific TCRtg mice, such as OT-I and OT-II mice, non-specific T cells were functionally suppressed by malaria parasite infection, and its suppression was partly induced by regulatory T cell-independent manner. To evaluate a severity of immune suppression upon malaria, malaria-infected mice were received *Listeria monocytogenes* and the ability to protect against bystander infectious agents was examined. Mice were infected with non-lethal murine malaria parasite *Plasmodium berghei* XAT, and then administered with a sub-lethal dose of *L. monocytogenes* EGD strain to examine the anti-Listeria protection. The control malaria non-infected group overcome a sub-lethal of Listeria, however, the

DOI: 10.1002/eji.201970400

malaria infected group could not overcome it, as shown that all mice died until day 9 of Listeria injection. In addition, the number of Listeria was about 1000 times higher in spleen of malaria-infected mice than that in the control non-infected mice. Furthermore, the amount of IFN-γ from T cells was significantly decreased in malaria-infected mice. Next, using Rag2-deficient mice lacking T and B cells and IFN-γ receptor-deficient mice, almost no difference in the number of Listeria in spleen between the malaria-infected and non-infected mice. Collectively, upon malaria infection, protective immune response against bacterial infection is significantly decreased, and the decrease ability of IFN-γ produced by T cells was suggested to lead to a decrease in their protective immunity against bacterial infection.

P1569

Effects of rapamycin and cyclosporine A on the ability of hASCs to generate regulatory T cells

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Background: Human adipose-derived stem cells (hASCs) are mesenchymal stem cells (MSCs) that can be obtained easily from adipose tissues. They have the capacity to differentiate into multiple cell lineages and possess many regenerative properties. hASCs have been shown to be effective in treating severe refractory acute graft-versus-host disease and hematological and immunological disorders, indicating that hASCs may have immunomodulatory function.
Regulatory (Treg) cells are key regulators of inflammation and important for immune tolerance and homeostasis.
The aim of this study was to evaluate the effect of hASCs cultured in the environment of immunosuppressive agents rapamycin (RAPA) and cyclosporine A (CsA) on the generation of regulatory T cells ($CD4^{+}CD25^{high}Foxp3^{+}$)*in vitro*.
Methods: Human hASCs were cultured in the presence of rapamycin and cyclosporine A or without drugs (control). To evaluate induction of regulatory T cells by these ASCs, mixed leukocyte reaction (MLR) was applied. At the end of MLR cultures regulatory T cells were identified by surface phenotype: $CD4^{+}CD25^{high}Foxp3^{+}$and analyzed by flow cytometry.
Results: We observed a significant increase in the percentage of $CD4^{+}CD25^{high}Foxp3^{+}$cells when hASCs were cultured with CsA and a small increase when hASC were cultured with RAPA.
Conclusions: We have shown that the immunosuppressive agents rapamycin and cyclosporine A may affect the ability of hASC to generate regulatory T cells.

This work was supported by Grant no. STRATEGMED2/267976/13/NCBR/2015.

P2148

TIDE-E: an online evaluator of tumor immune-suppressive function of gene sets

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Recent immunotherapy studies have generated an extensive collection of datasets, covering different aspects of cancer immune evasion. Integration of this data facilitates the development of cancer immunology research. Due to the complexity of such studies, it is challenging for cancer biologist,

DOI: 10.1002/eji.201970400

translational scientists and immuno-oncologists to utilize those publicly available data in their investigations. Here, we presented the TIDE-E (Tumor Immune Dysfunction and Exclusion - Evaluator) website, which integrates three categories of datasets, to help investigators make an informed decision based on the current best-published knowledge (http://tide.dfci.harvard.edu). The regulator prioritization module can help cancer biologist to identify which genes in their input gene set are clinically relevant and worth functional and mechanistic follow-ups. The biomarker evaluation module allows translational scientists to evaluate how does their biomarker work on many ICB cohorts and how does it perform in comparison with other published biomarkers. The biomarker consensus test aims to help oncologists to decide whether a patient will respond to ICB according to many known biomarkers. As the immunotherapy data becomes increasingly available, we foresee that the TIDE-E website will have a significant impact on the biomarker development and our understanding of how cancer escape immune attack.

P2149

Roles of HLA-G expression in MDSCs proliferation and the M1/M2 macrophage differentiation in tumor bearing murine model

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HLA-G can play multiple immunosuppressive roles which benefit tumor development and disease outcome. Previous studies revealed that HLA-G can bind to the receptors such as ILT2 and ILT4 expressed on myeloid-derived suppressor cells (MDSCs) and macrophages, and render the expansion of MDSCs and M1 to M2 macrophage differentiation. In this study, we established a tumor bearing murine model with the lung cancer cell line NCI-H446 and its HLA-G transfectant in the immune competent C57BL/6 mice. The roles of HLA-G in myeloid-derived suppressor cells (MDSCs) proliferation and M1/M2 macrophages differentiation were investigated. NCI-H446 was served as negative control, mice were sacrificed at 5th, 10th, 15th, 20th days, respectively. The tumor volume and weight, and the rejection time were documented, and the percentage of splenic CD11b+Gr1+ MDSCs, F4/80+CD80+ M1 and F4/80+CD206+ M2 macrophage of tumorbearing mice were analyzed by flow cytometry. Data showed that HLA-G expression could enhance the tumor cell proliferation. Tumor grew fast in first five days in tumor-bearing mice, and decreased later. Tumor volume in NCI-H446-G tumor-bearing mice is greater than the control group in each time point, and the tumor in NCI-H446-G mice was rejected slower than other groups. The percentage of splenic MDSCs in NCI-H446-G was significantly increased, and the ratio of M1/M2 in NCI-H446-G was much lower in tumor-bearing mice. In summary, our study revealed that HLA-G expression enhances the proliferation of MDSCs and decrease the ratio of M1/M2 in tumor bearing mice, which benefits the tumor development and delay the tumor rejection.

P2150

Tumor microenvironment following Gemcitabine treatment favors differentiation of immunosuppressive Ly6C^{high} myeloid cells

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Regulations of myeloid-derived suppressor cells (MDSC) by chemotherapy still remain elusive. Here, we explored the mechanisms by which multi-dose clinical regimen of Gemcitabine (GEM) treatment induce an ongoing inflammation and apoptotic cells which favor the differentiation of

DOI: 10.1002/eji.201970400

immunosuppressive Ly6C^{high} monocytic MDSC (M-MDSC). We found that repeated GEM treatment promoted the expansion and differentiation of M-MDSC although tumor development was delayed in E0771 tumor-bearing mice. Accordingly, IFN-γ-producing CD4 and CD8 T cells were significantly decreased in the tumor microenvironment (TME) of GEM-treated mice. The conditioned medium of GEM-treated tumor cells (GEM-CM) enhanced differentiation of mouse BM Ly6C^{high} cells into immunosuppressive M-MDSC. Cytokine profiling of GEM-treated E0771 cells identified GM-CSF as one of the most differentially expressed cytokines. Blocking of GM-CSF with neutralizing Ab could partially reduce immunosuppression of Ly6C^{high} cells induced by GEM-CM. Enhanced production of reactive oxygen species (ROS) and activation of NF-κB were observed in GEM-treated tumor cells. Treatment with the inhibitor of NF-κB signaling and mitochondrial targeted antioxidant could abrogate GEM-induced hyperexpression of GM-CSF in E0771 cells. Further, the phagocytic clearance of apoptotic tumor cells (efferocytosis) enhanced the immunosuppressive function of BM Ly6C^{high} myeloid cells. Together, our results define an undesired effect of GEM-induced up-regulation of GM-CSF and apoptotic cells in the TME which favor differentiation of immunosuppressive Ly6C^{high} myeloid cells.

P2151

Echinococcus granulosus laminated layer: characterization of immunoregulatory activity and proteins composition

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Echinococcus granulosus is a flat worm that causes cyctic echinococcosis The Laminated layer (LL, outside layer of the cyst), protect the metacestode from host immunity. However, little is known about it mechanism of action. In this sense, the aim of our study was to Investigate *in vitro* the immunomodulatory effect of LL on LPS-induced mice splenocytes and its protein characterization. Splenocytes were prepared from WT Balb/c mice and stimulated by LL extract and LPS. NO was measured in culture supernatant by Griess modified method. The cytokines (IFN-gamma, IL-1beta, TGF-beta, IL-10) and Foxp3 mRNA expression were measured by quantitative RT-PCR. Cell subsets were identified by Flow cytometry using antibody against CD4, Foxp3, IL-10 and IFN-gamma. For LL characterization, SDS-PAGE, Western blot and amino-acid analysis where used.
Our results revealed a significant decrease ($p< 0.01$) in nitric oxide (NO) production, IFN-gamma ($p< 0.001$), IL-1beta ($p< 0.05$) and TNFalpha mRNA expression was reduced. Indeed, we found that NO diminution correlate ($p< 0,01$) with IFN-gamma diminution. Interestingly, the expression of Foxp3 ($p< 0.05$), TGF-beta ($p< 0.01$) and IL-10 ($p< 0.05$) was significantly upregulated. In another hand, flow cytometry results from LL treated splenocytes culture showed increased number of CD4+/Foxp3+ and CD4+/IL-10+ cells. In contrast, the number of CD4+ /FN-gamma+ cells decreased in LPS+LL treated splenocytes compared to LPS treated.
The WB and SDS-PAGE revealed that the LL has an antigenic nature.The amino-acid-analysis identified proteins such as, Paramyosin and 14-3-3-protein-homolog from *E. granulosus.*
Our data demonstrate the immuno-regulatory and anti-inflammatory effects of hydatid laminated layer.

DOI: 10.1002/eji.201970400

P2152

Tumor-educated lung epithelial cells promote the pre-metastatic niche formation

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Pre-metastatic nichein distant organs contributes to organ-specific metastatic tropism and tumor metastasis. As the main component of alveolus, the role of host lung epithelial cells in the lung metastatic niche formation and organ-specific metastatic tropism need to be clearly defined. Using the spontaneous lung metastatic models, we find that lung epithelial cells can promote pre-metastatic niche formation via inducing immunosuppressive microenvironment. Activation of host lung epithelial cells TLR3 by tumor exsomal RNAs can initiate neutrophil recruitment and pro-metastatic inflammation. Mechanistically, tumor-derived exosomal RNAs, which are enriched in snRNAs, activate TLR3 in lung epithelial cells, consequently inducing chemokine secretion in the lung and promoting neutrophil recruitment. In human lung cancers, high expression of TLR3 in adjacent tissues is closely correlated with high level of neutrophil infiltration, both of which predicted poor survival of the patients.Besides, we also find that tumor-derived exosomes can promote the functional alternation of lung epithelial cells to secrete abundant anti-inflammatory cytokines (TGF-β, IL-10). These lung epithelial cells can inhibit the proliferation of $CD4^+$T cells and induce the production of regulatory T cells (Tregs) via IL-10. Thus, our study indicates a profound role of host cells in inducing inflammatory cascade in distinct organ and pre-metastatic niche formation, suggesting effective targets to prevent and treat tumor metastasis to the lung.

P2153

Direct red 80 and suramin inhibit human immune-mediated response to neonatal pig islet cells in vitro

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Pigs are being considered as an alternative source of islets for transplantation. Thus, it is essential to develop anti-rejection strategies to overcome the human immune-mediated response to pig islet cells. In this study, we investigated the immune response of individuals with type 1 diabetes to neonatal pig islet cells in vitro and determined the inhibitory effect of Diret Red 80 (DR80) and Suramin on the proliferation of human peripheral blood mononuclear cells (PBMC) after stimulation with pig islet cells. Human PBMC from individuals with type 1 diabetes (n=4-6) were stimulated with gamma irradiated pig islet cells or pig PBMC with or without various concentrations of DR80 or Suramin. After 7 days of culture, human PBMC proliferation was traced with carboxyfluorescein succinimidyl ester and analysed with FCS 5.0 software. Response of human PBMC after 3 days stimulation with Concanavalin A (Con A) was also measured.
DR80 inhibited proliferation of human PBMC when stimulated with pig islet cells or pig PBMC (IC_{50}~62.5 µg/ml). Suramin also inhibited human PBMC proliferation in response to stimulation with pig

DOI: 10.1002/eji.201970400

islet cells or pig PBMC but with higher concentration than DR80 (IC_{50}~125 to 250 µg/ml). Similar trend was observed when human PBMC were stimulated with Con A. DR80 inhibited the proliferation of human PBMC stimulated with Con A (IC_{50}~31.2 to 62.5 µg/ml). Suramin also inhibited Con A-induced proliferation of human PBMC but with higher dose (IC_{50}~62.5 µg/ml).
These results demonstrate the inhibitory effect of DR80 and Suramin on the in vitro human immune response to pig cells.

P2154

Macrophage-derived CCL22 promotes an immunosuppressive tumor microenvironment via IL-8 in malignant pleural effusion

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Immune dysfunction often occurs in malignant pleural effusion (MPE). In our previous study, TGF-β derived predominantly from macrophages plays an important role in impairing T cell cytotoxicity in MPE. Therefore, we aimed to investigate whether other immunoregulatory cells and factors mediated TGF-β secretion from macrophages, involved in the immunosuppressive microenvironment of MPE, and to provide clues for potential immune therapy for MPE as well. We found that CCL22 level in MPE was significantly higher than that in non-malignant pleural effusion. The high level of CCL22 was closely associated with poor survival in MPE patients with lung cancer. CCL22 was dominantly produced by tumor-associated macrophages (TAMs) in MPE. Meanwhile, TAM-derived TGF-β mediated CCL22 expression in TAMs via c-Fos. CCL22 promoted the recruitment of regulatory T cells (Tregs) in MPE. Lastly, Treg-secreted high level of IL-8 further induced TGF-β production from TAMs, and promoted the immunosuppressive tumor microenvironment in MPE. Our results indicate that macrophage-derived CCL22 plays an important role in the immunosuppressive tumor microenvironment via IL-8 in MPE.

P2155

PI-3K (Phosphotidylinositol-3-Kinase) and STK (Src Tyrosine Kinase) are important in signaling pathway of PMN effector responses following burn injury-mediated immunosuppression

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Key Polymorphonuclear neutrophils (PMN) signaling components induced by burn injury have been known but potential signaling elements upstream and downstream to these key components have not been definitely elucidated. These signaling pathways cooperatively modulate PMN adhesion, degranulation (oxidant release), cell shape change, apoptosis function and other effector functions. In this study we have identified a role of PI-3K by using its inhibitor Wortmannin, and STK inhibitor, AG556. Rats were treated intra-peritoneally with the signaling blockers before subjecting them to burn (30% TBSA-total body surface area) injury. PMNs were harvested from blood of rats 18hr past injury. Oxidant radical production was measured in PMNs after their stimulation with PMA (100ng/mL) using Isoluminol-enhanced luminometry. Adhesion was assessed by digital imaging of Calcein-loaded PMNs cocultured for 30 minutes with TNF alpha-stimulated rat microvascular (RLMVEC) endothelial cells. The results show a substantially decreased oxidant radical production in burn-treated (Wortmannin or AG556) PMNs as compared to untreated burn. The decrease in PMN oxidant radical production corroborated with decrease in PMN adhesion to the endothelial cells. The deactivation of the PMNs

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DOI: 10.1002/eji.201970400

oxidant radical production and adhesive responses with the PI-3K and STK blockers supports the concept that both PI-3K and STK are important in signaling pathway of PMN effector responses following burn injury.

P2156

Endogenous retrovirus-encoded syncytin-2 contributes to exosome-mediated immunosuppression of T cells

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Modulation of the activation status of immune cell populations during pregnancy depends on placental villous cytotrophoblasts (VCT) and syncytiotrophoblast (STB) layer. Failure in the establishment of this immunoregulatory function leads to pregnancy complications. Our laboratory has been studying Syncytin-2 (Syn-2), an endogenous retroviral protein expressed in placenta and on the surface of placental exosomes. This protein plays an important role in STB formation through its fusogenic properties, but also possesses an active immunosuppressive domain (ISD). Considering that Syn-2 expression is importantly reduced in preeclamptic placentas, we were interested in addressing its possible immunoregulatory effects on T cells. Activated Jurkat T cells and peripheral blood mononuclear cells (PBMCs) were treated with monomeric or dimerized version of a control or a Syn-2 ISD peptide. Change in phosphorylation levels of ERK1/2 MAP kinases was measured in Jurkat cells solely treated with the dimerized ISD peptide. Upon incubation with the dimerized Syn-2 ISD peptide, significant reduction in Th1 cytokine production was specifically measured by ELISA and Human Th1/Th2 Panel Multi-Analyte Flow Assay. To determine if exosome-associated Syn-2 could also have an immunosuppressive effect, placental exosomes were incubated with activated Jurkat T cells and PBMCs. Upon quantification of Th1 cytokines in the supernatants, T cell activation was severely reduced. Interestingly, exosomes from Syn-2-silenced VCT incubated with PBMCS were less suppressive when compared to exosome derived from VCT transfected with control siRNA. Our results suggest that Syn-2 could be an important immune regulator both locally and at the systemic level, via its association with placental exosomes.

P2157

The roles of myeloid-derived suppressor cells (MDSCs) in hepatocellular carcinoma

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Backgrounds and Aims: Emerging evidence confirms the immunosuppressive characteristics of myeloid-derived suppressor cells (MDSCs) which can promote the tumor growth and blockade immune-checkpoint. Considering various of phenotypes of MDSCs, we aimed to investigate the diverse roles of MDSCs in hepatocellular carcinoma (HCC).

DOI: 10.1002/eji.201970400

Methods: Myeloid-derived Suppressor Cells (MDSCs) derived from HCC patients were quantified using immunohistochemistry and fluorescence-activated cell sorting (FACS). The immunosuppressive functions of MDSCs and the underlying mechanisms of MDSCs in HCC were investigated in an in vivo and in vitro system.
Results: In the clinical study, we first investigated the enrichment of MDSC in the peripheral blood of HCC patients by fluorescence-activated cell sorting (FACS) compared with the PBMC of healthy donors ($P< 0.05$) and high levels of MDSC was related to the high recurrence rate and poor prognosis in HCC patients. We further compared the three major groups of MDSCs including PMN- (polymorphonuclear), M- (monocytic) and E- (early-stage) MDSCs in HCC. We next performed CD11b and CD8 immunohistochemical staining in tumors of HCC patients indicating that CD8+ T cells can be inactivated in tumor microenvironment. In an orthotopic mice model, the bulk of liver tumor was increased after MDSCs injection compared with the control.
Conclusion: These results described the immunosuppressive roles of MDSC which can promote tumor growth in HCC. We suggested targeting-MDSCs can be a further treatment to enhance the immunotherapy.

P2923

Experimental study on immune tolerance of rhesus monkey liver transplantation induced by immature dendritic cells comodified with CD40L extracellular gene

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In liver transplantation, interaction between CD40 and CD34 can promote DC maturation and expression of CD86 and CD1a which play an important role in cell immune. To identify stable expression of recombinant adenovirus vector expressing of rhesus monkey and the ability to inhibit T cells proliferation, we successfully constructed a recombinant adenovirus vector expressing rhesus monkey pAd-CMV-efCD40L with the extracellular gene of CD40L. After transfection, the results of flow cytometry showed that the maturity and purity of DC in experimental groups were 27.61% and 92.35%. Moreover, using mixed lymphocyte assay, PCR and Western blot the extracellular gene expression of CD40L and T cell proliferation were appraised. In addition, we demonstrated that recombinant adenovirus-mediated CD40L can be stable expression in immature dendritic cells (imDC), and inhibit T cell proliferation. **In conclusion, recombinant adenovirus-mediated CD40L infection of rhesus imDC was demonstrated its inhibitory effect on T cell proliferation and expression in dendritic cells. Understanding the role of CD40L in immune tolerance of liver transplantation induced by rhesus imDC will provide a new strategy for immune tolerance after clinical transplantation in the future.**

P2924

Multifunctional MnO_2 nanoparticle platform can improve the immune microenvironment of tumor

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Background: The rapid proliferation of tumor cells consume a large number of organic substances and produce many acid metabolites, which result in the acidic pH value (around 5-6) in various solid

DOI: 10.1002/eji.201970400

tumors and area near tumors. Rapidly proliferating and dividing tumor cells use the aerobic glycolysis and consume a large number of oxygen molecules, resulting in hypoxia in tumor microenvironment. Hypoxia and acidic microenvironment not only lead to the inactivation of immune cells in tumors, but also promote the angiogenesis and metastasis of tumors.
Materials and Methods: In this study, we designed a nano-scale hollow MnO_2 as a carrier (MnO_2 as a catalyst, which can decompose hydrogen peroxide in tumor microenvironment) to connect HIF-1a hypoxia factor antibody. Therefore, nano-MnO_2-HIF-1α antibody not only produces oxygen, but also improves the hypoxic environment. As delivery vectors, hollow MnO2 particles can deliver cytotoxic drugs, PD-1 antibodies or anti-angiogenesis drugs.
Results: In vitro studies in breast cancer cell line MCF-7 showed that the PH of MnO_2 culture medium increased significantly and the molecular oxygen level increased. Moreover, the antibody of MnO_2+doxorubicin+HIF-1α has obvious cytotoxicity, which is better than that of doxorubicin, MnO_2+doxorubicin or MnO_2+HIF-1α alone. In vivo studies showed that MnO_2 + doxorubicin + HIF-1α antibody had obvious anti-tumor effect.
Conclusion: The first multi-drug delivery platform using hollow MnO2 as carrier not only improves the effect of cytotoxic drugs, but also improves the immune microenvironment of tumors, so it is a feasible strategy to overcome drug resistance.

P2925

IDO inhibitor synergized with radiotherapy to delay tumor growth by reversing T cell exhaustion

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Recent studies suggest that radiotherapy (RT) can induce immune activation, which not only reduces the progression of tumors but also causes the release of tumor antigens. Studies have shown that indoleamine 2,3-dioxygenase (IDO) can cause T cell exhaustion and increase regulatory T cell (Treg) formation. Therefore, IDO inhibitors combined with radiotherapy may have a synergistic effect by relieving immunosuppression. Lewis lung cancer (LLC)-bearing mice were treated with the IDO inhibitor 1-methyl-tryptophan (1MT) and/or 10 Gy RT. Tumor size was measured by caliper every day, and flow cytometry was performed to measure the expression of DC maturation markers, inhibitory receptors (iRs), ligands, cytotoxic T cells and the Treg phenotypic markers. Quantitative real-time RT-PCR was used to evaluate the mRNA expression levels of IDO, PD-L1, PD-1, TIM-3, BTLA and Galectin-9. Treatment with 1MT or RT reduced the tumor growth compared to those of the control group, and combination therapy was more effective than either treatment alone. Flow cytometry showed an upregulation of CD80, CD86, and MHC II expression in spleen DCs and a concurrent downregulation of Treg in lymphocytes in the treatment groups. Compared to those of the controls, the levels of the iRs and ligands that affect DCs and T cells were both decreased in the treatment groups, while PD-L1, PD-1, TIM-3, BTLA and Galectin-9 expression were observed. IDO inhibition synergizes with RT can downregulate Tregs, iRs and ligands prevented T cell exhaustion. By activating DCs and T cells, this combination therapy can strongly boost antitumor immunity and inhibit tumor progression.

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DOI: 10.1002/eji.201970400

P2926

Effect of tumor microenvironment on TRAF6 expression in dendritic cells

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Background and objective: Tumor microenvironment (TME) is an important site for tumor cells to inhibit normal immune response. Dendritic cells play an important role in mediating immune response and can activate T cell response in TME, but with the progression of tumor, tumor infiltrating DC (TIDC) development and maturation is impaired by a variety of tumor-derived inhibitory factors. DC will change from immune activation to immunosuppression. However, the molecular mechanism of TME inhibiting DC maturation is not clear, and this study has carried out a preliminary exploration.
Methods: The supernatant of mouse breast cancer cell line 4T1 and human leukemia cell line MV4-11/primary leukemia cell were co-cultured with mouse bone marrow-derived DC (BMDC) and human peripheral blood-derived DC for 48 hours respectively. The expression of TRAF6 in DC was detected by RT-qPCR and western blot. The expression of costimulatory molecules in DC was detected by flow cytometry. BMDC treated with tumor cell culture supernatant was mixed with CFSE labeled mice T cells, and the proliferation of T cells was detected by flow cytometry.
Results: 1. The expression of TRAF6 in mRNA and protein level in DC was significantly decreased after treated with tumor cell culture supernatant.; 2. The expression of MHC-II, CD80 and CD86 in tumor microenvironment DC was decreased obviously; 3. T cell proliferation was inhibited.
Conclusion: Tumor microenvironment may inhibit the maturation of DC by inhibiting the expression of TRAF6 in DC.
Keywords: tumor microenvironment;dendritic cell;TRAF6

P2927

SALL4-mediated upregulation of exosomal miR-146a-5p drives T-cell exhaustion by M2 tumor-associated macrophages in HCC

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Emerging evidence indicates that cancer cell-derived exosomes contribute to cancer progression through the modulation of tumor microenvironment, but the underlying mechanisms are not fully elucidated. Here, we report that hepatocellular carcinoma (HCC)-derived exosomes could remodel macrophages by activating NF-κB signaling and inducing pro-inflammatory factors, and resulted in M2-polarized tumor-associated macrophages. In addition, expression of IFN-γ and TNF-α was inhibited, while the expression of inhibitory receptors such as PD-1 and CTLA-4 were upregulated in T cells by HCC-derived exosome educated macrophages. Data also revealed that HCC exosomes were enriched with miR-146a-5p and promoted M2-polarization. Further investigation demonstrated that the transcription factor Sal-like protein-4 (SALL4) was critical for regulation miR-146a-5p in HCC exosomes and M2-polarization. Mechanistically, SALL4 could bind to the promoter of miR-146a-5p, and directly control its expression in exosomes. Blocking the SALL4/miR-146a-5p interaction in HCC reduced the expression of inhibitory receptors on T cells, reversed T cell exhaustion, and delayed HCC progression in DEN/CCL_4-induced HCC mouse model. In conclusion, identification of a role of the exosomal SALL4/miR146a-5p regulatory axis in M2-polarization as well as HCC progression provides potential targets to for therapeutic and diagnostic applications in liver cancer. prevent liver cancer.

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DOI: 10.1002/eji.201970400

P2928

Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction

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T cells become dysfunctional when they encounter self antigens or are exposed to chronic infection or to the tumour microenvironment. The function of T cells is tightly regulated by a combinational co-stimulatory signal, and dominance of negative co-stimulation results in T cell dysfunction. However, the molecular mechanisms that underlie this dysfunction remain unclear. Here, using an in vitro T cell tolerance induction system in mice, we characterize genome-wide epigenetic and gene expression features in tolerant T cells, and show that they are distinct from effector and regulatory T cells. Notably, the transcription factor NR4A1 is stably expressed at high levels in tolerant T cells. Overexpression of NR4A1 inhibits effector T cell differentiation, whereas deletion of NR4A1 overcomes T cell tolerance and exaggerates effector function, as well as enhancing immunity against tumour and chronic virus. Mechanistically, NR4A1 is preferentially recruited to binding sites of the transcription factor AP-1, where it represses effector-gene expression by inhibiting AP-1 function. NR4A1 binding also promotes acetylation of histone 3 at lysine 27 (H3K27ac), leading to activation of tolerance-related genes. This study thus identifies NR4A1 as a key general regulator in the induction of T cell dysfunction, and a potential target for tumour immunotherapy

P2929

Blockade of IDO-kynurenine-AhR metabolic circuitry abrogates IFN-g-induced immunologic dormancy of tumor-repopulating cells

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Interactions with the immune system may lead tumorigenic cells into dormancy. However, the underlying molecular mechanism is poorly understood. Using a 3D fibrin gel model, we show that IFN-g induces tumour-repopulating cells (TRCs) to enter dormancy through an indolamine 2,3-dioxygenase 1 (IDO1)-kynurenine (Kyn)-aryl hydrocarbon receptor (AhR)-p27 dependent pathway. Mechanistically, IFN-g signalling triggers differentiated tumour cell apoptosis via STAT1; however, when IDO1 and AhR are highly expressed as in TRCs, IFN-g results in IDO1/AhR-dependent p27 induction that prevents STAT1 signalling, thus suppressing the process of cell death and activating the dormancy program. Blocking the IDO/AhR metabolic circuitry not only abrogates IFN-g-induced dormancy but also results in enhanced repression of tumour growth by IFN-g-induced apoptosis of TRCs both in vitro and in vivo. These data present a previously unrecognized mechanism of inducing TRC dormancy by IFN-g, suggesting a potential effective cancer immunotherapeutic modality through the combination of IFN-g and IDO/AhR inhibitors .

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DOI: 10.1002/eji.201970400

P2930

Multiple myeloma cell-derived IL-32γ increases the immunosuppressive function of macrophages by promoting indoleamine 2,3-dioxygenase (IDO) expression

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The interaction of multiple myeloma (MM) cells with macrophages (MΦs) contributes to the pathophysiology of MM. We previously showed that IL-32 is overexpressed in MM patients. The present study was designed to explore the clinical significance of IL-32 in MM and to further elucidate the mechanisms underlying the IL-32-mediated immune function of MΦs. Our results showed that high IL-32 expression in MM patients was associated with more advanced clinical stage. RNA-sequencing revealed that IL-32γ significantly induced the production of the immunosuppressive molecule indoleamine 2,3-dioxygenase (IDO) in MΦs, and this effect was verified by qRT-PCR, western blotting, and immunofluorescence. Furthermore, MM cells with IL-32-knockdown showed a reduced ability to promote IDO expression. As a binding protein for IL-32, proteinase 3 (PR3) was universally expressed on the surfaces of MΦs, and knockdown of PR3 or inhibition of the STAT3 and NF-κB pathways hindered the IL-32γ-mediated stimulation of IDO expression. Finally, IDO-positive IL-32γ-educated MΦs inhibited CD4+ T cell proliferation and IL-2, IFN-γ, and TNF-α production. Taken together, our results indicate that IL-32γ derived from MM cells promotes the immunosuppressive function of MΦs and is a potential target for MM treatment.

P2931

Erythropoeitin signaling in macrophages promotes dying cell clearance and immune tolerance

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The failure of apoptotic cell clearance is linked to autoimmune diseases, nonresolving inflammation, and developmental abnormalities; however, pathways that regulate phagocytes for efficient apoptotic cell clearance remain poorly known. Apoptotic cells release find-me signals to recruit phagocytes to initiate their clearance. One intriguing hypothesis suggeste the primary role of the find-me signals for priming rather than recruitment of phagocytes, however underlying mechanisms remain unknown. **Here we found that find-me signal sphingosine 1-phosphate (S1P) activated macrophage erythropoietin (EPO) signaling promoted apoptotic cell clearance and immune tolerance.** Dying cell-released S1P induced the expression of EPO, which is viatl for erythrocyte production, and its receptor (EPOR) in macrophages via hypoxia-inducible factor 1α. EPOR-deficient macrophages exhibited impaired apoptotic cell phagocytosis and an aberrant proinflammatory response. EPO enhanced apoptotic cell clearance through peroxisome proliferator activated receptor-γ (PPARγ). Furthermore, EPO increased macrophage PPARγ expression through activating JAK2/ERK/CEBPβ pathway. Moreover, macrophage-specific *Epor*$^{-/-}$ mice developed lupus-like symptoms, and interference in EPO signaling ameliorated the disease progression in lupus-like mice. In addition, treatment with PPARγ agonist ameliorated lupus-like symptoms in macrophage-specific *EPOR*$^{-/-}$ mice, but EPO intervention shows no protective effects on lupus-like symptoms in macrophage-specific *PPARγ*$^{-/-}$ mice. Thus, we have identified a pathway that regulates macrophages to clear dying cells, uncovered the priming function of find-me signal S1P, and found a role of the erythropoiesis regulator EPO in apoptotic cell disposal, with implications for harnessing dying cell clearance.

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DOI: 10.1002/eji.201970400

Immunometabolism

O201

Lactate inhibits ATP6V0d2 expression in tumor-associated macrophages to promote HIF-2α-mediated tumor progression

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Macrophages perform key functions in tissue homeostasis that are influenced by the local tissue environment. Within the tumor microenvironment, tumor-associated macrophages can be altered to acquire properties that enhance tumor growth. Here, we found that lactate, a metabolite found in high concentration within the anaerobic tumor environment, activated mTORC1 that subsequently suppressed TFEB-mediated expression of the macrophage-specific vacuolar ATPase subunit ATP6V0d2. Atp6v0d2-/- mice were more susceptible to tumor growth, with enhanced HIF-2α-mediated VEGF production in macrophages that display a more protumoral phenotype. We found that ATP6V0d2 targeted HIF-2α but not HIF-1α for lysosome-mediated degradation. Blockade of HIF-2α transcriptional activity reversed the susceptibility of Atp6v0d2-/- mice to tumor development. Furthermore, in a cohort of patients with lung adenocarcinoma, expression of ATP6V0d2 and HIF-2α was positively and negatively correlated with survival, respectively, suggesting a critical role of the macrophage lactate/ATP6V0d2/HIF-2α axis in maintaining tumor growth in human patients. Together, our results highlight the ability of tumor cells to modify the function of tumor-infiltrating macrophages to optimize the microenvironment for tumor growth.

O202

Lactate influx into human CD4+ T-cells, via SLC5A12, causes STAT3- and fatty acid synthesis-mediated IL17 expression and tissue retention

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The tissue microenvironment is a key determinant of pathology in both inflammatory disease and cancer. Accumulation of lactate in the tumour microenvironment, largely due to the accelerated metabolism of cancer cells, has been shown to skew the local immune response towards immune suppression favouring tumour growth. Immune cells that infiltrate the tissue microenvironments in inflammatory disorders find similarly harsh conditions, including scarce nutrients and high levels of lactate and other metabolites. Yet opposite to the tumour microenvironment, the inflamed tissue features high levels of inflammatory cytokines with impairment regulatory responses, contributing to the exacerbation of the chronic inflammatory disease process.
Here, we explored the response of CD4+ T-cells to lactate in the context of inflammation. We show that lactate accumulation at the inflamed tissue contributes, together with activating and inflammatory

DOI: 10.1002/eji.201970400

stimuli, to the up-regulation of the sodium-coupled lactate transporter SLC5A12 on human CD4+ T-cells. SLC5A12-mediated lactate influx into human CD4+ T-cells induces a plastic reshaping of their effector phenotype at the site of inflammation, resulting in increased IL-17 production via nuclear STAT3 signalling and enhanced fatty acid synthesis. It also leads to increased CD4+ T-cell retention at the inflamed tissue as a consequence of reduced glycolysis and enhanced fatty acid synthesis. Furthermore, we show that lactate/SLC5A12-induced metabolic reprogramming in CD4+ T-cells is a distinctive mechanism of the RA synovitis subset characterized by lymphocyte infiltration in the arthritic synovium. Finally, antibody-mediated blockade of SLC5A12 ameliorates the clinical scores in a murine model of CD4+ T-cell-driven arthritis.

O203

Sugar alcohols of polyol pathway serve as alarmins to mediate the local-systemic innate immune communication

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Interorgan immunological communication is critical to connect the local-systemic innate immune response and orchestrate a homeostatic host defense. However, the structure and function of interorgan communication networks remain unclear, particularly the factors and their roles in mediating innate immune signal transmissions. We find that *Drosophila* IMD (homologous to mammalian TNFR/NF-κB) response in guts locally can sequentially trigger a systemic IMD reaction in the remote organ, the fat body (analogous to the mammalian liver). Innate immune activation is usually associated with an increased glucose metabolism in inflammatory cells, but whether carbohydrate metabolites involve in gut-fat body immunological communication (GFIC) has not been explored. Herein we report that sugar alcohols of the polyol pathway in hemolymph are essential for the spatiotemporal regulation of GFIC in *Drosophila*. IMD activation in guts, either by enterobacterial infection or ectopic expression of IMD receptor (PGRP-LCx), elevated levels of sorbitol and galactitol in hemolymph. Detailed studies revealed that aldose reductase (AR) in hemocytes, the rate-limiting enzyme of the polyol pathway in the alternative glucose metabolism, was necessary and sufficient for the increase of plasma sugar alcohols. Sorbitol relayed GFIC by subsequent activation of metalloprotease 2 (mmp2), which cleaved PGRP-LC and activated IMD response in fat bodies. Thus, this work unveiled that GFIC relied on the intermediate activation of the polyol pathway in hemolymph, and AR provides a critical metabolic checkpoint in the global inflammatory response.

O204

Salt modulates cellular metabolism of regulatory T cells

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High-salt intake has been linked with shifts in the immune cell balance, mainly by promoting proliferation and activity of pro-inflammatory cells, such as T helper 17 (Th17) and M1 macrophages, and by impairing the functions of anti-inflammatory cells such as regulatory T cells (Tregs) and M2 macrophages. However, the precise molecular mechanisms that contribute to this phenomenon are still unknown. The role of metabolic regulation in shaping immune responses has gained increasing

DOI: 10.1002/eji.201970400

attention in recent years. Cellular metabolism is a vital process, which is essential for growth, survival and proliferation of every cell type, and can be greatly influenced by environmental factors such as diet. High-salt was previously shown to promote metabolic changes in M2 macrophages by decreasing their mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis necessary for their activation. So we hypothesize that this phenomenon is most likely extrapolated to other cell types such as Tregs. We analyzed changes on the metabolic pathways of human Tregs after high-salt exposure. Our results show significant salt-induced changes in both OXPHOS and glycolysis and since these changes are known to alter Treg suppressive function both *in vitro* and *in vivo*, we further hypothesize that the observed metabolic alterations might be linked to the loss of suppressive function seen in human Tregs upon high-salt challenge. Thus, the interference with these pathways may have the potential for targeting Tregs in salt-sensitive diseases.

O205

Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome

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Reciprocal interactions between the metabolic system and immune cells play pivotal roles in diverse inflammatory diseases, but the underlying mechanisms remain elusive. The activation of bile acidmediated signaling has been linked to improvement
in metabolic syndromes and enhanced control of inflammation. Here, we demonstrated that bile acids inhibited NLRP3 inflammasome activation via the
TGR5-cAMP-PKA axis. TGR5 bile acid receptorinduced PKA kinase activation led to the ubiquitination of NLRP3, which was associated with the PKA-induced phosphorylation of NLRP3 on a single residue, Ser 291. Furthermore, this PKA-induced phosphorylation of NLRP3 served as a critical brake on NLRP3 inflammasome activation. In addition, in vivo results indicated that bile acids and TGR5 activation blocked NLRP3 inflammasome-dependent inflammation, including lipopolysaccharide-induced systemic inflammation, alum-induced peritoneal inflammation, and type-2 diabetes-related inflammation. Altogether, our study unveils the PKA-induced phosphorylation and ubiquitination of NLRP3 and suggests TGR5 as a potential target for the treatment of NLRP3 inflammasome-related diseases.

O207

Cholesterol homeostatic regulator SCAP-SREBP2 integrates NLRP3 inflammasome activation and cholesterol biosynthetic signaling in macrophages

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Cholesterol metabolism has been linked to immune functions, but the mechanisms by which cholesterol biosynthetic signaling orchestrates inflammasome activation remain unclear. Here, we have shown that NLRP3 inflammasome activation is integrated with the maturation of cholesterol master transcription factor SREBP2. Importantly, SCAP-SREBP2 complex endoplasmic reticulum-to-Golgi translocation was required for optimal activation of the NLRP3 inflammasome both *in vitro* and *in vivo*. Enforced cholesterol biosynthetic signaling by sterol depletion or statins promoted NLPR3

DOI: 10.1002/eji.201970400

inflammasome activation. However, this regulation did not predominantly depend on changes in cholesterol homeostasis controlled by the transcriptional activity of SREBP2, but relied on the escort activity of SCAP. Mechanistically, NLRP3 associated with SCAPSREBP2 to form a ternary complex which translocated to the Golgi apparatus adjacent to a mitochondrial cluster for optimal inflammasome assembly. Our study reveals that, in addition to controlling cholesterol biosynthesis, SCAP-SREBP2 also serves as a signaling hub integrating cholesterol metabolism with inflammation in macrophages.

O208

The immune-endocrine relationship between diabetes and tuberculosis comorbidity: deleterious high production of cortisol and its correction by dehydroepiandrosterone

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Diabetes is a well recognized like a risk factor for Tuberculosis (TB), with a 3-fold susceptibility rate increase. Although diverse studies have found several immune abnormalities in DM that favor TB development, the immune-endocrine mechanisms of this comorbidity have not been studied. Both diseases are characterized by excessive glucocorticoid production, apparently by the overstimulation of 11-β-hydroxysteroid dehydrogenase type 1 (11-βHSD1). This enzyme has reductase activity for 11-ketosteroids products, favors the conversion of inactive cortisone to active cortisol, and it seems that in DM the high activity of 11-βHSD1 can increase cortisol production favoring insulin resistance and suppression of Th-1 response that could contribute to the high susceptibility of TB, also suggesting that blocking this enzyme could improve glucose metabolism and favors the protective Th-1 response in TB. This 11-βHSD1 blocking activity is exhibited by the adrenal steroid hormone dehydroepiandrosterone (DHEA) or its synthetic analog 16-alpha-bromoepiandrosterone (BEA, SD Chem), which is a modified androstane hormone without androgenic effects. In this work, this effect was studied using a BALB/c mouse model of DM-2 and TB, treating DM mice with BEA after two months of intratracheal infection with a high dose of *M. tuberculosis* reference strain H37Rv. This treatment produced significant reduction of hyperglycemia, liver steatosis, pulmonary bacillary loads and pneumonia. Thus, it seems that high 11-βHSD1 activity in DM and TB produce high cortisol production that is deleterious in the comorbidity DM/TB, and using glucocorticoid antagonist hormones such as DHEA or BEA is a beneficial and novel therapy for both diseases.

O361

CD73^{hi}adipose Treg subset prevents metabolic dysregulation

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Adipose regulatory T (Treg) cells maintain immune and metabolic homeostasis in young and healthy mice but may contribute to the exacerbation of age-related insulin resistance. We found that insulin signaling occurs through the HIF-1α-Med23-PPAR-γ axis to induce adipose Treg dysfunction and insulin resistance. This outcome is associated with the downregulation of CD73. To support these findings, Treg cells depleted of *Hif1a* or*Med23* havedecreased expression of PPAR-γ that in turn promotes the accumulation of CD73^{hi}adipose Tregs and physiologic adenosine levels to prevent metabolic dysregulation. Interestingly, in Type 2 Diabetes the expression of CD73 by circulating Tregs

DOI: 10.1002/eji.201970400

is downregulated. The adoptive transfer of CD73^{hi}adipose Tregs, and the administration of adenosine or an A_{2A}receptor agonist, could all improve insulin sensitivity in animal models. Our findings reveal the protective roles of CD73^{hi}Tregs in metabolic dysregulation and offer a new therapeutic perspective to treat Type 2 Diabetes through immunotherapy.

O362

Lipid droplet dependent fatty acid metabolism modulates the polarization of tumor associated macrophages

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Tumor-associated macrophages (TAMs) promote tumor growth and metastasis by suppressing tumor immune surveillance. However, the role of fatty acids in this process has been neglected for years, although it has been shown, that tumor cells prefer to release free fatty acids into the microenvironment. Our study now, reveals for the first time that the fatty acid enriched environment itself, is sufficient to induce the phenotype of TAMs.
In our study, we demonstrate that the immunosuppressive phenotype of TAMs is controlled by long chain fatty acid metabolism, specifically unsaturated fatty acids. Additionally, lipid droplets, which are enriched in the presence of unsaturated fatty acids were identified as essential organelles for fatty acid oxidation and represent effective targets to block *in vitro* polarization of TAMs and consequently tumor growth *in vivo*. In line, the very same lipid droplets, we found accumulated only in tumor infiltrating TAMs but not in benign tissue of colorectal cancer patients. In summary, we here define the mechanism how unsaturated fatty acids polarize myeloid cells to the classical suppressive TAMs and propose an alternative therapeutic strategy by targeting the pro-tumoral myeloid cells on a metabolic level.

O363

One-carbon metabolism supports S-Adenosylmethionine and histone methylation to drive inflammatory macrophages

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Activated macrophages adapt their metabolic pathways to drive the pro-inflammatory phenotype, but little is known about the biochemical underpinnings of this process.
Here, we find that lipopolysaccharide (LPS) activates the pentose phosphate pathway, the serine synthesis pathway, and one-carbon metabolism, whose synergism drives epigenetic reprogramming for the expression of interleukin-1β (IL-1β) but not tumor necrosis factor-α. Glucose-derived ribose and one-carbon units fed by both glucose and serine metabolism are synergistically integrated into the methionine cycle through *de novo* ATP synthesis, and fuel the generation of S-adenosylmethionine (SAM) during LPS-induced inflammation. Impairment of these metabolic pathways that feed SAM generation lead to anti-inflammatory outcomes, implicating SAM as an essential metabolite for inflammatory macrophages. Mechanistically,

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DOI: 10.1002/eji.201970400

SAM generation maintains a relatively high SAM/S-adenosylhomocysteine ratio to support histone H3 lysine 36 trimethylation for IL-1β production. We therefore identify a synergistic effect of glucose and amino acid metabolism on orchestrating SAM availability that is intimately linked to the chromatin state for LPS-induced inflammation.

O364

Resistance to Leishmania donovani infection is associated with host metabolism

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Being an obligate intracellular parasite, *Leishmania* amastigotes remain dependent on the host macrophages for nutrients. *Leishmania* require large amount of lipids and rest of nutrients for regular cellular functions for survival and replication. Besides this, the host cells undergo major metabolic changes while activation and transformation into their subsets, which might affect anti-leishmanial immune response. Based on this scientific rationale, we hypothesize that the resistance and susceptibility to *Leishmania donovani* might be due differential metabolism of the host cells. To examine the hypothesis, we selected four different inbred mouse strains- BALB/c, C57BL/6, FVB/N and 129/Sv- and checked their susceptibility to *Leishmania donovani*. It was observed that BALB/c mice were susceptible and had highest parasite load whereas 129/Sv was resistant and had the least parasite load. The splenocytes from the infected resistant and susceptible mice and infected peritoneal macrophages did show differences in cytokine and metabolic gene expression. The glycolysis and mitochondrial oxidative phosphorylation also showed significant differences. The host metabolism might favor parasite survival in two different ways: first, by providing essential nutrients and the second by dictating the transformation of immune cells into specific subsets which might help parasites to survive by producing pro-parasitic immune mediators. Ours is the first study that shows functional association between host metabolism and susceptibility to *Leishmania donovani* infection.

O365

Energy sensing directs the immunometabolic course of sepsis inflammation

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Sepsis inflammation substantially consumes energy and changes cellular NAD/NADH ratio, which is sensed in real time by sirtuin family (SIRTS) to reprograms inflammation and metabolism. To adapt pro-inflammation, SIRT 1, 6, and 3 activation switches the early anabolic effector immune phenotype to a catabolic immune repressor phenotype. Mechanistically, SIRT1 and 6 sense the increase in NAD/NADH ratio and epigenetically coordinate an immunometabolic co-switching of pro-inflammation to immune suppression and glycolysis to β-oxidation; simultaneously, SIRT1 and SIRT3 reprogram a biphasic energetics to support the inflammation adaptation. However, tryptophan-dependent de novo NAD biosynthesis sustains reductive to oxidative redox shift and fuels SIRT activation, which promotes an insurmountable gap between anabolic energy need and the catabolic energy supply in lethal sepsis. In applying this checkpoint concept to treatment, we have discovered two redox controlled and novel druggable checkpoints that reverse immune tolerance and prevent death: the SIRT1 inhibition by EX-527 and pyruvate dehydrogenase complex (PDHC) stimulation by dichloroacetate.
To resolve inflammation, we found that SIRT4 is the first recognized regulator of sepsis survival by readjusting both of nuclear and mitochondrial-derived metabolic inflexibility. First, SIRT4 reverses

DOI: 10.1002/eji.201970400

SIRT1-SIRT6-dependent epigenetic block on glucose oxidation that underlies catabolic sepsis. Second, SIRT4 reactivates PDHC-dependent glucose oxidation, restore the glycolysis/glucose oxidation ratio to narrow the gap between anabolic and catabolic bioenergetics.
In summary, our studies of sepsis inflammation support primary roles for energy sensing signaling in reprogramming immunometabolism and mitochondrial bioenergetics to direct sepsis resolution and clinical prognosis, and advance the checkpoint treatment concept for inflammation and immune therapeutics.

O366

24-nor-ursodeoxycholic acid ameliorates inflammation by reshaping mTOR proteome and immunometabolism sensing programs in CD8 T cells

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Aberrant T cell responses are key immunopathological factors driving inflammatory liver diseases. T cells undergo metabolic reprogramming during effector differentiation to accommodate changes in energy demands. 24-Nor-ursodeoxycholic acid (*nor*UDCA) is a novel therapeutic bile acid already used in phase III clinical studies for treatment of liver diseases, such as Primary Sclerosing Cholangitis (PSC), a devastating cholestatic liver disease lacking effective medication. Here we show that *nor*UDCA reduced $CD8^+$ T cell proliferation and lymphoblast formation by 53% compared to untreated controls ($p< 0.05$). Mass spectrometry (MS) analysis revealed that *nor*UDCA specifically modulated mTOR phosphoproteome network as evidenced by reduced phosphorylation of mTOR substrate RPS6(Ser235/236) by 43% ($p< 0.05$). Consequently, *nor*UDCA reduced abundance of mTOR regulated glycolytic enzyme hexokinase2 ($p< 0.05$). Subsequently, *nor*UDCA reduced abundance of effector molecules GranzymeB and IFNγ by 43% ($p< 0.005$) and 21% ($p< 0.05$), respectively and increased abundance of lymph node homing receptors, like CD62L and CCR7 ($p< 0.05$). Conversely, fatty acid (FA) oxidation was enhanced by *nor*UDCA as reflected by higher abundance of Cpt1a and Cpt2 ($p< 0.005$), while FA synthesis was reduced as shown by lower abundance of FA synthase. Supporting MS finding, in vivo *nor*UDCA dampened $CD8^+$ T cell liver infiltration and response in Mdr2 $(Abcb4)^{-/-}$model of PSC and LCMV model of $CD8^+$ T cell-driven hepatic immunopathology. Our data unravelled a novel immunometabolic mode of action of *nor*UDCA by reshaping mTOR proteome and phosphoproteome in $CD8^+$ T cell, thus contributes to *nor*UDCA therapeutic potency that may warrant further clinical applications in T cell-mediated diseases.

DOI: 10.1002/eji.201970400

O367

Fibrinogen-like protein 2 aggravates non-alcoholic steatohepatitis by enhancing inflammatory signaling in macrophages

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Introduction: Fibrinogen-like protein 2 (fgl2), which is critical for immune regulation in inflammatory state, leads to production of proinflammatory cytokines and hepatic injury. **Objectives:** This study aims to investigate the role of fgl2 in the pathogenesis of non-alcoholic steatohepatitis (NASH) and to explore the mechanisms.
Methods: The wild type and fgl2(-/-)C57BL/6 mice were subjected to methionine/choline-deficient (MCD) diet or high fat diet (HFD) for establishing NASH models. Liver injury and steatosis, were detected by Hematoxylin-eosin staining and Oil-red staining. *Bone marrow*-derived *macrophages* were stimulated with LPS or free fatty acids (FFA). The NF-*k*B, p38-MAPK and NLRP3 inflamasome signaling pathways as well as lipid metabolism related molecules were tested both in vivo and in vitro using RT-PCR and western blotting. The levels of proinflammatory cytokines in cell supernatant and homogenate of liver tissues were tested by ELISA.
Results: Compared with WT mice NASH models, inflammatory liver injury and steatosis were dramatically attenuated in fgl2-deficient test groups. In both liver tissues and *bone marrow*-derived macrophages from the NASH models, fgl2 deficiency resulted in reduced secretion of proinflammatory cytokines including TNF-a, IL-1b, MCP-1 and IL-6. Meanwhile, activation of NF-*k*B and p38-MAPK signaling pathways as well as expression of NLRP3, pro-caspase-1, caspase-1 and pro-IL-1b were significantly decreased. Genes involved in lipogenesis (SREBP-1c, HMGCR) were downregulated while those involved in lipometabolism (PPARa, PGC-1a) were upregulated in fgl2-deficient test groups.
Conclusion: Fgl2 promotes inflammation and steatosis in NASH by enhancing inflammatory activity of macrophages via multiple signaling pathways.

O368

Metabolic-immunoregulatory role of mTOR signaling in NAFLD-associated hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer and the second leading cause of cancer deaths worldwide. Non-alcoholic-fatty-liver disease (NAFLD) comprises a spectrum of disorders from simple steatosis to non-alcoholic steatohepatitis (NASH). Driven by the epidemics of obesity and diabetes, it is anticipated that NAFLD will become the most important cause of HCC. Activation of mammalian target of rapamycin (mTOR) pathway in NAFLD has been shown to promote deregulated lipid/glucose metabolism, immunosuppression and tumorigenesis. To fully decipher the immunoregulatory role of mTOR pathway in NAFLD and NAFLD-associated HCC, we performed immune cell profiling and signaling characterization in a high-fat-high-carbohydrate-diet (HFHC)-induced NAFLD murine model. Our results uncovered that the activation of mTORC1 signaling cascade and specific reduction in cytolytic natural killer T (NKT) cells in NAFLD liver, which subsequently enhanced tumor growth of orthotopically-implanted HCC tumor cells. Notably, treatment

DOI: 10.1002/eji.201970400

of the mTORC1/C2 dual kinase inhibitor vistusertib (AZD2014), currently undergone phase I/II clinical trials, not only abrogated mTORC1/SREBP2 signaling and cholesterol levels, but also repressed the cytolytic NKT cells in blood and liver leading to reduced tumorigenicity. Furthermore, NKT cells inactivation by a specific antibody targeting the CD1d receptor abolished anti-tumor effects of vistusertib. As we also observed correlations of upregulated mTORC1 signaling cascade, cholesterol level and abnormal NKT depletion in NAFLD-HCC patients, our results delineate the metabolic-immunosuppressive role of mTOR and provides insights into development of therapeutic strategies against the mTOR-reinforced NAFLD-associated HCC.
This project is supported by Collaborative Research Fund (C4045-18W) and the AstraZeneca Preclinical Oncology Research Program (2017).

P0376

Effect of splenectomy on morphology and function of islets and intestinal microbiome in mice

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The spleen as a main immune organ, plays an important role in maintaining immune homeostasis and preventing infection. Previous studies have reported that long-term risk of type 2 diabetes in traumatic patients after splenectomy is significantly increased. In this study, we established the mouse model of splenectomy, to research the metabolic and fecal bacteria abnormality with splenic deficiency. KM mice were randomly divided into SP (Splenectomy, n = 10), NC (Normal control, n = 10), Sham (Sham operation, n = 10). After 240 days post-treated, the feces, blood, pancreas, liver, kidney and small intestine tissues were collected. The body weight of SP mice increased ($P< 0.01$). Serum insulin and C-peptide levels were significantly higher than other groups ($P< 0.05$). Pancreatic islets atrophied; hepatocyte were slightly necrosed and inflammatory; massive glomerular atrophy, necrosis, inflammatory cell infiltration were in renal tissue; thickening of intestinal wall, thickening of mucosa with neutrophil infiltration were in small intestinal tissue. TGF-β was up-regulation, TLR-4, IL-6 were down-regulated ($p< 0.05$) in peripheral blood of SP. By 16SrRNA seq of fecal bacteria, the proportion of Firmicutes is reduced, while that of Bacteroides was increased in SP. Analysis of family and genus of bacterias shows that Ruminococcaceae, Bacteroides S24-7, Clostridiales, Lachnospiraceae, Oscillospira were significantly increased ($P< 0.01$); Comamonadaceae, Prevotellaceae, and Clostridiales were significantly reduced ($P< 0.01$). In conclusion, splenectomy might cause morphology of islet, abundance and species of intestinal microbial, and further induce insulin resistance, even diabetes, through changes of lymphocyte and immune factors.

P0377

Mass spectrometry analysis for MHC class I-associated immunopeptidomes defines obese fat-exclusive targets for proinflammatory $CD8^+$ T cells responses

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Adaptive $CD8^+$ T cells contribute to the initiation and propagation of obesity-induced visceral adipose tissue (VAT) chronic inflammation, which is critically linked to the development of metabolic diseases. Whether obese VAT-specific antigens generated as a result of obesity can drive the proinflammatory responses of $CD8^+$ T cells is still unknown. Here, we performed large-scale mass spectrometry to

DOI: 10.1002/eji.201970400

analyze MHC class I-associated immunopeptidome (MIP) isolated from VAT of lean and obese mice respectively, and revealed a distinct signature of obese VAT-exclusive MIPs source proteins. Of note, an obese VAT-exclusive octapeptide derived from lactate dehydrogenase (LDH), was identified as an immunogenic target, which elicited the proinflammatory responses of $CD8^+$ T cells. Furthermore, both the protein level and enzyme activity of LDH were significantly decreased in obese VAT when compared to that in lean VAT, indicating that the generation of LDH peptide might be due to the abnormal degradation of LDH in VAT under obese state. We also found that mRNA levels of constitutive proteasome and especially immunoproteasome were significantly increased in obese VAT samples relative to lean VAT, suggesting a hyperactive antigen processing machinery in obese VAT. Our findings suggest that the abnormal adipocyte metabolism under obese condition may generate obese VAT-exclusive immunogenic neo-MIP, which may drive the proinflammatory responses of $CD8^+$ T cells, representing an unrecognized mechanism underlying the obesity-induced VAT inflammation.

P0378

Glycogen metabolism regulates macrophage-mediated inflammatory responses

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Our current understanding of how sugar metabolism governs inflammatory pathways in macrophages is incomplete. Here, we show that glycogen metabolism is a key event that controls macrophage-mediated inflammatory responses. IFN-γ/LPS treatment stimulates macrophages to synthesize glycogen, which is then channeled through glycogenolysis to generate G6P and further through the pentose phosphate pathway to yield abundant NADPH, ensuring high levels of reduced glutathione for inflammatory macrophage survival. Meanwhile, glycogen metabolism also increases UDPG levels and the receptor P2Y14 in macrophages. The UDPG/P2Y14 signaling pathway not only upregulates the expression of STAT1 via activating RARβ but also promotes STAT1 phosphorylation by downregulating phosphatase TC45. Blockade of this glycogen metabolic pathway disrupts acute inflammatory responses in multiple mouse models. More significantly, this glycogen metabolism regulates inflammatory responses in septic patients. These findings reveal that glycogen metabolism in macrophages is a key regulator that provides potential strategies to combat acute inflammatory diseases.

P0379

Toll-like receptor 2 signaling improves CD8+ T cell metabolism, functionality and antiviral activity

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Toll-like receptor (TLR) 2 serves as a co-stimulatory molecule on activated T cells. However, it is unknown how the functionality and antiviral activity of CD8+ T cells are modulated by direct TLR2 signaling. Here, we studied the TLR2-mediated enhancement of TCR-driven CD8+ T cell activation *in vitro* and in woodchuck hepatitis virus transgenic mice. In vitro stimulation of purified CD8+ T cells showed that TLR2 agonist Pam3CSK4 directly enhanced the TCR-dependent CD8+ T cell activation. Transcriptome analysis revealed that TLR2 signaling increased expression of bioenergy metabolism-

DOI: 10.1002/eji.201970400

related genes in CD8+ T cells, such as IRF4, leading to improved glycolysis and glutaminolysis. This was associated with the upregulation of genes related to immune regulation and functions such as T-bet and IFN-γ. Glycolysis and glutaminolysis were in turn essential for the TLR2-mediated enhancement of T cell activation. Administration of TLR2 agonist Pam3CSK4 promoted the expansion and functionality of vaccine-primed, antigen-specific CD8+ T cells in both wildtype and transgenic mice and improved viral suppression. Thus, **TLR2 could promote CD8+ T cell immunity through regulating the energy metabolism.**

P0380

Mechanism of lactic acid enhancing murine tumor infiltrating myeloid-derived suppressor cells immunosuppressive function through glycolysis

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MDSCs play a critical role in tumor-associated immunosuppressive function, recent studies have confirmed that myeloid-derived suppressor cells (MDSCs) in tumor microenvironment have stronger immunosuppressive function than MDSCs in spleen, this may be related to metabolic reprogramming. But little is known about the metabolic status of MDSCs in the tumor microenvironment and its physiological role in metabolic reprogramming. In this study, we found that MDSCs in the tumor microenvironment have stronger glucose uptake capacity and higher glycolysis activity compared with MDSCs in the spleen of lung cancer mice xenograft model. The lactic acid in the tumor microenvironment mainly comes from the anaerobic glycolysis of tumor cells, after adding lactic acid treatment to the mouse spleen MDSCs culture system, the glycolysis activity and immunosuppressive function of MDSCs are simultaneously enhanced. Glycolysis inhibition by 2-deoxyglucose (2-DG) decreased the glycolysis and reduced the suppressive activities of these cells. Meanwhile, the results demonstrated that MCT/PI3K/AKT pathway was responsible for this function regulation. These results demonstrated that lactic acid regulates its immunosuppressive function through the glycolytic pathway and modulation of metabolism in immune cells can be an effective way to enhance anti-tumor effects.

P0381

Tolerated trained immunity: a novel mechanism of dietary fiber derived β-glucans for improving metabolic inflammation in obesity

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The metabolic inflammation exerts an essential role in triggering obesity, which increase risk for life-threatening diseases, such as stroke, heart diseases and certain cancers. However, the underlying mechanism of metabolic inflammation is still largely unknown. Trained Immunity, the latest advance on the function study of innate immune cells, may provide a new insight for the mechanism of metabolic inflammation in obesity. This study addressed whether dietary fiber derived β-glucans (1-3, 1-4 β-glucan from oats and 1-3, 1-6 β-glucan from mushroom) and their metabolites—short chain fatty acids (SCFAs) could improve the metabolic inflammation via trained immunity in high fat diet (HFD) induced obese mice. Both oat and mushroom β-glucan supplementation increased serum SCFAs, lowered body weight, improved glucose tolerance, intestinal permeability and inflammation in HFD mice. Butyrate also improved HFD-induced obesity and systemic inflammation, mechanically reflecting

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DOI: 10.1002/eji.201970400

beneficial effects of β-glucans supplementation. Furthermore, using GPR41$^{-/-}$ and GPR43$^{-/-}$ mice, butyrate's effects were found to be dependent on GPR41, but not GPR43. Interestingly, both oat and mushroom β-glucans induced an enhanced trained immunity, while butyrate induced a tolerated trained immunity in mouse and human monocytes/macrophages cell culture system *in vitro*. Consistent with the findings *in vitro* studies, the *ex vivo* monocytes/macrophages of oat and mushroom β-glucans supplementation in HFD mice exhibited a down-regulated inflammatory level with lower metabolic activities in compared with HFD mice. Collectively, this study demonstrated that tolerated trained immunity mediated by butyrate is a novel mechanism of dietary fiber derived β-glucans for improving metabolic inflammation in obesity.

P0382

Role of TIPE2 on the tumor-promoting effect of tumor-associated macrophages by regulating their aerobic glycolysis

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Tumor-associated macrophages (TAMs) have an M2 phenotype and can activate the AKT-mTOR pathway to initiate the process of aerobic glycolysis, rapidly synthesize and secrete negative regulators, and promote the malignant progression of tumors. The negative immune regulatory molecule TNFAIP8L2 (TIPE2) is highly expressed in myeloid immune cells and can promote macrophage polarization toward the M2 phenotype by activating the AKT pathway. However, the role of TIPE2 in the tumor-promoting effect of TAMs has yet to be elucidated. Firstly, we injected the mouse colon cancer cell Ct26 subcutaneously into mice and found the tumor grows slower and smaller in TIPE2$^{-/-}$ mice compared with WT ones. And this difference in tumor growth can be depleted by the intraperitoneal injection of the Colony Stimulating Factor 1 Receptor (CSF1R) antibody. Then we found under the stimulation of Ct26 culture supernatant, TAMs differentiated from TIPE2$^{-/-}$ BMDMs have impaired glycolysis and synthesize less negative regulators of VEGF and Arg1 compared with the WT TAMs. Mechanism study revealed that pre-treatment of AKT inhibitor MK2206 can significantly reduce the elevated glycolysis of WT TAMs, but not that of TIPE2$^{-/-}$ TAMs under the stimulation of Ct26 culture supernatant. Similarly, MK2206 can also lower the synthesis of VEGF and Arg1 of WT TAMs but not that of TIPE2$^{-/-}$ TAMs in the same condition. Taken together, these data suggested that TIPE2 can enhance the tumor-promoting effect of TAMs by regulating their aerobic glycolysis through activation of the AKT-mTOR pathway, which provided new therapeutic target to reverse the tumor-promoting effects of TAMs.

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DOI: 10.1002/eji.201970400

P0383

FABP5 regulates alternative activated macrophages and allergic airway inflammation by regulating metabolic reprogramming

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Alternative activated macrophages (M2) are positively correlated with allergic airway inflammation(AAI). Fatty acid binding protein 5 (FABP5), intracellular proteins, can affect cell lipid fluxes and metabolism. We have known that FABP5 is highly expressed in macrophages. However, it remains poorly characterized that the function of FABP5 in regulating allergic airway inflammation. In addition, it is unknown that the regulation we claimed whether can be associated with macrophage polarization and metabolic reprogramming. Here, we found that FABP5 deficient could significantly increase M2 polarization which was induced by IL-4/IL-13 in vitro. We found that compared to wild type mice, alternative activated macrophages in FABP5-deficient mice increased Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR). These indicated that FABP5 deficient could increase oxidative phosphorylation and glycolysis in M2 polarization. Mechanistically, FABP5 knockout increased M2 polarization through activating peroxisome proliferator activated receptor gama (PPARγ) dependent metabolism pathway. In vivo, using an ovalbumin (OVA)-induced mouse model of AAI, Lyzs-FABP5 knockout mice aggravated AAI significantly, which was accompanied with increased M2 polarization significantly in alveolar macrophages. Therefore, our work identifies that FABP5 may play an important role in attenuating allergic airway inflammation by regulating metabolic reprogramming.

P0384

Lack of FGF21 accelerates the Th17-IL-17 axis mediated transition from nonalcoholic steatohepatitis to hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) has been increasingly recognized in non-alcoholic steatohepatitis (NASH) before cirrhosis. In fact,FGF21 has recently emerged as a promising therapeutic candidate for the treatment of obesity and diabetes. However, the NASH related carcinogenesis and role of FGF21 played during NASH-HCC transition are largely unknown.
Aim: To investigate the effect of FGF21 on Th17-IL-17 axis during the NASH-HCC carcinogenetic transformation and to reveal the potential NASH-HCC mechanism.
Methods: NASH-HCC was induced in FGF21 knockout (KO) mice treated with DEN at aged 2 weeks at 100 mg/kg body weight (i.p.), and followed feeding with high fat diet (HFD,60% kcal% fat) or methionine/choline-deficient diet (MCD). Fenofibrate (50 mg/kg) was administered to induce FGF21. In *in vitro*studies were performed using shRNA-FGF21 knock down hepatocyte and HCC cell lines. The cellular and molecular events, in turn, white adipose tissue (WAT) lipolysis, insulin resistance (IR), release of free fatty acids (FFAs), Th17 cell infiltration and IL-17A production, and NASH-HCC initiation, were determined both *in vivo*and *in vitro*.
Results and conclusion: Increased WAT lipolysis, hepatic FFAs uptake, Th17 cells/IL-17A, and aberrant NF-κB and p53 signaling were found in NASH and HCC tissues (adipose, spleen and liver) from in FGF21-KO mice by Flow Cytometry, immunohistochemistry (IHC) and Western blot.

DOI: 10.1002/eji.201970400

Fenofibrate treatment significantly increased PPAR-α and native FGF21 protein levels in the liver tissues,and decreased the tumor volumes.Lack of FGF21 played a key role in hepatic lipid accumulation and subsequent up-regulated Th17-IL-17 signaling in liver and promoted NASH-HCC transition. This work was supported by IDeA from NIH number P20GM113226.

P0385

MondoA regulates metabolism and function of regulatory T cells in colorectal cancer microenvironment

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MondoA is a member of the Myc-superfamily transcription factors and regulates several genes involved in glucose and glutamine metabolism. MondoA is highly expressed in regulatory T cells (Treg). However, the role of MondoA in Treg remains to be elucidated. Here, we generated a Treg specific MondoA knockout mouse strain, MondoA$^{fl/fl}$ Foxp3-YFP-Cre (MondoA$^{\Delta Treg}$). We found that MondoA deficient Treg cells, under activated conditions, had an enhanced capacity to use glycolysis and exhibited impaired suppressive function. RNA-sequencing identified TXNIP as a MondoA target involved in metabolic reprogramming of Treg cells. We further induced inflammation associated colorectal cancer in MondoA$^{\Delta Treg}$ and control mice using the mutagenic agent azoxymethane (AOM) and the inflammatory agent dextran sodium sulfate (DSS). Interestingly, MondoA deficiency in Treg cells promoted colorectal carcinogenesis and increased pro-inflammatory cytokine IL17A production in tumor microenvironment. Moreover, IL17A elevation inhibited CD8 T cells proliferation and up-regulated PD-1 expression, thereby contributing to tumor infiltrated CD8 T cells exhaustion. Taken together, our data reveal for the first time the MondoA-TXNIP axis as the regulator of regulatory T cell metabolism and function in colon cancer microenvironment.

P0386

^{1}H NMR metabolic profiling and biomarker of gastric cancer patients with lymph node metastasis

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Gastric cancer (GC) is a malignant tumor worldwide. As primary pathway for metastasis, the lymphatic system is an important prognostic factor for GC patients. Although the metabolic changes of gastric cancer have been investigated in extensive studies, little effort focused on the metabolic profiling of lymph node metastasis (LNM)-positive or negative GC patients. We performed ^{1}H NMR spectrum of GC tissue samples with and without LNM to identify novel potential metabolic biomarkers in the process of LNM of GC. There was a clear separation between GC patients and normal controls (n=40). Moreover, GC patients were also well-classified according to LNM-positive (n=40) or negative (n=40). Totally 8 distinguishing metabolites were selected in the metabolic profiling of GC patients with LNM -positive or negative, suggesting the metabolic dysfunction in the process of LNM. According to further validation and analysis, surprisingly, we found the levels of BCAAs (leucine, isoleucine and valine) not only were increased in GC tissue specimens, but also were up-regulated during the progression of GC. Moreover, we also showed that BCAT1 was aberrantly activated in GC tissue

DOI: 10.1002/eji.201970400

samples. Knockdown or overexpression of the BCAT1 in GC cells led to sharp decrease or increase of cell proliferation, migration and invasion. To our knowledge, this is the first metabolomics study focusing on LNM of GC. The identified distinguishing metabolites showed a promising application on clinical diagnose and therapy prediction, especially BCAAs metabolism and BCAT1 may be as potential factors of diagnose and prognosis of GC patients with or without LNM.

P0387

Metabolome of pancreatic juice delineates distinct clinical profiles of pancreatic cancer and reveals a link between glucose metabolism and PD-1+ lymphocytes

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Objective: Pancreatic diseases are an urgent medical need with little advances in preoperative differential diagnosis, preventing rational selection of therapeutic strategies.
Design: The metabolomic profile of pancreatic juice in 40 patients with different pancreatic diseases was investigated by ^{1}H Nuclear Magnetic Resonance. Based on the profile obtained, the concentration of lactate in pancreatic juice was probed on a larger cohort of 94 patients. The correlation of tissue glycolysis with immune infiltration was analysed in pancreatic adenocarcinoma (PDAC) sections and the immune-metabolic axis modeled in preclinical models of PDAC.
Results: Metabolic variables in pancreatic juice discriminated PDAC from other less aggressive pancreatic diseases and identified metabolic clusters of patients with distinct clinical behaviors. PDAC specimens were overtly glycolytic, with significant accumulation of lactate. In human PDAC sections, high levels of the glucose transporter GLUT-1 correlated with tumor grade and a higher density of PD-1^{+} T lymphocytes, suggesting that PD-1^{+} cells accumulate in highly glycolytic tumors. In a preclinical model, PD-1^{+} CD8-TILs differently infiltrated PDAC tumors obtained from cell lines with different metabolic consumption and tumors metabolically rewired by knocking down the Phosphofructokinase (*Pfk-m*) gene displayed a decrease in PD1+ cell infiltration.
Conclusion: The metabolic profile of pancreatic juice discriminates distinct pancreatic pathologies and clinical profiles of PDAC. For the first time, we introduce pancreatic juice as a novel, valuable source of metabolic variables that could contribute to differential diagnosis, fostering the implementation of diagnostic, marker-based strategies to advance management of pancreatic diseases**.**

P0388

The effect of uric acid on AMP-activated protein kinase in mice

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Objective: AMP-activated protein kinase (AMPK) is a serine/threonine kinase which monitors and regulates an intracellular energy condition. Several recent reports have shown that AMPK activation protected against the development of atherosclerosis. Hyperuricemia is regarded as one of the risk factors of atherosclerosis, but the association of uric acid with AMPK hasn't been clear. In this study, we clarified the association of them.

DOI: 10.1002/eji.201970400

Methods: We utilized 2 types of uricase transgenic mice. Secretable uricase (ssUOX) or intracellular uricase (intUOX) transgenic mice express extracellular or intracellular uricase that lead to reducing levels from the uric acid of outside or inside of the cells than those of wild type C57BL/6, respectively. Two types of uricase transgenic mice, ssUOX Tg, intUOX Tg, and control mice were administered with 30% fructose solution as drinking water for 5 days. They were sacrificed and their leukocytes were collected. In another experiment, Apoe-/- mice were fed with high fat diet for 16 weeks with or without administration of allopurinol. Their leukocytes were also collected, and the activities of AMPK of the leukocytes were evaluated by western blot.
Result:] In fructose administration experiment, activities of AMPK were significantly increased in both types of uricase transgenic mice. In high fat diet experiment, activities of AMPK were increased in mice with allopurinol.
Conclusions: Decreasing uric acid levels by expressing uricase or inhibition of xanthine oxidase activated AMPK of leukocytes in mice. It suggests that uric acid has a role in development of atherosclerosis via regulation of AMPK activity.

P0965

Relationship of hypothalamic inflammation and protein energy wasting in patients with maintenance hemodialysis

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Objective: The aim was to explore the relationship between hypothalamus inflammation and PEW via analysis the correlation of hypothalamus T2 signal intensity and body composition on MHD.
Methods: 158 MHD aged 18-60 years with PEW who were regularly dialysis for more than 3 months in 11 hemodialysis centers analyzed.The bioelectrical impedance analyzer was used to measure the body, lean body mass, fat mass and body cell mass (BCM) .
Results: Compared with the control , the T2 relaxation time of MHD was significantly increased (33.15±1.95 vs53.36±3.18) ms, and the difference was statistically significant ($P< 0.05$). In diffusion weighted imaging, the apparent diffusion coefficient of maintenance hemodialysis was significantly lower than that of the healthy control group (2.09±0.08 vs 2.88±0.13)×$10^{-3}$$mm^2$/s, and the difference was statistically significant ($P< 0.05$).Multivariate linear regression analysis (R^2=0.74) showed that T2 relaxation time was positively correlated with BMI (β=0.350) and grip strength (=0.217) in dialysis , while fat mass (β=-0.228) and skinfold thickness (β=-0.163) were negatively correlated. No correlation between the apparent dispersion coefficient and PEW was found.
Conclusion: T2 relaxation time is associated with PEW with maintenance hemodialysis, which may be a new indicator to evaluate the hypothalamic inflammation in patients with maintenance hemodialysis.

P0966

Microglia activation in Hypothalamus contribute to renal ectopic fat deposition in diabetic nephropathy

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Chronic inflammation modulates the development of metabolic-related comorbidities like Type 2 diabetes. Recent evidence suggests that microglia activation in hypothalamus is a priority in the

DOI: 10.1002/eji.201970400

deregulation of energy homeostasis. Pattern of fat distribution is a major determinant for metabolic homeostasis. Decreased capacity of fat storage in adipose tissue may result in ectopic fat deposition in nonadipose tissues such as kidney. However, the cellular mechanism of hypothalamic inflammation, as well as its impact on ectopic fat deposition, is unclear. Here, we found that type 2 diabetes mice induced by STZ or db/db mice, is accompanied by microglial activation as manifested by microgliosis and pro-inflammatory cytokine up-regulation. Targeted depletion of microglia significantly attenuated hypothalamic inflammation, glutamate receptor expression in the paraventricular nucleus, plasma vasopressin level, kidney norepinephrine concentration and ectopic fat deposition. Furthermore, when microglia were pre-activated and transferred into the brains of orthoglycemic mice, there was a significantly prolonged press or response to intracerebroventricular injection of LPS; and inactivation of microglia eliminated these effects. These data demonstrate that microglia, the resident immune cells in the brain, are the major cellular factors in mediating hypothalamic inflammation and modulating neuronal excitation, which contributes to the ectopic fat deposition.

P0967

The IGF-1R inhibitor blocked pathological changes in DKD without up-regulating SOCS2 protein levels

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Objective: to explore the anti-inflammatory mechanism of Insulin-like growth factor-1 receptor inhibitor(IGF-1R) in diabetic nephropathy.
Methods: C57/BL6 male mice were fed high-fat diet for 8 weeks, then mice were injected 30mg/kg streptozotocin intraperitoneally to induce type 2 diabetes. When diabetes model is successful the different drugs were administrated to mice with diabetes (insulin 1～2 U/d i.h), (benazepril 10 mg/kg·d i.g) (IGF-1R inhibitor 30 mg/kg·d i.g). After 8 weeks of drugs administrated, all mice were euthanized to collect the kidney tissue collected kidney tissues, measured levels of inflammation and fibrosis using immunohistochemistry and in situ hybridization.
Results: upregulation ofIGF-1R was associated with increased urinary protein excretion rate and increased inflammatory infiltration, and the correlation was characterized by increased CD68+, F4/80+ cells, and increased TLR4, αSMA , SR expression. IGF-1R inhibitors reversed this change, with no significant changes in insulin. The insulin and IGF-1R inhibitors decreased the expression level of TLR4, αSMA and IGF-1, And insulin treatment increase the levels of suppressor of cytokine signaling 2(SOCS2)
Conclusion: inhibition of IGF-1R was a more effective choice for inflammation treatment than benazepril or insulin in DKD. The IGF-1R inhibitor blocked pathological changes in DKD without up-regulating SOCS2 protein levels.

P0968

Role of SOCS2 in renal tubular epithelial mesenchymal transition in type 1 diabetic nephropathy

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Suppressor of cytokine signaling (SOCS) is mainly induced by various cytokines, the dysregulation of cytokine signaling pathways is associated with the development and pathogenesis of a variety of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and type 1

DOI: 10.1002/eji.201970400

diabetes. Here, we investigated the role of SOCS2 in renal tubular epithelial to mesenchymal transition (EMT) in type 1 diabetic nephropathy (T1DN). An animal model of type 1 diabetes was established by directly destroying islet β cells by single high-dose intraperitoneal injection of streptozotocin (STZ) in C57/BL6 mice. After continuous administration for 8 weeks, serum, urine, and kidney tissue were collected for biochemical, pathological, and related protein and mRNA detection. Compared with the control group, fasting blood glucose and 24-hour urinary protein were significantly increased in the T1DN group, along with renal tissue structure destroyed and interstitial collagen fibers deposition significantly increased. The expressions of FN and SOCS2 in the renal tissue of T1DN group were significantly increased, and the expression of E-cad was greatly decreased. Moreover, the expression level of SOCS2 was positively correlated with the collagen fiber deposition and FN, and negatively correlated with E-cad in the T1DN mice kidneys. SOCS2 plays an important role in the EMT process of renal tissue at T1DN.

P0969

The effect of 1,25-$(OH)_2$-D_3 on protein expression of HIF-1α and TIMP in kidney diabetic nephropathy

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Suppressor of cytokine signaling (SOCS) is mainly induced by various cytokines, the dysregulation of cytokine signaling pathways is associated with the development and pathogenesis of a variety of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and type 1 diabetes. Here, we investigated the role of SOCS2 in renal tubular epithelial to mesenchymal transition (EMT) in type 1 diabetic nephropathy (T1DN). An animal model of type 1 diabetes was established by directly destroying islet β cells by single high-dose intraperitoneal injection of streptozotocin (STZ) in C57/BL6 mice. After continuous administration for 8 weeks, serum, urine, and kidney tissue were collected for biochemical, pathological, and related protein and mRNA detection. Compared with the control group, fasting blood glucose and 24-hour urinary protein were significantly increased in the T1DN group, along with renal tissue structure destroyed and interstitial collagen fibers deposition significantly increased. The expressions of FN and SOCS2 in the renal tissue of T1DN group were significantly increased, and the expression of E-cad was greatly decreased. Moreover, the expression level of SOCS2 was positively correlated with the collagen fiber deposition and FN, and negatively correlated with E-cad in the T1DN mice kidneys. SOCS2 plays an important role in the EMT process of renal tissue at T1DN.

P0970

The relationship between IL-2 and renal fibrosis of Type 1 diabetic nephropathy explored in mouse models

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In recent years, Interleukin-2 (IL-2) has been widely used in the treatment of autoimmune diseases, especially in type 1 diabetes (T1D). However, there is still no satisfactory result for diabetic nephropathy due to complications caused by T1D. The etiology of diabetic nephropathy is closely related to many factors, and fibrosis plays an important role in the initiation and development of diabetic kidney disease (DKD).The aim of this study was to investigate the relationship between IL-2 and type 1 diabetes nephropathy (T1DN) renal fibrosis by analyzing changes in IL-2 expression in a

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DOI: 10.1002/eji.201970400

T1DN mouse model. Functional and pathological markers, expression of epithelial marker E-cad, and myofibroblast transdifferentiation indicator α-SMA, matrix proteins component [Col-1, MMP9 and TIMP2] were systematically evaluated. The pathological results showed that glomerular hypertrophy, pyknosis and cirrhosis occurred, K-W nodules formed, basement membrane thickened, mesangial expansion, interstitial inflammatory cells infiltrated, and obvious fibrosis appeared in renal tissue of the model group, suggesting the occurrence of diabetic renal damage. Levels of IL-2 were significantly decreased in T1DN mice kidneys. Finally, the expression of IL-2 was negatively correlated with renal fibrosis markers and positively correlated with E-cad in T1DN mice kidneys. These results suggested that IL-2 might play an important role in DKD renal fibrosis, and its mechanism may be related to the fibrosis caused by immune inflammatory factors.

P0971

Clinical observation of combined blood purification in uremia with severe infection

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Methods: Thirty-five patients with serious infection and uremia were recruited from our hospital between October 2010 and October 2015 .They were randomly divided into three groups: group A (13cases) treated with hemodialysis combined with hemodialysis filtration (HD+HDF) group B (12 cases) treated with the hemodialysis combined with blood perfusion (HD+HP) and group C (10 cases) treated with hemodialysis combined with plasma pheresis (HD+PE), 2 weeks treatment. Before and after treatment,Inflammatory factor, such as c-reactive protein (CRP), interleukin 6 (IL-6), Calcitonin original (PCT) , biochemical indicator were recorded. Such as ,White blood cells(WBC),Neutrophils(N%),Platelet(PLT),Bun and Scr.
Results: Compare with group A and B,the levels of group C of CRP (9.0±1.9 pg/ml、9.2±1.6 mg/L、5.0±0.9 mg/L), IL-6(105.5±20.15 pg/ml 、95.00±22.10 pg/ml、60.5±16.11 pg/ml、), PCT(90.2±24.9 ug/L、88.5±20.8 ug/L、40.2±12.9 ug/L),WBC（11.4±9.20*109/L、 10.3±10.02*109/L、5.3±4.20 *109/L）；N%（80.1±36.6、82.0±40.1、65.1±35.4）decreased significantly and the levels of PLT (40.5±25.0*1012 /L、 55.8±20.1*1012 /L, 70.7±23.5*1012 /L)were increased in group A after treatment(all P< 0.05). Compare with group A, B and C, the levels of Bun(8.9±2.3 umol/l、8.2±2.1umol/l,、7.8±1.8 umol/l)and Scr（315.1±60.2 mmol/l、308.2±62.2 mmol/l、298.5±59.9 mmol/l ）decreased obviously after treatment.(all P>0.05). The recovery of patient conditions was most rapid in group C of three group.
Conclusion: The PE+HD may be more effective in the clearance of inflammatory mediators and improved the recovery of patient conditions with antibiotic encephalopathy leaded by cephalosporin , which can be popularized in clinic.

P0972

Imbalance of Th17/Treg cells and expression of cytokines in peripheral blood of Type 2 diabetic nephropathy rats

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Objective: To explore the relationship between Th17/Treg cell imbalance and related cytokines and the development of diabetic nephropathy.The proportion of Th17 cells in the peripheral blood of diabetic nephropathy group increased significantly (*P< 0.05*). The proportion of Th17 cells in diabetic

DOI: 10.1002/eji.201970400

nephropathy rats was positively correlated with UAE（*r=0.765，P＜0.001*）, and the proportion of Treg cells was negatively correlated with UAE（*r= -0.786，P＜0.001*）. The levels of Th17 cell related proinflammatory cytokines IL-23,IL-6 and IL-17 increased significantly in diabetic nephropathy rats (*P < 0.05*). The level of Treg cell related cytokines IL-10, TGF-β and IL-35 increased significantly (*P< 0.05*).
Conclusion: 1,Compared with the control group, there was an imbalance in the proportion of Th17/Treg cells in the peripheral blood of type 2 diabetic nephropathy rats. The levels of IL-23, IL-17 and IL-6 mRNA were increased in the Th17 cell related inflammatory cytokines, and the Treg cell related factors IL-10,TGF-β and IL-35 mRNA were down. 2,The proportion of Th17 cells in peripheral blood of rats with type 2 diabetic nephropathy was positively correlated with the level of 24h urinary albumin excretion in rats, and the proportion of Treg cells was negatively correlated with the level of 24h urinary albumin excretion in rats.3,Th17/Treg cell imbalance and its related cytokines expression may be involved in the occurrence and development of diabetic nephropathy.

P0973

PID1 promotes obesity-associated NAFLD via FABP4/ LRP1-PPARγ mediated pathway in liver and immune cells

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Obesity-associated gene PID1, phosphotyrosine interaction domain containing 1, contains a phosphotyrosine-binding (PTB) domain and was reported to be involved in obesity and insulin resistance. In this study, we established high fat diet (HFD) fed mice to investigate effects of PID1 on the progress of nonalcoholic fatty liver disease (NAFLD) complicated with insulin resistance and to explore the mechanism underlying lipid metabolism regulation in liver and immune cells. We observed that PID1overexpression decreased the level of oral glucose tolerance and insulin tolerance, accompanied with increased body weight and formation of lipid droplets in the liver. Concurrently, PID1 promoted secretion of pro-inflammatory cytokines IL-6, IL-12, TNF-α, MCP-1 IFN-1, suppressed regulatory T cells and myeloid-derived suppressor cells (MDSC) differentiation in the spleen. The hepatic histology results showed that PID1 reinforced liver steatosis and inflammatory cell aggregation compared with the HFD courtparts, the former of which was attenuated by blocking MDSC with Gr-1 antibody but not anti-CD4/CD8 antibody. We also found that inhibition of PID1 abrogated these effects. Further in vivo and in vitro studies showed that interference with PID1 profoundly down-regulated the transcription and protein expression of FABP4, but increased the level of PPARγ protein, as well as promoting migration of LRP1 to nucleus from the cell membrane, acting as a transcriptional co-activator of PPARγ in hepatocytes and MDSC. Collectively, our data indicated that PID1 promotes insulin resistance and steatosis by impeding metabolic homeostasis in liver and immune cells via acting on FABP4/ LRP1-PPARγ pathway, reshaping the function of liver and immune cells.

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DOI: 10.1002/eji.201970400

P0974

Tumorous niche induces NK exhaustion by disordering mitochondrial metabolism

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There is growing recognition that inappropriate metabolic reprogramming underlies many aberrant disease microenvironments, and that manipulation of intracellular metabolism can temper or enhance immunity. Thus, whether NK cells presenting abnormal reprogramming of metabolism in tumor microenvironments is a cause of immune escape is not clear.
In this study, 102 cases from hepatocellular carcinoma (HCC) patients were analyzed by RNA sequence, pathology, flow cytometry and cell culture in vitro. We found a significant enrichment of infiltrating T cells within the tumor tissues of HCC patients but not NK cells, as compared to the adjacent peritumoral tissues. It is interesting that the infiltrating NK cells enrich $CD56^{bright}$CD49a+ NK subset, like liver-resident NK. Nearly 50% of the entire healthy liver NK cell population is composed of functionally relevant $CD56^{bright}$ CD49a- NK cells, and almost all of the subset was lose. The infiltrating $CD56^{bright}$CD49a+ NK cells present exhausted phenotype and downregulated expression of efficacy molecules of CD11b, CD226 and IFN-γ. We also found a few of $CD56^{dim}CD16^{+}$ NK cells were infiltrating, which were similar to their peripheral circulating counterparts in transcription and phenotype, but the lower effect function and viability of infiltrating NK cells compared with normal liver or peripheral circulating counterparts.
Dynamic reprogramming of metabolism is essential for immune cell stable liveness and effector function. Gene Ontology profiling and metabolism analysis revealed the infiltrating NK cells bioenergetic insufficiencies and enrichment of metabolic oxygen specis leading to its subset variations and immune surveillance insensitivity.

P0975

Lipid metabolic pathways confer immunosuppressive function of myeloid-derived suppressor cells in tumor

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Myeloid-derived suppressor cells (MDSCs) play crucial roles in tumorigenesis and their inhibition is critical for successful cancer immunotherapy. MDSCs undergo metabolic reprogramming from glycolysis to fatty acid oxidation (FAO) and oxidative phosphorylation leading to lipid accumulation in tumor. Increased exogenous fatty acid uptake by tumor MDSCs enhance their immunosuppressive activity on T-cells whereby promoting tumor progression. Tumor-infiltrating MDSCs in mice may prefer FAO over glycolysis as their primary source of energy, and treatment with FAO inhibitors significantly improve anti-tumor immunity. This review highlights the immunosuppressive function of lipid accumulation and lipid signaling pathways in the context of MDSCs regulation of tumor microenvironment. The manipulation of these pathways can alter the function of these cells and therefore, could provide novel therapeutic approaches to enhance cancer immunotherapy.
This work was supported by Nanshan pilot team project (LHTD20160004), Start-up funding (CYZZ20180307154657923), the Shenzhen Basic Science Research Project

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(JCYJ20170413153158716 and JCYJ20170818164619194),the fourth talents project of Guangdong province (2014-1),Shenzhen special funds for industry of the future (Development and Reform Commission in Shenzhen [2015] Grant 971),Guangdong Provincial Research Award for Thousand Talents Program Scholars.

P0976

Daily pattern of feeding and rhythmic oscillation of TNFa production regulate synchrony of Plasmodium chabaudi stages with host circadian cycle

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Malaria is a devastating tropical disease and leading cause of death among children up to 5 years old. The development of *Plasmodium* involves a consecutive differentiation of ring-form trophozoites, to mature trophozoites, to schizonts and release of merozoites, synchronously. One of the big mysteries of the *Plasmodium* life cycle is the mechanism by which millions of parasites differentiate and proliferate synchronously. To address this question, we used the *P. chabaudi* model, in which the process of parasite synchronization parallels the host circadian cycle. We found that expression of carbohydrate metabolism and inflammatory genes in IFNg-primed leukocytes and liver cells are boosted in *P.chabaudi*-infected mice. We also found that expression of *TNFa* and plasma levels of circulating TNFa in *P.chabaudi*-infected mice peak at zeitgeber (ZT) 23 and associate with a marked hypoglycemia. Importantly, we observed that rhythmic hypoglycemia, at ZT 23, is associated with parasite arrest into non-replicative stages, whereas parasite proliferation and schizont formation occurred only at the time of food-intake period. In addition, we found that in IFNg$^{-/-}$, TNF receptor$^{-/-}$ and diabetic mice infected with *P.chabaudi*, the hypoglycemia was attenuated and parasite cell cycle no longer synchronized with host circadian cycle. Altogether, we propose that *Plasmodium* synchrony with host circadian rhythm is determined by rhythmic oscillation of circulating glucose levels that are influenced by TNFa and periodicity of host feeding habits.

P0977

In vivo neutrophil extracellular trap (NET) formation in obese mice during sepsis - role of leptin and IL-33

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Obesity is an immunometabolic state which impact on the outcome of sepsis remains controversial. Excess adipose tissue is associated with a higher leptin release which correlates with increased neutrophil numbers as well as IL-33 and neutrophil elastase (NE) presence. Moreover, IL-33 facilitates neutrophil migration during sepsis whereas NE is a component of neutrophil extracellular traps (NETs). Thus the aim of this study was to compare NET formation in obese and lean individuals during sepsis, and then establish a role of leptin and IL-33 in NET release. Obesity was studied in two models, in C57BL6/J mice fed on a high fat diet and in leptin mutants, ob/ob mice. Sepsis was induced by intraperitoneal injection of liposaccharide. Prior to sepsis, some mice were treated with either recombinant leptin or antibodies neutralizing leptin, or anti-IL33 antibody. Neutrophil infiltration and formation of NET was visualized with intravital spinning disk confocal microscopy. The obtained data revealed that neutrophils from obese mice release substantially less NETs in comparison with their

DOI: 10.1002/eji.201970400

lean littermates. Moreover, exogenous leptin increases formation of NETs without affecting neutrophil numbers, but only in lean mice. In contrast, neutralization of leptin reduces neutrophil numbers in lean mice, however, has no impact on NET release either in lean or obese animals. Blocking IL-33 production diminishes NET ejection independently of the metabolic state of mice. We conclude that the weak NET release during sepsis might directly result from the immunometabolic state of obese individuals. Study supported by National Science Centre of Poland, grant No. 2018/29/B/NZ6/00713.

P1570

Control of regulatory T cell suppressive function and metabolism by transketolase

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Regulatory T (Treg) cells play a pivotal role in maintaining immune homeostasis by suppressing self-destructive immune responses.Metabolic pathways such as glycolysis ,oxidative phosphorylation and fatty acid beta-oxidation have been reported to regulate Treg survival, function and migration. However, it remains unknown whether the pentose phosphate pathway (PPP) is required for the suppressive function, stability or survival of Treg cells. Here we newly identified an essential role of the pentose phosphate pathway in maintaining Treg cell suppressive function and metabolism. Among enzymes in PPP, transketolase (TKT) in the non-oxidative branch displayed the most prominent expression in Treg cells when compared to conventional $CD4^{+}$T cells. Treg specific deletion of TKT in mice led to a fatal auto-immune disease with increased serum immunoglobulin levels and massive organic lymphocytic infiltration at an early age, without affecting Treg cell number. Although Treg cell proliferation and survival was not altered by TKT deletion, TKT deficient Treg cells displayed a complete loss of suppressive function. RNA sequencing analysis revealed that deletion of TKT downregulated the expression of Treg-associated suppressive function genes but Foxp3 expression remained stable. Additionally, Seahorse metabolic analysis showed that loss of TKT reprogrammed Treg cell metabolism with decreased glycolysis and activated oxidative phosphorylation. Taken together, our findings have demonstrated that TKT is critical for maintaining Treg function and metabolism. The detailed underlying mechanism need to be further elucidated.

P1571

High expression of USP28 predicts poor disease-free survival in hepatocellular carcinoma

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Ubiquitination is a common protein post-translational modification progression. Abnormal expression and activity of USPs are closely related to the occurrence and development of various cancers. ubiquitin specific peptidase 28(USP28) as a novel de-ubiquitination hydrolytic enzyme plays an important role in DNA transcription, myc-mediated cell malignancy and cell cycle progression. However, the potential function of USP28 in hepatocellular carcinoma is still ill-defined. To unravel this unknown function, USP28 expression in 90 HCC and HCC cell lines were evaluated. The mRNA expression of USP28 was significant lower in HCC tissues than that in their para-carcinoma tissues (P=0.000). Although USP28 cytoplasm expression in HCC tissues was no statistical expressed (P=0.235), its nucleus immunohistochemical staining was significantly upregulated in cancer tissues (1.42±1.98 vs 0.24±0.48, P=0.001). Moreover, USP28 was significantly positive correlated with the

DOI: 10.1002/eji.201970400

pathological grading (r= 0.439, P=0.000), tumor cell membrane (r=0.259, P=0.015) and HCC recurrence (r=0.224, P=0.034) of the patients. Besides, high USP28 was correlated with a worse disease-free survival, and USP28 expression could serve as an independent predictor of disease-free survival. In conclusion, our study revealed that USP28 was overexpression in hepatocellular carcinoma, and high USP28expression promote the development and progression of HCC.

P1572

The effect of Alpinia oxyphylla Miq.Extract(AOE) on diabetic nephropathy progression: miRNA signature analysis

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Objective: The objectives of this study are to investigate protective effect of Alpinia oxyphylla Miq.Extract in miRNA regulation on the pathological mechanism of diabetic nephropathy.
Methods: The mice were divided into DB/DB H_2O,db-/db-AOE 500 mg/kg and db-/db-H_2O. Euthanized mice were dissected and miRNA was extracted from liver and kidney, and confrmed by qRT-PCR. Biochemical Indicator parameters were measured using commercial kits.Cluster analysis was used to predict target genes.
Results: After 8 weeks AOE treatment, impaired kidney function was ameliorated in db-/db-AOE group by significantly reduced urea nitrogen, urinary creatinine, and urinary microalbumin, MDA ($P < 0.05$). Furthermore, AOE led to significantly lower concentrations of blood glucose ($P< 0.05$), serum triglyceride ($P< 0.05$), and serum cholesterol in db-/db- mice, while elevated the concentration of glutathione and increased the activities of the antioxidant enzymes superoxide dismutase and peroxidase ($P< 0.05$) was also detected in the livers. Importantly, we determined miRNAs expression patterns, 19 identified miRNAs, were statistically significantly differentially regulated in the livers of DB/DB mice group, and 7 were differentially regulated in the livers of db-/db-AOE500 mice group, compared to db-/db-H_2O mice group. Additionally, 17 and 13 miRNAs were statistically identified as differentially regulated in kidney of the DB/DB and db-/db- AOE mice, respectively, compared to the untreated db-/db- mice.
Conclusion: These founding suggest that the potential of AOE as a medicinal anti-diabetes treatment through changes in the expressions of specifc miRNAs.
Keywords: Alpinia oxyphylla Miq. Extract;Diabetic nephropathy;miRNA.

P1573

Implications of the gut microbiota in analyzing the progression of diabetic nephropathy treatment by Alpinia oxyphylla Miq. extract

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Objective: This study aimed to evaluate the efficacy of Alpinia oxyphylla Miq. Extract (AOE) administration in the treatment of diabetic nephropathy(DN) based on gut microbiota analysis.
Methods: The mice were divided into 3 groups with DB/DB H_2O,db-/db-AOE and db-/db-H_2O. Physiological and biochemical indicators were measured weekly, including: concentration of blood glucose, urea nitrogen, urinary creatinine and urinary microalbumin. After the end of 8-week period, the mice were euthanized by chloral hydrate anesthesia. The morphology of renal tissue was

DOI: 10.1002/eji.201970400

observed by HE staining. The serum levels of SOD, O^{2-} and H_2O_2 were determined. The feces of 3 groups were collected, and gut microbiota was examined.
Results: The content of blood glucose, urea nitrogen, urinary creatinine and urinary microalbumin were significantly reduced in db-/ db-AOE mice as compared to the db-/db-H_2O group. In addition, content of H_2O_2, O^{2-} ($P < 0.05$) and SOD inhibition rate were significantly reduced in db-/ db-AOE mice, which indicate significantly improvement for pathological status of kidney.Identification of gut microbiota shows db-/db-AOE intragastric significantly induced the proportion of bacteroides and firmicutes compared with db-/db-H_2O group. The abundance of Akkermansia was also increased dramatically while helicobacter pylori was significantly inhibited in db-/ db-AOE group.
Conclusion: Our results suggest that AOE might have the potential as possible pharmacological agent against DN by suppressing renal oxidative stress and regulating gut microbiota.
Keywords: Alpinia oxyphylla Miq. Extract ; Diabetic nephropathy; gut microbiota.

P1574

Protective effects of paederia scandens extract on rheumatoid arthritis mouse model by modulating gut microbiota

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Objective: This study aimed to investigate the possible influences of P. scandens on the progression of rheumatoid arthritis (RA), inflammatory responses and gut bacterial communities in RA mouse model.
Materials and methods: Collagen-induced arthritis (CIA) mice were treated with P. scandens extract (PSE) for 24 days. Then, the levels of pro-inflammatory cytokines in serum were detected, and the intestinal microflora was detected by Illumina HiSeq method.
Result: Compared with the vehicle group, PSE significantly alleviated paw swelling and reduced arthritis score. Histological examination of soft tissue of ankle joint demonstrated PSE can effectively inhibit tissue fibrosis and inflammatory cell infiltration. High levels of TNF-alpha, IL-1beta, IL-6, IL-7 and IL-23PSE in serum of RA mice were severely inhibited. In addition, PSE treatment help restore gut microbial ecosystem altered in RA mice including decreasing relative abundance of inflammatory related microorganisms, Desulfovibrio, Mucispirillum, Helicobacter, and Lachnospiraceae.
Conclusion: These results indicate that PSE has therapeutic effect in RA mice with CIA and shows potential as an anti-arthritis reagent.
Keywords: Paederia scandens , Rheumatoid arthritis , Inflammatory responses , Gut bacterial communities .

P1575

Selenium regulates the differentiation and immune function of human dendritic cells by selenoproteins

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DOI: 10.1002/eji.201970400

Selenium is a trace element essential for mammals and humans, which can regulate the immune function of macrophages, B cell and T cell by SelK, or SelR or other selenoproteins. However, the effect of selenium on human DCs differentiation and immune function is still unclear. First, we studied the mRNA levels of selenoproteins in monocyte, imDCs, and mDCs by real-time PCR. The results showed that there were different expression levels of selenoproteins and were down- or up-regulated in monocyte, imDCs, and mDCs. Moreover, the effect of selenium on the selenoproteins, surface markers and cytokines expression levels in human DCs were also investegated. The results demostrated that some of the selenoproteins and the CD80, CD86, IL12p35 and IL12p40 were up-regulated in imDCs and mDCs after treatment with 0.1 µM selenium, while those in 0.05 and 0.2 µM selenium treated group were down-regulated and CD11c was up-regulated. In addition, the antiphagocytic ability, migration ability, and mixed lymphocyte reaction were detected. The results indicated that the antiphagocytic ability was suppressed in 0.2 µM selenium treated group, and 0.1 µM selenium could improve antigen phagocytosis in imDCs. The migration ability of imDCs and mDCs was inhibited after treatment with 0.05 and 0.2 µM selenium, and that in 0.1 µM selenium treated groups was improved. And the T cell proliferation was inhibited after 0.2 µM selenium treatment, which was improved in 0.1 µM selenium treated groups. In conclusion, selenium coulde regulate the differentiation and immune function of human DCs by selenoproteins.

P1576

Differential cholesterol metabolism in $CD4^+$ and $CD8^+$ T cells

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Membrane lipids are critical for the signaling and function of lymphocytes, of which cholesterol is an indispensable cell membrane component and regulates cell functions in various aspects. Previous data has demonstrated that cholesterol promotes T cell activation. However, it is still not known whether cholesterol metabolism is differentially regulated in $CD4^+$ and $CD8^+$ T cells. Interestingly, we found that $CD8^+$ T cell contained more cholesterol than CD4+ T cells and the synthesis and uptake of cholesterol in $CD8^+$ T cells were more active than $CD4^+$ T cells. Cholesterol inhibitors could inhibit the proliferation and survival of activated $CD8^+$ T cells with higher degree than $CD4^+$ T cells. Activated LDLR deficient $CD8^+$ T cells had much severe defects in proliferation and function than $CD4^+$ T cells. Since we detected lower surface level of TCR complex and CD28 but higher cell signaling in $CD8^+$ T cells, we hypothesized that higher cholesterol level could facilitate the clustering and co-localization of these surface receptors, which then supports $CD8^+$ T cell activation. So, the higher cholesterol levels might be required for the faster proliferation and enhanced cell signaling (AKT-mTOR) of $CD8^+$ T cells. In summary, our results indicate that cholesterol plays more important roles in $CD8^+$ T cells, which makes $CD8^+$ T cells more sensitive to the manipulation of cholesterol metabolism.

P1577

Recovery of butyrate-induced SIRT1 depletion restores the Th17/Treg balance in necrotizing enterocolitis

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DOI: 10.1002/eji.201970400

Although SIRT1 metabolism-dependent modulation plays a critical role in immune responses, particularly inflammation, its role in necrotizing enterocolitis (NEC) is unknown. Using a formular-fed plus enteric bacteria and hypoxia induced experimental NEC model in which mice develop proinflammation and necrosis due to an imbalance caused by diminished tolerogenic Fox3$^+$ regulatory (Treg) cells and increased proinflammatory interleukin-17 (IL-17)-producing T helper 17 (Th17) in intestinal tissues, we found that a marked deficiency of SIRT1 and elevation of butyrate when compared with breast-fed controls. Neonatal NEC specimens had similar alterations in levels of SIRT1 and butyrate. **Further analyses revealed that butyrate could inhibit SIRT-1 expression *in vitro*.** Intriguingly, butyrate supplementation promoted the excessive inflammatory responses and decreased the levels of SIRT1, whereas supplementation SIRT1 agonists reduced the inflammatory responses. **The benefit of SIRT1 agonists treatment was mediated by restoring of Th17/Treg balance, which were restored due to downregulation mTOR-HIF-1α pathway, a central regulator of cellular metabolism.** Consistent with previous findings, lack of HIF-1α resulted in diminished Th17 development but enhanced Treg differentiation and protected mice from intestinal inflammation. **Taken together, these results revealed a novel mechanism by which butyrate drive intestinal proinflammation and necrosis and highlighted SIRT1 activators may directly target Th17/Treg homeostasis through regulation metabolic checkpoint kinase mTOR-HIF-1α pathway.**

P1578

Alisertib enhanced intratumoral CD8$^+$ T cells function by mitochondrial STAT3 mediated respiratory chain

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Intratumoral tumor-specific activated CD8$^+$ T cells harboring antitumor immune surveillance function predict metastasis and clinical outcome in human colorectal cancer (CRC), and affect immune checkpoint inhibitor therapies. However, the mechanisms by which introtumoral CD8$^+$ T cells are recruited and controlled remain unclear. Our recent results that in CT-26 xenograft tumor model, blockade of Aurora-A with its selective inhibitor alisertib slowed the tumor growth in association with an increased in infiltration, activation and expansion of introtumoral CD8$^+$ T cells as well as their IL-10RA mRNA expression. Interestingly, antibody-mediated blockade of IL-10Ra dramatically attenuated the anti-tumor effects of alisertib in parallel with a decrease in the number of introtumoral CD8$^+$ T cells. Furthermore, the levels of phosphorylated forms of STAT3 in these CD8$^+$ T cells mediated by alisertib were reduced in the administration of anti-IL-10Ra antibody. Of note, alisertib-induced IL-10 enhanced CD8$^+$ T cells migration and confered CD8$^+$ T cells much more mitochondrial cristae as well as higher concentration of mitochondrial electron transport chain I through STAT3 mitochondria localization, at least partially. Additionally, alisertib potentiated the action of anti-PD-1 antibody in a CT-26 murine xenograft model. Taken together, alisertib could be useful for restoring tumor-specific intratumoral CD8$^+$ T cell function. The combined administration of alisertib and immune checkpoint agents to patients with CRC warrants further investigation.

DOI: 10.1002/eji.201970400

P1579

Tumor suppressor ZHX2 inhibits NAFLD and related HCC via blocking LPL-mediated lipid uptake

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Mounting evidences strongly suggest non-alcoholic fatty liver disease (NAFLD) as an increasingly important etiology of hepatocellular carcinoma (HCC). However, the underlying mechanism remains largely unclear. Here, we investigated the role of tumor suppressor Zinc-fingers and homeoboxes 2 (ZHX2) in the progression of NAFLD to HCC. Mice were maintained with methionine-choline deficient (MCD) diet, high fat diet (HFD), or streptozotocin (STZ)-HFD to induce NAFLD or NAFLD-HCC. Lipidomics was measured by liquid chromatography-mass spectrometry (LC-MS). ZHX2-mediated transcriptional regulation was evaluated by luciferase reporter and ChIP assays. We found that ZHX2 expression was significantly decreased in fatty liver tissue, especially in liver with NAFLD-HCC. **ZHX2 overexpression disturbed lipid homeostasis of cultured HCC cells and significantly inhibited lipid deposition in hepatocytes both in vitro and in vivo**. Moreover, **ZHX2 inhibited uptake of exogenous lipids through transcriptional suppression of lipid lipase (LPL), leading to retardate growth of HCC cells**. Importantly, **LPL promoted proliferation of HCC cells, and LPL overexpression significantly reversed ZHX2-mediated inhibition of HCC cell proliferation, xenograft tumor growth, lipid deposition and spontaneous liver tumor formation in STZ-HFD fed mice**. Consistently, IHC staining demonstrated the negative correlation of ZHX2 with LPL in HCC cohort. In conclusion, ZHX2 protects hepatocytes from abnormal lipid deposition in NAFLD through transcriptional repression of LPL, which further retards cell growth and inhibits the progression of NAFLD to HCC. These findings illustrate a novel mechanism of NAFLD progression to HCC.

P1580

Intestinal microbiota contributes to altered glucose metabolism in simulated microgravity mouse model

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Exposure to space environment induces alterations in glucose and lipid metabolisms that contribute to muscular atrophy, bone loss, and cardiovascular disorders. Intestinal microbiota is also changed but its impact on spaceflight-related metabolic disorder is not clear. We investigated the relationship between glucose metabolic changes and gut dysbiosis in hindlimb unloading (HU) mouse model, a well-accepted ground-based spaceflight analog. Impaired body weight gain, glucose intolerance, and peripheral insulin resistance were found in 2-4-week HU mice. However, the levels of food intake and serum corticosterone were comparable between HU and ground control mice. HU mice had reduced abundance of gut *Bifidobacterium* spp. and *Akkermansia muciniphila* as early as 1-3 days of HU. The ground control mice that were co-housed with HU mice showed similar patterns of dysbiosis and metabolic changes. Compared to the controls, higher levels of plasma LPS-binding protein, altered transcription of *Tnfa* and glucose metabolism-related genes in the liver were observed in HU mice. The supplementation of *Bifidobacterium* spp. suppressed endotoxemia and liver inflammation, improved glucose tolerance in HU mice. The results indicate a causal relationship between dysbiosis and altered glucose metabolism in HU model. It emphasizes the importance of evaluating intestinal microbiota in astronauts and its effect on glucose metabolism. It also demonstrates the necessity of taking probiotics to maintain a balanced micro-ecosystem during distant space travel.

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DOI: 10.1002/eji.201970400

P1581

MEmOrY-T - dissecting the *m*etabolic-*e*pigenetic control of the adaptive immune response in *o*besity inflamma*t*ion

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Obesity is a major burden to public health, worldwide. Individuals with obesity are at increased risk of several health conditions including type 2 diabetes, dyslipidaemia, liver steatosis, hypertension, cardiovascular disease, osteoarthritis, as well as cancer.
A strong link between obesity and chronic systemic inflammation underpinning obesity-driven pathology emerged. We recently showed that immunization following high-fat diet activates a PI3K p110d-Akt pathway, particularly via the saturated fatty acid (FA) palmitate, leading to $CD4^+$ T cell differentiation into inflammatory effector memory cells (Cell Metabolism 2017).
This led to a key question: do fasting and loss of weight protect us from obesity-driven inflammation? If so, is the effect immediate, i.e. weight-loss = protection, or is any beneficial effect delayed? We started to investigate the hypothesis that obesity and diet high in saturated FAs cause long-term reconfiguration of the epigenetic landscape of $CD4^+$ T cell DNA leading to harmful inflammatory response, well beyond the point of weight-loss. This is not unconceivable considering that recent studies showed that cigarette smoking has broad impact on genome-wide methylation that persists and could drive smoke-related diseases even 30 years after smoking cessation.
Here, we will discuss our published data and present our new, unpublished observations
By answering this critical question, MEmOrY-T is pushing the boundaries of our knowledge and precept, unveiling the mechanistic interactions between metabolism and epigenetics that determine the long-term inflammatory response orchestrated by effector memory T cells. This is important since such immune-mediated response might have a crucial impact on obesity-driven disease, long after weight-loss.

P2158

Glycolytic activation of peritumoral monocytes fosters immune privilege via PFKFB3-PD-L1 axis in human hepatocellular carcinoma

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PD-L1 expression on antigen-presenting cells (APCs) is essential for T cell impairment, and PD-L1-expressing macrophages may mechanistically shape and therapeutically predict the clinical efficacy of PD-L1/PD-1 blockade. Despite the clinical success fostered by immune checkpoint inhibitors, the mechanisms underlying PD-L1 upregulation in human tumor microenvironments remain an enigma. In present study, we found that monocytes significantly enhanced the levels of glycolysis at the peritumoral region of human HCC. The activation of glycolysis induced PD-L1 expression on these cells and subsequently attenuated CTL responses in tumor tissues. Mechanistically, tumor-derived soluble factors, including hyaluronan fragments, induced the upregulation of a key glycolytic enzyme, PFKFB3, in tumor-associated monocytes. This enzyme not only modulated the cellular metabolic switch but also mediated the increased expression of PD-L1 by activating the NF-κB signaling pathway in these cells. Consistently, the levels of $PFKFB3^+CD68^+$ cell infiltration in peritumoral tissues were negatively correlated with overall survival and could serve as an independent prognostic factor for the survival of patients with HCC.
Conclusions: Our results reveal a mechanism by which the cellular metabolic switch regulates the pro-tumor functions of monocytes in a specific human tumor microenvironment, and PFKFB3 might be

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DOI: 10.1002/eji.201970400

a potential therapeutic target for not only cancer cells but also tumor-associated monocytes in human HCC.

P2159

Fatty acid receptor GPR120 promotes breast cancer chemoresistance by upregulating ABC transporters expression and fatty acid synthesis

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Chemoresistance is the major cause of neoadjuvant treatment failure in breast cancer patients. Despite recent progress, the mechanism underlying chemoresistance remains to be further defined. Expression of G protein-coupled receptor 120 (GPR120) was analyzed by immunohistochemistry in the biopsies of primary breast cancer who subsequently underwent preoperative neoadjuvant chemotherapy. In vitro and in vivo loss- and gain-of -function studies were performed to reveal the effects and related mechanism of GPR120 signaling pathway in the chemoresistance of breast cancer cells.We identified that GPR120, a receptor for long-chain fatty acids, was important for the acquisition of chemoresistance in breast cancer cells. We showed that GPR120 expression was positively associated with clinical response to neoadjuvant chemotherapy in patients. In breast cancer cells, GPR120 enhanced the de novo synthesis of fatty acids that served as GPR120 ligands to activate GPR120 signaling via a feedback mechanism. Upregulated GPR120 signaling rendered cells resistant to epirubicin-induced cell death by upregulating ABC transporters expression and thus decreasing the intracellular accumulation of epirubicin. Akt/NF-κB pathway was responsible for the GPR120-mediated expression of ABC transporters leading to modulation of the concentration of chemotherapeutic drugs in cells. The functional importance of GPR120 in chemoresistance was further validated using epirubicin-treated tumor xenografts, in which we showed that blockade of GPR120 signaling with AH7614 or GPR120-siRNA significantly compromised chemoresistance. Our results highlight that GPR120 might be a promising therapeutic target for breast cancer chemoresistance.

P2160

Effect of a polarized Th2 on high-fat-diet induced obesity

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Immune cells are involved in steatosis and chronic inflammation of white adipose tissue induced by high-fat-diet (HFD) in obese conditions. Since Th2 levels were elevated in subjects with overweight patients, we investigated the effect of Th2 on the white adipose tissue hyperproliferation and steatosis related to high fat diet induced obesity. We used magnetic beads to sort out Th2 cells from LATY136 mice, which are characterized by overactivation and secretion of a large number of interleukins - 4,5,6,10 and 13. Th2 cells tail vein injection significantly attenuated HFD-induced weight gain, adipocytes hyperproliferation, insulin resistance, glucose intolerance and steatosis. However, serious liver fibrosis was observed in Th2 treatment mice upon HFD. Th2 protects against adipocytes hyperproliferation and maintains glucose tolerance in the context of high fat diet challenge. Moreover, we provided the evidence that Th2 modulates diet-induced fat accumulation dysfunction through secreting IL-10. Mechanistically, we demonstrated that interleukin 13 produced by Th2 can significantly aggravate hepatic steatosis caused by HFD. Therefore, Th2 cells have a dual role in the metabolic changes caused by high-fat diets. Our data reveal the potential possibility of polarized Th2

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DOI: 10.1002/eji.201970400

supplement for treating high fat diet induced obesity and hepatic steatosis related to the several cytokines secretion of Th2.

P2161

Role of a lipid metabolite in shaping colorectal cancer immune microenvironment

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Colorectal cancer (CRC) ranks high for both incidence and mortality rate, but current immunotherapy is only limited to some subtypes of CRC, which is directly related to tumor immune microenvironment. Thus, it is of great importance to elucidate the complicated CRC immune microenvironment development. Previous evidence indicates that pro-inflammatory Th17 cells contribute to CRC progression, however, the underlying mechanism remains obscure. Here we found that CRC cell line conditioned medium (CM) specifically promotes Th17 differentiation but not other CD4 subsets like Th1 and Treg. Using advanced mass spectrometry technique, we detected substantial accumulation of a novel lipid metabolite in CRC CM, which is an important intermediate from cholesterol synthesis pathway and directs Th17 differentiation. Furthermore, we identified the critical enzyme that is responsible for this metabolite accumulation in CRC CM and regulation of the enzyme level by either genetic manipulations or small molecule inhibitors indeed modulates Th17 differentiation as predicted. Consistently, according to public TCGA library data, the expression of this enzyme is remarkably reduced in CRC samples, presumably leading to the accumulation of the downstream lipid metabolite. In line with that, elevation of this metabolite was further confirmed in human CRC biopsies as well as tumor interstitial fluid, which is likely correlated with Th17 population in CRC immune microenvironment. Taken together, our results uncover the mechanism of Th17 accumulation during CRC progression and provide the potential implication for improving CRC immunotherapy.

P2162

Resistin increases PASMCs proliferation and contributes to PAH via PI3K/AKT signaling pathway and PPARγ /PGC1α axis involving mitochondrial biogenesis and metabolic dysfunction

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Background: Increasing evidence suggests that PAH vascular cells show features similar to a paradigm of cancer, such as suppression of mitochondrial glucose oxidation and increased glycolysis. Resistin is a member of the family of resistin-like molecules(RELM, also named FIZZ), found to play a function on metabolic disease and areimplicated in the development of PAH. We aim to study whether Resistin could alsoincrease PASMCs proliferation in PAH by regulation its mitochondrial biogenesisglucose metabolism.
Methods and Results: We treated human PASMCs (hPASMCs) with recombinanthuman Resistin (rhResistin). The result showed hPASMCs was proliferation by Brduassay. PPARγ and PGC1α mRNA and protein were down-regulated by QPCR and WB.Mitochondrial fusion genes and proteins MFN1

DOI: 10.1002/eji.201970400

and MFN2 were also down-regulated and mitochondrial fission related genes and proteins DRP1 and TFAM were up-regulated.Furthermore, the result shows hResistin could up-regulate glycolysis related genes(GLUT1, HK2, PKM2) and down-regulate mitochondrial oxidative phosphorylation(OXPHOS) related gene (CS, COX5A) expression. Furthermore, the cell respirationoxygen consumption rate（OCR) and extracellular acidification rate (ECAR) weremeasured by Seahorse XF Analyzer. We found the rate of OXPHOS was decreased andthe rate of glycolysis was increased after rhResistin treatment. After we inhibitedPI3K/Akt pathway by LY294002 and/or applied PPARγ agonist rosiglitazone (RGZ), allof the above effects were reversed.
Conclusion: hResistin could up-regulated hPASMCs proliferation by activatingPI3K/Akt signaling pathway and inhibiting PPARγ/PGC1α axis and then increasingmitochondrial biogenesis, glycolysis and decrease mitochondrial oxidativephosphorylation (OXPHOS).

P2164

Anti- versus pro-inflammatory metabololipidome upon cupping treatment

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Cupping treatment is an ancient and traditional physical approach to improve health and maintain homeostasis. However, its underlying mechanism remains elucidated. Polyunsaturated fatty acids (PUFAs) and the derived metabolites play critical roles in inflammation and tissue catabasis. In the present study, a nude mouse model mimicking the cupping treatment in humans was established by administrating four cupping sets on the back skin for 15 minutes. UPLC-MS/ MS was performed to determine the PUFA metabolome in mice skin and blood before and after cupping treatment. The significantly changed lipids were administered in macrophages to assess the production of pro-inflammatory cytokines IL-6 and TNF-α by ELISA. Our results demonstrated that numerous anti-inflammatory lipids (e.g. PGE1, 5,6-EET, 14,15-EET, 10S,17S-DiHDoHE, 17R-RvD1, RvD5 and 14S-HDoHE) were increased and serveral pro-inflammatory lipids (e.g. 12-HETE and TXB2) were deceased in the skin and plasma post cupping treatment. Cupping treatment reversed the LPS-stimulated IL-6 and TNF-α expression in mouse peritoneal exudates. Moreover, 5,6-EET, PGE1 decreased the level of TNF-α, while 5,6-EET, 5,6-DHET downregulated IL-6 production in macrophages. Importantly, 14,15-EET and 14S-HDoHE inhibited both IL-6 and TNF-α induced by lipopolysaccharide (LPS). 17-RvD1, RvD5 and PGE1 significantly reduced the LPS-initiated TNF-α, while TXB2 and 12-HETE further upregulated the LPS-enhanced IL-6 and TNF-α expression in macrophages. This study identified the anti- versus pro-inflammatory metabololipidome upon cupping treatment, and suggested potential PUFA-derived lipid mediators that function as diganostic biomakers and therapeutics compounds in cupping treatment.

DOI: 10.1002/eji.201970400

P2165

Factors influencing the response to urate-lowering therapy in patients with gout

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Objectives: To explore factors influencing the response to urate-lowering therapy in gout patients .
Method: The patients with gout were collected and reviewed in First Afiliated Hospital of Nanchang university from June 2017 to June 2018. According to the dosage of urate lowering drug in reached treat-to-target patients, they were divided into sensitive group, mid-sensitive group and resistant group. The difference was analyzed on Gender, age, duration of the disease, BMI, serum uric acid, serum creatinine, urea nitrogen, ESR, CRP, uratoma, family history, smoking, alcohol consumption and comorbidity with hypertension, diabetes, hyperlipidemia, atherosclerosis, coronary heart disease, stroke in three groups.
Results: A total of 123 patients were included in this study with 115 males and 8 females. Sensitive group (n=29) included 11 allopurinol user (100mg/d, 9 phenbromarone (50m/d), 9 febuxostad user(40mg/d). Mid-sensitive group (n=44) included 14 allopurinol usrer(100-300mg/d) group, 9 phenbromarone user(50-100mg/d), 21 febuxostat user（40-80mg/d）. 50 patients in resistance group included 8 allopurinol user (more than 300mg/d), 8 phenbromarone (more than 100mg/d), 19 febuxostat (more than 80mg/d) and 15 Combination treatment. Univariate regression analysis showed family history, duration, BMI, highest serum uric acid value, serum creatinine value, ESR, CRP, diabetes and chronic renal function failure had associated with dosage of ULT (P< 0.05). Multifactor regression analysis showed that family history, course of disease, BMI, and diabetes correlated to response of ULT in gout (P< 0.05).
Conclusion: Gout patients with family history, long duration, high BMI and comorbidity with diabetes mellitus had poor response to uric acid lowering treatment.

P2166

Glycometabolism pattern of peripheral blood immune cells in myasthenia gravis patients

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Background and aims: Immunometabolism represents a newly identified checkpoint of effector functions in autoimmune diseases, and various subtypes of immune cells exhibit discrepant cellular metabolism patterns. We investigated the correlation between glycometabolism patterns of different immune cells in myasthenia gravis (MG) patients and metabolism key regulators of the PI3K/AKT/mTOR-HIF-1α signaling pathway and expected to provide potential immunometabolism reprogramming checkpoints and therapeutic targets of MG.
Methods: We recruited pre-treatment MG patients to extract peripheral blood mononuclear cells (PBMCs) and sort $CD19^+$B cells, dendritic cells (DCs), $CD4^+$T cells, $CD8^+$T cells, $CD4^+CD25^+$ regulatory T cells (Tregs) and $CD4^+CD25^-$T cells using immune-magnetic beads. T helper cells (Th-) Th1, Th2 and Th17 cells were sorted using flow cytometry. We extracted the total RNA of PBMCs and sorted cells, performed real-time PCR to detect the expression levels of PI3KCA, AKT-1, mTROC1, HIF-1α, GLUT1, hexokinase (HK), fructose phosphokinase (FPK) and pyruvate kinase (PK), and detected the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of the ex-vivo fresh sorted cells using the Seahorse XF^e96 Extracellular Flux Analyzer. In addition, we compared the glycolysis levels of all these cells from the same MG patients. And *in vitro* experiment, we observed the changes of glycolysis levels by using rapamycin and PX-478 (selective mTOR and HIF-1α inhibitor, respectively) in cultured PBMCs and B cells from MG patients.

DOI: 10.1002/eji.201970400

Results: Compared to HC, the frequency of Th2 decreased and Th1, Th17 increased significantly in MG patients. The expression of key enzymes of glycolysis and HIF-1α were significantly higher in B cells, DCs, Tregs, $CD4^+CD25^-$T cells, Th1 and Th17 cells in MG patients, except PBMCs, Th2 and $CD8^+$T cells, and the measurement of ECAR and OCR in the sorted cells confirmed the metabolic status of them. In MG patients, the B cells and DCs showed higher glycolysis and glycolytic capacity than $CD8^+$ T cells, $CD4^+$T cells and its subsets. In vitro, the increased glycolysis levels of B cells, rather than PBMCs could be inhibited by rapamycin and PX-478.
Conclusions: Different subtypes of immune cells in MG patients exhibit different glycometabolism patterns. mTOR-HIF-1α signaling may be the immunometabolism reprogramming checkpoint and therapeutic targets of MG.
Keywords: Myasthenia gravis; immune cells; glucose metabolism; HIF-1α; mTOR

P2167

Targeting lipid metabolism transferases DGAT1 and DGAT2 in MDSCs enhance the potential of cancer immunotherapy

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Myeloid-derived suppressor cells (MDSCs) play crucial roles in tumorigenesis and their inhibition is critical for successful cancer immunotherapy. How lipid metabolism regulates MDSCs function remain elusive and require more robust investigations. In this study, we demonstrate that the lipid accumulated in MDSCs from bone marrows (BM), spleens and tumors of tumor-bearing mice or in-vitro tumor explanted supernatant(TES) induced BM-MDSCs was via the utilization of exogenous fatty acids (FAs). Taking up unsaturated FAs, not saturated FAs enhanced the suppressive role of MDSCs in the tumor microenvironment by up-regulating ROS level and downregulating SOD3 expression. Increased Diacylglycerol O-transferases (DGAT1 and DGAT2) expression in tumor-induced MDSCs contribute to lipid accumulation and its suppressive activity, thus mediating cancer progression. These findings highlight the critical role of lipid metabolism in promoting tumor tolerance. Hence, suggesting a novel therapeutic strategy of targeting DGAT1 and DGAT2 in MDSCs to enhance the potential of cancer immunotherapy.

This work was supported by Nanshan pilot team project (LHTD20160004), Start-up funding (CYZZ20180307154657923), the Shenzhen Basic Science Research Project (JCYJ20170413153158716 and JCYJ20170818164619194), the fourth talents project of Guangdong province (2014-1), Shenzhen special funds for industry of the future (Development and Reform Commission in Shenzhen [2015] Grant 971), Guangdong Provincial Research Award for Thousand Talents Program Scholars.

DOI: 10.1002/eji.201970400

P2168

Purification of brain antigens and its reaction with the patient sera with Alzheimer disease

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Background: Alzheimer´s disease is one cause of progressive perception failure in the elderly. This Disease, is a disorder of the CNS that affects approximately 1-6% of people over 65 years.
Methods: The study included two categories of response. The one is Precipitation reactions between a brain antigen in animal models and the antibody in the serum , the procedure was performed in 2 methods ,immunoelectrophoresis and counter-immunoelectrophoresis.Because of the absence of a clear precipitation lines , result was not acceptable. Another class of reaction is flocculation, in which the procedure is similar to the VDRL test. Serial dilutions of sera (1/40,1/20,1/10,1/5) were performed ,with using this method the semi-quantitative autoantibodies titration was performed.
Results: Undiluted serum of all patients showed the significant positive response to this test, while this figure is indicated in the control group was 29.51 % (P=0.00011) , control group showed a positive response only to a dilution of 1/10, while 3.44 % of the patients showed a positive response to a dilution of 1/40. No significant differences between male and female patients were seen in the incidence of positive or negative responses. As well ,titration in patients was not significantly related to age.
Conclusion: These data indicated that Increased titers of anti-lipid antibodies against antigens of brain in Alzheimer´s patients compared to healthy controls and Autoimmunity may be said that at least one of the factors involved in the genesis and progression of the disease.
Key Words: CNS: central nervous system , VDRL:Vernal Disease Research Laboratory

P2169

Adipose tissue macrophage specific deletion of src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) improves glucose homeostasis in obese insulin resistant model

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Src homology 2 domain-containing protein tyrosine phosphatase (SHP-1) is a non-receptor protein tyrosine phosphatase expressed predominantly in the hematopoietic cell. It is a known regulator of various signaling pathways including immune cells proliferation and activation. Improved glucose utilization & insulin sensitivity in viable motheaten (*Ptpn6*$^{me\text{-}v/me\text{-}v}$) mice expressing low levels of functionally deficit SHP-1 protein introduced the metabolic regulatory function of SHP-1. Keeping in mind, the role of SHP-1 in macrophage functions, we speculated that SHP-1 might play an important role in polarization of adipose tissue macrophages, the hallmark of obesity-induced insulin resistance. Our study reported that during diet induced obesity the expression of SHP-1 significantly increases in adipose tissue resident macrophages. The inflammatory milieu triggers macrophage activation and high levels of SHP-1 expression by activated macrophages (M1 cells). ATM-specific deletion of SHP-1 using glucan particle loaded siRNA improves glucose homeostasis by modulating M1/M2 macrophage population and subset of macrophage phenotype markers in adipose tissue leading to metabolic improvement in the obese insulin-resistant model. Thus, glucan particle based targeted silencing of SHP-1 recognized an unrevealed function of adipose tissue-specific SHP-1 in modulating adipose tissue physiology and macrophage phenotype and the molecular mechanism behind the improved metabolic character in SHP-1 deficient mice.

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DOI: 10.1002/eji.201970400

P2170

Nlrp1b1 negatively modulates obesity-induced inflammation by promoting IL-18 production

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Obesity-induced inflammation, triggered by lipid-mediated activation of the Nlrp3 inflammasome, results in glucose metabolism alterations and type 2 diabetes. This knowledge has been generated using animals deficient for any of the different components of this inflammasome (Caspase-1, Asc or Nlrp3) in the C57BL/6 background. Unlike C57BL/6 mice, which carry allele 2 of the *Nlrp1b* gene (*Nlrp1b2*), Balb/c mice that carry allele 1 (*Nlrp1b1*) are less prone to develop alterations in the glucose metabolism when fed with a high fat diet. However, the molecular bases for these metabolic differences are unknown. Here we show that the *Nlrpb1* allele down regulates the adipose tissue inflammatory response attenuating glucose intolerance and insulin resistance in obese C57BL/mice. Our results indicate that the positive effects of the Nlrp1b1 inflammasome on glucose tolerance and insulin sensitivity involve IL-18 production and points out that differential expression of allelic variants of genes coding for inflammasome components might control susceptibility or resistance to develop diabetes in obese individuals.

P2932

Study on the relationship between serum folic acid, vitamin B12 with multiple sclerosis and neuromyelitis optica

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Objective: By comparing the patients with multiple sclerosis and neuromyelitis optica with healthy controls of the levels of serum folic acid and vitamin B12, to explore if there is a difference between serum folic acid and vitamin B12 of the patients with MS and NMO with healthy people.
Methods: Collected 76 patients that diagnosed with MS and 80 patients that diagnosed with NMO (MS group and NMO group) from 2014 to 2019 in our hospital. Physical examination patients admitted to our hospital were set as control group, among which 56 cases were MS control group and 102 cases were NMO control group. All patients were collected fasting blood and test the serum folic acid and vitamin B12 levels in each group.
Results: Two independent sample t tests were conducted for MS group, NMO group and control group respectively: serum folic acid and vitamin B12 of MS group were lower than those of control group, and the difference was statistically significant ($P< 0.05$).Serum vitamin B12 level in NMO group was slightly higher than that in control group, but the difference was not statistically significant ($P>0.05$).The serum folic acid level in NMO group was lower than the control group, and the difference was statistically significant ($P< 0.05$).
Conclusion: Serum folic acid and vitamin B12 levels were decreased in MS patients and serum folic acid levels decreased in NMO patients. Serum folic acid and vitamin B12 levels may be associated with MS and NMO.

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DOI: 10.1002/eji.201970400

P2933

Perforin attenuates nonalcoholic fatty liver disease in high-fat diet-induced mice

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Perforin, secreting by cytotoxic lymphocytes, was a cytotoxic pore-forming protein. However, whether it also played a role in high fat diet-induced non-alcoholic fatty liver disease is still to be investigated. We demonstrated that in the absence of perforin, these mice had significantly aggravated high fat diet-induced non-alcoholic fatty liver disease, with extremely high levels of IFN-γ+CD4+ T cells in liver. Furthermore, Ifngr1 &Perforin double knock-out mice prevented high fat diet-induced non-alcoholic fatty liver disease, indicating a critical role of IFN-γ in this pathological process. Depletion of CD4+ T cells of perforin knock-out mice improved high fat diet-induced non-alcoholic fatty liver disease. Further studies defined naïve CD4+ T cell from perforin knock-out mice had an intrinsically elevated ability of IFN-γ production. In conclusion, these findings support the protected of perforin in non-alcoholic fatty liver disease and suggest that perforin in inflamed liver was one possible avenue for therapeutic intervention.

P2934

PD-L1 is required for gonadotropin synthesis and secretion

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PD-L1 checkpoint blockade is used to treat certain types of tumor in clinic. However, there are few reports on the effects of anti-PD-L1 therapy on the secretion of gonadotropins (follicle-stimulating hormone, FSH; luteinizing hormone, LH) in pituitary. Our study showed that PD-L1 co-expressed with FSHβ and LHβ in the adenohypophysis of adult male mice but less expressed in the neurohypophysis. The expression level of PD-L1 is significantly increased in the pituitary of 60-day-old male mice compared to 35-day-old male mice.However, the expression level of PD-1 is extremely low in the pituitary. Further, the *in vivo* experiments show that PD-L1 antibody (*InVivo*Plus anti-mouse PD-L1, Bio X Cell) treatment results in a significant decrease in the expression of *FSHβ* and *LHβ* in mouse pituitary accompanying with insufficient secretion of FSH and LH. The *in vitro* experiments show that the phosphorylation level of p38 MAPK is significantly decreased in LβT2 cells treated with PD-L1 inhibitor BMS-202, which results in the decreased expression of *FSHβ* and *LHβ*. In-depth study of the regulatory function and molecular mechanism of PD-L1 in pituitary gonadotropin secretion will contribute to the clinical safety assessment of anti-PD-L1 therapy.

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DOI: 10.1002/eji.201970400

P2935

Decline in serum progastrin releasing peptide predicts objective response to chemotherapy in patients with small cell lung cancer

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We evaluated the usefulness of serum proGRP and NSE level as biomarker for treatment monitoring and prognostic factor in patients with SCLC. Patients who were firstly diagnosed pathologically in the First Affiliated Hospital of University of Science and Technology of China and measured serum proGRP and NSE level by Elecsys ProGRP Assay consecutively were enrolled in the study. Totally 120 SCLC patients who visited in the hospital for tissue diagnosis were enrolled. For treatment responsive patients, proGRP level decreased markedly after the first cycle of therapy and kept a continued momentum of decline during treatment. But for unresponsive patients, no obvious decline was observed. Receiver operating characteristic (ROC) curves of rate of proGRP decline had higher therapeutic efficiency on SCLC. The value of cutoff of proGRP decline was 55.02%, and the AUC was 0.9645. We speculated that the rate of proGRP decline could be a better predict therapeutic response to chemotherapy in patients with small cell lung cancer.

P2936

Sperm-associated antigen 9 is upregulated in hepatocellular carcinoma tissue and enhances QGY cell proliferation and invasion in vitro

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The incidence and mortality rates of hepatocellular carcinoma (HCC) are higher in China compared with in other countries. Further research is required in order to improve the diagnosis and treatment of HCC. Sperm-associated antigen 9 (SPAG9) protein has been revealed to serve an important function in cancer progression; however, the underlying mechanisms remain to be elucidated. The present study investigated the expression level of SPAG9 in HCC tissues using quantitative-polymerase chain reaction, immunohistochemistry and western blotting, and the results demonstrated that SPAG9 was overexpressed in HCC tissues compared with the adjacent non-cancerous tissues. To explore the potential mechanisms underlying SPAG9 in HCC, the effect of SPAG9 on cell proliferation, cell cycle, migration and invasion capacities were investigated in the QGY HCC cell line by RNA interference. It was revealed that inhibition of SPAG9 mRNA in QGY cells significantly inhibited the expression level of SPAG9 compared with the control.
Depletion of SPAG9 expression decreased cell proliferation ($P< 0.01$) and increased the percentage of cells in the G1/G2 cell cycle phase. The percentage of cells in the S phase was decreased, and cell migration and invasion capabilities *in vitro* were reduced ($P< 0.01$). In summary, the results of the present study suggested that SPAG9 was upregulated in HCC and may serve an important function in cancer cell proliferation, differentiation and invasion. Whether SPAG9 is a potential diagnostic marker and therapeutic target of human HCC requires additional study.

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DOI: 10.1002/eji.201970400

P2937

Pioglitazone enhances anti-tumor efficacy of iNKT cells via promoting their lipid biosynthesis in tumors

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Dysfunction of iNKT cells in tumor microenvironment would hinder their anti-tumor efficacy, and the underlying mechanisms remain unclear. Here, we demonstrate that tumor microenvironment suppresses IFN-γ production in iNKT cells via inhibiting lipid biosynthesis. Increased lipid biosynthesis was detected in iNKT cells after activation, and that was controlled by PPARγ. Fatty acid synthesis was required for their optimal IFN-γ production. Tumor microenvironment reduced expression of PPARγ and consequently diminished fatty acid synthesis and IFN-γ production in intratumoral iNKT cells. Importantly, PPARγ agonist Pioglitazone (PIO), a TZD drug for type 2 diabetes, successfully restored IFN-γ production in tumor-infiltrating iNKT cells from human patients and mouse models. Combination of PIO and αGC treatments significantly enhanced iNKT cell-mediated anti-tumor immune responses and prolonged survival of tumor-bearing mice. Our studies provide a new strategy to augment the anti-tumor efficacy of iNKT cell-based immunotherapies via promoting their lipid biosynthesis.

P2938

Immunometabolism regulates TCR recycling and iNKT cell functions

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Invariant natural killer T (iNKT) cells are innate-like T lymphocytes that express an invariant T cell receptor (TCR), which recognizes glycolipid antigens presented on CD1d molecules. These cells are phenotypically and functionally distinct from conventional T cells. When we characterized the metabolic activity of iNKT cells, consistent with their activated phenotype, we found that they had much less mitochondrial respiratory capacity but increased glycolytic activity in comparison to naïve conventional CD4+ T cells. After TCR engagement, iNKT cells further increased aerobic glycolysis, which was important for the expression of interferon-γ (IFN-γ). Glycolytic metabolism promoted the translocation of hexokinase-II to mitochondria and the activation of mammalian target of rapamycin complex 2 (mTORC2). Inhibiting glycolysis reduced the activity of Akt and PKC, which inhibited TCR recycling and accumulation within the immune synapse. Diminished TCR accumulation in the immune synapse reduced the activation of proximal and distal TCR signaling pathways and IFN-γ production in activated iNKT cells. Our studies demonstrate that glycolytic metabolism augments TCR signaling duration and IFN-γ production in iNKT cells by increasing TCR recycling

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DOI: 10.1002/eji.201970400

P2939

The relationship between chemokines CXCL12, CCL2, CCL5 and preeclampsia

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Objective: To analysis the relationship between the expression of the chemokines and the umbilical artery flow in preeclampsia (PE)
Methods: Thirty-nine PE women delivered in the shengjing hospital were selected, and thirty-five women with corresponded gestational weeks were enrolled as control. All the women were singleton primiparas. They didn't have infectious or other complicated diseases. Enzyme linked immune sorbent assay (ELISA) was used to measure the concentrations of CXCL12, CCL2 and CCL5 in serum. The systolic/diastolic ratio of umbilical artery was detected on the same day of blood collection.
Results: The level of CCL2 and CCL5 were significantly higher while the level of CXCL12 was significantly lower in PE group than those in the control group ($P< 0.05$). The CCL2 level was related to that of CXCL12 and CCL5 ($P< 0.01$), and the CXCL12 level was related to the S/D ratio ($P< 0.05$) in the PE group.
Conclusions: The chemokines might play a role in the formation of maternal-fetal interface during pregnancy. The abnormal level of chemokines CXCL12, CCL2 and CCL5 in PE might increase the adhesion of mononuclear macrophages, T cells to vascular endothelial cells, and cause damage in vascular endothelial cell and excessive inflammatory response at maternal-fetal interface, which might lead to the clinical characters of PE.
Keywords: Chemokine, preeclampsia, umbilical artery flow

P2940

Rab32 deficient regulates hepatic lipid metabolism through inhibition of early stages of autophagy

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Recent evidences suggested that Hepatic Lipid could be degraded by a specific autophagy-related process. Several GTPases have been observed to associate tightly with the lipid metabolism in liver. The objectives of this research are to determine whether GTPases Rab32 regulate lipid metabolism in liver and clarify the molecular mechanism of Rab32 in regulation hepatic lipid metabolism. Here, we demonstrated that Rab32 knockout mice (Rab32$^{-/-}$) exhibit lipid storage in liver which was also found in zebrafish with Rab32 knockout. Then the hepatocyte-specific Rab32 (Rab32$^{f/f}$; Alb-Cre) knockout in mice was generated and found lipid accumulation in liver as the Rab32$^{-/-}$ mice. Both the primary hepatocytes from Rab32$^{f/f}$; Alb-Cre mice and AML12 cell line with Rab32 knockout in vitro showed the number and size of LDs per cell were significantly increased compared to wild-type group. Second, there is no obvious difference of the expression levels of RNA about lipogenesis, fatty acid (FA) uptake, FA oxidation and secretion in liver tissue between Rab32$^{f/f}$; Alb-Cre mice and C57BL6/J wild type mice. But inhibition of autophagy was found in tissue section of liver from Rab32$^{f/f}$; Alb-Cre mice. Impaired autophagy flux was also found in AML12 cell line with Rab32 knockout. Third, primary hepatocytes from the C57BL6/J wild type and Rab32$^{f/f}$; Alb-Cre mice were treated with inhibitor of different stages of autophagy. The result showed Rab32 involved in the autophagy at an early stage.

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DOI: 10.1002/eji.201970400

These observations provide a function of Rab32 in the regulation of lipid homeostasis through autophagy degradation system.

P2941

Intestinal tract is an important organ for lowering serum uric acid in rats

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Kidney was recognized as the dominant organ for uric acid excretion. The study would demonstrate intestine was an even more important organ for serum uric acid (SUA) lowering. Sprague-Dawley rats were treated normally or with antibiotics, uric acid, adenine, or inosine of the same molar dose for 5 days. Rat's intestine was equally divided into 20 segments except cecum. Uric acid in serum and intestinal juice was assayed. Total RNA in the initial intestine and at the end ileum was extracted and sequenced. Protein expression of xanthine dehydrogenase (XDH) and urate oxidase (UOX) was tested by Western blot. The effect of oral UOX in lowering SUA was investigated in model rats treated with adenine and oxonate for 5 days. SUA in the normal rats was 20.93±6.98 µg/ml, and total uric acid in the intestinal juice was 308.27±16.37 µg, which is two times more than the total SUA. The uric acid was very low in stomach juice, and attained maximum in duodenum and then declined all the way till the intestinal end. The level of uric acid in the initial intestinal tissue was very high, where XDH and most of the proteins associated with bicarbonate secretion were up-regulated. In addition, SUA was decreased by oral UOX in model rats. The results suggested that intestinal juice was an important pool for uric acid, and intestine was an important organ for SUA lowering. The mechanism was associated with uric acid synthesis and secretion in the upper intestinal tract, and reclamation in the lower.

P2942

TSPO deficiency promotes glioma growth and malignancy by enhancing aerobic glycolysis

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The mitochondrial translocator protein (TSPO) has received significant attention since its function in controlling mitochondrial cholesterol import for steroidogenesis was refuted using both in vivo and in vitro genetic Tspo-deficiency models. TSPO is highly expressed in various human cancers. However, its role in tumorigenesis remains controversial. Here, we show that TSPO regulates mitochondrial morphology and controls the balance between oxidative phosphorylation and aerobic glycolysis in glioma cells. Loss of TSPO resulted in more fragmented mitochondria, increased glucose uptake and lactic acid conversion, decreased oxidative phosphorylation and enhanced aerobic glycolysis. TSPO deficiency also promoted glioma angiogenesis and tumor formation in intracranial xenografts from mouse glioma cells and a spontaneous mouse glioma model. This study provides novel clues for defining the role of TSPO in regulating mitochondrial function, glioma metabolism and tumorigenesis.

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DOI: 10.1002/eji.201970400

P2943

Fibrinogen-like protein 2 controls sepsis catabasis by interacting with resolvin Dp5

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The role of coagulation on resolution of inflammation remains elusive. Here we report the temporal regulation of soluble (s) and transmembrane (m) fibrinogen-like protein 2 (Fgl2) during sepsis and show that both sFgl2 and mFgl2 correlate with the outcome. The expression and ectodomain shedding of Fgl2 are promoted by miR-466l and metalloproteases (ADAM10 and ADAM17) during inflammation resolution. Deficiency of Fgl2 enhances polymorphonuclear neutrophil (PMN) infiltration but impairs macrophage (MΦ) maturation and phagocytosis and inhibits the production of n-3 docosapentaenoic acid (DPA)-derived resolvin 5 (RvDp5). In contrast, administration of sFgl2 blunts PMN infiltration, promotes PMN apoptosis and RvDp5 biosynthesis. By activating ALX/FPR2, RvDp5 enhances sFgl2 secretion via ADAM17 and synergistically accelerates resolution of inflammation. Importantly, anti-coagulant dabigatran increases sFgl2 which promotes RvDp5 production and improves sepsis survival. These results uncover a novel endogenous programmed mechanism linking prothrombinase and resolution of inflammation and sheds new light on clinical anti-sepsis treatments.

Microbiome and Immune System

O077

Microbiota diversity controls the intravascular immune response during sepsis

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Microbiota-immune homeostasis is essential to maintain health, while dysbiosis is linked to immune dysfunction and disease, including susceptibility to infections. Emerging evidence suggests that critically ill patients with sepsis display severe dysbiosis of their gut microbiota, marked by reduced species diversity. However, the impact of dysbiosis on host defense and immunopathogensis in sepsis has not been studied. To investigate the role of microbiota diversity during sepsis, *Staphylococcus aureus* USA300 was administered intravenously to germ-free mice or gnotobiotic mice with engineered microbiota of minimal diversity (5 bacteria) or moderate diversity (12 bacteria). Germ-free and gnotobiotic animals with minimally-diverse microbiota showed impaired clearance of blood-borne bacteria, resulting in disseminated infection compared to gnotobiotic mice with moderately diverse or conventional (specific pathogen-free, SPF) mice. Mass cytometry-based immune profiling across compartments involved in systemic bacterial clearance (blood, liver, and spleen) identified abnormal populations of critical intravascular immune cells (neutrophils, monocytes, and liver Kupffer cells) in germ-free mice compared to animals with moderately-diverse or SPF microbiota. Using high resolution *in vivo* imaging of the host response in septic mice, we observed defective intravascular immunity in germ-free animals, including impaired neutrophil trafficking (adhesion and crawling) and effector functions (phagocytosis and neutrophil extracellular trap [NET] production), and impaired Kupffer cell-mediated phagocytosis of circulating pathogens. Together, this dysbiosis-induced impairment of

DOI: 10.1002/eji.201970400

intravascular immunity promoted impaired clearance of bacteria, and hematogenous dissemination of infection. Our results demonstrate that dysbiosis and reduced microbiota diversity are mechanistically linked to impaired systemic intravascular immunity in sepsis, and defective host defence against bacterial dissemination.

O078

Comparisons of gut microbiota profiles between wild-type and HLA-DRB1*12:02 transgenic C57BL/6J mice

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Background and aims: Intestinal flora plays a key role in maintaining homeostasis in the host. Studies have shown that genetic factors can influence the composition of intestinal flora. We had performed a genome-wide association study for HBV-related acute-on-chronic liver failure (ACLF) and identified *HLA-DRB1*12:02* as the top susceptible HLA allele associated with ACLF. This study was to investigate the difference of intestinal microflora between wild C57BL/6J mice and HLA-DR (HLA-DRB1*12:02 allele) transgenic C57BL/6J mice.
Methods: The composition and structure of intestinal flora between the two kinds of mice were analyzed based on 16S rDNA high-throughput sequencing.
Results: Our results showed that the intestinal flora of HLA-DRB1*12:02 transgenic mice were significantly different from those of wild-type C57BL/6J mice, and the Lachnospira and Gastraophilales were the characteristic intestinal flora in DRB1*1202 mice. When the two groups mice were separate-housed, the results showed that together with DRB1*1202, mother effect and cage variables explained 66% of the fecal microbiota variation, and DRB1*1202 explained 32% of the difference. After co-housed, the abundances of Lachnospira and Gastraophilales bacteria from HLA-DRB1*12:02 transgenic mice remain increased, and HLA-DRB1*12:02 gene explained 23% of the difference.
Conclusions: This study revealed the effect of HLA-DRB1*12:02 gene on intestinal flora of C57BL/6J mice, which implicates the functional significance among HLA class II molecule, intestinal flora and pathogenesis involved in HBV-ALCF.

O079

Systemic translocation of Staphylococcus drives autoantibody production in HIV disease

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Background: The mechanism of autoantibody production in HIV remains unknown.
Results: Seasonal influenza vaccination induces autoantibody production (e.g., IgG antinuclear antibody (ANA) and anti-double-stranded DNA antibody (anti-dsDNA)) in viral-suppressed antiretroviral therapy (ART)-treated HIV+ subjects ($P< 0.05$, Wilcoson test), but not in healthy controls ($P>0.05$). These autoantibodies were not derived from antigen-specific B cells but from activated "bystander" B cells using single-cell assay and purified polyclonal ANAs from plasma. To explore the mechanism of autoantibody generation in HIV+ subjects, plasma level of microbial products, gene expression profile of B cells, and B cell receptor (BCR) repertoires were analyzed. We found that autoantibody production was directly correlated with increased plasma microbial translocation ($P< 0.05$, Spearman Correlation test); the patients with high autoantibodies had skewed B cell repertoires

DOI: 10.1002/eji.201970400

and upregulation of genes related to innate immune responses to microbial translocation. By analyzing circulating microbial 16S rDNA in plasma, the relative abundance of Staphylococcus was found to be associated with autoantibody production in HIV+ subjects. Finally, we found that injection of heat-killed Staphylococcus aureus promoted germinal center B cell responses and autoantibody production in mice ($P < 0.05$, Wilcoson test), consistent with the notion that autoantibody production in HIV+ patients is triggered by microbial products.
Conclusions: Our results showed that translocation of Staphylococcus can promote B cell activation through enhancing germinal center response and induces autoantibody production. It uncovers a potential mechanism linking microbial translocation and autoimmunity in HIV+ disease and provides a strong rationale for targeting Staphylococcus to prevent autoantibody production.

O080

Immune virome of gut

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Gut luminal IgA in humans has been shown to differentially profile colitogenic bacteria from non-colitogenic bacteria. Whether such differential labelling exists for the gut virome/phageome is unknown. We sought to profile (a) the endogenously associated IgA phageome from the human gut virome, and (b) the peripheral IgG immune repertoire to human and bovine gut bacteriophage. Bacteriophage were isolated from the faeces of (a) self-declared healthy human donors and (b) adult female lactating Holstein-Friesian cattle. Isolated immunoglobulin labelled bacteriophage were sequenced by targeted whole metagenome sequencing using the Illumina platform. Following removal of human and bovine genomic reads, sequences were assembled into putative viral contigs using the aon metaSPAdes assembler. Taxonomic classification of reads was performed against the NCBI viral RefSeq database. We identified endogenously associated IgA bacteriophage from known and unknown viral families. Both human and bovine IgG associated bacteriophage populations could be isolated and demonstrated significant variation in diversity between sources. The taxonomical classification of the sequences were populated with bacteriophage from Myoviridae, Podoviridae, Gemycircularvirus, Herpesviridae, Microviridae, Sphoviridae and Gokushovirinae. The unselected phageome from the donor human panel and a single cow yielded over 2000 putative viral contigs. In conclusion, this small study demonstrates that the bacteriophage can be IgA associated in the human gut and that the peripheral IgG repertoire has reactivity against a select group of definable bacteriophage. The majority (98%) of the immunoglobulin associated bacteriophage are currently unclassified.

LB061

Contribution of the microbiome to maintenance of the CD4:CD8 ratio in inbred and congenic mouse strains

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The gut microbiome is known to play an integral role in the regulation of the immune system. Previous studies indicate that species diversity and overall abundance of commensal bacteria in the gut influences immune system function by altering total T cell counts and the ratio of $CD4^+$ to $CD8^+$ T cells in peripheral lymphoid organs and blood. In normal individuals, the CD4:CD8 ratio is greater than one.

DOI: 10.1002/eji.201970400

However, the congenic B10.A mouse strain in our conventional facility consistently demonstrates an inverted CD4:CD8 ratio in a sex- and age-dependent manner, in contrast to both the parental C57Bl/10 strain and B10.A kept under SPF conditions. To determine whether this phenomenon is due to the composition and diversity of the gut microbiome affecting the homeostasis of the CD4:CD8 ratio, we altered the microbiome by supplementing the diet with the prebiotic inulin and looked for changes in the number of Peyer's patches and changes in the CD4:CD8 gut lymphoid organs, and blood in both B10.A and C57Bl/10 strains. In addition, we followed the changes in the microbiome upon treatment. We find that the mice in our facility have lower microbiome diversity and very different composition from mice kept at SPF conditions. The inulin treatment significantly increased the CD4:CD8 ratio in the blood of the C57Bl/10 mice, but not the B10.A mice. In addition, males of both strains had a significant increase in Peyer's patch number, while the females had a significant increase in the CD4:CD8 ratio in the Peyer's patches.

P0389

Probiotics Lactobacillus reuteri abrogates immune checkpoint blockade-associated intestinal inflammation by inhibiting group 3 innate lymphoid cells

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Objective: ICB-associated colitis is quite frequent and severe in immune checkpoint blockade immunotherapy. Studies have shown that the gut microbiome is closely related to the body´s immune function. Therefore, this study attempts to analyze the changes of microbiome in the body of patients with colitis, the effect of the microbiome on the intestinal inflammation and explain the mechanism, which will provide new ideas for mitigating intestinal inflammation.
Methods: 1. Withing DSS, ICB-associated colitis mouse model were established to simulate ICB treatment.2. Based on 16S rRNA metagenomic sequencing, the intestinal flora were analyzed. 3. Vancomycin and *Lactobacillus reuteri* both used to determin the effect of *Lactobacillus reuteri* on colitis mice. 4.Flow cytometry was used to analysis of immune cells, and innate lymphoid cells (ILCs) in the colon of mice to investigate the mechanism of *Lactobacillus reuteri* alleviating intestinal inflammation in mice.
Result: 1. ICB administration aggravated mouse DSS colitis.2, ICB-related colitis is closely related to intestinal flora change.3. Vancomycin significantly aggravated intestinal inflammation in mice. Intravenous *Lactobacillus reuteri* can significantly reduce intestinal inflammation in mice And *Lactobacillus reuteri* does not affect the anti-tumor effect of ICB. 4. Mechanically, the protective effect of L. reuteri was associated with the decreased distribution of ILC3s, and has no significant relationship with ILC1s lymphocytes and ILC2s cells.
Conclusion: Our study highlights the immunomodulatory mechanism of gut microbiota and suggests that manipulating gut microbiota by administrating L. reuteri to mitigate the autoimmunity caused by ICB.

DOI: 10.1002/eji.201970400

P0390

Effects of fecal microbiota transplantation on a pristane-induced lupus mouse model

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Background: The correlations between SLE(systematic lupus erythematosus) and gut microbiota colonization have been receiving much attention during recent years. The mechanism and complex mutual network accounting for the disturbances of the microbial composition are not fully understood. There are limited data regarding microbial practices for SLE,such as probiotics, prebiotics and fecal microbiota transplantation, to support the process from bench to beside.
Method: Oral gavage of feces from lpr or mpj mice to pristane-induced lupus model mice, regular and dynamic evaluation of weight, proteinuria and antibody titers during the observation period. Spleen, lymph nodes, intestine and kidneys are collected for flow cytometry or histopathology analysis after sacrifice.
Result: There exist significant differences in proteinuria between two groups. The level of dsDNA is higher in the lpr feces gavage group than that in the mpj feces gavage group. Renal histopathology from lpr feces gavage group exhibits more severe inflammatory infiltration and immune complex deposition. Likewise, besides more serious local inflammation, intestinal permeability is also increased in lpr feces gavage group. Flow cytometry analysis of isolated lymphocytes from intestine showed B220 or a B cell-relevant difference could be a potential marker accounting for the phenotype differences.
Conclusion: Lpr feces gavage group showed accelerated course or aggravating conditions of disease. It reveals the dynamic relevance between the gut microbiome and pristane-induced lupus models, which lays the foundation for the mechanism researches and the following clinical treatments in SLE.

P0391

Bifidobacterium lactis BB-12 attenuates the aging of macrophage induced by D-Galactose and promotes the M2 macrophage polarization

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Immunosenescence comprises a set of dynamic changes occurring to both, the innate as well as the adaptive immune system. Increasing evidences indicate that macrophage aging plays an important role in innate and adaptive immunosenescence. However, the function and polarization change of aging macrophage has not been fully evaluated, and the effective method to delay macrophage senescence are still poorly understood. In this study, we found that D-galactose (D-gal) can clearly promoted J774A.1 macrophage senescence, and induced the differentiation of macrophage M1 polarization. Furthermore, Bifidobacterium lactis BB-12 could significantly inhibit J774A.1 macrophage senescence induced by D-gal. The IL-6 and IL-12 level in the BB-12 groups were markedly decreased compared with that in the D-gal group, and the M2 marker, IL-10 and Arg-1 mRNA level was increased in the BB-12 group. Moreover, BB-12 could inhibit the p-STAT1 expression and promote the p-STAT6 expression. In sum up, our results showed that BB-12 could clearly attenuate the J774A.1 macrophage senescence and induce M2 macrophage polarization, indicated the potential of BB-12 to slow down immunosenescence and inflamm-aging.

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DOI: 10.1002/eji.201970400

P0392

Proteus mirabilis isolated from mesenteric lymph nodes of CT26-tumor-bearing mice treated with chemotherapy and antibiotics affect anti-tumor immune responses

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Recent findings show that using antibiotics impairs the efficacy of cancer immunotherapies and chemotherapies via eliminating commensals. However, a sterile gut seems impossible by using antibiotics in patients with cancer. Moreover, chemoresistant pathogens may be selected by antibiotics. Whether these remaining and expanding pathosymbionts affect the efficacy of chemotherapy for cancer is yet not known. We treated mice that were transplanted with subcutaneous CT26 tumor with intraperitoneal injections of four cycles of 5-fluoriuricil (5-FU) (4 injections per cycle) and drinking water that contained ampicillin, colistin E, and streptomycin. We found that 5-FU could not inhibit tumor growth at the fourth cycle of 5-FU in the combination group, whereas, it effectively delayed the tumor growth in mice treated with 5-FU alone. After treatment, we isolated mesenteric lymph nodes for aerobic and microaerophilic culturing and performed 16S rRNA sequencing for bacterial colonies. We found that *Escherichia coli* were cultured from the lymph nodes of antibiotic group, *Klebsiella pneumonia* from the 5-FU group, and *Proteus mirabilis* from the combination group. *Proteus mirabilis* were neither resistant to 5-FU compared with *Klebsiella pneumonia* nor resistant to antibiotics compared with *Escherichia coli*. We isolated splenic lymphocytes and found that compared with the 5-FU group or antibiotic group, the cytotoxicity of splenic lymphocytes against CT26 was reduced and percentages of $CD44^{lo}CD62L^{+}$ and $CD44^{hi}CD62L^{-}$ $CD8^{+}$ T cells were decreased in the combination group. Translocated *Proteus mirabilis* in the combination group might affect the efficacy of 5-FU by regulating immune responses but not by metabolizing 5-FU.

P0393

Dietary 1-3, 1-4 β-glucan improves neuroinflammation and cognitive impairment via gut-brain axis in high-fat diet induced obesity

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A high-fat (HF) diet induced obesity increases the risk for neuroinflammation, cognitive impairments and Alzhemer's Disease via alteration of gut microbiota-brain-axis. 1-3, 1-4β-glucan, a dietary fibre, is fermented by gut microbiota in to short-chain fatty acids (SCFAs), which show antiinflammation effects. However, the potential protective effects and mechanism of β-glucan in prevention of obesity induced neuroinflammation and cognitive impairment is unknown. In this study, C57/BL mice were divided into five groups: control diet (lab-chow, LC); β-glucan supplementation in LC; HF diet; β-glucan supplementation in HF diet; antibiotics intervention combined with β-glucan supplementation in HF diet for 14 weeks. We found that β-glucan prevented recognition memory impairment induced by HF diet. Concurrently, we found that in HF diet, β-glucan inhibited gliosis (activation of microglia and astrocyte) and inflammatory cytokines expression, increased synaptogenesis markers (BDNF, PSD95) and improved synapse morphology in the hippocampus and prefrontal-cortex, two important brain regions for cognition. At phylum level of gut microbiota, Bacteroidetes was increased after β-glucan supplementation with significantly increased of SCFAs in plasma. In colon, β-glucan prevented HF diet-induced proinflammatory macrophage accumulation and impaired tight junctions. Importantly, oral antibiotics intervention distorted β-glucan in improving cognition in HF diet, suggesting β-glucan improving cognition via gut microbiota. Furthermore, SCFAs administration improved cognition

DOI: 10.1002/eji.201970400

declined in obese mice, while the beneficial effects disappeared in GPR41(SCFAs receptor) knockout mice. Overall, these behavioral and neurochemical findings suggest that 1-3, 1-4 β-glucan could be a validated prebiotics to prevent alteration of gut-brain-axis to limit neuroinflammation and cognitive decline in HF diet-induced obesity.

P0394

HBV promotes the expresion of interleukin-34 in hepatoma cells to induce the differentiation of monocytes into M2 macrophages

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Interleukin-34 (IL-34) is a newly identified cytokine that contributes to the differentiation of monocyte into macrophage. In addition, it has been reported that IL-34 is associated with hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). However, whether IL-34 is involved in the differentiation of monocyte mediated by HBV in viral related HCC tissues is still unknown. We found that HBV could promote the expression of IL-34 at gene and protein levels in HCC cells. Among the viral genes in HBV genome, only HBV X (HBX) gene was found to facilitate the increase of IL-34 mediated by HBV in hepatoma cells. In addition, in order to investigate the effect of HBV on the differentiation of monocytes regulated by HCC cells, the HCC cells with or without HBV were co-cultured with monocytes. Compared to control cells, HBV positive HCC cells could significantly induce the differentiation of monocytes into M2 Macrophages. Furthermore, after the HBV positive HCC cells were treated with IL-34 short hairpin RNA (shRNA) and neutralizing antibody. The number of M2 Macrophages mediated by HBV-related HCC cells was inhibited. Taken together, these results indicate that HBV could induce the expression of IL-34 in HCC cells and the induced IL-34 by the virus contributes to the differentiation of monocytes into macrophages with M2 phenotype, and the expression IL-34 mediated by HBV is dependent on HBX.

P0395

Urinary Tract infections - *E. coli* survival by inflammation, adhesion and degradation of host cell membranes

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Urinary Tract infections (UTIs) are common, usually resulting from ascent of enteric *E.coli* microbes via the urethra. Uropathogenic *E. coli* (UPEC) behaving as commensals in the gut cause the majority of UTIs. However, UPEC do not metabolise urine and the metabolic factors allowing for their survival is poorly understood. We sought to determine if UPEC induce inflammation allowing for bacterial metabolism of ethanolamine, a host cell breakdown product, as a selective advantage during UTI. We analysed 103 clinical UTI samples by conventional culture, ELISA, genomic sequencing, HPLC, microscopy and qRT-PCR. We found evidence of ethanolamine in infected urine and *E. coli* metabolism thereof via the Eut operon mRNA expression ($P < 0.01$). Active immune responses to the UPEC was evident by high urine levels of IL1b, IL-6 and IL-8 ($P < 0.01$). Microscopic cytopsin analysis of UTI urine revealed *E. coli* adherent to host epithelial cell membranes. Genomic analysis of the

DOI: 10.1002/eji.201970400

UPECs identified ethanolamine metabolism operon (*Eut*) as a core genomic requirement for infection while virulence factors were not seen to be essential for patient infection. *In vitro* bacterial culture and mutation studies of these clinical isolates revealed that ethanolamine confers a growth advantage to UPEC. Co-culture of the UTI isolates with the bladder epithelium cell line HT1376 revealed their adherent nature and the requirement for an intact operon for cell invasion. This data demonstrates that UPECs induce inflammation, adhere and metabolise host cell membranes via the *Eut* operon. Interruption off this metabolic pathway could be exploited in developing therapeutics.

P0396

An epigenetic link between the gut microbiome and severity in allergic lung inflammation

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The gut microbiome is a driving force behind the development of atopic susceptibility and severity. We previously demonstrated that treatment of newborn mice with low-doses of the antibiotic vancomycin alters gut microbiome diversity with a marked loss of bacteria that produce short-chain fatty acids (including butyrate). Vancomycin-induced gut dysbiosis enhances T_H2 responses to lung allergens due to altered dendritic cell trafficking and activation. Dietary butyrate supplementation reverses this T_H2-skewed pro-inflammatory phenotype.
We have now found that engraftment of total bone marrow (BM) from vancomycin-treated, dysbiotic mice transfers the enhanced T_H2 response phenotype to normobiotic recipients. These data suggest epigenetic reprogramming of donor hematopoietic cells from dysbiotic mice is maintained through long-term engraftment of normobiotic recipients.
Butyrate is known to exert some of its effects on target cells by inhibiting histone deacetylases (HDACs) with consequent effects on gene expression. Indeed, unique regulatory states (H3K27ac marks) persist in the genome of hematopoietic stem and progenitor cells purified from normobiotic mice transplanted with dysbiotic mouse BM. Single-cell RNA sequence analyses identified a distinct stem/progenitor cell transcriptomic signature in dysbiotic mice that is reversed by butyrate supplementation. We conclude that the gut microbiome sculpts gene expression in blood progenitor cells with long term effects on immune response.

P0978

Zymosan promotes Candida albicans adhesion, proliferation and IL-1β production of oral squamous cell carcinoma

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Background: Oral squamous cell carcinoma (OSCC) is the most common type of head and neck squamous cell carcinoma (HNSCC), and the interaction between *Candida albicans* and oral cancer remains unclear.
Methods: Cell proliferation assay was performed with CCK-8 to evaluate the effect of zymosan on OSCC cell lines, and potential mechanism was explored by quantitative real-time PCR, immunofluorescence assay and western blot. Cell adhesion assay was conducted to determine the adhesion of *Candida albicans* to OSCC cells, and related gene expression and protein was detected. Also, the proinflammatory cytokines including IL-6, IL-8, TNF-α and IL-1β from OSCC cells were

DOI: 10.1002/eji.201970400

determined by ELISA.
Results: In current study, zymosan (ZYM) from fungi cell wall promotes proliferation of OSCC cell lines including WSU-HN4, WSU-HN6 and CAL-27 by CCK-8 assay via TLR2/MyD88 pathway. By cell adhesion assay, the number of *Candida albicans* per oil field significantly increased in ZYM-treated OSCC cells compared to controls. When treated with zymosan, OSCC cells secreted significantly more pro-inflammatory cytokine IL-1β, which could enhance inflammation in oral cancer microenvironment.
Conclusion: Zymosan from fungi cell wall promotes proliferation, *Candida albicans* adhesion and IL-1β production of oral squamous cell carcinoma.

P0979

SHP2 deficiency promotes Staphylococcus aureus pneumonia following influenza infection

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Secondary bacterial pneumonia is common following influenza infection and can result in high mortality. However, it remains unclear about the underlying molecular mechanisms for the increased susceptibility to secondary bacterial infection. Here, we report that SHP2, a widely expressed Src homology 2 domain-containing phosphatase, is critically involved in regulating the host defense response against secondary bacterial pneumonia. Mice harboring the SHP2 deletion in macrophages (*LysM*$^{Cre/+}$*:Shp2*$^{flox/flox}$) displayed impaired antibacterial immunity and high mortality compared with control mice (*Shp2*$^{flox/flox}$) in postinfluenza *Staphylococcus aureus* pneumonia. The attenuated antibacterial ability was associated with the induction of type I interferon and suppression of chemo-attractants KC and MIP-2, which reduced the infiltration of neutrophils into the lung upon secondary bacterial invasion. Additionally, enhanced polarization to alternatively activated macrophages (M2 phenotype) in conditional SHP2 knockout mice was observed, which contributed to the susceptibility to secondary bacterial infection. Further *in vitro* analyses consistently demonstrated that SHP2-deficient macrophages were skewed towards an M2 phenotype and exhibited the decreased production of inflammatory cytokines. Moreover, SHP2 modulated the inflammatory response to secondary bacterial infection via interfering with NF-κB and IRF3 signaling in macrophages. Collectively, our findings reveal that the SHP2 expression enhances the host immune response and prompts bacterial clearance in postinfluenza *S. aureus* pneumonia

P0980

Dynamic analysis of the immunological characteristics of children with lupus nephritis

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Objective: To investigate the correlation between immune indicators and SLEDAI score of lupus children.
Methods: A retrospective analysis was made of 42 children diagnosed as lupus nephritis (LN) including 22 cases received DNA immune adsorption treatment and 20 cases received traditional treatment.
Results: 11 cases of which were male, 31 cases were female. Average age:11.7±1.89years old;renal biopsy in 32 cases, accounting for 76.2%. (1)With the course of treatment and mitigation, the mean value of B cells declined; each period of treatment, the percentage of B cells still significant higher

DOI: 10.1002/eji.201970400

than the control group (P< 0.01);(2)The percentage of CD8+cell in children with SLE is significantly higher than in the healthy control group, the differences were statistical significance (P< 0.05). (3)The value of complement were significantly increased after treatment. (4) After immunoadsorption treatment, ANA, ds-DNA and the level of immunoglobulin had obvious decreased(P< 0.01) ; Compared to the lymphocyte subsets between this two groups , there were no differences in the complements of the lymphocyte subsets between this two groups .(5)Our study also find that there will be a downtrend in the numbers of natural killer cells , the complements of C3 and C4 after the treatment of three years later . The level of immunoglobulin IgG and IgA crosscurrent.
Conclusion: It is patency immunologic derangement in the SLE children. The level of immunoglobulin IgG and IgA in SLE patients is significantly corelated with SLEDAI , which can be used as an index to evaluated the activity of SLE disease.

P0981

Variations in gut microbiota associated with the importance of PMN-MDSCs by 16S rRNA gene sequencing in mice aGVHD after allo-HSCT

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Acute graft-versus-host disease (aGVHD) is one of the fatal and refractory complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT) . Polymorphonucler myeloid-derived suppressor cells (PMN-MDSCs) as a major part of MDSCs play a role of immunosuppressive activity and balance immune homeostasis in aGVHD and other pathological conditions. While gut microbes have been shown the important in estimating patients' condition, litter research exists directing the microbiome changes in allo-HSCT with or without PMN-MDSCs.
In this study, we used an integrated approach of 16S rRNA gene sequencing to analyze the importance of PMN-MDSC on the variations in gut microbiota in aGVHD and its immunosuppressive function.
A total of 6 phyla were identified in the fecal microbial by 16S sequencing in day 14 and 28 in a murine model of aGVHD. Firmicutes, Bacteroidetes, Verrucomicrobia and Proteobacteria are the main components of the fecal microbial composition of mice after allo-HSCT, and the ratio showed differences in with or without PMN-MDSC aGVHD mice. The mice received without PMN-MDSCs bone marrow performed the most severe aGVHD combining fewer Verrucomicrobia, Firmicutes and Proteobacteria proportions while the data showed statistically significant differences at D28. Though Aminicenantes possess less ratio, it was markedly increase in D28 comparing with D14 in mice without PMN-MDSCs aGVHD model denoting that it could be interrelated with the progress of aGVHD. Our result indicate for the first time that PMN-MDSC not only protect the gut microbiota at the abundance level and promote predominant bacterium but also balance the immune homeostasis in aGVHD.

DOI: 10.1002/eji.201970400

P0982

Altered gut microbiome contribute to the cognitive impairment in human AD

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Background: Alzheimer's disease (AD) is one of the main causes of disability in elder people worldwide. Animal studies indicated that intestinal microbiome (IM) was involved in neuroinflammation and the pathogenesis of AD, however, the IM study in human remains scarce. In this study, we aimed to identify the alteration of IM and bacteria-derived short-chain fatty acids (SCFAs) in human AD (Pre-onset stage amnestic mild cognitive impairment, aMCI; and AD) compared with healthy controls (HC), and evaluate their association with clinical characteristics.
Methods: A total of 97 subjects (33 AD, 32 aMCI, and 32 HC) were recruited. The IM composition was detected by the 16S ribosomal RNA Miseq sequencing, and SCFAs were measured by gas chromatography-mass spectrometer. Clinical scores (including MMSE, MoCA and CDR) were used to evaluate the severity of cognitive impairment.
Results: Among bacterial taxa, SCFA-producing Firmicutes was significantly decreased, whereas pro-inflammatory Proteobacteria was highly enriched in AD. Moreover, the KEGG results showed decreased modules related to immune system in AD. Meanwhile, fecal SCFAs progressively decreased from HC to aMCI and AD. Importantly, significant correlations were observed between the clinical severity scores of AD and the altered microbiomes or SCFAs. Additionally, the differing models based on the altered microbiota and SCFAs could effectively discriminate aMCI and AD from HC and also AD from aMCI.
Conclusions: Distinct microbiota and the related SCFAs were associated with the progress of human AD. These novel findings will give new clues to understand the disease and provide new diagnosis and therapeutic strategy for AD.

P0983

The effect of Salmonella enterica serovar Derby on host and gut microbiota

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Recent epidemic studies demonstrated that *Salmonella enterica* serovar Derby (*Salmonella* Derby) has become the predominant *Salmonella* serovar isolated from pork world-widely, which seriously threats the public health. Contaminated pork is a common source of human *Salmonella* Derby isolates across the world. Antibiotics treatment is commonly adopted to control *Salmonella* contamination in pig farms; however, the use of antibiotics can cause raising of antibiotic resistant bacterial strains, therefore, we need to explore alternatives to antibiotics. Recent studies have demonstrated that certain members of the gut microbiota and their metabolites can play key roles, negatively or positively, in the pathogen infection process. To identify the intestinal symbiotic bacteria that could prevent the infection of *Salmonella* Derby, we selected two representative strains of *Salmonella* Derby from our *Salmonella* Derby strain collections which contain more than 400 isolates collected from

DOI: 10.1002/eji.201970400

multiple cities spanning several years, and determined how the two strains interacted with the gut microbiota during the infection process using a mouse model. We treated the C57BL/6J female mice with *Salmonella* Derby at the dose of 10^8CFU or with phosphate buffer solution as control, and found that mice infected with *Salmonella* Derby gained significant less weight. Although infection of the two strains caused different levels of inflammation responses, they caused similar changes in the composition of the gut microbiota, particularly, the relative abundance of Lactobacillus and Clostridiaceae were dramatically altered at the early stage of *Salmonella* Derby colonization. The roles of the gut microbiota in the *Salmonella* Derby infection will be discussed.

P0984

The immune suppressive role of DC-derived IL-27 in hepatocellular carcinoma via the modulation of gut microbiota

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IL-27 can drive the expression of co-inhibitory receptors on T cells, such as PD-1, TIM-3 and podoplanin, which may inhibit T cell functions during chronic inflammation and tumor development. However, the role of IL-27 in regulating anti-tumor immunity is still controversial. Here, we identified a tumor-promoting role of DC-derived IL-27 in murine hepatocellular carcinoma models. IL-27-p28 conditional knockout (KO) in DCs had significantly reduced tumor growth compared with the wild type (WT) controls. The numbers of T cells and NK cells were increased in the tumor microenvironment and the productions of IFN-gamma and TNF-alpha in T cells and NK cells were also enhanced in IL-27 KO mice compared with the WT mice. Moreover, deficiency of IL-27 in DCs did not affect the expression of co-inhibitory receptors on T cells from naïve mice, but reduced the expression level of PD-1 on tumor infiltrating T cells. Interestingly, when WT mice received fecal microbiota transplant from IL-27 KO mice, the tumor growth was significantly reduced. Therefore, DC-derived IL-27 may regulate anti-tumor immune response through the modulation of gut microbiota. Furthermore, we discovered that the commensal bacteria, bifidobacterium and akkermansia, were increased in IL-27 deficient tumor-bearing mice compared with the WT controls. These two strains were found to be increased during anti-PD-L1 or anti-PD-1 antibody therapy and may promote anti-tumor immunity. Thus, our results demonstrated that DC-derived IL-27 could promote tumor growth by suppressing anti-tumor immune responses possibly through the modulation of gut microbiota.

P0985

The functional interplay between the intestinal microbiota, T cells, and immunoglobulins

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Aims: Mucosal and systemic sites are traditionally considered compartmentalized; however, recent research suggests that the intestinal microbiota stimulates systemic immunoglobulins (Igs). While there is typically little translocation of commensals into circulation, select gram-negative bacteria in healthy mice can disseminate systemically and induce systemic IgG. It is unclear how cognate $CD4^+$ T cell (Th) help influences systemic Ig induction, and how this is modulated by the intestinal microbiota. This project aims to investigate the functional microbiota-T cell-Ig triangle in a defined gnotobiotic mouse model.
Methods: Experiments are performed in the germ-free facility of the International Microbiome Centre (IMC) at the University of Calgary. A genetically modified commensal *Escherichia coli* (*E. coli*) that

DOI: 10.1002/eji.201970400

expresses a defined Th cell epitope (gp61) is i.v. injected to induce a systemic immune response. Ig bacterial surface coating is measured by bacterial flow cytometry.
Results: Mice primed with gp61 *E. coli* produce significantly more IgG2b and IgG2c than mice primed with WT *E. coli*. Gnotobiotic mice primed with gp61 *E. coli* exhibit significantly less coating of *E. coli* in vitro by both IgG2b and IgG2c than monocolonized mice primed with gp61 *E. coli.* These experiments will be repeated with transferred gp61-specific SMARTA $CD4^+$ T cells or $CD4^+$ T cell depletion.
Conclusions: Bacteria that express a defined Th cell epitope induce a more robust systemic Ig response than WT *E. coli* that can broadly bind commensal *E. coli* that lack gp61. A more complex gut microbiota dampens systemic Ig induction by bacteria with a defined Th cell epitope.

P1582

High-throughput sequencing for analysis of diversity of gut microbiota across stages of colorectal carcinogenesis

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In recent years, there is growing evidence that microbiota can modulate the immune system of host by locally and systematically, gut microbiota plays a key role in human health and disease; and in turn, immune system may alter the intestinal microbiota at varying degrees. We learn from the article that the development and behavior of immune cells are largely dependent on the metabolites of bacterial flora.
Colorectal cancer (CRC) is a multi-factorial and multi-stage involved disease, which has presented a considerable disease burden worldwide. The gut microbiota has been demonstrated to influence the development of colorectal cancer.
Understanding how intestinal flora regulates the immune system of patients with colorectal cancer (CRC) can provide new perspective for the treatment of colorectal cancer.
Our research investigated the structural changes of gut microbiota in patients with colorectal polyps, adenoma and colorectal cancer(CRC), we aimed at figuring out the specific function of microbial communities in human gut microbiota at different stages of colorectal tumorigenesis, furtherly exploring the relationship between dysbiosis in gut microbiome and the immune system of patients with colorectal cancer (CRC).

P1583

Transcriptionally active lung microbiome and its association with bacterial biomass and host inflammatory status

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DOI: 10.1002/eji.201970400

Alteration of the lung microbiome has been observed in several respiratory tract diseases. However, most previous studies were based on 16S ribosomal RNA and shotgun metagenome sequencing; the viability and functional activity of the microbiome, as well as its interaction with host immune systems, have not been well studied. To characterize the active lung microbiome and its associations with host immune response and clinical features, we applied metatranscriptome sequencing to bronchoalveolar lavage fluid (BALF) samples from 25 patients with chronic obstructive pulmonary disease (COPD) and from nine control cases without known pulmonary disease. Community structure analyses revealed three distinct microbial compositions, which were significantly correlated with bacterial biomass, human Th17 immune response, and COPD exacerbation frequency. Specifically, samples with transcriptionally active *Streptococcus*, *Rothia*, or *Pseudomonas* had bacterial loads 16 times higher than samples enriched for *Escherichia* and *Ralstonia*. These high-bacterial-load samples also tended to undergo a stronger Th17 immune response. Furthermore, an increased proportion of lymphocytes was found in samples with active *Pseudomonas*. In addition, COPD patients with active *Streptococcus* or *Rothia* infections tended to have lower rates of exacerbations than patients with active *Pseudomonas* and patients with lower bacterial biomass. Our results support the idea of a stratified structure of the active lung microbiome and a significant host microbe interaction. We speculate that diverse lung microbiomes exist in the population and that their presence and activities could either influence or reflect different aspects of lung health.

P1584

Clinical significance of serum tumor marker levels in pulmonary tuberculosis patients receiving an initial treatment

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The diagnosis of pulmonary tuberculosis (PTB) is a complicated and time-consuming process. The diagnosis of PTB was associated with serum tumor markers. The purpose of this study is to explore the clinical significance of serum tumor marker levels in patients with PTB. This study was designed as a case control study with 240 subjects, who visited the second affiliated hospital of Hainan Medical University. This study was divided into three groups: Group 1 consisted of 60 patients with initial treated PTB; Group 2 included 60 patients with lung adenocarcinoma; and Group 3 consisted of 120 healthy subjects. The serum levels of CA125, CA19-9, CA153, CEA, and SCC were detected by the electrochemical fluorescence immunoassay method. The serum levels of CA125 in the initial treatment of patients with PTB declined from (24.51 ± 12.84) U/mL to (15.15 ± 8.47) U/mL after the standardized treatment ($P < 0.05$). Logistic regression analysis showed that CA125 and CA19-9 were introduced to model as the main effect. The ROC analysis revealed that area under curve was 0.93 . The sensitivity, specificity in serum, and ideal boundary point were found to be 0.892, 1.000, and 0.30. A combined detection of serum levels of CA125 and CA19-9 and the combined diagnostic model can identify PTB and lung adenocarcinoma. The serum level of CA125 can be used as a bio-marker for assessing the progression and treatment effectiveness of patients with PTB.

This work was financially supported by the Department of Health of Hainan Province(2013058).

DOI: 10.1002/eji.201970400

P1585

Preventive consumption of green tea provided long-term protection against HFD-induced obesity by modifying gut microbiota

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Green tea could reportedly modify gut microbiota and benefit on High-Fat-Diet(HFD) induced obesity. In this study, we designed three parallel experiments. In the first experiment, one group of lean mice was fed a Standard-Fat-Diet (SFD)+water and a second group of lean mice was fed a SFD+GT for five weeks. Then HFD+water were administered to both groups for an additional five-week period. The SFD+water group became obese, while the SFD+GT group did not. In the second experiment, when lean mice were replaced by obese mice, both the SFD+water and the SFD+GT group gained weight. During the third experiment, the obese mice were given a GT+HFD or a water+HFD for additional 10-week, the GT+HFD group gained much less weight than the water+HFD group. We found that the anti-obesity effect by GT consumption always accompanied with microbiota structure modification and the inhibition of increasing HFD-induced Firmicutes to Bacteroidetes ratio. Hence, GT consumption by lean mice could prevent obesity after stop drinking GT, while the pre-existing obese mice couldn't get such benefit. We hypothesized that this difference depended upon gut microbiota. GT consumption for only a period of time might help lean mice build a persistent protective microbiota structure for the prevention effect; but obesity prior to GT consumption has destroyed the gut microbiota and abolished this effect. Therefore, to gain the long-term protect effect from GT, the precondition is drinking GT before getting fat; otherwise, GT could only prevent the pre-obese mice from weight gain by continuous consumption.

P1587

Hepatitis B virus infection alters gut microbiota composition in mice

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Background: Gut microbiota composition is known to be related to the progression of Hepatitis B virus (HBV)-related liver cirrhosis in humans, the outcome of HBV infection in mice, and HBV e-antigen seroconversion in nucleot(s)ide analogue-treated patients. The dynamic alteration of the gut microbiota following HBV infection was investigated in this study.
Methods: Hydrodynamic injection with pSM2/HBV or pAAV/HBV1.2 plasmids yielded a mouse model with virological and immunological features comparable to acute or chronic HBV infection in humans. The composition of gut microbiota in control mice and mice with acute or chronic HBV infection was analyzed using the Illumina MiSeq platform.
Results: We found that changes in gut microbiota composition, including the total operational taxonomic unit (OTU) countsand Shannon-Weaver index,were significantly delayed in mice with HBV infection. Furthermore, the ratio of *Bacteroidetes*and*Firmicutes*was stable in control mice, whereas remarkable dynamic patterns were observed in mice with HBV infection. In addition, the expression of IFN-γand PD-L1 in the colon was found to be upregulated early in mice with acute HBV infection, while PD-L1 expression in the colon of mice with chronic HBV infection was upregulated later.
Conclusion: These data indicate that HBV infection could hamper the development of the gut microbiota community and dynamically change the gut *Firmicutes/Bacteroidetes*ratio. Interestingly, the dynamic changes in*Lactobacillus* and *Bifidobacterium*were found to differ in acute or chronic HBV infection, which may be related to the IFN-γ mediated immune response. These data improve our understanding of the relationship between gut microbiota and HBV infection.

DOI: 10.1002/eji.201970400

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Maternal diet impacts on the development of offspring immune response to potential allergens

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There is evidence that maternal diet can influence their offspring's immune system development. This is important because of the link between the composition of the gut microbiome and the development of allergies in children.
The objectives of this study were 1. To determine the contributions that the maternal diet compared to the offspring diet made to the development of allergic airways inflammation and, 2. If these changes correlate with changes in the microbiome composition.
C57Bl/6 mice were fed either semi-pure control diet or a semi-pure diet containing 10% SunGold kiwifruit throughout pregnancy and pre-weaning (8 weeks). At 3 weeks post-birth the offspring were weaned onto the same diet as their mothers or crossed over onto the opposite diet for 3 weeks before being challenged with intranasal house dust mite.
Offspring mice of kiwifruit consuming mothers had less lung immune cells with no allergic challenge (baseline conditions) independent of their diet ($P< 0.05$). Kiwifruit diet also resulted in changes in the lung and gut microbiomes in both the mothers and the offspring. We also found that kiwifruit consuming offspring mice had the greatest immune response to allergen exposure if their mothers consumed the control diet ($P< 0.05$) compared to all other mother-offspring diet combinations. This suggests that having a dietary mismatch between the offspring and the mothers increased the allergic lung responses in the offspring. This study also highlights the impact of maternal diet on the development of the immune system in offspring.

P2171

Anti-diabetic activity of *Ganoderma lucidum* polysaccharides via reversion of disturbed gut microbiota and metabolism as well as immunomodulation

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Type 2 diabetes mellitus (T2DM) as a complex metabolic disease currently damages people's quality of life and causes severe economic problems. Growing evidences show that gut microbiota has a great impact on the development of T2DM. Meanwhile, the accompanying chronic and low-grade inflammation, in most cases, is strongly correlated with the occurrence, progression and complications of T2DM. *Ganoderma lucidum* polysaccharides (GLP) are a kind of polysaccharides from Chinese medicinal plant with putative anti-diabetic activity. In this study, the effects of GLP on gut microbiota and host metabolites in high-fat diet and streptozotocin-induced T2DM rats were examined as well as the serumal inflammatory factors including malonaldehyde (MDA), IL-1β, IL-6 and C-reactive protein (CRP). Administration of GLP alleviated emaciation, hyperglycemia, hyperlipidemia and insulin resistance significantly in T2DM rats. Moreover, our results showed that GLP reduced the level of harmful bacteria, such as *Aerococcus*, *Ruminococcus*, *Corynebactrium* and *Proteus*, and increased

DOI: 10.1002/eji.201970400

the abundance of *Blautia*, *Dehalobacterium*, *Parabacteroides* and *Bacteroides*. The PICRUSt analysis indicated GLP could restore the disturbed amino acids metabolism, carbohydrates metabolism, inflammatory substances metabolism and nucleic acid metabolism of gut bacterial community in T2DM rats, which aligned mostly with the metabolomics analysis. Notably, the determined MDA, IL-1β, IL-6 and CRP of T2DM rats reverted to normal levels after GLP treatment, potentially reflecting the modification of aberrant inflammatory conditions. Hence, the therapeutic efficacy of GLP on T2DM rats was achieved by regulating the disordered gut microbiota and its related metabolic disturbance as well as improving the low-grade inflammation of hosts.

P2172

Proinflammatory and autoimmunogenic gut microbiome in systemic lupus erythematosus

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Gut microbiota is closely implicated with the development and function of host immune system. Systemic lupus erythematosus (SLE), an autoimmune disease with a multi-facet nature, has long been suggested to associate with gut dysbiosis, but knowledge is limited from small sample size and 16s rRNA-based studies. To shed new light on the role of microbiota in SLE development, we analyzed comprehensively the taxonomical and functional changes in the fecal samples of 117 treatment-naïve SLE patients excluding the influence of medications by whole-genome metagenomic shotgun sequencing. Post-treatment feces from 52 patients mentioned above were also collected for comparison. The relative abundance of distinct enriched species between groups were correlated with clinical indices. In-depth SNP-based analysis revealed an oral-microbiome origin for two marker species, strengthening the importance of bacterial translocation in disease development. We further demonstrated experimentally that molecular mimicry of SLE-specific autoantigens such as Sm by the peptides of SLE-enriched species might trigger autoimmune T-cell responses in vitro. Collectively, our large-scale gut metagenomic study of treatment-naive SLE patients by deep sequencing, along with experimental verification, provides new insights into the plausible causal relationship between microbiota and SLE.

P2173

Probiotics confers protection against RSV by activating antiviral responses of alveolar macrophage through resetting lung microbiota

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RSV is a leading cause of upper and lower respiratory tract infections in young children. The lung microbiotas are critical in shaping the immune system and in maintaining homoeostasis in lung. The gut microbiota has been shown to influence pulmonary immunity through metabolites, while whether regulation the gut microbiota modulate lung microbiota remains to be determined. Herein a probiotic mixture exerted efficacy in modulating gut microbiota was used to explore whether regulation the gut microbiota by probiotics can modulate lung microbiota, which consequently regulates lung immunity in RSV infected mice.

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DOI: 10.1002/eji.201970400

Our result showed probiotics administration markedly reduced lung RSV replication and reduces RSV-induced lung pathology. The microbiota analysis showed that probiotics not only resumed the gut microbiota, but also increased the abundance of SCFA-producing bacteria, at the phylum, genus and species level. The level of SCFAs increased markedly upon treatment with probiotics. Moreover, probiotics not only resumed the gut microbiota, but also increased the abundance of *Corynebacterium* and *Lactobacillus* at genus and species, which consequently induces production of type I IFNs in alveolar macrophages through activating RIG-I and TLR3 pathway. Furthermore, we demonstrated that the regulation of lung microbiota by probiotics was dependent on gut microbiota metabolite SCFAs. Consequently, to the best of our knowledge we have for the first time demonstrated potency of probiotics in treating RSV infections most likely through regulating lung microbiota and immune responses.

P2174

Gut microbiome alterations in children with juvenile idiopathic arthritis

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Aim: To characterize gut microbiome in patients with juvenile idiopathic arthritis (JIA) and evaluate the potential role of the microbiome to serve as non-invasive biomarkers for JIA.
Methods: A total of 40 JIA patients and 42 healthy controls, aged from 1 to 16 years, were enrolled in the study in Zhejiang province, China, from March to August in 2018. All fecal samples, six JIA synovial fluids, and six normal saline samples were collected for 16S rRNA sequencing.
Results: A total of 39 fecal samples, six JIA synovial fluids, and six normal saline samples were sequenced successfully. Shannon-Wiener Diversity Index in JIA patients was smaller than that in controls. The Bray-Curtis dissimilarity differed between the two groups. At the genus level, the relative abundance of Anaerostipes, Dialister, Lachnospira, and Roseburia decreased in JIA patients compared with those in controls. Of the four genera, three were butyrate-producing genera. Several genera in JIA were associated with clinical indices. Random forest model based on genera could be used to differentiate JIA patients from controls; the area under the ROC curve was 0.779. Thirteen genera identified by the random forest had the potential to serve as biomarkers. 16S rRNA could not be detected in JIA synovial fluid.
Conclusions: Gut microbiome was altered in JIA patients, with the characteristic of decreased abundance of butyrate-producing genera. Thirteen genera had the potential to serve as biomarkers. 16S rRNA could not be detected in JIA synovial fluid.
Keywords: juvenile idiopathic arthritis; microbiome; butyrate; biomarker; synovial fluid

DOI: 10.1002/eji.201970400

P2175

Pseudomonas aeruginosa adaptions in cystic fibrosis patients with G551D-CFTR mutations treated with ivacaftor

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Background: Ivacaftor therapy is effective in treating cystic fibrosis (CF) patients with G551D-CFTR mutation. It improves the lung environment, thus affecting colonizing microorganisms, like *Pseudomonas aeruginosa* (*P.au*). But litter is known about the selection pressure of Ivacaftor therapy to the populations of *P.au* in CF lungs. Here, we designed an observational study to reveal the effect of Ivacaftor therapy on genetical and phenotypic adaptations of *P.au* in CF patients by comparing several microbiological markers before or after Ivacaftor therapy.
Methods:We archived data of 12 G551D CF patients and sputum samples were collected at 0,3,6,9,12 months after commencement of Ivacaftor therapy. *P.au* isolates were got from sputum samples and examined for (i) SNP analysis, (ii) antibiotic susceptibility test to 9 different drugs, (iii) virulence factors, like protease .
Results:By 12 months of Ivacaftor therapy, significant reduction was found in density of *P.au*, but no patients eradicated *P.au*. Freq-seq results showed no clear pattern after Ivacaftor therapy, however, diversity of mutants decreased. Antibiotic susceptibility adaptions behaved differently by patients but all *P.au* isolates showed increased susceptibility to tobramycin. Protease quantification results reported that clinical *P.au* isolates produced less protease than wild-type *P.au.*
Significances: Our study was the first to research both genetical and phenotypical adaptions of *P.au* to Ivacaftor therapy for G551D CF patients. Our results showed that the genetical and antibiotic susceptibility adaptions were diverse among patients, guiding to personalized and combined therapies for Ivacaftor treated G551D CF patients.

P2176

Microbiome composition in bowel and mesenteric lymph nodes is associated with bacterial translocation in experimental cholestasis

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Bacterial translocation in patients with cirrhosis is an important triggering factor for infections and mortality. In the bile duct ligation (BDL) model, crucial players of bacterial translocation are still unknown. This study aims to determine the interrelation between microbiome composition in the colon, mesenteric lymph nodes, and liver, as well as the local inflammatory microenvironment in the BDL model. Materials and methods. Liver damage was assayed by Masson trichrome staining, and hepatic enzymes. The diversity of microbiota in colon stools, mesenteric lymph nodes, and liver was determined by 16S rRNA pyrosequencing. Cytokine expression in mesenteric lymph nodes was

DOI: 10.1002/eji.201970400

analyzed by qRT-PCR. Results. Our results show that Proteobacteria was the predominant phylum found to translocate to mesenteric lymph nodes and liver in cirrhotic rats. Bile duct ligation induces a drastic intestinal dysbiosis, revealed by an increased relative abundance of Sarcina, Clostridium, Helicobacter, Turicibacter, and Streptococcus genera. However, beneficial bacteria, such as Lactobacillus, Prevotella and Ruminococcus were found to be notably decreased in BDL groups. Mesenteric pro-inflammatory (TNF-α, IL-1β, IL-6, TLR-4) and regulatory (TGF-β, Foxp3, and IL-10) molecules at 30 days post-BDL were significantly increased. Conversely, TGF-β and Foxp3 were significantly augmented at 8 days post-BDL. Conclusion. Dysbiosis in the colon and mesenteric lymph nodes is linked to an imbalance in the immune response; therefore, this may be an important trigger for bacterial translocation in the BDL model.

P2177

Type I interferon driven gut dysbiosis and insulin resistance

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Chronic inflammatory mechanisms are implicated in obesity and associated insulin resistance. Previous clinical and preclinical studies have identified a critical role of type I IFNs in these clinical contexts. Interestingly, these contexts have also been linked to derangements in gut microbial consortia. We aimed at validating pathogenetic role of type I IFN in loss-of-function (LOF) and gain-of-function (GOF) contexts in wild type C57BL/6 mice by assessing the nature of gut dysbiosis, serum metabolome and underlying immunocellular dynamics. In LOF condition, created by systemic blockade of the type I IFN receptor IFNAR1 using a monoclonal antibody, high fat diet-fed (HFDF) mice failed to gain weight and develop insulin resistance. Alternatively, in GOF condition, wherein systemic IFNa abundance was achieved by infection with an adenovirus expressing mouse IFNa, normal diet-fed (NDF) mice developed systemic insulin resistance. On genus-level assessment of gut microbial consortia, using 16s-rRNA gene V3/V4 region sequencing of fecal DNA, we documented significant differences, with defined microbial signatures for LOF (that mimicked NDF mice) and GOF (mimicking HFDF mice) conditions. These were associated with coherent changes in relative abundance of microbe-derived metabolites in the sera. Studies involving fecal transplant from LOF and GOF conditions to wild type mice, to validate the role of gut dysbiosis in inducing insulin resistance and exploring the influence of microbe-derived metabolites on systemic immune dynamics are ongoing. These studies are gathering critical insights into the role of type I IFNs on diet-induced insulin resistance as well as revealing novel therapeutic cues for relevant clinical contexts.

P2178

Allergen exposure modulates the gut microbial community composition and metabolite fingerprint

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DOI: 10.1002/eji.201970400

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Introduction: The modified microflora hypothesis suggests that environmental factors, such as family size, pets or medical interventions, shape the host microbiome which leads to increased susceptibility to develop allergies. Here, we ask if exposure to allergens in order to induce experimental allergy or tolerance in the lung affects the gut microbiome.
Methods: We tested the impact of ovalbumin-induced allergic airway inflammation and orally-induced allergen-specific tolerance on the composition and function of the intestinal microbiome in mice. The microbial communities in cecal samples were assessed with Fourier transform infrared spectroscopy (FT-IR) and Illumina MiSeq sequencing of 16S rRNA gene amplicons. Additionally, the metabolic pattern in serum was analyzed with hydrophilic interaction chromatography/mass spectrometry (HILIC-MS) and FT-IR.
Results and discussion: FT-IR measurements indicated shifts in the gut microbiome and serum metabolite fingerprint of allergic and tolerized mice compared to naïve mice. Analysis of 16S rRNA gene sequence data indicated an increase in relative abundances of the families *Prevotellaceae* and *Rikenellaceae* in cecum of both allergic and tolerized mice compared to sham controls, while *Bacteroidaceae* were reduced in tolerized mice compared to allergic mice and sham controls. HILIC-MS revealed a distinct metabolite pattern in serum of sensitized mice, which exhibited reduced levels of L-carnitine and its alkylated forms compared to sham-controls, supporting previous studies showing that supplementation of L-carnintine reduces the severity of asthma in children.
Characterizing the mechanisms responsible for microbial changes might pave the way for novel intervention strategies to prevent or treat allergic diseases targeting the gut microbiota.

P2944

Prevent drug resistance among oral yeast from inpatients in Hainan and multiple origins of fluconazole resistance in Candida tropicalis

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Yeast infections are among the most common diseases caused by opportunistic pathogens in both immunocompromised and immunocompetent hosts, and most opportunistic yeast pathogens are common residents of the oral mucosa. As a result, understanding the oral microbiota has broad implications for the prevention and management of human infectious diseases. However, relatively little is known about the ecological and geographic patterns of genetic variation and drug susceptibility profiles of oral yeasts. The objective of this study was to investigate species, genotype, and antifungal susceptibility profiles of yeasts from the oral cavities of inpatients on Hainan Island. A total of 677 inpatient mucosal specimens were collected in this study. Among the 677 oral swabs, 446(65.9%) were found to contain yeasts. 459 strains of yeast belonging to 14 species were isolated from oral cavities of these 446 inpatients, the three most prevalent species were *Candida albicans* (231), *Candida glabrata* (99), and *Candida tropicalis* (66). The in vitro antifungal drug susceptibility analyses found that 33.3% strains had different degrees of drug resistance, and more than half of these strains were resistant to two or more antifungal drugs. Among these yeast species, we focused on Candida tropicalis and analyzed its fluconazole resistance mechanism. Mantel test identified no significant correlation between the diploid sequence types at the six loci and fluconazole susceptibility, non-synonymous mutation was found in the ERG11 gene of fluconazole-resistant strain vs. fluconazole-susceptible strain but with the same MLST genotype, consistent with multiple origins of fluconazole resistance in the Hainan population of *Candida tropicalis*.

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DOI: 10.1002/eji.201970400

P2945

Expression, purification and crystallization trials of Helicobacter pylori collagenase HpPrtC

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Objective: To express, purify and screen out crystallization conditions of *Helicobacter pylori* collagenase HpPrtC.
Methods: A recombinant plasmid was constructed for production of HpPrtC in *E. coli*. The expressed protein was purified with a three-step chromatography strategy using an Ni^{2+} affinity column, an anion exchange column and a gel filtration column. Primary crystallization conditions were screened using Hampton Research crystallization kits.
Results: The *hp0169* gene encoding HpPrtC, a member the U32 collagenase family was amplified by PCR and inserted into pET-28a. The resultant plasmid pET-28a-*hp0169* was then transformed into BL21 (DE3) for IPTG-induced protein expression. The destination protein was purified using an ÄKTA pure. As detected by SDS-PAGE, the recombinant HpPrtC protein was purified to 95% of purity with a yield of roughly 12 mg/L. A crystallization condition, 0.2 M $MgCl_2$, 0.1 M Tris, pH 8.5 and 3.4 M hexanediol was found out of ca 500 conditions using a protein crystallization robot.
Conclusions: Prokaryotic expression of HpPrtC led to soluble production of this protein, which could be crystallized. Our experimental results have established a good starting point for further study of HpPrtC structure and functions.
Keywords: *Helicobacter pylori*；*hp0169*；HpPrtC；Collagenase；crystallizition

P2946

FAM96A plays a protective role in DSS induced colitis by influence gut bacterias

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FAM96A is an evolutionarily conserved intracellular protein that is involved in maturing the Fe/S proteins iron regulatory protein 1 (IRP1) and the mitochondrial related apoptosis of gastrointestinal stromal tumor cells. However, the physiological relevance of FAM96A in intestinal homeostasis and colitis remains unexplored. Here we show that Fam96a-deficiency in mice results in increased susceptibility to DSS-induced colitis. Fam96a-/- mouse exhibit gut microbial dysbiosis, increased crypt height, elevated proliferation and apoptosis, increased goblet cells, functional deficits in intestinal permeability and dysregulation of tight junction proteins. After cohousing, these differences between WT and Fam96a-/- colons were abrogated, indicating a potential role of Fam96a in regulating gut microbiota via IECs through several commensal-dependent antimicrobial genes. Importantly, transplantation of the fecal microbiota from Fam96a-/- mice to WT recipient mice was sufficient to promote intestinal inflammation in the WT mice. Taken together, our study suggests that Fam96a plays a vital role in maintaining gut homeostasis by regulating gut microbiota.

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DOI: 10.1002/eji.201970400

P2947

Role of CCR7-CCL21 signaling in chemotactic migration of PD-1+ T lymphocytes in the ovarian cancer microenvironment

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Ovarian cancer (OC) is one of the most common causes of death from gynecological cancers due to poor therapeutic response. Immune checkpoint inhibitors targeting PD-1 have revolutionized the treatment of various cancers. However, only a minority of patients with OC responds to this therapy. To investigate the underlying mechanism of this differential responsiveness, we analyzed the expression of PD-1 and CCR7 in T cells from patients with OC, and explored the chemotactic migration of PD-1+T cells. The expression of CCR7 in CD45+CD3+ T cells, and their co-expression with PD-1 in tumor tissues and peripheral blood were analyzed by flow cytometry. Further the effect of the tumor microenvironment (TME) on the chemotactic migration of CD45+CD3+PD-1+CCR7+T cells was simulated in vitro. Our results suggest that the expression levels of PD-1 and CCR7 in OC tissues were higher than those in control tissues ($P < 0.05$). In subset of CD45+CD3+ T cells from OC tissues, the co-expression of PD-1 and CCR7, as well as the proportion of PD-1 expressing cells in the CCR7+T cell population, were higher in tumor tissues compared to those of in peripheral blood ($P < 0.05$ and $P < 0.01$, respectively).High expression of CCL21 in the TME has an important activity for the chemotactic migration of CD45+CD3+PD-1+CCR7+T cells from the peripheral blood. Our study suggests that modulating the CCR7-CCL21 pathway is a possible strategy for restoring immune responses in the TME, thereby improving the prognosis of OC patients.

P2948

Alternation of tongue coating microbiota in gastric cancer patients

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Aim: The aim of our study was to investigate the alternation of the tongue coating microbiota, serum inflammatory factors, serum metabolites in the patients with gastric cancer (GC).
Methods: Here enrolled a population with 181 GC patients and 112 healthy controls. Propensity score matching (PSM) was used to collect 66 GC patients and 66 controls by matching age, gender, and six lifestyle factors. Tongue coating microbiota was profiled by 16S rRNA and 18S rRNA gene sequencing for bacteria and fungi, respectively. Serum metabolites were analyzed by ultraperformance liquid chromatography quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF/MS) and the serum inflammatory factors were detected by electro-chemiluminescence immunoassay (ECLIA).
Results: Comparing the microbiome construction (both bacteria and fungi) between gastric cancer patients and healthy controls before and after PSM, we observed that bacteria were relatively more stable than fungi under the influence of diet and lifestyle in the LEfSe analysis. In genus level, 39 bacteria and 17 fungi (LDA>2, $p < 0.05$) presented to be significantly different among GC patients and

DOI: 10.1002/eji.201970400

healthy controls. Furthermore, four GC-protective bacterial genera (*Porphyromonas*, *Peptostreptococcus, Peptococcus*, *Parvimonas*) were negatively associated with serum IL-17α and four specific serum metabolites including LysoPE(18:1(11Z)/0:0), LysoPE (20:1(11Z)/0:0), PA (20:4(5Z,8Z,11Z,14Z)e/2:0) (|r|>0.3, p< 0.01).
Conclusion: Here presented a comprehensive landscape of the tongue coating microbiota, metabolites and inflammatory factors in GC patients, and the alternation of tongue coating bacteria was irrelevant to the lifestyle.

P2949

HIV-1 Tat enhances purinergic P2Y4 receptorsignaling to mediate inflammatory cytokine production and neuronal damage via PI3K/Akt and ERK MAPK pathways

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Background: HIV-associated neurocognitive disorders (HANDs) afflict more than half of HIV-1 positive individuals. The transactivator of transcription (Tat) produced by HIV virus elicits inflammatory process and is a major neurotoxic mediator that induce neuron damage during HAND pathogenesis. However, activated astrocytes are involved in neuroinflammation and neuronal damage. Purinergic receptors expressed in astrocytes participate in a positive feedback loop in virus-induced neurotoxicity. Here, we investigated that whether P2Y4R, a P2Y receptor subtype, that expressed in astrocyte participates in Tat-induced neuronal death *in vitro* and *in vivo*.
Methods: Soluble Tat protein was performed to determine the expression of P2Y4R and proinflammatory cytokines in astrocytes using siRNAtechniquevia real time PCR, Western blot andimmunofluoresence assays. Cytometric bead array was used to measure proinflammatory cytokine release. The TUNEL staining and MTT cell viability assay were analyzed for HT22 cell apoptosis and viability, cresyl violet staining for the death of hippocampal neurons*in vivo*.
Results: We found that Tat challenge increased the expression of P2Y4R in astrocytes. P2Y4R signaling in astrocytes was involved in Tat-induced inflammatory cytokine production via PI3K/Akt and ERK1/2-dependent pathways. Knockdown of P2Y4R significantly reduced inflammatory cytokine production and relieved Tat-mediated neuronal apoptosis *in vitro*. Furthermore, *in vivo* challenged with Tat, P2Y4R knockdown mice showed decreased inflammation and neuronal damage, especially in hippocampal CA1 region.
Conclusions: Our data provide novel insights into astrocyte-mediated neuron damage during HIV-1 infection and suggest a potential therapeutic target for HANDs.

P2950

The mechanism of clostridium butyricum and short-chain fatty acids alleviate mouse insulin resistance and inflammation induced by high-fat feeding

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Obesity is currently one of the most serious public health problems worldwide because of its increasing prevalence and contribution to serious metabolic disorders, including type 2 diabetes. Gut microbiota transplantation experiments suggest a causal relationship between the gut microbiota and obesity development.

DOI: 10.1002/eji.201970400

Aim: We explored whether obesity-associated gut microbial species *Clostridium Butyricum* linked to changes in metabolic disorders as well as the effect of its fermentation product short chain fatty acid (SCFAs) among the procedure.
Methods: The male C57BL/6J mice were fed chow diet or high-fat diet (HFD) for 14 weeks followed by 8 weeks intragastrical administration of *Clostridium Butyricum* or sodium chloride. The systemic inflammation was assessed by immunohistochemistry, flow cytometry and real-time PCR. The richness of the bacterial community was detected by 16S rRNA gene sequencing and OTUs (α-diversity). The effects of SCFAs on insulin resistance and GPR41/43 signaling were tested by western blot.
Results: Gastrointestinal treatments by *Clostridium Butyricum* significantly alleviated systemic inflammation, insulin resistance, lipid ectopic deposition and intestinal flora disorder caused by HFD. *Clostridium Butyricum* treatment altered the composition of the intestinal microbiota of HDF fed mice, which accompanied by improved intestinal barrier function, lowed gut inflammation. These effects were positively associated with the activation of GPR41/43 signaling.
Conclusion: *Clostridium Butyricum* and SCFAs alleviate insulin resistance and inflammation caused by HFD may via activating the GPR41/43 signaling.
Keywords: *Clostridium Butyricum,* short chain fatty acid (SCFAs), insulin resistance, systemic inflammation.

P2951

The correlation study on *Helicobacter pylori* infection and metabolic syndrome in adult check-up subjects

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Previous studies found that chronic inflammation caused by *Helicobacter pylori* (*H. pylor*i, *Hp*) may lead to gastrointestinal tube damage. But the reports about the correlation between metabolic syndrome (MS) and *H. pylori* were less and controversia. So we randomly selected 2411 adult healthy subjects from medical examination center of the Second Hospital of Hebei Medical University for the detection of *H. pylor*i infection and metabolic syndrome, Laboratory examination items include ^{13}C urea breath test, body mass index (BMI), Systolic blood pressure and diastolic blood pressure (mmHg), fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). We found that in the positive population of *H. pylori* infection, the prevalence of metabolic syndrome is higher than that of *H. pylori* negative population. But the BMI was not significant difference between *H. pylori* positive and negative subjects. **Our research shows that the prevalence of metabolic syndrome was increased in adult check-up subjects of *H. pylori* infection. *H. pylori* infection was significantly associated with systolic blood pressure, diastolic blood pressure, fasting blood glucose, TC, LDL-C. *H. pylori* infection may participate in the occurrence and development of metabolic syndrome, is an important pathogenic factor of metabolic syndrome.**

DOI: 10.1002/eji.201970400

Systems Biology and Immunology

O405

Population immunosequencing depicts the commonality and diversity of human T cell receptor repertoire

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We provide and analyze the largest baseline human T cell receptor (TCR) repertoire dataset to date, deriving from over ten thousand individuals. The total diversity of TCR repertoire in human species determines the ability and homeostasis of its adaptive immune system to fight pathogens in the environment. It was speculated that the diversity of αβ T-cell receptor (TCR) in body is up to 10^{12} , however, the total TCR number in human population is difficult to be reliably estimated without a large sample pool. We have obtained tens of billions of TCR β chain sequences, enabling investigation on population TCR repertoire at unprecedented massive level. We estimated the population TCR clonotype number by different statistical approaches. We found the commonality of human TCR repertoires by identifying high frequencies of shared clonotypes, and delineated a subset of universally shared clonotypes. The dynamics and stability of individual TCR repertoire were evaluated from 5-year longitudinal samples. Further, the massive individuals and consecutive sampling also help us figure out whether TCR repertoire data can serve as personal genetic identifiers. Answering these fundamental questions would no doubt enrich our understanding of the adaptive immune system, in health and disease.

O406

Individual T cell receptor landscape shaped by host and environmental factors

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Adaptive immunity protects the host from the invading environmental pathogens, encodes exposure history to a large diverse collection of B cell receptors and T cell receptors, namely immune repertoire, influenced by both host genetic make-up and external factors. So far, it's not clear that how genetic and non-genetic factors influence immune repertoire and to what extend they contribute to formation of immune diversity, which needs further investigation to elaborate individual difference of immune response. To address this, we collect a large multi-omics dataset consisting of whole-genome sequencing, TCR repertoire, HLA typing, gut metagenome, metabolites and clinical laboratory tests from a discovery cohort of 1300 individuals and a validation cohort of 1500 individuals, then integrate ensemble of genomics and statistical learning techniques to analyze their contribution to TCR diversity and their interaction with TCR repertoire. Major histocompatibility complex (MHC) polymorphisms, as well as zinc-finger proteins, show strong trans associations with both variable and joint gene segment usage of TCR, suggesting negative and positive selection on T cells in thymus. Regulatory regions near TCR germlines also show strong cis associations with gene segment usage, indicating potential to bias combination. Collectively, genetic background, microbiome, hormones, vitamins, trace elements and amino acids, constituting a large trans-omics network, manifest extensive impacts on TCR diversity and clonotypes, which provide clues for avoiding decreasing the capacity of immune system, and prevention and therapy of immune related disease.

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DOI: 10.1002/eji.201970400

O407

Linking multi-modal single-cell RNA sequencing with targeted long-read sequencing reveals the clonal and transcriptional profile of lymphocytes

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High-throughput single-cell RNA Sequencing is a powerful technique for gene expression profiling of complex and heterogeneous cellular populations such as the immune system. However, these methods only provide short-read sequences from one end of a cDNA template, making them poorly suited to the investigation of gene-regulatory events such as alternative RNA splicing, adaptive immune responses or somatic genome evolution. To address this challenge, we have developed a method that combines targeted long-read sequencing with short-read based transcriptome profiling of barcoded single cell libraries generated by droplet-based partitioning. We use Repertoire And Gene Expression by Sequencing (RAGE-Seq) on 7,138 lymphocytes sampled from the primary tumour and draining lymphnode of a breast cancer patient to accurately characterize full-length T-cell (TCR) and B-cell (BCR) receptor sequences and transcriptional profiles. With this method we show that somatic hypermutation, alternate splicing and clonal evolution of lymphocytes can be tracked across tissue compartments, defining signatures of expanded clones. Furthermore, we simultaneously demonstrate the utility of this methodology on human samples tagged with + 100 DNA barcoded antibody markers (CITE-seq), where we additionally show CD45 isoform splicing profile of individual immune cells.

O408

Effect of opuntiol and its silver nanoparticle against in-vitro and in-vivo atherogenesis

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Atherosclerosis is an immunomodulatory disease, characterized by the accumulation of macrophage-derived foam cells accompanied with the production of a multiple cytokines predominantly regulated by Nuclear Factor κB Pathway. In present study, murine macrophages were incubated with oxidized low-density lipoprotein (ox-LDL; 50µg/ml) followed by *in-vitro* treatment with OP (1, 5 & 10µg/ml) and OP-AgNPs (0.5, 1 & 3µg/ml). Oil Red O staining, real-time PCR and ELISA were performed to observe the effect of treatment against foam cell formation, cytokines (TNF-α, IL-1β and IL-6) and NFkB expressions, respectively. In addition, 8-wk-old ApoE$^{-/-}$ mice fed HFD for 10 weeks, further treated with different doses of OP and OP-AgNPs. At 16th week, aortic root sections from ApoE$^{-/-}$ mice were examined for lesion area, plaque composition, cellular infiltration and cytokines expressions. OP and OP-AgNPs treatment markedly reduced atherosclerotic lesion area ($p < 0.001$), vulnerability of the lesion and maximal stenosis ($p < 0.001$) in ApoE$^{-/-}$ mice, in addition to dose-dependent inhibition against foam cell formation. Interestingly, OP and OP-AgNPs treatment not only inhibited the mRNA expressions of ox-LDL-induced TNF-α (63-85%), IL-1β (55-78%) and IL-6 (59-83%) cytokine production in foam cells but also dramatically reduced cellular infiltration, cytokine mRNA expressions and NF-κB levels in ApoE$^{-/-}$ mice. Taken together, OP and OP-AgNPs potentially interfered with ox-LDL-induced foam cell formation and cytokine production including TNF-α, IL-1β and IL-6 *via* NF-κB

DOI: 10.1002/eji.201970400

signaling thereby protecting ApoE$^{-/-}$ mice from HFD-induced atherosclerosis, thus can be considered for the development of novel anti-atherogenic agent to encounter the future challenges in cardiovascular ailments.

P0397

Kawasaki disease shock syndrome: clinical characteristics and possible use of IL-6, IL-10 and IFN-γ as biomarkers for early recognition

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Background: This study was to learn more about the clinical features and evaluate the role of cytokines in the pathogenesis of KDSS.
Methods: We collected clinical and laboratory data for all patients with KDSS (KDSS, n=27) in our hospital from Jan 2014 to Oct 2017. For patient with KDSS, we randomly identified 43 patients with KD as control subjects (KD, n=43). Clinical features, laboratory evaluations were collected. Cytokines in serum were assayed using flow cytometric bead array.
Results: The patients with KDSS were older age (43.41±31.42 vs 28.81±21.51 months, $P< 0.05$), longer duration of fever (10.63±5.12 vs 6.98±2.45 days, $P< 0.05$), higher WBC count, CRP, ESR, and lower hemoglobin and albumin, more refractory to IVIG therapy, more CAAs, aseptic meningitis, and longer duration of hospitalization than patients with KD (all $P< 0.05$). The levels of serum IL-6 , IL-10, TNF-α and IFN-γ in KDSS patients were significant higher than KD patients (all $P< 0.05$). ROC curves showed that 66.7 pg/ml of IL-6, 20.85 pg/ml of IL-10 and 8.35 pg/ml of IFN-γ had sensitivity and specificity for identifying KDSS as 85.2% and 62.8 %; 66.7% and 83.7 %; 74.1% and 74.4 % respectively. No fatality was recorded in this series.
Conclusions: KDSS were characteristic as more cytokine production and prone to developing IVIG non-responsiveness and CAAs. KD patients with IL-6 above 66.7 pg/ml, IL-10 above 20.85 pg/ml, and IFN-γ above 8.35 pg/ml suggested higher risk for KDSS.

P0398

Screening value of neutrophil to lymphocyte ratio in patients with heart failure caused by dilated cardiomyopathy

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Objective: The aim of this study was to assess wherease the level of peripheral blood neutrophil to lymphocyte ratio (NLR) are associated with New York Heart Association (NYHA) functional class in patients with dilated cardiomyopathy (DCM).
Methods: A total of 264 patients with DCM were recruited from September 2015 to December 2017, with 236 healthy individuals as the control group. The NLR between DCM patients and healthy controls were analyzed, and its diagnostic value was evaluated using the ROC curve. Besides, we assessed the relationship between NLR levels and New York Heart Association (NYHA) functional classes in patients with DCM .
Results: The NLR of DCM patients was 2.92 ± 1.44 and healthy controls was 1.65 ± 0.51. As NYHA functional classes increased, NLR ($P < 0.001$) also increased significantly. The NLR cutoff value predicting DCM was 1.94 with 73.1% sensitivity and 75.0% specificity and the area under the curve for

DOI: 10.1002/eji.201970400

DCM was 0.809 (95% CI :0.772-0.846).
Conclusions: NLR can be used as a valuable indicator for DCM patients. NLR is associated with the severity of chronic heart failure in patients with DCM.

P0399

The macromolecule Nocardia rubra cell wall skeleton suppresses cervical cancer growth by inducing apoptosis

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Nocardia rubra cell wall skeleton (N-CWS), a natural molecular compound, mainly used to cure cervical erosion in clinical, and has been reported to exert inhibitory effects on cancer cells. Cervical cancer has the highest morbidity rate and the most common cancer in women. Thus, it is urgent to identify an effective agent to treat this disease. The aim of the present study was to elucidate the anti-tumor functions and potential mechanism of N-CWS on HeLa cells. Cellular proliferation was assessed using an MTS assay and cloning formation. Apoptosis rates measured by flow cytometry via annexin V/PI double staining and observed by a fluorescence microscope via TUNEL/DAPI double staining. The protein and mRNA levels of caspase-3, caspase-9 and B-cell lymphoma-2 (Bcl-2) were assessed by western blot and qRT-PCR analysis. N-CWS inhibited the growth of HeLa cells and induced apoptosis in a dose- and time-dependent manner. N-CWS treatment also increased the expression of caspase-3 and caspase-9, but decreased levels of Bcl-2. In brief, the results of our study indicated that N-CWS maybe an effective drug candidate which possess the most promise in further cervical cancer therapy.

P0986

KDM5D regulates the migration of NSCLC via inhibiting p38 phosphorylation

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Epigenetic dysregulation has a major contributor to tumorigenesis. Histone methylation is an established mechanism of epigenetic regulation that is dynamically modulated by histone methyltransferases and demethylases. Our results showed that Kdm5d decreased in lung cancer. But the role and mechanism of demethylase KDM5D in non-small cell lung cancer (NSCLC) remains unknown. Here we found that knockout KDM5D significantly promoted cell migration. Overexpressed KDM5D inhibited cell migration. We also found that knockout KDM5D could increase p38 and ERK phosphorylation. Furthermore, there is an interaction between p38 and KDM5D. We therefore speculate that KDM5D may regulate the migration of NSCLC via inhibiting the activity of MAPK signaling pathway. In addition, knockout KDM5D reduce migration-associated genes expression of ADAMTS8. However, how KDM5D promoting ADAMTS expression and inhibiting p38 phosphorylation need further clarified. The project will contribute to elucidate the underlying mechanisms of demethylases in NSCLC migration and shed new light on the novel strategy and potential target for clinical treatment and drug development of NSCLC.

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DOI: 10.1002/eji.201970400

P0987

The role of peripheral blood CD19 + B cells FOXO1 mRNA and miR-155 from patients with systemic lupus erythematosus

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Objective: To investigate the expression of transcription factor FOXO1 mRNA and miR-155 in patients with SLE peripheral blood CD19+B cells and the correlation with clinical indexes.
Method: We chose 41 patients with SLE according to the criteria of the American College of Rheumatology were recruited from Rheumatology and Immunology Department, Anhui Provincial Hospital, and twenty sex-and-age-matched healthy controls were recruited.Real-time PCR to detect miR-155 and FOXO1 mRNA in CD19 + B cells.
Results: (1) Decreased expression of FOXO1mRNA with patients from SLE compared with healthy controls. Significant differences were found in active SLE compared with healthy controls ($Z = -3.39$, $p < 0.001$), while no significant differences were found between active and stable SLE ($Z = -1.24$, $p = 0.26$). (2) Increased expression levels of miR-155 with patients from SLE compared with healthy controls .There was significant differences were found between active SLE and healthy controls ($Z = -4.63$, $p = 0.001$). (3) Negative correlation was found between FOXO1mRNA expression and miR-155 in SLE patients ($r = -0.380$, $p = 0.014$). (4) miR-155mRNA levels were negatively correlated with RBC ($r = 0.452$, $p = 0.04$).(5) The expression of foxo1mRNA and clinical parameters detected no significant correlation ($p > 0.05$).
Conclusion: There was correlation between FOXO1mRNA and miR-155 in peripheral blood CD19 + B cells and disease activity from SLE patients, which may influence the differentiation of B cells involved in the pathogenesis of SLE.

P0988

PTPN21, a member of the protein tyrosine phosphatase family, involves in the proliferation and differentiation of human bone marrow-derived mesenchymal stem cells

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Introduction: Bone marrow-derived mesenchymal stem cells (BM-MSCs), as a crucial component of the bone marrow microenvironment, support the hematopoiesis of the bone marrow. Alterations in the characters of BM-MSCs are involved in many malignant hematological diseases. Previous studies have illustrated that protein tyrosine phosphatases (PTPs) act as key regulators in various cellular processes of stem cells including proliferation, differentiation et al. Our previous study revealed that the expression of PTPN21, a member of PTPs, was up-regulated in acute lymphoblastic leukemia (ALL), and the expression level was associated with the pathogenesis and prognosis. However, its role in BM-MSCs has not been explored. The aim of this study is to clarify how PTPN21 regulate BM-MSCs and its potential mechanism.
Results: BM-MSCs with PTPN21 upregulation acquired inhibited proliferation and cell cycle phenotype, promoted osteogenesis and adipogenesis, and activated senescence through upregulation of P21 and P53. In addition, the crosstalk with their typical target cells including immunocytes and tumor cells changed dramatically. PTPN21 up-regulated BM-MSCs had an impaired immunosuppressive function, and a higher migration number of tumor cells and vascular endothelial

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DOI: 10.1002/eji.201970400

cells in a transwell co-culture system.
Conclusions: The expression level of PTPN21 in BM-MSCs was related to their cellular activity and function; BM-MSCs with high expression of PTPN21 were deficient in cell viability and immunosuppressive ability, but promoted bone marrow metastasis of tumor cells.

P1589

Research of clinical features with an MYH9-related disorder

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Objective: To investigate the clinical features of 2 deaf patients in a family with an MYH9-related disorder, to search for candidate mutated genes related to deafness and to define the gene mutation sites associated with deafness in this family.
Materials and methods: Detailed medical histories, physical examinations, biochemical examinations, audiological testing and immunofluorescence assays of the MYH9 protein in the peripheral blood were performed. Genomic DNA was extracted from peripheral blood samples from the 2 patients; more than 100 known genes associated with deafness were sequenced by whole exome sequencing, and the results were confirmed by Sanger sequencing.
Results: All affected members in this family had the typical triad of giant platelets, thrombocytopenia and neutrophil inclusion bodies, and the pedigree analysis revealed autosomal dominant inheritance. In this study, the 2 deaf patients also suffered from anemia, hyperlipidemia and elevated levels of liver enzymes; the characteristics of their deafness were postlingual deafness, late-onset and progressive hearing loss, and medium- and high-frequency dominant sensorineural hearing loss. The MYH9 gene pathogenic mutation site c.G4546C:p.V1516L was detected by whole exome sequencing, and no other pathogenic mutations of known genes involved in deafness were found.
Conclusions: The deaf phenotype of 2 patients in this family may be related to the MYH9 pathogenic mutation p.V1516L.and this report confirms that the whole exome sequencing technique is an effective molecular diagnostic tool, which can providing valuable information for the genetic counseling of deaf patients.

P1590

A mathematical modelling of initiation of dendritic cells-induced T cell immune response

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Dendritic cells (DCs) are the most potent specialized antigen-presenting cells as now known, which play a crucial role in initiating and amplifying both the innate and adaptive immune responses. Immunologically, the motilities and T cell activation capabilities of DCs are closely related to the resulting immune responses. However, due to the complexity of the immune system, the dynamic changes in the number of cells during the peripheral tissue (e.g. skin and mucosa) immune response induced by DCs are still poorly understood. Therefore, this study simulated dynamic number changes of DCs and T cells in this process by constructing several ordinary differential equations and setting the initial conditions of the functions and parameters. The results showed that these equations could simulate dynamic numerical changes of DCs and T cells in peripheral tissue and lymph node, which

DOI: 10.1002/eji.201970400

was in accordance with the physiological conditions such as the duration of immune response, the proliferation rate and the motilities of DCs and T cells. This model provided a theoretical reference for studying the immunologic functions of DCs and practical guidance for the clinical DCs-based therapy against immune-related diseases.
Keywords : dendritic cell, immune response, T cells, mathematical model, ordinary differential equations

P2179

The crosstalk between local and systemic immunity in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) based on immune-related genes, immune cells and cytokines

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common urinary system disease in male, but the relationship between local and systemic immunity in CP/CPPS is not well understood. We used RNA-seq to detect immune-related genes (IRGs) in prostate tissues from benign prostate hyperplasia (BPH) with CP/CPPS patients and tissues from BPH patients, and found that highly expressed IRGs were mostly enriched in the pathways of Leukocyte migration, cell chemotaxis and Cytokine-cytokine receptor interaction. Further, mass spectrometry was used to detect the classification of peripheral blood mononuclear cells (PBMCs) in CP/CPPS. The proportion of NK cells, myeloid DCs and classical monocytes in CD45+ cells of PBMC from CP/CPPS patients was significantly up-regulated in CP/CPPS than that in normal controls. Meanwhile, the expression levels of MIP1a, IL-1b, TNFa, and MCP-1 were predominantly higher in CD45+ cells of CP/CPPS patients than those of the controls. In addition, protein levels of MIP1a, IL-1b, TNFa and MCP-1 in serum and expressed prostatic secretion (EPS) were largely increased in CP/CPPS group than that in controls. The content of IL-1b, TNFa and MCP-1 in the EPS of CP/CPPS patients were greatly more than that in serum, with the cytokine levels in serum and prostatic secretion being positively correlated, while the content of MIP1a was opposite. These results indicate a certain relationship between local and systemic immunity in CP/CPPS. Immune cells in the peripheral circulation may infiltrate into the prostate tissue, and cytokines in the prostate tissue may also enter the peripheral circulation.
Keywords: CP/CPPS; RNA-seq; mass spectrometry

P2180

Characterization of the immune cell system profile and activation status among Moroccan patients with non-small cell lung cancer

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DOI: 10.1002/eji.201970400

It has been well demonstrated that the progression of Non Small Cell Lung Cancer (NSCLC) is not only related to the genetic alterations and paracrine signaling within the tumor microenvironment, but also by a systemic effects. This later has been demonstrated to contribute significantly to metastasis. The present study aimed to highlight the effect of NSCLC in the remodeling of the peripheral immune cells distribution and the activation of the immune system among Moroccan patients.
We analyzed the percentage of T cells subsets (CD3+, CD4+ and CD8+), B cells, NK cells in peripheral blood, and expressions of the activated molecules CD38, HLA-DR in circulating CD8+ T cells and CD25+ in CD4+ cells in 70 NSCLC cases and 100 healthy controls (HC) using flow cytometry.
Results showed a significant increase in numbre of leucocytes, granulocytes, platelets and T lymphocytes $CD8^+$ in patients than HC (P=0,0001; P=0,0003, P=0,001 ; P=0,015, respectively). However, patients had a significant decrease of the percentage of T $CD4^+$ and B lymphocytes compared to HC (P=0,001; P=0,0002, respectively). Furthermore, the immune system activation was found significantly pronounced among patients than HC, as per the expression of $CD8^+$/$HLADR^+$ and $CD4^+$/$CD25^+$ markers (P=0,002; P=0,001, respectively).
Our results report significant differences in immune cells profiles between patients and HC which could give help in understanding in immunopathology of lung cancer. Further works at functional level are highly recommended to depict well the role of each specific immune cell in lung cancer development.

P2952

Atad3a suppresses Pink1-dependent mitophagy to maintain homeostasis of hematopoietic progenitor cells

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Although deletion of certain autophagy-related genes has been associated with defects in hematopoiesis, it remains unclear whether hyperactivated mitophagy affects the maintenance and differentiation of hematopoietic stem cells (HSCs) and committed progenitor cells. We found that targeted deletion of the gene encoding the AAA+-ATPase Atad3a hyperactivated mitophagy in mouse hematopoietic cells. Affected mice showed reduced survival, severely decreased bone-marrow cellularity, erythroid anemia, B cell lymphopenia and expanded myeloid cells. Those phenotypes were associated with skewed differentiation of stem cells, B lymphocyte progenitor cells, erythroid and myeloid progenitor cells. Mechanistically, Atad3a interacted with the mitochondrial channel components Tom40 and Tim23 and served as a bridging factor to facilitate appropriate transportation and processing of the mitophagy protein Pink1. Loss of Atad3a caused accumulation of Pink1 and activated mitophagy. Notably, deletion of Pink1 in Atad3a-deficient mice significantly ´rescued´ the mitophagy defect, which resulted in restoration of the progenitor and HSC pools. Our data indicate that Atad3a suppresses Pink1-dependent mitophagy and thereby serves a key role in hematopoietic homeostasis.

P2953

Interplay of DNA methyltransferase 1 and TAZ contributes to lactate-induced immune evasion of lung cancer

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DOI: 10.1002/eji.201970400

The activity of transcriptional co-activator with PDZ binding domain (TAZ) protein is strongly implicated in the immune evasion of lung cancer and is influenced by tumor metabolism. High levels of lactate concentration in tumor microenvironment due to metabolic reprogramming are inversely correlated with patient overall survival. Here we investigated the role of lactate in regulation of activity of TAZ and showed glycolysis-derived lactate efficiently increased TAZ expression and activity in lung cancer cells. We demonstrated that the ROS generated by lactate-fueled OXPHOS in mitochondria activated AKT, and thereby inhibited GSK3b/ b-TrCP-mediated ubiquitination and degradation of DNA methyltransferase 1 (DNMT1). Upregulation of DNMT1 by lactate caused hypermethylation of TAZ negative regulator of LATS2 gene promoter, leading to TAZ activation. Moreover, TAZ binds to the promoter of DNMT1 and is necessary ofr DNMT1 transcription. Our study reveals a molecular mechanism of DNMT1 in linking tumor metabolic reprogramming to the Hippo-TAZ pathway and functional significance of the DNMT1-TAZ feedback loop in the migratory and invasive potential of lung cancer cells.

P2954

Correlation of between TGF gene polymorphism and multiple myeloma

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To investigate the relationship between transforming growth factor gene(TGF) single nucleotide polymorphism and multiple myeloma.The case control study was performed ,
55 patients with MM and 55 healthy control were selected.The genotype and allele detections were performed by adopting the polymerchase chain reaction and the ligase detection respectively.Then the sequencing based typing was further conducted.There were no statistically significant fifferences in the two loci genotype frequencies,allele frequencies and genotype frequencies in joint haploid typing between the MM group and the control group.SNP of TGF gene has no obvious correlation with the occurrence of MM and subtypes.

Mucosal Immunology

O017

Early compartmentalization of immune cells in human fetal tissues revealed by high-dimensional mass cytometry

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The human fetal immune system must protect the infant against the sudden exposure to a large variety of pathogens upon birth. While it is known that the fetal immune system develops in sequential waves, relatively little is known about the composition of the innate and adaptive immune system in the tissues. Here, we applied high-dimensional mass cytometry to profile the immune system in human fetal liver, spleen and intestine. With Hierarchical Stochastic Neighbor Embedding (HSNE) we distinguished 177 distinct immune cell clusters, including both previously identified and novel cell clusters. Principal component analysis (PCA) indicated substantial differences between the composition of the immune system in the different organs. Through dual-tSNE we identified tissue-specific cell clusters, which were found both in the innate and adaptive compartment. To determine the

DOI: 10.1002/eji.201970400

spatial location of tissue-specific subsets we developed a 31 antibody panel to reveal both the immune compartment and surrounding stromal elements for analysis of snap-frozen tissue samples with imaging mass cytometry. With HSNE-based pixel analysis, imaging mass cytometry reconstructed the tissue architecture and allowed both the characterization and determination of the location of the various immune cell clusters within the tissue context. Moreover, it further underpinned the distinctness of the immune system in the tissues. Thus, our results provide evidence for early compartmentalization of the adaptive and innate immune compartment in fetal spleen, liver and intestine. Together, our data provide a unique and comprehensive overview of the composition and organization of the human fetal immune system in several tissues.

O018

Acyloxyacyl hydrolase promotes pulmonary defense by preventing endotoxin tolerance in alveolar epithelial cells and alveolar macrophages

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Both alveolar epithelial cells and alveolar macrophages play important roles in host defense against pulmonary infections. Pre-exposure to Gram-negative bacterial lipopolysaccharide (LPS) leads to endotoxin tolerance in macrophages. We have found previously that intestinal commensal bacterial LPS desensitizes alveolar epithelial cells, making them resistant to allergen stimulation. Acyloxyacyl hydrolase (AOAH), a highly conserved host lipase, degrades and inactivates LPS. We found that *Aoah*$^{-/-}$ alveolar macrophages were hypo-responsive to LPS stimulation in vitro. In response to intranasally instilled LPS and *Pseudomonas aeruginosa* (PA), *Aoah*$^{-/-}$ mice had reduced epithelium- and macrophage-derived chemokine/ cytokine production and diminished neutrophil recruitment. After PA infection, *Aoah*$^{-/-}$ mice had greater and prolonged body weight loss and higher bacterial burden than Aoah+/+ mice. Antibiotic treatment that reduced intestinal commensal Gram-negative bacteria largely normalized airway innate responses in *Aoah*$^{-/-}$ mice. Furthermore, *Aoah*$^{+/+}$*Tlr4*$^{-/-}$ mice and *Aoah*$^{-/-}$*Tlr4*$^{-/-}$ mice had similar innate response to PA stimulation. These findings suggest that gut commensal LPS desensitizes alveolar epithelial cells and alveolar macrophages in a TLR4-dependent manner and compromises pulmonary immunity. By inactivating commensal LPS, AOAH thus promotes host defense against pulmonary infections.

O019

Galectin-4 secreted by intestinal epithelial cells protects the intestine by inducing bacterial chaining and restricting bacterial motility

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Galectins are a member of animal lectins with conserved carbohydrate-recognition domains (CRDs) that recognize galactosides. They exist in the cytosol and nucleus, but also in extracellular space, although they do not have a classical signal peptide. Recent studies have identified the ability of galectin-4 and -8 to bind to and kill bacteria. We showed that a number of different galectins, especially galectin-4, were present in the secretions from freshly isolated intestines of mice. We found endogenous galectin-4 was bound to certain populations of commensal bacteria recovered from mouse colon. We chose four strains of bacteria: *Escherichia coli* O19ab, *Escherichia coli* O86, *Salmonella enterica* Cubana and *Salmonella enterica* Worthington for further study. We found that

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DOI: 10.1002/eji.201970400

recombinant human galectin-4 bound to these types of bacteria through its CRDs. However, galectin-4 did not affect bacterial survival, but induced chaining and clustering. The formation of chains was from the divided cells failing to separate into individual daughter cells. Galectin-4 was enriched at the cell-cell junctions and bridged the daughter cells through binding to lipopolysaccharides. Furthermore, the chaining of *E. coli* O19ab was observed in the ligated ileal loops of transgenic mice expressing human galectin-4. Moreover, galectin-4 restricted bacterial motility significantly. When mice were subjected to oral challenge with *E. coli* O19ab, after *Salmonella typhimurium* infection, the number of *E. coli* O19ab was lower in ileum and spleen of wild-type mice compared to galectin-4 knockout littermates. Our data suggest that galectin-4 protects the host by inducing bacterial chaining and restricting bacterial motility.

O020

RIPKX plays a protective role in idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal parenchymal lung disease with limited therapeutic options, which affects millions of patients worldwide. The pathogenesis of IPF is influenced by the orchestration of epithelial, mesenchymal, and immune interactions. RIPKX is a member of the receptor-interacting protein kinase(RIPK) family, which are known to play a number of roles in the immune system.
Microarray datasets have shown that RIPKX is highly expressed in normal lung tissues compared with other organs. However, RIPKX was found to be less expressed in lung tissue samples isolated from human IPF patients as well as bleomycin-induced pulmonary fibrosis in mice, relative to non-diseased controls. Mice with global RIPKX deficiency (RIPKX$^{-/-}$) displayed enhanced susceptibility to bleomycin challenge, with significantly increased inflammation and augmented fibrosis, compared with wild-type mice. Bone marrow chimera experiments indicated that RIPKX deficiency in stromal cells, rather than bone marrow-derived cells, promoted fibrosis. Impressive upregulation of β-catenin and β-catenin targeted genes(Fibronectin, Spp1) revealed aberrant activation of Wnt signaling in bleomycin challenged RIPKX$^{-/-}$mice. Therefore, RIPKX may play an important regulatory role in stromal cells including epithelial cells and fibroblasts in pulmonary fibrosis.

O021

Crohn´s disease risk gene, Galectin-9 prevent intestinal inflammation via regulating ER stress

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The epithelium in gastrointestinal tract form a barrier which is the first line defense of immune system to against commensal bacteria and invading pathogen. To maintain the barrier function of epithelium, the homeostasis of intestinal Lgr5^{+} stem cell and Paneth cell are critical. Lgr5^{+} stem cell could differentiate into Paneth cell, which responsible to produce anti-microbial peptide and provide essential niche signal to Lgr5^{+} stem cell. Interestingly, several studied showed Crohn's disease (CD) risk genes (Xbp1, Atg16l1) are associated with dysregulated Paneth cell homeostasis and ER stress. Galectin-9 (Gal-9), was recently reported as a risk gene for CD in a genome wide association study (GWAS). However, it remains unclear how Gal-9 is involved in intestinal immune-regulation. We first determined Gal-9 is predominantly expressed in intestinal crypts, where stem cells and anti-microbial Paneth cells are located. Further analysis indicated Gal-9 deficiency led to disrupted homeostasis at

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DOI: 10.1002/eji.201970400

steady state. Also, Gal-9$^{-/-}$ crypts showed impaired anti-bacterial ability. Next, we found after dextran sulfate sodium (DSS) treatment, Gal-9$^{-/-}$ mice showed higher mortality, accompanied with more severe inflammation and UPR response. Furthermore, the inflammatory condition and ER stress of DSS treated Gal-9$^{-/-}$ mice can be ameliorated by transplantation with WT organoid which is consistent with our in vitro findings that defective function of Gal-9$^{-/-}$ organoid could be restored and ER stress could be relieved by supplying recombinant galectin-9. Together, our findings therefore provide evidence that polymorphism of galectin-9 gene might contribute to accumulation of ER stress, leading to dysfunction of epithelial barrier and intestinal inflammation.

O022

Suppression of IL-17F, but not of IL-17A, provides protection against colitis by inducing Treg cells through modification of the intestinal microbiota

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The cytokines IL-17A and IL-17F have 50% amino-acid identity and bind the same receptor. IL-17A is reported to protect host from colitis by maintaining intestinal tight-junction, but the potential function of IL-17F in intestinal immunity have remained obscure. Here, by administrating IL-17A, IL-17F and IL-17A&F deficient (*Il17a*$^{-/-}$, *Il17f*$^{-/-}$, *Il17a*$^{-/-}$*Il17f*$^{-/-}$) mice with DSS, or by transferring CD25^{-} CD45RB^{hi}CD4^{+} T cells from these mice into lymphocyte-deficient *Rag2*$^{-/-}$ mice, we investigated the influence of these IL-17 family members on the development of intestinal inflammation. By sequencing analysis of ribosomal RNA in 16S region, we identified the modification of fecal microbiota from these mutant mice. We found that *Il17f*$^{-/-}$ mice resisted chemically induced colitis, but *Il17a*$^{-/-}$ mice did not, and that *Il17f*$^{-/-}$ CD45RB^{hi}CD4^{+} T cells induced milder colitis in lymphocyte-deficient *Rag2*$^{-/-}$ mice, accompanied by an increase in intestinal regulatory T cells (T_{reg} cells). *Clostridium* cluster XIVa in colonic microbiota capable of inducing T_{reg} cells was increased in both *Il17f*$^{-/-}$ mice and mice given transfer *Il17f*$^{-/-}$ T cells, due to decreased expression of a group of antimicrobial proteins. There was substantial production of IL-17F, but not of IL-17A, not only by naive T cells but also by various colon-resident cells under physiological conditions. Furthermore, antibody to IL-17F suppressed the development of colitis, but antibody to IL-17A did not. These observations show the immune function of IL-17F is different from that of IL-17A in the intestine, and suggest that IL-17F is an effective target for the treatment of colitis.

O023

Perinatal programming of mucosal stromal cell identity by the lymphotoxin pathway regulates adult B cell responses

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Redundant mechanisms support IgA responses to intestinal antigens. These include multiple priming sites (mesenteric lymph nodes (MLN), Peyer´s patches (PP) and isolated lymphoid follicles) and various cytokines (BAFF, APRIL, TGFβ) that promote class switch to IgA, even in the absence of T cells. In spite of these numerous back-up mechanisms, vaccination against enteric pathogens such as Rotavirus has limited success in some populations. Genetic and environmental signals experienced

DOI: 10.1002/eji.201970400

during early life are known to condition immune cells within the gut environment, yet the mechanisms for how these exposures promote or dampen IgA responses against gut pathogens remains unclear. In this study, we have used Rotavirus infection to follow antigen-specific IgA responses through time and in different gut associated lymphoid tissue compartments. Using genetic and pharmacological approaches, we found that lymphotoxin-beta receptor (LTβR) signalling *in utero* programs intestinal IgA responses in adulthood by affecting antibody class switch recombination to IgA and subsequent generation of IgA antibody-secreting cells within the MLN. In addition, *in utero* LTβR signalling dictates the phenotype and function of MLN stromal cells in order to support IgA responses in the adult, and deletion of LTβR in CCL19-expressing stromal cells ablated the IgA response to Rotavirus in the gut. Collectively, our studies uncover new mechanistic insights into how *in utero* signalling events impact mucosal immune responses during adulthood.

O024

Platelet activating factor receptor regulates colitis-induced pulmonary inflammation through the NLRP3 inflammasome

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Extra-intestinal manifestations (EIM) are common in inflammatory bowel disease (IBD). One such EIM is sub-clinical pulmonary inflammation, which occurs in up to 50% of IBD patients. In animal models of colitis, pulmonary inflammation is driven by neutrophilic infiltrations, primarily in response to the systemic bacteraemia and increased bacterial load in the lungs. Platelet activating factor receptor (PAFR) plays a critical role in regulating pulmonary responses to infection in conditions, such as chronic obstructive pulmonary disease (COPD) and asthma. We investigated the role of PAFR in pulmonary EIMs of IBD, using dextran sulfate sodium (DSS) and anti-CD40 murine models of colitis. Both models induced neutrophilic inflammation, with increased TNF and IL-1β levels, bacterial load and PAFR protein expression in mouse lungs. PAFR expression was localized to epithelial cells and neutrophils and associated with neutrophil extracellular traps formation in the lung tissue of DSS colitis animals. We have identified that epithelial cells, macrophages and neutrophils are the major cellular source of PAFR by flow cytometry. Antagonism of PAFR using CV6209 decreased lung neutrophilia, TNF and IL-1β in an NLRP3 inflammasome-dependent manner in DSS-induced model of colitis. Lipopolysaccharide (LPS) from phosphorylcholine (ChoP)-positive bacteria induced NLRP3 and caspase-1 proteins in human alveolar epithelial cells, however antagonism of PAFR prevented NLRP3 activation by ChoP. Amoxicillin reduced bacterial populations in the lungs and reduced NLRP3 inflammasome protein levels, but did not reduce PAFR in DSS-induced model of colitis. These data suggest a role for PAFR in microbial pattern recognition and NLRP3 inflammasome signalling in the lung.

DOI: 10.1002/eji.201970400

LB024

Oral sodium butyrate induces substantial intestinal changes in IL-17 / IFNγ and stabilizes intestinal tight junction proteins in an experimental model of cholestasis

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Introduction: Dysregulation of the intestinal epithelial barrier is part of the complications of cirrhosis and it is intimately related to bacterial translocation and death by bacterial infections. Butyrate regulates anti-inflammatory processes, importantly those related with intestinal barrier function. However, its effect in experimental liver fibrosis is unknown.
Aim. Evaluate butyrate's effect in the intestinal inflammatory response and tight junction proteins in experimental cholestasis.
Material and methods: Wistar rats were ligated in the common bile duct (BDL) for 8 or 15 days. Butyrate was intraperitoneal (500 mg/kg) or orally (1%) administered, respectively. Control groups with BDL were used. IL-17, IL-10 and IFNγ gene expression in ileum and TGFβ, IL-6 and collagen I in liver was analyzed by qRT-PCR. Tight junction proteins: occludin, ZO-1 and claudin-1 were analyzed by western blotting from ileum homogenates.Histopathological analysis from liver and different ileum sections were assessed.
Results: Butyrate IP administration significantly decreased IL-17 while promoting IL-10 and IFNγ expression in comparison with the control group; however, ileum tight junction proteins remained unchanged except for a significant decrease in claudin-1 (p=0.03). At the hepatic level, no difference was found. On the other hand, orally administered butyrate drastically decreased IL-17 (p=0.17) and IFNγ (p=0.004) in ileum. In the same intestinal section IL-10 increased as well as occludin (p=0.05) and claudin-1 (p=0.02) regard the control group. Liver TGFβ, IL-6 and collagen I decreased without statistical significance.
Conclusions: Chronic oral administration of sodium butyrate is efficient in modulating intestinal cytokines that directly impact in the tight junction proteins.

LB025

Protective effect of S-IgA and its fractions against EPEC intestinal infection in gnotobiotic piglets

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DOI: 10.1002/eji.201970400

Secretory IgA (S-IgA) contributes to protecting mucosal surfaces from pathogenic bacteria attachment. It is not clear, if S-IgA interacts with microorganisms through antigen-specific Fab moiety, or antigen-nonspecifically through remaining protein backbone, or through N-linked oligosaccharides. Enteropathogenic *E. coli* (EPEC) strain O55 causes lethal infection in gnotobiotic piglets and represents widely used model to study interaction of microorganisms with host intestinal epithelia. O55 attaches to epithelia through several adhesion systems including pili, attaching and effacing complex, and others adhesins. The establishment of EPEC infection is initiated by binding of bacterial type 1 pili to α-D-linked mannoses on epithelial cells surface N-linked oligosaccharides.
Here we tested the protective effect of S-IgA preparations - specifically Fab fragment, Fc fragment with attached secretory component (Fc fragment), and protective effect of S-IgA with enzymatically removed surface N-linked oligosaccharides - against *E. coli* O55 challenge in gnotobiotic piglets. Germ free miniature Minnesota piglets were orally infected at age of four days with 5x10exp5 CFU of *E. coli* O55. Bacteria were administered separately or together with S-IgA preparations at equimolar dose corresponding to 6.7 mg of S-IgA. The symptoms (fever, anorexia, somnolence, diarrhea, ataxia, and tremor), bacteria colonization, cytokine production and post mortem histology was recorded. Piglets, which received *E. coli* O55 together with S-IgA, Fab fragments or Fc fragments, exhibited reduced severity of enteritis symptoms, reduced immune cells infiltration of ileum lamina propria and reduced production of proinflammatory cytokine synthesis and serum levels.

Supported by Czech Science Fundation grant 17-11275S and CZ.02.1.01/0.0/0.0/16_025/0007397.

LB062

Bacillus thuringiensis Cry1Ac toxin induces MAPKs activation and allergenic effects in intestinal and immune cells

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The *Bacillus thuringiensis* biopesticide Cry1Ac toxin can be expressed by genetically modified plants for human and animal consumption. Cry1Ac is not toxic for vertebrates; however, it produces immunologic effects. We evaluated the allergenic potential with an intestinal allergic model, binding capacity, signaling induction and activation in human epithelial colonic cells and macrophages.
Materials and methods: An ovalbumine (OVA) murine alimentary allergic model was used to assess the allergenic potential of Cry1Ac toxin. Mice were sensitized intragastrically with: i) vehicle phosphate-buffered saline, ii) OVA, iii) OVA plus CT iv) Cry1Ac or v) OVA plus Cry1Ac. Seven weeks after intragastric challenge; IgE, Eosinophiles productions and Histopathologic changes were evaluated at intestinal and systemic level. Fluorescein labeled Cry1Ac interacted with HCT116 human colonic cells and Raw 264.7 murine macrophages. Protein binding with its target was measured after 1 h with confocal microscopy. Western blot was used to identify MAPKs activation.
Results: The immunized groups; Cry1Ac, OVA/Cry1Ac or OVA/CT developed moderate allergic reactions, induced significant IgE response. Interestingly, also showed colonic lymphoid hyperplasia, this response has been associated with food allergy and intestinal inflammation in humans. The toxin Cry1Ac-FITC binds the human epithelial cell surface and activates ERK, JNK and p38. The toxin binds and it internalizes macrophages and ERK, JNK and p38 are activated, furthermore, it favors IL-6, MCP-1 and TNF-α liberation.
Conclusion: Cry1Ac is not inocuos, it binds intestinal and immune cells. The toxin induces MAPKs activation, and it showed moderately allergenic effects. More importan, Cry1Ac provoke intestinal lymphoid hyperplasia**.**

DOI: 10.1002/eji.201970400

LB063

Identification and characterization of Der p 37, a new house dust mite allergen for component-specific diagnosis

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Background: House dust mites (HDM) are important elicitors of allergic reactions worldwide.
Objective: To identify new important HDM allergens for diagnosis and immunotherapy of HDM allergic patients.
Methods: A *D. pteronyssinus* expression cDNA library was screened with IgE antibodies from HDM allergic patients and a cDNA coding for a new mite allergen, designated Der p 37, was expressed in *Escherichia coli*. The localization of Der p 37 in mites was investigated by immunogold-transmission electron microscopy (TEM). IgE reactivity of HDM-sensitized subjects to Der p 37 was tested using ImmunoCAP ISAC technology and allergenic activity of Der p 37 was determined using human FcεRI-transfected rat basophilic leukaemia (RBL) cells. CD4+ T cell responses to Der p 37 were analysed in PBMC cultures from allergic and non-allergic individuals by CFSE dilution assay.
Results: rDer p 37 represents a 26 kDa protein with homology to chitin-binding proteins but without capacity to bind chitin. The allergen showed no cross-reactivity to other *D. pteronyssinus* allergens with chitin-binding domains (Der p 15, 18, 23) nor to proteins from other tested allergen sources. Rabbit Der p 37-specific antibodies localized the allergen in the peritrophic membrane of the midgut and in faecal pellets. The recombinant allergen bound IgE from 28% of HDM allergic Austrian patients and induced dose-dependent basophil activation. Der p 37 induced CD4+ T cell proliferation in Der p 37-sensitized as well as non-sensitized subjects.
Conclusion: The new HDM allergen, Der p 37 should be included into the panel of HDM allergens for component-specific diagnosis.

LB099

The role and mechanism of E3 ubiquitin ligase Smurf2 in promoting inflammatory bowel disease by regulating IL-17 signaling of intestinal epithelial cells

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Inflammatory bowel disease (IBD) has become a global disease that endangers human health. Exploring new molecule targets and the specific molecular mechanisms has always been the core goal of IBD research. The pro-inflammatory factor IL-17 has been reported to be involved in the development of IBD. The regulation of IL-17 signaling pathway by E3 ubiquitin ligase Smurf2 in the pathogenesis of IBD have not been reported. Our preliminary results showed that Smurf2 was specifically highly expressed in intestinal epithelial cells (IECs) of IBD patients, suggesting the potential role of Smurf2 in the pathogenesis of IBD throug regulating the function of IECs. Smurf2 IEC specific knockout mice (Smurf2flox/flox Pvillin Cre) were resistant to DSS-induced IBD compared to their control wild-type littermates. Cellular data showed that deficiency of Smurf2 in IECs resulted in

DOI: 10.1002/eji.201970400

downregulation of IL-17-mediated inflammation and signal transduction. Next, we will further clarify the function of Smurf2 in IBD and its specific molecular mechanism. The prospected results might offer new opinions to further understanding of the pathogenesis of IBD and provide potential new therapeutic targets for the IBD diagnosis and treatments.

P0400

Nutrient sensing by the intestinal epithelium orchestrates mucosal antimicrobial defense via translational control of Hes1

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Metabolic programs and host defense are highly integrated to ensure proper immune responses during stress. Central to these responses, mTOR regulates immune functions by sensing and integrating environmental cues, yet how these systems are coordinated at the intestinal surface remains undefined. We show that the antimicrobial peptide α-defensin is functionally sustained during nutrient deprivation due to regulation of defensin-processing enzyme MMP7 by microbiota- and host-derived factors. Unlike other antimicrobial peptides, the MMP7-α-defensin axis remains active during nutrient fluctuations, providing essential protection against enteric pathogens. Sustained Mmp7 expression requires the microbiota and is mediated by de-repression of the transcription activator Atoh1 upon attenuation of the transcriptional repressor Hes1 in intestinal epithelial cells. Hes1 levels are regulated via mTOR and controlled translationally, constituting a metabolism-translation-transcription loop. Disrupting this loop by supplying nutrients paradoxically compromises anti-bacterial defense. Together, these results uncover a regulatory circuit that couples host nutrient status to epithelial anti-microbial immunity.

P0401

Transcriptional atlas of intestinal immune cells reveals that neuropeptide α-CGRP orchestrates ILC2 responses

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Signaling abnormalities in immune responses in the small intestine can trigger chronic type 2 inflammation, involving interaction of multiple immune cell types. To systematically characterize this response, we analyzed 58,067 immune cells from the mouse small intestine by single-cell RNA-seq

DOI: 10.1002/eji.201970400

(scRNA-seq) at steady state and after induction of a type 2 inflammatory reaction to ovalbumin (OVA). Analysis revealed broad shifts in both cell type composition and cell programs in response to the inflammation, especially in group 2 innate lymphoid cells (ILC2s). Among the key transcripts associated with an inflammation-induced program in intestinal KLRG1$^+$ ILC2s was exon 5 of *Calca*, which encodes the alpha-calcitonin gene-related peptide (α-CGRP). α-CGRP antagonized IL-25-induced activation of intestinal KLRG1$^+$ ILC2s and reduced ILC2 frequency in an OVA reaction model. α-CGRP activated a cyclic AMP (cAMP) response, which suppressed ILC2 proliferation. In homeostasis, α-CGRP was predominantly expressed by two subsets of ChAT$^+$ enteric neurons, and genetic perturbation of α-CGRP increased the proportion of intestinal KLRG1$^+$ ILC2s and the number of Tuft cells. Our work highlights a model where α-CGRP-mediated neuronal signaling is critical for suppressing ILC2 expansion and maintaining homeostasis of the type 2 immune machinery.

P0402

BLT1 in dendritic cells promotes Th1/Th17 differentiation and its deficiency ameliorates TNBS-induced colitis

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Leukotriene B4 (LTB4) synthesis is enhanced in the colonic mucosa in patients with inflammatory bowel disease (IBD), BLT1, a high affinity receptor for LTB4, has been reported showing no effect on the progression of dextran sodium sulfate (DSS)-induced colitis which mostly relies on innate immunity. However, if BLT1 has an effect on the adaptive immune mechanisms of IBD is still unknown. Here, we used BLT1 knockout mice and trinitrobenzene sulfonic acid (TNBS)-induced colitis model to demonstrate a critical role of BLT1 in adaptive immune mechanisms of colitis. We found BLT1$^{-/-}$ mice displayed an alleviated severity of TNBS-induced colitis with less body weight loss and infiltrating cells in lamina propria. Deficiency of BLT1 in DCs led to a decreased production of proinflammatory cytokines including IL-6, TNF-α and IL-12. The impaired cytokines production by BLT1$^{-/-}$ DCs subsequently led to decreased Th1 and Th17 differentiation both *in vitro* and *in vivo*. We further performed a conditional DC reconstitution experiment to test if it is the BLT1 in DCs plays the major role in regulating the pathogenesis of TNBS-induced colitis, and the results did show that deficiency of BLT1 only in DCs can also lead to the significantly reduced disease severity. The mechanistic study demonstrated that BLT1 regulated proinflammatory cytokines production through Gαi βγ subunit-phospholipase Cβ (PLCβ)-PKC pathway. Our work demonstrated that BLT1 could control Th1 and Th17 differentiation by regulating DC-derived proinflammatory cytokines production in colitis, indicating BLT1 may serve as a potential drug target for adaptive immunity mediated IBD.

P0403

Gastric subserous vaccination with *Helicobacter pylori* vaccine: an attempt to establish tissue-resident CD4$^+$ memory T cells and induce prolonged protection

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Tissue-resident memory T (T_{RM}) cells are enriched at the sites of previous infection and required for enhanced protective immunity. However, the emergence of T_{RM} cells and their roles in providing protection are unclear in the field of *Helicobacter pylori* (*H. pylori*) vaccinology. Here, our results

DOI: 10.1002/eji.201970400

suggest that conventional vaccine strategies are unable to establish a measurable antigen (Ag)-specific memory cell pool in stomach; in comparison, gastric subserous injection of mice with micro-dose of Alum-based *H. pylori* vaccine can induce a pool of local $CD4^+$ T_{RM} cells. Regional recruitment of Ag-specific $CD4^+$ T cells depends on the engagement of Ag and adjuvant-induced inflammation. Prior subcutaneous vaccination enhanced this recruitment. A stable pool of Ag-specific $CD4^+$ T cells can be detected for 240 days. Two weeks of FTY720 administration in immune mice suggests that these cells do not experience the recirculation. Immunohistochemistry results show that close to the vaccination site, abundant $CD4^+$ T cells locate on epithelial niches, independent of lymphocyte cluster. Paradigmatically, Ag-specific $CD4^+$ T cells with a phenotype of $CD69^+CD103^-$ are preferential on lymphocytes isolated from epithelium. Upon *Helicobacter* infection, $CD4^+$ T_{RM} cells orchestrate a swift recall response with the recruitment of circulating antigen-specific Th_1/Th_{17} cells to trigger a tissue-wide pathogen clearance. This study investigates the vaccine-induced gastric $CD4^+$ T_{RM} cells in a mice model, and highlights the need for designing a vaccine strategy against *H. pylori* by establishing the protective $CD4^+$ T_{RM} cells.

P0404

Lactobacillus maintains healthy gut mucosa by producing L-Ornithine

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Gut mucosal layers are crucial in maintaining the gut barrier function. Gut microbiota regulate homeostasis of gut mucosal layer via gut immune cells such as RORγt (+) IL-22(+) ILC3 cells, which can influence the proliferation of mucosal cells and the production of mucin. However, it is unclear how gut microbiota execute this regulation. Here we show that lactobacilli promote gut mucosal formation by producing L-Ornithine (L-Orn) from arginine (Arg). L-Orn increases the level of aryl hydrocarbon receptor (AhR) ligand L-kynurenine (L-Kyn) produced from tryptophan (Trp) metabolism in gut epithelial cells, which in turn increases RORγt (+)IL-22(+) ILC3 cells. Human REG3A transgenic mice show an increased proportion of L-Orn producing lactobacilli in the gut contents, suggesting that gut epithelial REG3A favors the expansion of L-Orn producing lactobacilli. Our study implicates the importance of a crosstalk between Arg metabolism in Lactobacilli and Trp metabolism in gut epithelial cells in maintaining gut barrier.

P0405

IgA targeting phosphoprotein prevents measles virus from evading type I IFN signaling

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Immunoglobulin A (IgA) plays a unique role in inhibiting intracellular viral replication during its transporting through mucosal epithelial cells. We found a monoclonal IgA antibody 7F1-IgA against phosphoprotein (P) of measles virus (MV) inhibited the intracellular replication of MV in Caco-2 cells but not in interferon-deficient Vero-pIgR cells grown in a two-chamber transwell system, indicating the involvement of interferon pathway in 7F1-IgA-mediated antiviral activity. Further study revealed that transcytosis of 7F1-IgA across the MV-infected Caco-2 cells enhanced the production of IFN-β and the expression of IFN-stimulated genes, rendering Caco-2 cells with higher anti-viral immunity. Additional study showed that 7F1-IgA specifically interacted with MV-P inside the MV-infected Caco-2 cell and

DOI: 10.1002/eji.201970400

prevented MV-P from inhibiting the phosphorylation of JAK1 and STAT1. Furthermore, the intraepithelial interaction between 7F1-IgA and viral phosphoprotein resulted in an earlier and stronger phosphorylation of JAK1 and STAT1, and consequently a more efficient nuclear translocation of STAT1, which is essential for the activation of type I interferon pathway in MV-infected Caco-2 cells. Taken together, **our data reveal that IgA against phosphoprotein can prevent a virus from evading type I IFN signaling and confer host epithelial cells efficient innate antiviral immunity.** Findings in this study provide insights into a unique antiviral mechanism of IgA antibody, in which IgA intracellularly intervenes in the warfare between viral replication and cellular interferon response, resulting in the reinforcement of innate immune response.

P0406

The role of CCR5 in the maintenance of intestinal immune homeostasis

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The intestinal epithelium provides a crucial barrier against external pathogens. Specialized immune cells play an important role in maintaining intestinal integrity. In order to analyze the impact of individual cell populations on sustaining intestinal integrity, we created a novel mouse model, which allows us to identify, sort, or specifically delete individual cell subsets based on the expression of the chemokine receptor type 5 (CCR5). In this mouse model, we replaced the CCR5 coding region with a primate diphtheria toxin receptor (pDTR) and eGFP fusion, so that the cells carrying this allele are sensitive to non-inflammatory apoptosis via diphtheria toxin (DT). The *Ccr5* locus was further modified with a *loxP-3XSV40pA-loxP* element located within the first intron such that pDTReGFP should be expressed only in cells that expressed CRE-recombinase. With this model, we mapped the CCR expression file in gut associated lymphoid tissue (GALT)and showed that administration of DT lead to a complete loss of $CCR5^+$ cells in corresponding cell subsets in DTR^+ Cre^+ mice. Interestingly, sustained loss of $CCR5^+$ cells induced the disruption of intestine integrity, which we found was due to the loss of hematopoietic $CCR5^+$ macrophages and nonhematopoietic $CCR5^+$ cells. Finally, we showed that the administration of a broad-spectrum antibiotic cocktail prevented the occurrence of illness, indicating its dependency of gut microbiome. All together, we generated a novel and flexible mouse model for identifying or killing $CCR5^+$ cells, characterized the key importance and mechanism of CCR5 in the maintenance of intestinal immune homeostasis.

P0407

Interplay among lung dendritic cell subsets in the regulation of eosinophil recruitment

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DOI: 10.1002/eji.201970400

Allergic asthma is often chronic in nature. Eosinophil infiltration is a hallmark of allergic asthma. It has been shown that the eosinophil infiltration is essential for type 2 immune responses. How the initial eosinophil recruitment is regulated by lung dendritic cell (DC) subsets during the memory stage after allergen challenge is unclear. Here, we found that eosinophils started to accumulate as early as 1.5 days after the first OVA challenge both in the lung and Balf. Lung $CD11c^+$ DCs were essential for the initial eosinophil accumulation during the memory stage in chronic asthmatic inflammation after challenge. $CD103^+$DCs (cDC1s) and $CD11b^+$ DCs are two major DC subsets in the lung. Lung cDC1s from OVA challenged mice during the memory stage were both necessary and sufficient for the induction of lung eosinophilia. Further more, lung cDC1s directly attract eosinophils by secreting CCL17 and CCL22. We sought to investigate how cDC1-mediated eosinophil migration is modulated. We found that inducible nitric-oxide (NO) synthase-dependent NO from lung $CD24^-CD11b^+$ DC2s is critical for this early event via promoting CCL17 and CCL22 production by lung cDC1s. During late phase responses after allergen challenge, lung $CD24^+$ cDC2s inhibit eosinophil recruitment through secretion of TGF-β1, which impairs the expression of CCL17 and CCL22. These data reveal that different lung $CD11b^+$ DC subsets modulate lung cDC1-mediated eosinophil recruitment dynamically, through secreting distinct soluble factors during the memory stage of chronic asthma after allergen challenge in the mouse.
Supported by grants (2015CB943203, 2018WS136, NSFC81401328, NSFC31500715, ZR2013HL064, ZR2015HQ021).

P0408

Interleukin-33 facilitates allergic asthma occurrence by increasing host susceptibility to antigen

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It has been suggested that interleukin-33 (IL-33) plays an important role in the development of asthma. In order to determine whether IL-33 could increase host susceptibility to antigen and explore the mechanism, a murine model of asthma was established through intraperitoneal sensitization and nasal inhalation challenge with sub-threshold *Dermatophagoides farinae* (Der f) and with/without recombinant IL-33. Airway resistance, total cells in bronchoalveolar lavage fluid (BALF) and pulmonary inflammation were compared between groups of mice. Intranasal challenge with sub-threshold Der f or IL-33 alone did not cause increases in airways resistance or inflammatory cellular infiltration. In contrast, however, airways resistance and inflammatory cellular infiltration into airways were significantly elevated in the combined group of mice. In addition, we explored the effects of IL-33 on the proliferation and on the ability of RAW264.7 cell (mouse macrophage cell line) to capture antigens *in vitro*. The results showed that IL-33 promoted proliferation and the capture of Der f by macrophage. These findings suggest that IL-33 is able to increase host susceptibility to antigen involved in the pathogenesis and occurrence of asthma through enhancing proliferation and ability of macrophages to capture antigens.
Keywords: Asthma; Interleukin-33; *Dermatophagoides farinae*

DOI: 10.1002/eji.201970400

P0409

IL-3 protects from colitis by recruiting splenic reservoir neutrophils

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Interleukin-3 (IL-3), a hematopoietic growth factor, was recently described to play a key role during inflammatory diseases. However, its role during inflammatory bowel disease remains unknown. Here, we showed that IL-3-deficient mice were more sensitive to colitis compared to control. During the early phase of colitis, WT mice, but not IL-3$^{-/-}$ mice, exhibited a rapid influx of neutrophils in colon, which conferred protection and involved CCL5-producing CD3^{+} cells. Interestingly, splenectomy in WT mice led to increased severity after 1 day of colitis but had no effect in IL-3$^{-/-}$ mice and could be reversed by adoptive transfer of splenic neutrophils. WT mice displayed also increase number of neutrophils and hematopoietic stem and progenitor cells in spleen during colitis compared to naive mice and IL-3$^{-/-}$ mice. In addition, IL-3 was found to be expressed in colon by a non-hematopoietic population and in spleen by innate response activator B cells. In conclusion, the study reveals that IL-3 protects from colitis by driving the rapid deployment of splenic neutrophils into the colon and shows that IL-3 sustains this neutrophils influx by promoting splenic extramedullary hematopoiesis. Altogether, these results suggest that IL-3 administration may be a therapeutic approach during colitis.

P0410

3,4-Dihydroxyphenylethylamine mediated signalling limits regulatory T cells suppressive activity and their migration toward gut mucosa

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Evidence from inflammatory colitis mouse models and from samples obtained from inflammatory bowel diseases (IBD) patients has indicated that gut inflammation in IBD is driven mainly by the inflammatory effector CD4^{+}T-cell subsets T-helper 1 (Th1) and Th17. On the other hand, regulatory CD4^{+}T-cells (Treg), a suppressive subset of lymphocytes that plays a crucial role in maintaining intestinal homeostasis in healthy conditions, seems to be dysfunctional in IBD. After isolation from IBD gut mucosa, Treg retain their suppressive activity *in vitro*, suggesting that Treg function is attenuated just *in situ*by mediators present in the inflamed gut mucosa. Importantly, cells residing in healthy gut mucosa are exposed to high 3,4-Dihydroxyphenylethylamine (DA) levels upon homeostasis, nevertheless, there is a marked reduction of intestinal DA in inflamed gut mucosa in IBD patients and in animal models of inflammatory colitis. Thereby, we studied the role of DA receptors (DR) in Treg function in a model of inflammatory colitis in mouse. The results show that DR-signalling in Treg is selectively triggered by low-DA levels found in inflamed gut mucosa, however it was abrogated when exposed to high-DA levels found in the gut mucosa upon homeostasis. Moreover, DR-signalling attenuated Treg suppressive activity in vitro and in vivo. Furthermore, DR-signalling reduced the acquisition of gut-tropism by Treg and consequently, strongly decreased Treg infiltration into the gut mucosa. Thus, our findings show that DR-signalling in Treg represents a major pathogenic mechanism dampening Treg-activity and promoting gut inflammation.

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DOI: 10.1002/eji.201970400

P0411

A fast and robust protocol for immunomagnetic isolation of human CD45+ leukocytes from samples with variable start frequencies

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The study of immune cell function in non-lymphoid tissue and tumors has emerged as an exciting research area, with the promise to provide novel strategies for the treatment of immune disorders, infectious diseases and cancer. A major challenge in the field is the isolation of leukocytes from tissues and tumors due to their structural complexity and compositional variability. We describe here a new protocol for the isolation of particle-free human CD45+ leukocytes from varied sample sources. Using the EasySep™ Release Human CD45 Positive Selection Kit, leukocytes are labeled with antibody complexes linked to magnetic particles and separated using an EasySep™ magnet. The isolated cells are then released from the magnetic particles by resuspension in EasySep™ Release Buffer and a final magnetic separation step. Cell separation performance was assessed using defined mixtures of human leukocytes and mouse thymocytes representative of the leukocyte frequencies found in human tissue and tumor samples. Following isolation, human CD45+ purities were 96.9 ± 1.5% and 99.6 ± 0.3% when starting from 0.7 - 2.2% (n=11) and ≥10% (n=11) leukocytes, respectively. We have also assessed our CD45+ isolation kit from tissues of humanized NSG™ mice, expanding the application of our protocol to highly relevant disease and cancer models. The EasySep™ Release Human CD45 Positive Selection Kit provides a robust method for high purity isolation from diverse tissues and starting frequencies, generating particle-free cells ready for downstream applications in as little as 45 minutes.

P0412

Antibody and cytokine responses to viral infections (Aujeszky´s disease virus -ADV-) in piglets after early mucosal vaccination

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Newborn piglets are highly susceptible to viral infections causing important losses to porcine industry. The appropriate age for vaccination before weaning has not been determined yet. Aujeszky´s disease virus (ADV) is a porcine herpervirus 1, which infects the respiratory tract cells and is lethal before weaning. Respiratory and genital secretions mediate ADV transmission. Current intramuscular (IM) vaccination protocols, usually applied at weaning, induce poor mucosal immunity, frequently failing to prevent and control the disease. Thus, we studied a systemic/mucosal route of vaccination before weaning using an inactivated ADV vaccine in two and fourteen day-old groups of SPF miniature Vietnamese pigs (n=6 each), measuring the specific anti ADV antibody (ELISA) and mRNA cytokine (qPCR) responses in systemic and mucosal samples. Our results showed that the systemic (serum) ADV-specific IgG and IgA responses were similar in both age groups and evident one week after the mucosal immunization. The IgA response was higher in mucosal secretions than the IgG one and sustained after an intranasal boost. Moreover, PBMC and mononuclear lung cells, from immunized piglets, increased IFN-γ, IL-4 and IL-10 mRNA expression after *in vitro* stimulation with ADV,

DOI: 10.1002/eji.201970400

regardless of age of immunization. Our data suggest that unweaned piglets are able to mount systemic and mucosal antibody and cytokine responses regardless the age of early mucosal vaccination; therefore, protection, in virus susceptible animals, may be induced using this protocol.
Acknowledgments: Partial financial support by ICyTDF (267/09) and CONACYT (60941) Mexico. Technical assistance by Laura Zapata, Antonia Cobá (INIFAP-SAGARPA, México) and Enrique Chávez.

P0413

Clinical periodontal parameters and C-reactive protein levels during aggressive periodontitis patients treatment

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Aim: Aggressive periodontitis is periodontal disease which is characterised by severe destruction of the periodontal attachment apparatus and by the rapidness of this process. CRP is a plasma protein that reflects the extent of the acute phase response to inflammation.
Materials and methods: All patients (N=45) were examined prior to the initiation of periodontal treatment. Patients were divided into two groups. Group GEN (N=23) consisted of patients with generalised form of aggressive periodontitis and group LOC (N=22) consisted of patients with localised aggressive periodontitis. CRP levels in the peripheral blood samples and clinical periodontal parameters were determined in patients with aggressive periodontitis during the first twelve months of periodontal treatment.
Results: Results of this study have shown decreasing values of CRP as well as BOP and PD indices in the course of periodontal treatment.
Conclusions: Unchanged levels of CAL index in the course of periodontal treatment, especially in patients with generalised aggressive periodontitis, corresponds to healing of periodontium and careful deep scaling and root planing that was performed as periodontal treatment.
CRP levels decreased in the course of periodontal treatment in a similar way as BOP and PD indices; therefore it is possible to use this marker to follow patient's periodontal health in specific conditions. Nevertheless, further studies are needed to clarify this association and the associated confounding factors.
Acknowledgement: This study was supported by projects nr. NV19-08-00070 and 17-30753A (Ministry of Health, Czech Republic) and by project PROGRES Q29/1LF (First Faculty of Medicine, Charles University, Czech Republic).

P0989

MZB1 promotes the secretion of J chain-containing dimeric IgA and is critical for the suppression of gut inflammation

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IgA is the most abundantly produced antibody in the body and plays a crucial role in gut homeostasis and mucosal immunity. IgA forms a dimer that covalently associates with J chain, which is essential for IgA transport into the mucosa. How the dimer formation of IgA and its J chain association are regulated, however, remains largely unknown. Here we demonstrate that the marginal zone B and B-1

cell-specific protein (MZB1) interacts with IgA through the α heavy chain tailpiece dependent on the penultimate cysteine residue and prevents the intracellular degradation of α-light chain complexes. Moreover, MZB1 promotes J chain binding to IgA and the secretion of dimeric IgA. MZB1-deficient mice are impaired in secreting large amounts of IgA into the gut in response to acute inflammation and develop severe colitis. Oral administration of a monoclonal IgA significantly ameliorated the colitis, accompanied by normalization of the gut microbiota composition. The present study identifies a molecular chaperon that promotes J chain binding to IgA and reveals an important mechanism that controls the quantity, quality and function of IgA.
Keywords: MZB1, IgA, J chain, molecular chaperon, mucosal immunity

P0990

A novel cytokine like molecule, FAM3D, is essential for colon homeostasis, inflammation, and microbiota balance

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The physiological homeostasis of gut mucosal barrier is maintained by both genetic and environmental factors and its impairment leads to pathogenesis such as inflammatory bowel disease. Here, we demonstrate that family with sequence similarity 3, member D (FAM3D, mouse Fam3D), a novel chemotactic cytokine like molecule, was highly expressed in mouse gastrointestinal tract and enhanced phosphorylation of Akt, P38 and Erk1/2 via activating G-protein coupled formyl peptide receptors (Fprs) in murine colon cancer cell line CT26. Deficiency of Fam3D was associated with impaired integrity of mouse colonic mucosa, reduced anti-microbial peptide production and increased sensitivity to chemically induced colitis and carcinogenesis. Pretreatment of *Fam3D*$^{-/-}$ mice with antibiotics significantly reduced the severity of chemically induced colitis, suggesting deficiency in Fam3D led to dysbiosis in the colon. This was confirmed by observations that wild type (WT) mice co-housed with *Fam3D*$^{-/-}$ mice adopted increased sensitivity to chemically induced colitis, with skewed composition of fecal microbiota associated with Fam3D-deficiency. These results demonstrate the essential role of Fam3D in protection of colon mucosal homeostasis by controlling microbiota balance.

P0991

HCQ alleviates 5-FU-induced intestinal inflammation through inhibiting TLR9-dependent DNA sensing pathway

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Chemotherapies are known often to induce severe intestinal mucositis but the underlying mechanisms remain controversial**.** This study aims to investigate whether Hydroxychloroquine (HCQ) suppresses 5-FU-induced intestinal mucositis through inhibiting toll-like receptor (TLR) 9-dependent DNA sensing pathway. Positive correlation between the concentration of dsDNA and dose of 5-FU-induced intestinal toxicity was observed. Then, the effects of HCQ on 5-FU-induced intestinal mucositis were

DOI: 10.1002/eji.201970400

investigated *in vivo* and *vitro*. *In vivo* in 5-FU-treated mice, HCQ treatment markedly attenuated loss of body weight, severity of diarrhea, intestine shortening, and destruction of small intestine, inhibited expressions of TLR9 and nuclear factor-kappa B (NF-κB) in small intestine and pro-inflammatory cytokine interleukin-1β (IL-1β) secretion in serum, and reduced the number of macrophages in small intestine. *In vitro*, bone marrow-derived macrophages (BMDMs) were cultured, transfected with calf-thymus DNA(CT-DNA) and treated with HCQ. We found HCQ efficiently inhibited TLR9 expression, translocation of NF-κB to nucleus, and IL-1β secretion in the supernatant in CT-DNA stimulated BMDMs. These results provide a new insight into the mechanism of chemotherapy induced intestinal mucositis and indicate HCQ may be used as a strategy against intestinal mucositis during 5-FU chemotherapy.

P0992

C-di-AMP as mucosal adjuvant enhances immune responses induced by Mycobacterium tuberculosis ESAT-6 in mice model

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Despite advances in new vaccine candidates, tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) remains one of the most deadly killers of infectious diseases. Effective and safe vaccine is essential for TB control. It is reported that adjuvant can significantly improve the immunogenicity of subunit vaccine. The bis-(3′,5′)-cyclic dimeric adenosine monophosphate (c-di-AMP), a second messenger molecule produced only by bacteria and archaea, has been reported as a mucosal candidate adjuvant with antigens such as Ovalbumin and β-galactosidase. In this study, the vaccine candidate antigen of Mtb ESAT-6 (early secreted antigen target 6kDa) was administered with c-di-AMP as adjuvant by intranasal route to BALB/c mice. The results showed that ESAT-6 with c-di-AMP induced significantly higher specific IgG titer than that of ESAT-6 alone. ESAT-6 with c-di-AMP induced higher antigen-specific secretory IgA, which reflects the induction of local mucosal immune responses. In addition, ESAT-6 with c-di-AMP administration induced stronger cellular immune responses than that of ESAT-6, including enhanced lymphocyte proliferation, higher cytokines transcription and more cytokines production of IFN-γ, IL-2 and IL-10. After mice challenged with Mtb intravenously, co-administered c-di-AMP group mice showed reduced bacterial burdens in spleen and lung compared with ESAT-6 group. These results indicated that c-di-AMP could enhance the immunogenicity of subunit vaccines as mucosal adjuvant and showed application potential for TB vaccines.

Foundation: National Science and Technology Major Project (2018ZX10302302002-004), National Natural Science Foundation of China (81671638), Key Research and Development Foundation of Shaanxi province (2017ZDXM-SF-022)

P0993

Involvement of GPR84 in the alveolar macrophage subtypes switch during ARDS

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DOI: 10.1002/eji.201970400

The pathogenesis of Acute respiratory distress syndrome (ARDS), a kind of acute pulmonary disease, is closely related to the imbalance of pulmonary microenvironment's immune homeostasis, in which alveolar macrophages (AMs) are indispensably involved. During ARDS process, AMs participate in the immune responses and tissue recovery, regulate functions of different cell populations and rescue the pulmonary immune homeostasis in the end. In the study, we confirmed that AMs dominated the interaction with LPS in alveolar niches *in vivo* and shew transformation from $CD11b^{lo}$ to $CD11b^{hi}$ status in LPS-induced ARDS mouse model. $CD11b^{hi}$ AMs exhibited pro-inflammatory phenotype with higher secretion of IL-6, IL-12, TNF-α and phagocytic capacity. Metabolic-sensing GPR84 was highly expressed on ARDS lung tissues and $CD11b^{hi}$ AMs. Mice treated with potential GPR84 antagonist PBI-4050 and *Gpr84*$^{-/-}$ mice both shew alleviated ARDS symptoms with intact pulmonary histology, well-balanced air-blood barrier, limited respiratory burst and cytokine storm, which may be attributed to the downregulated switch of AM subtypes in *Gpr84*$^{-/-}$ mice. *Gpr84*$^{-/-}$ AMs displayed significantly downregulated expressions of ARDS-related cytokines, phagocytic capacity *in vitro* and lower LPS interaction *in vivo*, which hinted GPR84 may regulate TLR4-related pathways. We found that *Gpr84*$^{-/-}$ AMs downregulated expressions of CD14 and lipopolysaccharide-binding protein (LBP) in response to LPS challenge via Akt/Erk-Stat3. In conclusion, GPR84 regulated the LPS-induced ARDS process with their involvement in the switch of AM subtypes via Akt/Erk-Stat3-CD14/LBP pathway, which means GPR84 maybe a potential therapeutic target of ARDS.

P0994

Colon epithelial cell-derived Igk promotes murine colitis and colitis-associated colon carcinogenesis

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It is widely accepted that B cells and plasma cells are the only source of immunoglobulins (Igs), but our group have confirmed that non-B cells, including the colon epithelial cells, can produce and secret Igs under physiological condition. However, the role of Igk in regulating intestinal homeostasis remains unexplored. In this study, we found that colon epithelial cells could produce free Igk light chain (Ig FLC) with high level and the expression and secretion of Ig FLC could be upregulated by NF-kB. Interestingly, Ig FLC also upregulated NF-kB pathway and induced inflammation by promoting production of IL-6 or IL-8. The peptide F991, a functional blocker of Ig free light chain, significantly inhibited inflammation induced by Ig FLC in vitro. Markedly, F991 abrogated the development of diarrhea and tumor burden, elevated survival rate in the AOM-DSS model of colitis-associated colon carcinogenesis (CAC). Furthermore, F991 also inhibited the activation of the inflammasome by reducing the expression of cleaved caspase-1 and the maturation of IL-1β and IL-18. Importantly, intestinal epithelial cell (IEC)-specific knockout of Igk significantly reduced the susceptibility to DSS-induced acute colitis, changed the gut microbial landscape and reduced the abundance of Lachnospiraceae family and Rikenellaceae family. Upon the treatment of broad-spectrum antibiotics to deplete gut microbiota, the disease severity between $Igk^{-/-IEC}$ mice and their littermates tends to be similar. The results suggested that IEC-derived Igk altered the gut microbiota and promoted the progression of colitis and colitis-associated colon carcinogenesis.

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DOI: 10.1002/eji.201970400

P0995

Sodium chloride exacerbates dextran sulfate sodiuminduced colitis by tuning proinflammatory and antiinflammatory lamina propria mononuclear cells through p38/MAPK pathway in mice

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Aim: To investigate the influence of high salt on dextran sulfate sodium (DSS)-induced colitis in mice and explore the underlying mechanisms of this effect.
Methods: DSS and NaCl were used to establish the proinflammatory animal model. We evaluated the colitis severity. Flow cytometry was employed for detecting the frequencies of Th1, macrophages and Tregs in spleen, mesenteric lymph node and lamina propria. The important role of macrophages in the promotion of DSS-induced colitis by NaCl was evaluated by depleting macrophages with clodronate liposomes. Activated peritoneal macrophages and lamina propria mononuclear cells(LPMCs) were stimulated with NaCl, and proteins were detected by western blotting. Cytokines and inflammation genes were analyzed by enzyme-linked immunosorbent assay and RT-PCR, respectively.
Results: The study findings indicate that NaCl up-regulates the frequencies of CD11b+ macrophages and CD4+IFN-γ+IL-17+ T cells in lamina propria in DSS-treated mice. CD3+CD4+CD25+Foxp3+ T cells, which can secrete high levels of IL-10 and TGF-β, increase through feedback in NaCl- and DSS-treated mice. Furthermore, clodronate liposomes pretreatment significantly alleviated DSSinduced colitis, indicating that macrophages play a vital role in NaCl proinflammatory activity. NaCl aggravates peritoneal macrophage inflammation by promoting the expressions of interleukin (IL)-1, IL-6 and mouse inducible nitric oxide synthase. Specifically, high NaCl concentrations promote p38 phosphorylation in lipopolysaccharide- and IFN-γ-activated LPMCs mediated by SGK1.
Conclusion: Proinflammatory macrophages may play an essential role in the onset and development of NaCl-promoted inflammation in DSS-induced colitis. The underlining mechanism involves up-regulation of the p38/MAPK axis.

P0996

Intestinal lamina propria CD4$^+$T cells promote bactericidal activity of macrophages via galectin-9 and Tim-3 interaction during Salmonella typhimurium infection

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The intestinal immune system is crucial for protection from pathogenic infection and maintenance of mucosal homeostasis. Here, we studied the intestinal immune microenvironment in a *Salmonella typhimurium* intestinal infection mouse model. Intestinal lamina propria macrophages are the main effector cells in innate resistance to *intracellular* microbial pathogens. We found that *S. typhimurium* infection augmented Tim-3 expression on intestinal lamina propria CD4$^+$T cells and enhanced galectin-9 expression on F4/80$^+$CD11b$^+$ macrophages. Moreover, CD4$^+$T cells promote the activation and bactericidal activity of intestinal F4/80$^+$CD11b$^+$ macrophages via the Tim-3/galectin-9 interaction during *S. typhimurium* infection. Blocking the Tim-3/galectin-9 interaction with a-lactose significantly attenuated the bactericidal activity of intracellular *S. typhimurium* by macrophages. Furthermore, the Tim-3/galectin-9 interaction promoted the formation and activation of inflammasomes, which led to caspase-1 cleavage and IL-1b secretion. The secretion of active IL-1b further improved bactericidal

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DOI: 10.1002/eji.201970400

activity of macrophages and galectin-9 expression on macrophages. These results demonstrated the critical role of the crosstalk between $CD4^+$T cell and macrophages, particularly the Tim-3/galectin-9 interaction, in antimicrobial immunity and the control of intestinal pathogenic infections.

P0997

Bifidobacterium alters gut microbiota and modulate the functional metabolism of regulatory T cells in the context of CTLA-4 blockade

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There is a great deal of interest and excitement recently in understanding the mechanism of probiotics in modulating immune checkpoint therapy-induced toxicity. Our work provides insights on the mechanism of the immunomodulatory function of a commonly used probiotics, *Bifidobacterium*, in CTLA-4-induced gut immunopathology. We find that *Bifidobacterium* ameliorate colitis in CTLA-4 blockade condition by altering the composition of gut microbiota, a Treg cell-dependent process. We further identify colitis-ameliorating strains of both *Bifidobacterium* and *Lactobacillus* species. The altered microbiota by *Bifidobacterium* enhance the suppressive function and mitochondrial metabolism of intestinal Treg cells, in-turn maintaining immune homeostasis. Our findings have implications in the regulatory mechanism for probiotics in immune checkpoint-induced adverse events.

P0998

Cholinergic modulation of anti-bacterial innate immune responses in the intestinal mucosa

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We have previously demonstrated that cholinergic stimulation by a specific and irreversible inhibitor of acetylcholinesterase (AChE), paraoxon, improved survival in mice following an oral infection with virulent *Salmonella enterica* serovar Typhimurium (*S. typhimurium*). However, paraoxon is an organophosphorous compound unsuited for human use. In this study, we investigate the efficiency of rivastigmine, an FDA-approved inhibitor of AChE, on murine mucosal defenses against the same strain of *Salmonella*. BALB/c mice received daily subcutaneous injections of saline (control) or rivastigmine (2 mg/kg) for 3 weeks. At the end of treatment, half of the animals in each group were sacrificed and the other half infected with SL1344, a virulent strain of *S. typhimurium*, and then either sacrificed three days later or followed for survival. Our results showed that at day 3 post-infection, bacterial loads in rivastigmine-treated animals were lower in systemic organs (spleen and liver) but higher in the intestinal content and feces compared to saline group. Rivastigmine induced degranulation of goblet and paneth cells in the intestine of treated-animals. Immunohistochemical staining demonstrated a significant increase in the thickness of mucin layer in rivastigmine-treated mice. Moreover, rivastigmine rendered mice more resistant to SL1344 infection. These data suggest that cholinergic stimulation with rivastigmine increases the defense mechanisms at the level of the intestinal mucosa, inhibiting bacterial translocation from intestine to systemic organs which leads to enhanced protection against a lethal bacterial infection.

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DOI: 10.1002/eji.201970400

P0999

The tyrosine kinase Tec regulates Th17 cells in the intestine

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CD4$^+$T helper cells are divided into specialized subsets. Among these subsets, Th17 cells secrete the hallmark cytokine IL-17A and mediate host defense against pathogenic extracellular bacteria and fungi. Under physiologic conditions, Th17 cells reside at mucosal sites like the intestinal lamina propria, where they function as a first line of defense.
Here we show that tyrosine kinase expressed in hepatocellular carcinoma (Tec) regulates intestinal Th17 cells under homeostatic conditions and in a T-cell driven colitis model. Our data implicate Tec as a modulator of *in vivo*Th17-cell plasticity and as a rheostat of inflammatory Th17-driven conditions.

P1000

Mechanisms of immune defense induced by cholinergic stimulation in the intestinal mucosa

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The intestinal mucosa plays a crucial role in preventing microbial infections using mechanical, chemical and cellular mechanisms that control the entry and dissemination of pathogenic microorganisms. The mucosal innate immune system represents the first line of defense against microorganisms that invade the intestinal tract. Our previous findings demonstrated that an inhibitor of acetylcholinesterase (AChE) improves the response to oral infection by virulent *S. typhimurium*. This highlights an important link between the cholinergic nervous system and the immune response to infection in the gastro-intestinal tract. In this study, we investigated the underlying mechanism(s) for the enhanced protection at the intestinal barrier. Histological analysis revealed that at the mechanical level, cholinergic stimulation induced a thickening of the luminal mucin layer and, at the cellular level, a redistribution of lymphoid cells within the intestinal mucosa. A more detailed study of the different immune cell populations present in the two intestinal mucosal compartments, lamina propria and epithelium, was performed by multi-color flow cytometry. The findings indicate robust alterations in cell composition as a result of increased cholinergic stimulation. Our data demonstrate the modulatory effect that the cholinergic pathway can exert on the innate immune system at the intestinal mucosal barrier.

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DOI: 10.1002/eji.201970400

P1001

A novel role of IL-22 in regulating epithelial MHC molecule expression and antigen presentation during viral infection

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We discovered that IL-22 is a potent Endoplasmic Reticulum (ER) stress suppressor in respiratory epithelial cells (RECs). However, locally elevated IL-22 during respiratory viral infection could increase viral replication by promoting epithelial specific viral protein synthesis via dampening the stress pathways. Supporting our hypothesis, recombinant IL-22 (rIL-22) treatment increased viral load in HeLa cells infected with human Respiratory Syncytial Virus and Human Rhinovirus *in vitro*. The increased viral load was associated with decreased expression of ER stress markers, *Grp78* and spliced *Xbp1*. In the high-dose pneumovirus of mouse (PVM) neonatal infection model, rIL-22 administration prior to infection led to ~80% mortality before 6 day-post-infection (dpi). Similarly, during low-dose PVM infection, rIL-22 treatment increased epithelial specific viral load at 3 dpi and exacerbated lung pathology at 10 dpi. In the same model, in the absence of IL-22 (IL-22 neutralisation or using IL-22RA-deficient mice) there was a reduction in viral load and lung injury at 10 dpi. RNA-seq analysis was conducted on sorted RECs in PVM infected mice. Gene-ontology analysis revealed that genes involved in protein processing in ER related to antigen processing/presentation were upregulated in RECs in the absence of IL-22. We demonstrate a direct role of IL-22 in modulating MHC class I/II expression on RECs. Our data suggests that blocking endogenous IL-22 could limit viral replication and immunopathology by promoting viral antigen processing, upregulation of viral antigen presentation in RECs. IL-22 can be therapeutically manipulated at appropriate times to help limit respiratory viral infection.

P1002

Loss of PKM2 in Lgr5^{+} Intestinal stem cells promotes colitis-associated colorectal cancer

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The regulatory properties of pyruvate kinase M2 isoform (PKM2), the key glycolytic enzyme, influence altered energy metabolism including glycolysis in cancer. In this study, we found that PKM2 was highly expressed in patients with ulcerative colitis or colorectal cancer (CRC). We then investigated the effectiveness of conditionally ablating PKM2 in Lgr5^{+} intestinal stem cells (ISC) using a mouse model of colitis-associated CRC (AOM plus DSS). Tamoxifen-inducible Lgr5-driven deletion of PKM2 in ISC (PKM2$^{\Delta Lgr5}$-Tx) significantly promoted tumor incidence and size in the colon and lower body weight

DOI: 10.1002/eji.201970400

compared with findings in vehicle-treated mice (PKM2$^{\Delta Lgr5}$-Veh). Histopathologic analysis revealed considerable high-grade dysplasia and adenocarcinoma in the colon of PKM2$^{\Delta Lgr5}$-Tx mice while PKM2$^{\Delta Lgr5}$-Veh mice had low- and high-grade dysplasia. Of note, loss of PKM2 was associated with dominant expression of PKM1 in Lgr5^{+} ISC and their progeny cells. Further, the organoid-forming efficiency of whole cancer cells or Lgr5^{+} cells obtained from colon polyps of PKM2$^{\Delta Lgr5}$-Tx mice was significantly increased when compared with PKM2$^{\Delta Lgr5}$-Veh mice. Cancer organoids from PKM2$^{\Delta Lgr5}$-Tx mice exhibited increased oxidative phosphorylation and a shift of metabolites involved in energy metabolism. These findings suggest that loss of PKM2 function in ISC promotes colitis-associated CRC. We therefore propose that alteration of the glycolytic enzyme in ISC for therapeutic strategies targeting colitis-associated CRC must be approached with caution.

P1591

Microanatomy of gut-associated lymphoid tissues in ulcerative colitis studied by mass cytometry and imaging mass cytometry

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Little is known of the features of the gut-associated lymphoid tissues (GALT) in the inflammatory bowel diseases (IBD): Crohn's disease (CD) and ulcerative colitis (UC), though interestingly, the appendicectomy at a young age has been indicated to be protective of UC development later in life. We investigated immune cell phenotypes in GALT in UC patients compared to healthy individuals by mass cytometry (CyTOF) and the microanatomy of GALT in appendix in UC and healthy controls by imaging mass cytometry (IMC). With these single cell analysis methods, we observed several significant differences between GALT in health and in UC. We observed a consistent proportionate enrichment of the CD27^{-}IgD^{-} non-conventional memory B cell subset in GALT and reduction in blood together with a lack of normal germinal centre structure and B cell/T cell zonation in GALT in UC. Whereas the ratio of CD4:CD8 T cells was unaltered in UC compared to health, an increase in the proportion of naïve CD8 T cells was observed in UC. Subepithelial dendritic cells were more abundant in UC compared to controls. Our results highlight a potentially important role of GALT in IBD and further studies may identify potential new targets for the development of therapeutics.
Funded by Crohn's and Colitis UK, KC Wong Education Foundation and SHMCHFP Science Foundation (20174Y0224)

P1592

Non-hematopoietic STAT6 induces epithelial tight junction dysfunction and promotes intestinal inflammation and tumorigenesis

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Enhanced gut permeability due to dysregulated epithelial tight junction is often associated with inflammatory bowel diseases (IBD), which have greater risk for developing colorectal cancer. STAT6 activation was detected in inflamed colonic epithelium of active IBD patients, suggesting a role of

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DOI: 10.1002/eji.201970400

epithelial STAT6 in colitis development. Here, we demonstrated that non-hematopoietic STAT6, but not hematopoietic STAT6, induced DSS-induced colitis and subsequent tumorigenesis. This could be due to the enhancing-effect of STAT6 on gut permeability and microbiota translocation via interruption of epithelial tight junction integrity. Mechanistically, long myosin light chain kinase (MLCK1) was identified as a target of STAT6, leading to epithelial tight junction dysfunction and microbiota-driven colitis. Furthermore, neutralization of IL-13, which was primarily derived from type 2 innate lymphoid cells (ILC2) in a microbiota-dependent way, inhibited epithelial STAT6 activation and improved gut permeability and DSS-induced colitis. Importantly, pharmacological inhibition of STAT6 reduces murine intestinal tumor formation, and tumoral p-STAT6 levels positively correlated to the clinical stage and poor prognosis of human colorectal cancer. Thus, our study reveals a direct role of STAT6 in the disruption of epithelial tight junction integrity and colitis development, and suggests STAT6 as a potential therapeutic and prophylactic target for IBD and colitis-associated cancer.

P1593

Type 2 inflammation suppression by T regulatory cells attenuates decrease the eosinophil recruitment in mucosa of chronic sinusitis

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Type 2 inflammation predominant and eosinophilic chronic rhinosinusitis with nasal polyps (CRSwNP) prones to recurrence. Our studies aims to determine the roles of Tregs in controlling Type 2 inflammation to inhibit eosinophil filtration in CRSwNP. 134 Nasal polyps, 67 ostiomeatal complex from CRS, and normal nasal tissues from 62 controls were collected to analyze Tregs' subtypes and function, the effective T cell subsets percentages, and the expressions of cytokine profiles via immunofluorescence staining, flow cytometry, RT-qPCR, ELISA, and/or H&E staining. The effects of Tregs on Th2 and Th17 cell were determined in eosinophilic chronic sinusitis (ECRS) mice model. It was confirmed that the CRSwNP displayed the feature of Th2 and Th17 cells-mediated inflammation, accompanying with an increased level of eosinophilic infiltration and the eosinophil cationic protein (ECP), with a decreased frequency of Treg cells. Furthermore, the percentages of $CD4^+CD25^+CD127^{low}$Treg and $CD4^+CD25^+Foxp3^+$Treg were only decreased in the polyps of CRSwNP but not in the paired peripheral blood. The CRSwNP possessed the decreased $Nrp1^+$Tregs and $Helios^+$Treg, and low TGF-β and IL-10 expressions in Tregs. The ECRS mice showed similar inflammatory characteristics to CRSwNP patients. The adoptive transfer of $CD4^+CD25^+Foxp3^+$ Treg cells significantly decreased the inflammatory cytokines, eosinophilic chemotactic factors in the mucosa of the ECRS mice without alteration of the immunity balance in the peripheral blood and spleen. In conclusion, CRSwNP showed high Th2 and Th17 inflammation and defective Tregs. The iTreg may correct the imbalance between immune tolerance and effect via limiting the recruitment of eosinophil of mucosa in CRS.

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DOI: 10.1002/eji.201970400

P1594

IL-19 Up-regulates mucin 5AC production in patients with chronic rhinosinusitis via STAT3 pathway

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The mucin gene MUC5AC is highly expressed both in chronic respiratory inflammatory diseases and inflammatory bowel disease where mucin secretion is regulated by members of the interleukin IL-20 subfamily. This study was conducted to determine the roles and mechanisms of IL-19, a member of the IL-20 subfamily, in regulating MUC5AC production in chronic rhinosinusitis (CRS). We analyzed the expression of mucin and MUC5AC in the nasal mucosa of patients with CRS by periodic acid Schiff (PAS) staining and immunohistochemical examination. Real-time quantitative PCR, ELISA, confocal microscopy and western blotting were used to measure MUC5AC expression in primary human nasal epithelium cells (PHNECs) stimulated with recombinant human IL-19 (rhIL-19), IL-19 receptor siRNA transfection or control. The involvement of the STAT3 signaling pathways was examined by the use of cryptotanshinone (CRY, an inhibitor of STAT3). Mucin and MUC5AC were significantly increased in mucosa of CRS with/without nasal polyps compared to mucosa isolated from control who had no CRS, but there were no significant differences between these two groups. Pretreatment with rhIL-19 up-regulated the expression of MUC5AC levels in PHNECs. Knockdown of IL-20R2 and pretreatment with CRY attenuated MUC5AC production induced by rhIL-19. We propose that IL-19 up-regulates MUC5AC-induced mucin production via the STAT3 pathway in CRS, highlighting the important role IL-19 may play in mucin production in chronic respiratory diseases.

P1595

Batf2 differentially regulates tissue immunopathology in type 1 and type 2 diseases

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Basic leucine zipper transcription factor ATF-like 2 (BATF2) belongs to a family of transcription factors critical in sterile and infectious inflammations. A role was first ascribed to this factor in the development of CD8+ dendritic cells, then a possible role was suggested as an inducer of type-1 inflammatory responses. More recently, this factor has been shown to inhibit immuno-pathological Th17 response during Trypanosoma cruzi infection through the suppression of IL-23. Given its tight association with immune polarization, this factor is increasingly suggested as a druggable target for host-directed therapies to combat infections. In the present report, a role during the most debilitating Human helminth infection, schistosomiasis, became envisageable when we noted that mice responded to the trapped eggs of the parasite by up-regulating the expression of Batf2 in the affected intestinal tissue. Batf2 is shown to be important to limit untoward immune responses and small intestinal fibro-granulomatous inflammation during murine schistosomiasis. Although the Batf2 was found to be

DOI: 10.1002/eji.201970400

crucial during acute schistosomiasis, it was however identified as detrimental to type 1 infections such as Mycobacterium tuberculosis (Mtb) and Listeria monocytogenes. This study therefore report on a regulatory role of Batf2 on the host immune responses to Type 2 (schistosomiasis) and Type 1 (TB and listeriosis).

P1596

High-dimensional mass cytometry reveals the immune cell landscape in untreated inflammatory bowel disease

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Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the intestine. IBD studies have shown alterations in the cellular immune system. However, a comprehensive analysis of the cell composition in biopsies from patients across all major immune lineages simultaneously was lacking. In IBD-suspected patients, we took paired biopsies (N=142) from ileum and colon (both inflamed and uninflamed mucosa) and blood samples in 38 patients and 23 controls. Single-cell suspensions were analyzed with a 36-mass cytometry antibody panel, and the dataset was analyzed with Hierarchical tSNE.
Altogether, we identified 309 cell clusters from the intestinal dataset containing 3.4 million cells in a data-driven manner. Controls clustered separately from patients, ileums separate from colons and affected segments separate from unaffected segments. However, affected samples from the different subgroups of IBD (Crohn's disease and ulcerative colitis) were mostly intermixed. We observed a considerable interindividual variation, indicative of unique individual 'immune fingerprints'. Also, 21 subsets were significantly different between IBD-affected samples and IBD-unaffected samples/controls. Several CD4+ T cell clusters correlated with myeloid cell and NK cell clusters and were upregulated in IBD-affected segments, while CD8+ T cell and TCRgd cell clusters were upregulated in unaffected samples. Finally, disease-associated CD4+ T cells were detectable in blood. Our study provides evidence that a coordinated cellular network of both innate and adaptive immune cell types is implicated in IBD. Together with the evidence for the unique individual-specific composition of the intestinal immune system, this may aid in the development of more effective and personalized patient care.

P1597

Repeated antigen painting induces tolerogenic cells in sublingual mucosa

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Sublingual immunotherapy (SLIT) applied for the allergen-specific immune suppression is performed by repeated antigen (Rpt Ag)-painting to sublingual mucosa (SLM). Previously we found that Rpt Ag-painting to SLM exhausted typical $CD11c^+$ DC and increased round-type macrophage-like $CD206^{hi}$ cells. In order to study whether $CD206^+$ cell dominant status induced by Rpt Ag painting are related to T cell suppression, we examined T cell responses after FITC painting to sublingual mucosa to induce $CD206^{hi}$ cells, and painted OVA to induce OVA-specific T cell responses. Rpt Ag-painting inhibited OVA-specific $CD4^+$ T cell responses and reciprocally enhanced the ratio of both IL-10^+ cells and $Foxp3^+$ Tregs in regional lymph nodes. These results imply that $CD206^{hi}$ cell dominant status induces

DOI: 10.1002/eji.201970400

suppression of Ag-specific T cell responses. In order to know how CD206^{hi} cells suppress T cell immune responses, we investigate the phenotypic and genetic characters of CD206^{hi} cells. CD206^{hi} cells showed CD11b^{+}, MHCII^{lo}, CD86^{lo}, PD-L1^{hi}, Gr-1^{int}, Ly6C^{+}, and CD64^{hi} phenotype, suggesting that antigen presenting capacity of CD206^{hi} cell is limited compared to conventional DCs. Microarray analysis revealed CD206^{hi} cells preferentially expressed molecules related to immune homeostasis and tolerance, especially B7 family of immune checkpoint molecules. In addition, several M2-related genes were highly detectable in these CD206^{hi} cells. These results suggest that CD206^{hi} cells induced by Rpt Ag-painting may contribute to oral mucosal tolerance.

P1598

TNFR1 facilitates necroptotic cell death in type 2 diabetes mellitus mice alveolar macrophages infected with Mycobacterium tuberculosis

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Previously, we developed a mouse model of type 2 diabetes mellitus (T2DM) using streptozotocin and nicotinamide and found that T2DM mice are susceptible to *Mycobacterium tuberculosis* (*Mtb*) infection. We also found that alveolar macrophages from T2DM mice were more permissive to *Mtb* growth ex vivo compared to non-diabetic controls. In the current study, we determined the defective mechanisms that make T2DM mice alveolar macrophages more susceptible to Mtb infection. Mtb infected alveolar macrophages from T2DM mice produced more TNF-α (973.8 ± 13.3 pg/ml vs. 614.6 ± 27.2 pg/ml) and less apoptotic (4.7 ± 1.8% vs. 28.6 ± 2.3%) compared to Mtb infected non-diabetic control mice. Mtb infected alveolar macrophages from T2DM mice expressed higher levels of TNFR1 (12.8 ± 0.5 vs. 2.09 ± 0.01) and markers of necroptosis RIPK1 (11.5 ± 0.57 vs. 2.94 ± 0.20), RIPK3 (16.1 ± 0.58 vs. 2.89 ± 0.2) and MLKL (9.2 ± 0.56 vs. 1.58 ± 0.72) compared to Mtb infected alveolar macrophages from non-diabetic control mice as determined by real-time PCR (confirmed by Western blot and confocal microscopy). Anti-TNFR1 antibody treatment of alveolar macrophages from T2DM mice before or after Mtb infection reduced RIPK1, RIPK3 and MLKL expression and reduced the bacterial burden in in vitro. Our findings demonstrate that T2DM induces TNFR1 mediated necroptosis of alveolar macrophages (CD11c+F4/80+ cells) upon Mtb infection. Studies are underway to determine the in vivo relevance of our current findings to Mtb growth in T2DM mice.

P1599

Antigen-specific tissue-resident memory T cells in respiratory system were generated following intranasal vaccination of mice with BCG

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Tissue resident memory T cells (Trm) are different from effector memory T cells (Tem) and central memory T cells (Tcm) and contribute to the protective immunity against local challenges. Currently, we found that CD4^{+} and CD8^{+} Trm cells in nasal mucosal, trachea, lungs and lavage fluids were heterogeneous on the expression of CD69 and CD103 as well as the production of cytokines including IFN-γ and IL-17. After intranasal vaccination of mice with BCG, antigen-specific CD4^{+} and CD8^{+} Trm cells expressed cytokines following the stimulation with BCG and persisted for more than hundred days in nasal, trachea and lungs. At the same time, mice were infected intranasally with live BCG, which showed that vaccinated mice cleared up live BCG faster than non-vaccinated mice in the

DOI: 10.1002/eji.201970400

respiratory system. Taken together, our data demonstrated that intranasal vaccination of mice with BCG could induce antigen-specific $CD4^+$ and $CD8^+$ Trm cells in respiratory system and have the ability to provide protection against pulmonary reinfection.

P1600

Control of colonic stem cells and their niche by high-fat-diet

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Dietary signals are known to modulate stemness and tumorigenicity of intestinal progenitors. Previous other studies demonstrated that high-fat-diet (HFD) enhanced numbers of $Lgr5^+$ intestinal stem cells (ISCs) and further promoted primary and metastatic colon cancer. However, little is known how obesity affects ISCs niche which might be involved in increasing tumorigenesis of colon. In this study, we found that histopathological scores were enhanced in the colon of HFD-fed mice in comparison to those of purified diet (PD)-fed one in a steady state. We confirmed that HFD feeding resulted in highly enhanced susceptibility to AOM-DSS-induced colorectal cancer. Since deep crypt secretory cells, one of the niches supporting ISCs maintenance in the colon, were not altered by HFD feeding, we next focused on the function of mesenchymal cells (MSCs). Of note, predominant growth of colon organoids was shown in the presence of MSCs isolated from HFD-fed mice in comparison to those from PD-fed mice. The fact that Wnt2b secretion by colon PDGFR-α^+ MSCs were significantly higher in HFD-fed mice than those in PD-fed mice, indicating that MSCs might play a crucial role on tumorigenesis in the colon of obese mice. Importantly, growth of organoids obtained from normal and cancer ISCs were activated in the presence of HFD-derived MSCs. Furthermore, HFD-induced free-fatty-acid (FFA) was indispensable factor to promote colon organogenesis. Taken together, these results demonstrate that colon PDGFR-α^+ MSCs and FFA might be critically involved on modulation of cancer stem cells in obesity.

P1601

Cross-talk between mucin degrading bacteria and intestinal stem cells controls epithelial development

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Mucin degrading bacteria are closely located in intestinal epithelium; however, how they associate differentiation and development of intestinal stem cells (ISCs) is not elucidated. To address an exact role of mucin degrading bacteria on the gut homeostasis, mice were fed with *Akkermansia muciniphila*, which is specialized to mucin degradation. We found that administration of *A. muciniphila* increased thickness of mucus layer, paradoxically. Mice fed *A. muciniphila* showed enhanced self-renewal and differentiation of $Lgr5^+$ ISCs, and increased numbers of lysozyme$^+$ Paneth cells and $Muc2^+$ goblet cells. Wnt3-related signaling was more expressed in crypt region of the small intestine of mice fed *A. mucinphila*. In addition, numbers of $Lgr5^+Ki67^-$ cells in crypt region, which might differentiate to secretory lineage cells, were signifciantly increased in mice fed *A. muciniphila* in comparison with control mice. Of note, higher levels of acetate and propionate were found in cecum contents of mice fed *A. muciniphila* than control mice. *Ex vivo* study revealed that organoids treated with cecum contents obtained from *A. muciniphila*-fed mice exhibited larger size and these effects were diminished by co-culture with Gpr41/43 antagonists. These findings suggest that mucin degrading bacteria *A.*

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DOI: 10.1002/eji.201970400

muciniphila boosts the numbers and regeneration capacity of ISCs using their metabolites and contributes to maintain intestinal homeostasis.

P1602

Sox8 is essential for M-cell maturation to accelerate IgA response at the early stage after weaning in mice

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Microfold (M) cells residing in the follicle-associated epithelium (FAE) of the gut-associated lymphoid tissue are specialized for antigen uptake to initiate mucosal immune responses. The molecular machinery and biological significance of M-cell differentiation, however, remain to be fully elucidated. Here, we demonstrate that Sox8, a member of the SRY-related HMG box transcription factor family, is specifically expressed by M cells in the intestinal epithelium. The expression of Sox8 requires activation of RANKL-RelB signaling. Chromatin immunoprecipitation and luciferase assays revealed that Sox8 directly binds the promoter region of *Gp2* to increase *Gp2* expression, which is the hallmark of functionally mature M cells. Furthermore, genetic deletion of *Sox8* causes a marked decrease in the number of mature M cells, resulting in reduced antigen uptake in Peyer's patches. Consequently, juvenile *Sox8*-deficient mice showed attenuated germinal center reactions and antigen-specific IgA responses. These findings indicate that Sox8 plays an essential role in the development of M cells to establish mucosal immune responses.

P1603

Osteoprotegerin-dependent M cell self-regulation balances the gut immunity and infection

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Microfold (M) cells are responsible for antigen uptake to initiate intestinal immune responses in gut-associated lymphoid tissue (GALT). Receptor activator of nuclear factor-kappa B ligand (RANKL) is essential for the differentiation and the function of M cells. Although follicle-associated epithelium (FAE) covering GALT is continuously exposed to RANKL from stromal cells in the subepithelial dome region, only a subset of epithelial cells in FAE differentiates into M cells (5-10%). This fact suggests that the M cell differentiation in FAE is restricted. However, the molecular machinery restricting the M-cell number as well as its physiological significance are not clear. Here, we show that M cells express osteoprotegerin (OPG), a soluble decoy receptor of RANKL, to suppress the differentiation of adjacent FAE cells into M cells. OPG deficiency increased the number of M cells in FAE, and enhanced commensal-specific immunoglobulin production. Concordantly, the ablation of OPG ameliorated disease symptoms (i.e. diarrhea and bloody stool) in DSS-induced colitis. Meanwhile, the OPG-deficient mice were highly susceptible to infection with *Salmonella*, which invades host tissue through M cells. Thus, the OPG-dependent self-regulation of M-cell differentiation is essential for maintaining the equilibrium between infectious risk and immunosurveillance on the mucosal surface.

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DOI: 10.1002/eji.201970400

P2181

γδT Cells contribute to th2-biased inflammatory profiles in eosinophilic chronic rhinosinusitis with nasal polyps

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Background: Eosinophilic chronic rhinosinusitis with nasal polyps (ECRS) is a condition linked with skewed T-helper 2 (Th2) polarization, poor treatment outcomes, and high recurrence tendency. Although γδT cells have been reported to induce Th2-type immune responses and eosinophilic infiltration in several diseases, their role in ECRS has not been fully explored. We aimed to evaluate the association of γδT cells with the Th2-biased inflammatory profiles in ECRS.
Methods: Tissue nasal samples obtained from patients with chronic rhinosinusitis with nasal polyps (CRSwNP) (51 eosinophilic and 48 non-eosinophilic), 50 patients with chronic rhinosinusitis without nasal polyps (CRSsNP), and 58 control subjects were examined for γδT cells, inflammatory markers and eosinophils using HE, RT-qPCR, ELISA, Immunofluorescence and flow cytometry. In parallel, studies were also conducted in an ECRS murine model induced by anti-γδT cells neutralizing antibody administration.
Results: γδT cells expression was significantly increased in tissues from patients with ECRS compared with non-ECRS, CRSsNP and control subjects. Moreover, inflammatory markers including Th2 proinflammatory cytokines (IL-4, IL-5, IL-13), GATA3, eosinophil cationic protein (ECP), eotaxin levels were also increased in nasal tissues of patients with ECRS and Vγ1$^+$ γδT cells mRNA expression was positively correlated with Th2 cytokines, GATA3 and ECP. In the ECRS murine model, anti-Vγ1$^+$ γδT antibody treatment reduced the infiltration of eosinophils and expression of Th2 cytokines, GATA3 and ECP in nasal mucosae.
Conclusion: The results of this study suggest that γδT cells play a crucial role in the Th2-biased inflammatory profiles and eosinophilic nasal tissue accumulation in patients with ECRS.

P2182

Origin-dependent functional heterogeneity of the TCRαβ$^+$ CD8αα small intestinal intraepithelial lymphocytes

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According to the canonical model, TCRαβ intraepithelial lymphocytes are divided into two main types, A and B, induced and natural IELs respectively. The “activated yet resting” phenotype indicates that polarization towards both groups requires encounter of the cognate antigen by the type A and B IEL progenitors (IELp). It is generally accepted that CD8αα (natural) IELs differentiate directly from CD4+CD8αβ+ CD8αβ+ (TP) thymocytes, which TCRs recognized self MHC/peptide complexes with high affinity but avoided deletion through downregulation of co-receptors (CD4-CD8-TCRαβ+) to become postselected DN IELp. However, a possible alternative, TCR-dependent, developmental pathway caused by engagement of microbial antigens was recently described for CD8αα IEL. In this

DOI: 10.1002/eji.201970400

work we are addressing mechanisms underlying peripheral recruitment of the TCRαβ+ CD8αα IELs from peripheral CD4, and CD8αβ T cells. Our data indicate on possible origin-dependent imprint in the gene expression profiles, and origin-dependent functional specialization of the IELs developed from two different types of peripheral T cells (CD4 or CD8αβ). Thus, based on these data, we hypothesize that the origin of CD8αα IEL might dictate the scope of action and interaction mechanisms of these cells with small intestinal flora in the process of maintaining the mucosal barrier.

P2183

The role of alpha 2-macroglobulin in the low-grade inflammation of Irritable Bowel Syndrome

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The Irritable Bowel Syndrome (IBS) is characterized by a low-grade inflammation where increased mucosal mast cell activation play a central role. Alpha-2 macroglobulin (A2M) is an inflammatory regulator that inhibits protease activity and binds IL-6 and IL-8, affecting its bioavailability and function. The role of A2M in IBS has not being explored before.
Objective: To determine differences in A2M plasma level between IBS´s patients and control subjects (CS) and its correlation with inflammatory response and mast cell´s number at intestinal mucosa.
Methods: Patients with IBS (Rome III) (n=25, 14 SII-D; 6 SII-C; 3 SII-M; 2 SII-I) and SC (n=18) were assessed for A2M, IL-6 and IL-8 plasma level by ELISA; and mast cell's number at colonic mucosa by immunofluorescence microscopy. Statistics: Mann-Whitney/ANOVA; Spearman correlation; significance $p< 0.05$.
Results: A significant increase of A2M, IL-6 and IL-8 plasma level was observed in patients with IBS vs CS (A2M: IBS:2127±107, CS:1832±163pg/ml, $p< 0.0001$; IL-6: IBS:2.350±2.198, CS:0.808±0.724pg/ml, $p< 0.0074$; IL-8: IBS:10.24±4.221, CS:7.167±5.136pg/ml, $p< 0.0209$); as well as the number of colonic mast cells (IBS:17.40±10.39; HC:11.94±6.54 mast cell/hpf, $p=0.0474$). There was no correlation between the A2M and IL-6, IL-8 and mast cell's number.
Conclusion: The elevated levels of A2M, inflammatory cytokines and mast cell number observed in IBS´s patients suggest a activated immune response process which is controlled by an A2M independent mechanism. Future studies aimed at evaluating the role of A2M in the low-grade inflammation in IBS will allow us to propose this mechanism in the pathogenesis of IBS. FONDECYT Nº1181699

P2184

TRAIL suppresses gut inflammation and inhibits colitogenic T cell activation in experimental colitis via an apoptosis-independent pathway

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DOI: 10.1002/eji.201970400

Background: TRAIL induces cell apoptosis by transducing apoptosis signals. Although the actual biological role of TRAIL remains to be elucidated, recent accumulating evidence demonstrated that TRAIL regulates autoimmune inflammation and immune cell homeostasis, suggesting a novel immunoregulatory role of TRAIL in autoimmune diseases. The purpose of this study was to address the therapeutic effects and immunoregulatory role of TRAIL in autoimmune gut inflammation.
Methods: TRAIL was administered to mice with dextran sodium sulfate (DSS)-induced colitis to explore possible roles of TRAIL in the development and pathogenesis of autoimmune colitis and its therapeutic potential in inflammatory bowel disease. TRAIL-treated colitogenic T cells from wild-type or TRAIL-receptor (TRAIL-R) KO mice were further adoptively transferred into Rag1 KO mice to induce autoimmune colitis.
Results: TRAIL significantly suppressed gut inflammation and reduced the severity of colitis in a DSS-induced colitis model, and the TRAIL-mediated anti-inflammatory effects were dependent on interactions with the TRAIL-R. Furthermore, the suppression of gut inflammation was not due to induction of apoptosis in colonic T cells, dendritic cells, or epithelium cells by TRAIL. In contrast, TRAIL inhibited activation of colitogenic T cells and development of gut inflammation in an adoptive transfer-induced colitis model. The anti-inflammatory effects of TRAIL on colitis were abolished when T cells from TRAIL-R KO mice were adoptively transferred, suggesting that TRAIL regulates autoreactive colitogenic T cell activation in the development of gut inflammation.
Conclusion: TRAIL effectively inhibited colonic T activation and suppressed autoimmune colitis, suggesting a potential therapeutic application of TRAIL in human inflammatory bowel disease.

P2185

Impact of Fms-like tyrosine kinase 3 ligand (Flt3L) on *Chlamydia*-specific immunity

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Wetested the hypothesis that the impact of the Fms-like tyrosine kinase 3-ligand (Flt3L; FL) on recombinant *Vibrio cholerae*ghost(rVCG) vaccine-induced chlamydial immunity is influenced by route of vaccine delivery. Female mice were immunized rectally (IR) or intramuscularly (IM) with a rVCG-based *Chlamydia* vaccine with and without FL or control.Vaccine evaluation revealed high levels of CD4+ T cell-mediated and humoral immune responses were elicited in mice, irrespective of route of vaccine delivery. Unexpectedly, co-administration of vaccine with FL enhanced specific Th1-type cytokine levels and T cell proliferation following IR but not IM immunization. While administration of vaccine with FL enhanced IgA antibody responses following both immunization routes, IgG2c responses were not enhanced following IR delivery. The vaccine-induced immune effectors protected mice against live *Chlamydia* infection irrespective of route of vaccine administration, with the IR-delivered FL regimen having a protective advantage. Further evaluation showed protection afforded by the FL-adjuvanted vaccine was facilitated by CD4+ T cells, as indicated by reduction in the intensity and duration of genital chlamydial shedding by naïve mice following adoptive transfer of immune CD4+ T cells.Taken together, comparable protective immunity, which is enhanced by co-delivery with FL, is elicited in the female genital tract after mucosal and systemic administration, highlightingthe effective immunostimulatory function of FL at both mucosal and systemic sites. However, the differential modulation of protective immunity afforded by IR-delivered FL-adjuvanted vaccine indicates the immunomodulatory impact of FLon chlamydial-specific immunity is influenced by route of vaccine administration.

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DOI: 10.1002/eji.201970400

P2186

The gut microbiome-mediated proinflammatory macrophages recruitment to promote colon tumorigenesis

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Colorectal cancer (CRC) represents the one of the most common cancer in the world. Emerging evidence suggests that microbial dysbiosis is involved in the development of CRC, while the potential mechanisms that orchestrate microbiota-driven chronic inflammation fostering tumorigenesis remain unclear. We found the gut microbiome could inducing a pre-cancerous inflammatory milieu, with an increasement of proinflammatory macrophages and enhanced secretion of inflammatory cytokines. Mechanistically, proinflammatory macrophages accumulation was regulated by CCL2 expression, which was controlled by the microbiome-mediated Toll-like receptor (TLR) 4 activation in epithelial cells. Bacteria-derived lipopolysaccharides were considered as a trigger for CCL2, as TLR4 signaling inhibition or antibiotic treatment notably influenced its level and reduced tumor load. Our work provided a crosstalk between gut microbiota and immune responses in colon tumorigenesis, and may have possible implications for therapeutic treatment targeting microbiota in the future.

P2187

A single-cell decipher of human uterine leukocytes at early pregnancy

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Immune tolerance at the feto-maternal interface plays a key role in pregnancy maintenance. The failure in immune regulation is tightly associated with abnormal pregnancy outcomes such as recurrent spontaneous abortion (RSA). During pregnancy, multiple maternal immune cells, including decidual NK cells, macrophages, T cells and DC cells, gather in uterine mucosa and form a complex cross-talk network with placental trophoblasts. Despite the pivotal roles of immune tolerance for fetal development in utero, detailed understanding of the cellular subtypes and their molecular characteristics of human uterine leukocytes remains lacking. Here we profile the transcriptomes of nearly 30,000 single leukocytes from first-trimester decidua of normal and RSA pregnancies. A total of 13 different cell subsets with unique molecular properties and spatial distribution at the feto-maternal interface are identified. Further data analysis reveal in-depth recognitions of the roles of human decidual leukocytes in reproductive success, and illustrate a blueprint to reveal the immune etiology of RSA.

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DOI: 10.1002/eji.201970400

P2188

Detection of bacterial DnaK and enolase specific antibodies in serum of patients with chronic rhinosinusitis with nasal polyps (CRSwNP)

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Background: The statuses of antibodies against heat shock protein 70 (HSP70) and enolase in chronic rhinosinusitis (CRS) are unclear yet. We hypothesized that concentrations of antibodies against bacterial HSP70（DnaK）and enolase increased in serum of CRS.
Objective: To detect bacterial DnaK- and enolase- specific antibodies in serum of chronic rhinosinusitis with nasal polyps (CRSwNP).
Methods: Molecular biological and ELISA techniques were employed to obtain recombinant DnaK, enolase of *Staphylococcus aureus* (*S.a*) and *Streptococcus pneumonia* (*S.p*) to measure the existence of specific IgG and IgE antibodies in sera of CRSwNP.
Results: The concentrations of IgG antibodies against bacterial DnaK and enolase were elevated in sera of CRSwNP although elevation of *S.a* enolase IgG was not statistically significant higher than those of controls. The concentrations of IgE against *S.a* DnaK and *S.p* enolase were significantly greater in CRSwNP. Levels of IgG and IgE antibodies against these proteins between the bacterial species were positively correlated to each other. Up-to 60% of antigen-antibody specificity of *S.p* was neutralized by using whole proteins extracts derived from *S.a,* and vice versa, suggesting the high homology of these proteins between different bacterial species.
Conclusions: Our data suggests that the presences of bacterial DnaK- and enolase-specific IgG and IgE antibodies in serum of CRS and of high homology of these proteins between different species raise the possibility that these antigens and antibodies may play important roles in the pathogenesis of the disease through “molecular mimicry”.
Keywords: chronic rhinosinusitis, *streptococcus pneumonia*, *staphylococcus aureus*, HSP70, enzyme

P2189

Classic heterodimeric and single-domain heavy chain immunoglobulin (Ig) A are co-expressed in the Arabian camel

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Adaptation of the humoral immune system of the dromedary has resulted in the unique capacity to simultaneously synthetize 2 variants of IgG, i.e. classic heavy-light-chain heterodimer and single-domain homodimeric heavy chain antibody. Heavy chain variant lacks γ1 domain in the constant region ($C_H\gamma1$) which is required for dimerization with light chains. No data existed with regard to IgA. Using RT-PCR, cloning and sequencing we have observed that in camel third eyelid tissue this peculiarity of adaptive immunity - namely the presence and absence of α1 domain in the constant region ($C_H\alpha1$) - is also present in mucosal IgA. Polyclonal rabbit antibodies were raised against 3 different domains of the constant heavy (C_H) region of IgA, i.e. $C_H\alpha1$, $C_H\alpha2$, and $C_H\alpha3$ and used to perform immunoblots on lacrimal gland tissue and serum of dromedaries. Polyreactive antibodies were visualized with a secondary HRP-conjugated goat anti-rabbit antibody. Two IgA species of estimated

DOI: 10.1002/eji.201970400

Mw 52 and 60 kDa were observed in protein lysates of third eyelids using anti-$C_H\alpha2$ and anti-$C_H\alpha3$ antibody, whereas the 60 kDa species was detected with the anti-$C_H\alpha1$ antibody only confirming co-expression of conventional and homodimeric heavy chain IgA. By contrast, only the higher molecular weight species was circulating in serum. We conclude that beside IgG the dromedary has acquired the distinctive potential to combine and differentially regulate the synthesis of classic as well as heavy chain IgA which seem both required for mucosal immune defense such as immune exclusion and opsonization.

P2190

Human mucosal MAIT cell distribution and its response to bacterial mucosal infection

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Mucosal-associated invariant T (MAIT) cells are innate-like, unconventional T cells that are widely distributed through the human body, including peripheral blood and mucosal surfaces. These cells are restricted to the non-polymorphic, highly evolutionarily conserved MHC class Ib molecule, MHC-class I related protein, MR1. Despite being found in many different tissues in the host, MAIT cells in peripheral blood and MAIT cells found in the mucosal tissues (mucosal MAIT cells) share many similar features. However, a clear understanding of how MAIT cells function and whether the tissues that they reside in play a role in determining their function is still lacking. Therefore, our aim was to investigate MAIT cell distribution and characterize their T cell receptor (TCR) usage of MAIT cells in the gastrointestinal mucosal tissue. Our results provide a fundamental understanding of MAIT cell biology within the human host and how they respond in the presence of a bacterial mucosal infection.

P2191

Evaluation of human T and B cell immune responses in nasopharynx-associated lymphoid tissue (NALT) to live attenuated oral vaccine against rotavirus

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Background: Mucosal vaccines are an effective way of immunization against enteric pathogens. These vaccines have shown to stimulate a local production of antigen-specific antibodies and the development of long-term B and T memory cells promoting both a mucosal and systemic protective immune response. Despite vaccine effectiveness in reducing disease burden, there are significant differences in vaccine efficacy between developed and developing countries, although the immunological mechanisms remain undefined.
Aims: To evaluate the immunogenicity of live attenuated oral rotavirus vaccine using an *ex vivo* cell culture model with human tonsillar tissues, a key mucosal compartment mediating mucosal immunity, and examine the effect of pre-existing immunity on vaccine-induced immune response.

DOI: 10.1002/eji.201970400

Methods: 56 immunocompetent patients referred to adenotonsillectomy were included in this study. Serum levels of rotavirus-specific antibodies were measured by ELISA. Mononuclear cells from tonsil tissues were isolated and stimulated with Rotarix vaccine. T cell proliferation levels and antibody response were measured by flow cytometry and ELISA respectively.
Results: Rotavirus-specific IgG and IgA were detected in serum from recruited patients. A positive correlation was observed between serum rotavirus-specific antibodies and patients age. Rotavirus-specific antibodies were also detected in tonsillar cell supernatants following vaccine stimulation. There was no significant difference in the mucosal B cell antibody production between children and adults, yet a positive correlation was found against serum rotavirus-specific antibody titres. Vaccine stimulation elicited a CD8+ and CD4+ T cell proliferative response in children. Studies are ongoing to analyse the effect of pre-existing immunity on vaccine-induced mucosal T and B cell responses.

P2192

HIV and the colorectal mucosa - investigating the early interactions of HIV with mucosal target cells *in situ*

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There is no vaccine for HIV. Antiretroviral therapy has helped reduce transmission rates, but alone is not enough to combat this significant global health issue. As such, we need to develop strategies to block transmission of the virus to complement current therapies. This requires an in-depth understanding of early viral pathogenesis across the human anogenital tract, of which there is limited data.
In this study we infected human colorectal explants and examined HIV spread within the mucosa within minutes to hours post-infection. To do this we combined RNAscope with high-parameter microscopy to compare HIV-uptake and transfer kinetics across multiple known HIV target cells including Dendritic Cells (DC), Macrophages and T-cells, all in a single tissue section.
Our results show that both DCs and CD4 T-cells are able to take up HIV rapidly, within 30min post-infection, however macrophage involvement does not occur until 2h post-infection. Furthermore, we have observed HIV in association with DC-T cell conjugates with the frequency of these contacts increasing with time. We devised several image analysis algorithms which show that in fact, the majority of HIV resides within DC-T cell conjugates within the mucosa early post-infection. Although previously hypothesised, to our knowledge this is the first demonstration of DC involvement in early viral transfer to T-cells within the mucosa.
Overall, we show that initial HIV dissemination is rapid, involving multiple cell-types which may interact to facilitate viral spread.

P2193

Role of autophagy in *Mycobacterium tuberculosis* growth restriction by kupffer cells

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DOI: 10.1002/eji.201970400

Alveolar macrophages (AMs) are the first cells encountered by TB pathogen and acts as a primary defense against *Mtb* in the lung. Studies have shown that liver macrophages (Kupffer cells; KCs) can control *Mtb* growth but, it is not clear how KCs completely eliminate mycobacterial infections. Thus, identifying these mechanisms will facilitate development of immunomodulatory strategies to boost AM mediated immunity to *Mtb* and also to prevent progression of active disease from latent tuberculosis infection. In the current study, we compared *Mtb* growth and cytokine production in murine AMs and KCs and found three-fold higher CFU in AMs compared to KCs indicating KCs are more efficient in inhibiting *Mtb* growth. Differences in *Mtb* growth restriction were not due to differences in Toll-like receptors 2 and 4 expression, cytokine production, M1/M2 paradigm or apoptosis. Western blot analysis revealed that *Mtb* infected KCs expressed more than double the amount of LC-3B protein than *Mtb* infected AMs. This was confirmed by Flow cytometry. In addition, confocal microscopy imaging indicated increased expression of ATG-5, ATG-7 and Beclin-1 and distinctly enhanced LC-3B puncta formation in *Mtb* infected KCs compared to *Mtb* infected AMs. Later, we found significantly enhanced *Mtb* CFU in KCs transfected with LC-3B, ATG-5, ATG-7 and Beclin-1 siRNA compared to KCs transfected with control siRNA ($p< 0.01$; 0.006; 0.01&0.002) respectively. Our results provide evidence that enhanced autophagy mechanism efficiently restricts *Mtb* growth in KCs. Studies are underway to determine in vivo relevance to our current findings using mouse model of *Mtb* infection.

P2194

Homing receptors on B lymphocytes and plasma cells in human colostrum, and their association with infectious diseases during pregnancy

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Background: Breastfeeding has protective attributes associated with the reduction of the risk of neonatal infections. Colostrum is the first milk secreted after birth, and its rich in many immune factorsand nutrients. Previous studies have shown that the cells in the lactating mammary gland express α4β7 and α4β1 integrins, but there is no clear information about the expression of these adhesion molecules on the surface of B lymphocytes and plasma cells contained in the colostrum. The understanding of the homing of these cells to the mammary gland may be essential to develop prevention strategies to confer newborn protection that could be applied to their mothers during pregnancy. **Aim:** To describe the presence of the homing receptors (integrinsβ7 and β1) on B lymphocytes and plasma cells in colostrum, and their association with infections episodes during pregnancy.
Methods: Cells from the colostrum of thirteen lactating women were collected. The expression of the homing receptors was determined on B cells (CD19+) and plasma cells (CD138+) by flow cytometry.
Results: Six mothers had infectious episodes of the upper tract during pregnancy, and five mothers did not have infections. Women that suffered respiratory diseases during pregnancy had increased expression of the integrinsβ7 and β1 on B lymphocytes and plasma cells.
Conclusion. The results suggest that the presence of respiratory mucosal infections during pregnancy increased the number of B cells with β7 and β1 integrins in human colostrum.
This work was supported by grants from CONACYT (900) and SEP-CINVESTAV (255053).

DOI: 10.1002/eji.201970400

P2955

Dysbiosis contributes to neuronal inflammation through microbial regulation of Th17 cells in the intestine

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Environmental factors are believed to contribute to the increased prevalence of autoimmune diseases, although commensal gut microbiota has been to show multifarious effects on intestinal and extra-intestinal immune homeostasis and accumulating evidence implicates intestinal metabolites as crucial mediators of gut-brain axis cross-talk. however, how intestinal metabolites regulate intestinal immune cells to affect neuronal system is still largely unknown. Here, we found wild type mice cohoused with dysbiotic mice get severer phenotype of experimental autoimmune encephalitis (EAE), a mouse model for neuronal autoimmune disease multiple sclerosis. We also found altered metabolites profile during dysbiosis, among which we identified a significantly upregulated metabolite which promotes Th17 differentiation in vitro, and the deficient cells of the receptor for this metabolite showed impaired Th17 production. Moreover, IL-17A$^{-/-}$ mice cohoused with dysbiotic mice didn't show elevated EAE phenotype, suggested Th17 produced IL17 is critical for the cohoused EAE phenotype. Thus, gut microbiota contributes to neurological disease pathogenesis by producing metabolites to induce pathogenic Th17 cells and promoting proinflammatory cytokine expression.

P2956

A T helper 17 cell specific long non-coding RNA lncT$_H$17-1 regulates T$_H$17 mediated autoimmune pathogenesis

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IL-17-producing T helper 17 (T$_H$17) cells play vital role in host defense against pathogens, including extracellular bacteria and fungi, but also result in autoimmune inflammation, including multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease. While the epigenetic regulation of pathogenic T$_H$17 cell is yet to be resolved. Long non-coding RNA (lncRNA) is one of non-coding RNA family transcripts, with length exceeding 200nt. Recently more and more reports focused on the function of lncRNA in immune system, especially in innate immunity, whereas lncRNA function in adaptive immunity is still largely unknown. Based on RNA-seq screening of different CD4$^+$ T helper cell subsets sorted from reporter mice, including T$_H$1, T$_H$2, T$_H$9, T$_H$17 and T regulatory (Treg) cells. We identified a long non-coding RNA lncT$_H$17-1 is specifically expressed in T$_H$17 cells, and regulated by the IL-6-STAT3 axis. Deficiency of lncT$_H$17-1 decreased T$_H$17 cell differentiation in vitro, but not T$_H$1 and Treg. Meanwhile our in vivo data demonstrated that, compared with WT, deficiency of lncT$_H$17-1 in CD4 T cells ameliorate mouse colitis post CD45RB^{hi} naïve CD4 T cells transfer into Rag1$^{-/-}$mouse. Furthermore, deficiency of lncT$_H$17-1 decresed proinflammatory cytokine production, including IL-17A and IFNγ, and accompany with upregulation of anti-inflammatory cytokine IL-10. Mechanically, we find lncT$_H$17-1 binds to RNA helicase DHX9 to promote the binding between DHX9 and STAT3. Thus we identify a long non-coding RNA lncT$_H$17-1 as a regulator of pathogenic T$_H$17 cells, and provide a promising diagnostic biomarker for autoimmune disease.

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DOI: 10.1002/eji.201970400

P2957

T cells become exhausted in the process of malignant transformation of oral leukoplakia

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Objective: To explore the potential immune mechanism underlying the malignant transformation from oral leukoplakia (OLK) to oral squamous cell carcinoma (OSCC), the characteristics of infiltrating T lymphocytes in OLK and OSCC were identified and analysed.
Methods: The expression of CD4, CD8, CD25, Ki67, PD-1, TIM-3 was examined using multiple immunofluorescence in serial sections of paraffin-embedded tissue samples of OSCC, and its corresponding OLK from 35 patients. The persentage of positive lymphocytes was evaluated. The expression of the immune cells among OLK and its malignant transformation was compared by paried t test.
Results: We found that the proportion of CD4+ T cells and CD8+ T cells decreased from OLK to OSCC, while activated T lymphocytes (CD25+ T cells) were increased. There was a higher level of exhausted T cells (PD-1+TIM-3+ T cells) when OLK progressed into OSCC. There were no significant differences in T cell proliferation ($Ki67^{+}$) between the two groups.
Conclusion: The activated and exhausted T cells are involved in the malignant transformation of oral leukoplakia.

P2958

BMP-2 from macrophage affect the expression of ciliay protein in airway epithelial cell after cigarette smoke exposure

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COPD is a common disease with high morbidity and mortality. Cigarette smoking is the leading cause of COPD. The characteristics of COPD is macrophage infiltration and airway remodeling which cause the impaired mucociliary clearance. The decrease of cillia account for part of the improper mucociliary clearance in addtion to increased mucus secretion. This study focused on how macrophages affect the expression of ciliary protein of airway epithelial cells.
In vivo, acute or chronic cigarette smoke exposure both resulted in the decrease of ciliary protein and the increase of mucin in the airway of mice. At the same time, macrophages in the lung increased significantly.
In vitro, low concentration of cigarette smoke extract（CSE） cause the increased expression of ciliary protein of Beas-2B cells and high concentration of CSE has the inverse effect. The BMP receptors expression of Beas-2B cells was significantly increased with the stimulation of CSE. Adding recombinant human BMP-2 protein to medium could decrease the expression of ciliary protein of Beas-2B cells in a concentraion dependent manner. Thp-1 cells showed an inhibitory effect on ciliary protein expression when were co-cultured with Beas-2B in transwell, which was augmented by CSE. However, the inhibitory effect can be partly block when Beas-2B cells were pretreated with BMP receptor blockage LDN or when Thp-1 cells were interfered with BMP-2 siRNA before co-culture. The results indicated that BMP-2 from macrophages binding to its receptor was involved in decrease of ciliary protein expression in airway epithelial cells after cigarette smoke exposure.

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DOI: 10.1002/eji.201970400

P2959

IL-7 and RANKL/OPG system in DSS-induced mouse colitis

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Inflammatory bowel disease (IBD) is a recurrent chronic non-specific intestinal inflammatory disease, including Ulcerative colitis (UC) and Crohn´s disease (CD). In recent years, the incidence of IBD in China has been increasing year by year. Studies have shown that the onset of IBD is associated with damage to the intestinal mucosal barrier and the persistence of multiple inflammatory factors. Interleukin-7 (IL-7) secreted by intestinal epithelial cells regulates the survival and proliferation of mucosal lymphocytes. It also affects the role of the RANKL/OPG system in inflammatory diseases by binding to the IL-7 receptor. However, the role of the IL-7 and RANKL/OPG systems in the intestinal mucosal barrier of IBD remains to be further investigated.
The animal model of IBD was constructed, and the recombinant cytokine IL-7 was used to intervene. Explore the role of the IL-7 and RANKL/OPG systems in the colitis model.
This study found that the RANKL/OPG system is involved in the pathogenesis of DSS-induced colitis in mice.And Recombinant cytokine IL-7 solution can partially inhibit the expression of RANKL and OPG in colon tissue, and to some extent alleviate DSS-induced inflammation of colitis.

P2960

The role of PPARγ agonists in DSS-induced colitis in mice

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Background: Defects in mucus and intestinal epithelia can lead to intestinal inflammation in colitis. Reduced peroxisome proliferator-activated receptor gamma (PPARγ) in the mucosa may contribute to inflammation. However, the roles of PPAR in the intestinal barrier remain poorly understood.
Methods: Chronic colitis was induced in BALB/c mice by administration of dextran sulfate sodium (DSS) for 11 days. Three days before DSS treatment, mice were treated with the PPARγ agonist rosiglitazone (RSG) and the same volume of normal saline（200ul）orally at 20 mg/kg/day.
Results: The mice in the DSS group and the DSS+NS group was poor condition with the obvious weight loss the shortened colon,and the DAI and HI scores were significantly higher than the control group.Compared with the model group, the levels of TNF-α and IL-6 in the serum of the DSS+RSG group were decreased. The expression of TLR4、NFκB、MMP7 were decreased in the colon tissue of DSS+RSG group than those in DSS + NS or DSS group. Moreover, tight junctions were up-regulated in DSS+RSG mice.
Conclusions: Our results demonstrate that activated PPARγ could inhibit TLR4/NFκB signaling pathway and regulate the expression of various inflammatory factors, meanwhile PPARγ agonists can inhibit the expression of MMP7 and improve the intestinal barrier function under inflammatory conditions.

DOI: 10.1002/eji.201970400

P2961

Amino acid transporter Slc38ai promotes IL1β production in classical activation macrophages in DSS induced colitis mice

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Amino acid transporters (AATs) are membrane-bound transport proteins that mediate the transfer of amino acids into and out of the cell or cellular organelles. While the dysregulation of AATs leads to metabolic reprogramming, contributing to the pathogenesis of cancer and immune disease. However, most of the AATs is poorly described. In our previous study, we focused one AAT, Slc38ai(identified) in the macrophage. We found that Slc38ai was drastically induced in LPS and IFNγ activated macrophage compared with unstimulated or IL4 and IL13 activated alternative macrophages. Slc38ai knockdown or knockout experiments from classical activated macrophages and sequent expression profiling demonstrated important roles for regulation of immune responses, including proinflammatory cytokines, TNF, IL1β, and iNOS. Using the dextran sodium sulfate (DSS) induced colitis model that relies heavily on the innate immune system, we have now found a significantly elevated Slc38ai in colon infiltrated macrophages. We bred the Slc38ai knockout mice and induced colitis with DSS to identify any potential protective or pathogenic function for Slc38ai in this model. We found that Lack of the *slc38ai* gene in mice had better performances in both clinical and histology tests when compared to littermate mice. Mechanistically, we found that the Slc38ai mainly acts in classical activated macrophages. Interestingly, it was involved in IL1β cleave via inflammasome activation and susceptibility to colitis. In summary, we demonstrated that elevated Slc38ai promoted IL1β production in macrophages, which turned out to be a key causative factor to mediate macrophage activation and a potential therapeutic target to colitis.

P2962

Bactericidal permeability-increasing protein gene knockout aggravates dss inducing ulcerative colitis in mice

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Bactericidal Permeability-Increasing Protein (BPI) and BPI autoantibody are related to human inflammatory bowel disease.In our laboratory, we prepared BPI gene knockout mice. Mice with ulcerative colitis were made using 5%DSS drink for 5 days. We found that BPI knockout mice showed weight loss, shortened colonic length, increased intestinal bleeding, decreased colonic epithelial cells and goblet cells, increased inflammatory cell infiltration, compared with the control group. The level of MPO, IL-5 and MCP-1 increased in the colon tissues of BPI knockout mice. The level of peripheral blood endotoxin and LBP increased in the serum of BPI knockout mice. The results showed that BPI gene knockout weakened the colon barrier of endotoxin in mice, and when ulcerative colitis occurred, the intestinal mucosal damage was aggravated, resulting in the aggravation of the symptoms of ulcerative colitis in BPI gene knockout mice.

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DOI: 10.1002/eji.201970400

P2964

B7-H3 exacerbates the chemoresistance of colorectal cancer by targeting cell division cycle 25A

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Colorectal cancer is one of the most common malignancies, and chemoresistance is one of the key obstacles in the clinical outcome. Here, we found that B7-H3, an important costimulatory molecule, could effectively enhance the resistance to a chemotherapeutic drug (oxaliplatin or 5-fluorouracil). Furthermore, we showed that the overexpression of B7-H3 enhanced chemoresistance by reducing the G2/M phase arrest in a cell division cycle 25A (CDC25A)-dependent manner. Silencing CDC25A or treatment with CDC25A inhibitors could reverse the B7-H3-induced chemoresistance of cancer cells. Moreover, we found that both B7-H3 and CDC25A were significantly upregulated in CRC samples compared with normal adjacent tissues and that the levels correlated with tumor stage. CDC25A was positively correlated with B7-H3 expression in this cohort. Taken together, our findings provide an alternative mechanism by which CRC cells can acquire chemoresistance via the B7-H3/CDC25A axis.
Keywords: colorectal cancer; chemoresistance; B7-H3; CDC25A

P2965

Non-canonical RIG-I signaling maintains IELs via IL-15

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As an intracellular sensor for viral pathogens, RIG-I signaling plays an important role in intestinal defense and is closely associated with the development of colorectal cancer. However, the mechanisms of how RIG-I maintains the intestinal homeostasis and regulates the susceptibility of intestinal diseases are still unclear. Here, we show that the numbers of intestinal intraepithelial lymphocytes (IELs) are significantly decreased in *Rig-I*$^{-/-}$ mice. Further study showed that RIG-I in antigen presenting cells (APCs) can maintain IELs via an interferon-independent, but MAVS-IRF1-IL-15 axis dependent manner. Notably, the recovery of IELs restores the colitis susceptibility of MAVS deficient mice to dextran sulfate sodium. Collectively, our results indicate that *non*-canonical RIG-I signaling support the homeostasis of IELs and regulate mucosal immunity.

P2966

Dynamic change of microbiota in dexamethasone-induced insulin resistance

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The gut microbiota plays a vital role in metabolic diseases such as obesity, insulin resistance and type 2 diabetes. In this study, dexamethasone was used to induce insulin resistance. Male SD rats received daily intraperitoneal injections of dexamethasone (1.0mg/kg) for 7, 14, 21, and 28 days. The dynamic changes of gut microbiota was analyzed by 16S rRNA sequencing. Our results showed that

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DOI: 10.1002/eji.201970400

dexamethasone induced an significant increase in ALT, AST, HOMA-IR and insulin levels. After dexamethasone challenge, there were 209 species in7d group, 259 species in 14d group, 221 species in 21d group and 307 species in 28d group. All the four groups share 209 identical species. The LEfSe analysisshowed that the relative abundance of *Lachnospiraceae*,*Oscillospira* and *Ruminococcus* in 7d group was significantly higher than that of 14d, 21d and 28d groups (LDA>2); the relative abundance of *Formicilis*,*Gemmiger*,*Corynebacterium*, *Actinomycetales*,*Corynebacteriaceae* and *Aerococcus* in 14d was significantly higher than that in 7d, 21d and 28d groups (LDA>2); the relative abundance of *Clostridium*and *Clostridiaceae* in 21d group was significantly higher than that in 7d, 14d and 28d group (LDA>2); the relative abundance of *Clostridium and Flavefaciens* in 28d group was significantly higher than that of 7d, 14d and 21d groups (LDA>2). The study showed that dexamethasone-induced insulin resistance has a different effect on microbiota at different times. The relative abundance of *Actinobacteria,Bacteroidetes,Firmicutes, Proteobacteria* and *Verrucomicrobia* is related with dexamethasone-induced insulin resistance.

P2967

Phosphatase Shp2 exacerbates intestinal inflammation by disrupting macrophage responsiveness to interleukin-10

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Inflammatory cytokines produced by activated macrophages largely contributes to the pathological signs of inflammatory bowel disease (IBD). Interelukin-10 (IL-10) is the predominant anti-inflammatory cytokine in the intestine and its therapeutic efficacy for IBD has been clinically tested. Nevertheless, how the function of IL-10 is regulated in intestinal microenvironment remains unknown, which largely hinders the further development of IL-10-based therapeutic strategies. Here, we found that the expression of phosphatase Shp2 was increased in colonic macrophages and blood monocytes from colitis patients compared to those from healthy controls. Shp2 deficiency in macrophages protected mice from colitis and colitis-associated colon cancer. Mechanistically, Shp2 disrupted IL-10/STAT3 signaling and its dependent anti-inflammatory response in human and mouse macrophages. Furthermore, a Shp2-inducing role of TNF-α was unveiled in our study. Collectively, our work identifies Shp2 as a key factor disrupting intestinal immune tolerance and hopefully will be helpful in the future exploitation of IL-10 immunotherapy for IBD.

P2968

γδ T cells: crosstalk between mircobiota, chronic inflammation and colorectal cancer

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Increasing evidence suggests intestinal microbiota dysbiosis and chronic inflammation contribute to colorectal cancer development. γδ T cells represent a major innate immune cell population in the intestinal epithelium and are involved in the maintenance of gut homeostasis, inflammation regulation

DOI: 10.1002/eji.201970400

and carcinogenesis. The important contributions of γδ T cells are positioned to: (i) perform protective role in the context of barrier damage and pathogenic microorganisms translocation; (ii) exert either pro- or anti-inflammatory effects in different inflammatory stages; (iii) boost the crosstalk between immune cells and tumor microenvironment, inducing a cascade of suppressive immune responses. Understanding the crucial role of γδ T cells contribute to manipulating these cells during the colorectal cancer sequence to improve the efficacy of tumor therapy.

Regional Immunology and Disease

O101

Unfolded protein response regulates cancer-induced splenic myelopoiesis and suppressive myeloid cell generation

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Tumors can induce profound extramedullary hematopoiesis (EMH) in the spleen to support the immune escape and disease progression of cancer. We have recently shown that the tumor-associated splenic EMH is mediated by a novel GM-CSF-expressing hematopoietic stem/progenitor cell (HSPC) population. These splenic HSPCs are functionally distinct from their bone marrow counterparts and are committed to generating immunosuppressive myeloid cells (***JCI***, 2018). However, the mechanism and key regulatory molecules of the tumor-associated splenic myelopoiesis remain unclear. In the present study, we found that HSPCs in the spleen of HCC-bearing mice underwent a cellular endoplasmic reticulum (ER) stress response, which triggered a series of downstream signals and regulated HSPC functions. Through activating the PERK-ATF4-C/EBPβ pathway, ER stress response ultimately led to the up-regulation of GM-CSF expression in splenic HSPCs, and promoted their differentiation to myeloid-derived suppressor cells (MDSCs). IL-6 secreted by splenic stromal cells were found responsible for inducing the ER stress in splenic HSPCs. In addition, IL-6-inducd ER stress and activation of PERK pathway was also crucial for human umbilical cord blood-derived HSPCs differentiation into MDSCs, consistent with the findings in the mouse model. These results indicate that the ER stress response, as a key event mediating the functional alteration of tumor-associated splenic HSPCs, plays a critical role in mediating tumor-promoting splenic myelopoiesis.These findings not only illustrate the key molecular pathways regulating tumor-induced splenic HSPCs, but also provide novel potential targets for cancer therapy that restrains the tumor-promoting myeloid response at its source.

O102

γδT cells suppress liver fibrosis via strong cytolysis and enhanced NK cell-mediated cytotoxicity against hepatic stellate cells

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DOI: 10.1002/eji.201970400

Liver fibrosis is the excessive accumulation of extracellular matrix proteins, resulting from maladaptive wound healing responses to chronic liver injury. γδT cells are important in chronic liver injury pathogenesis and subsequent liver fibrosis; however, their role and underlying mechanisms are not fully understood. The present study aims to assess whether γδT cells contribute to liver fibrosis regression. Using a carbon tetrachloride (CCl_4)-induced murine model of liver fibrosis in wild-type (WT) and γδT cell deficient (TCR$\delta^{-/-}$) mice, we demonstrated that γδT cells protected against liver fibrosis and exhibited strong cytotoxicity against activated HSCs. Further study show that chronic liver inflammation promoted hepatic γδT cells to express NKp46, which contribute to the direct killing of activated hepatic stellate cells (HSCs) by γδT cells. Moreover, we identified that an IFNγ-producing γδT cell subset (γδT1) cells exhibited stronger cytotoxicity against activated HSCs than the IL-17-producing subset (γδT17) cells upon chronic liver injury. In addition, γδT cells promoted the anti-fibrotic ability of conventional natural killer (cNK) cells and liver-resident NK (lrNK) cells by enhancing their cytotoxicity against activated HSCs. The cell crosstalk between γδT and NK cells was shown to depend partly on co-stimulatory receptor 4-1BB (CD137) engagement. In conclusion, our data confirmed the protective effects of γδT cells, especially the γδT1 subset, by directly killing activated HSCs and increasing NK cell-mediated cytotoxicity against activated HSCs in CCl_4-induced liver fibrosis, which suggest valuable therapeutic targets to treat liver fibrosis.

O103

PHACTR1 mediates atherosclerosis by promoting endothelial inflammation as a novel PPARγ co-repressor

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Atherosclerosis is a disease with the narrowing and hardening of arteries caused by lipid deposition, initiated by endothelial inflammation. Atherosclerotic plaques tend to form in areas of low shear stress, such as bifurcation, branches and high curvature of vessels. However, the mechanotransduction in response to shear stress in endothelial cells (ECs) is still obscure.
Several GWASs (genome-wide association studies) revealed that phosphatase and actin regulator 1 (PHACTR1) is highly correlated with atherosclerosis. However, physiological functions of PHACTR1 *in vivo* have not been reported. We generated PHACTR1 knockout mice using CRISPR/Cas9 system. No embryo lethal or developmental defects were observed. Notably, aortic oil red O staining showed that aorta lesion area decreased by 38.2% in *Phactr1*$^{-/-}$*Apoe*$^{-/-}$ mice. Western blot demonstrated that PHACTR1 was highly expressed in ECs but not in smooth muscle cells or murine macrophages. We detected EC inflammation *in vitro* and found that depletion of *Phactr1* inhibited VCAM-1 and ICAM-1 expression and Thp1 adhesion. Importantly, PHACTR1 shuttled to nucleus from cytoplasm under disturbed flow detected by immunostaining. To investigate the mechanism of PHACTR1 in atherosclerosis, RNAseq of aortic ECs was conducted. GO enrichment analysis showed that PHACTR1 affected transcription-related biological functions, and transcription factor analysis revealed that PPARγ was the most relative transcription factor. PPARγ-PHACTR1 interaction was confirmed by co-immunoprecipitation and PHACTR1 overexpression reduced PPARγ luciferase activity.
In conclusion, PHACTR1 is crucial for atherosclerosis by mediating endothelial inflammation in areas with low shear stress. Targeting PHACTR1-PPARγ interaction will be a promising therapeutic strategy for atherosclerosis.

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DOI: 10.1002/eji.201970400

O104

The involvement of IFN-γ and CXCR3 in the CD8$^+$ T-cell-mediated regression of squamous cell carcinoma

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Cutaneous squamous cell carcinoma (SCC) is one of the most common cancers, with a high risk of mortality if left untreated. The "gold standard" treatment for SCC is surgery; however, this does not prevent tumour recurrence, and patients with one SCC have an increased incidence of secondary SCCs. An alternative way to treat SCCs and induce long-term protective immunity is through immunotherapy. This strategy is specifically suitable because SCCs carry a high mutational load and are considered to be highly immunogenic. To better understand the immune mechanisms important for the regression of SCC, we have created an SCC cell line derived from UV-irradiated HPV38 E6/E7 FVB mice. This cell line forms tumours when injected into immune-suppressed mice, which subsequently regress following the removal of immunosuppression. Preliminary data using this tumour model show that both IFN-γ and CXCR3 are critical for SCC regression. Neutralizing IFN-γ *in vivo* impaired CD8$^+$ T-cell infiltration into the tumour site and resulted in uncontrolled tumour growth. The anti-tumour effect of IFN-γ was not the result of a direct cytotoxic effect on SCC cells, but did impact upon the induction of MHC I expression and CXCL10 secretion. In IFN-γ-associated immune-surveillance, CXCL10/CXCR3 signaling is important for CD8$^+$ T-cell recruitment. *In vivo* blockade of the CXCL10 receptor- CXCR3, also reduced the number of CD8$^+$ T-cells infiltrating into tumours and delayed tumour regression. Together, the data strongly suggest a key role for IFN-γ and CXCR3 in the CD8$^+$ T-cell-mediated control of SCC regression.

P0414

Nicotine attenuates osteoarthritis pain and matrix metalloproteinase-9 expression via the α7 nicotinic acetylcholine receptor

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Osteoarthritis (OA) is a degenerative joint disease that causes chronic disability among the elderly. Despite recent advances in symptomatic management of OA by pharmacological and surgical approaches, there remains a lack of optimal approaches to manage inflammation in the joints, which causes cartilage degradation and pain. In this study, we investigated the efficacy and underlying mechanisms of nicotine exposure in attenuating joint inflammation, cartilage degradation and pain in a mouse model of OA. A mouse model of OA was induced by injection of monosodium iodoacetate (MIA) into the knee joint. Cell culture models were also used to study the efficacy and underlying mechanisms of nicotine treatment in attenuating symptoms of OA. Nicotine treatment reduced mechanical allodynia, cartilage degradation and the upregulation of metalloproteinase-9 (MMP-9), a hallmark of joint inflammation in OA, in mice treated with MIA. The effects of nicotine were abolished by the selective α7 nicotinic acetylcholine receptor (α7-nAChR) blocker MLA. In RAW264.7 cells and murine primary bone marrow-derived macrophages, nicotine significantly inhibited MMP-9 production induced by LPS. In addition, nicotine significantly enhanced PI3K/Akt and inhibited NF-κB translocation from the cytosol to the nucleus in an α7-nAChR-dependent manner, suggesting that nicotine acts on α7-nAChRs to inhibit MMP-9 production by macrophages through modulation of the PI3K/Akt-NF-κB pathway. Our results provide novel evidence that nicotine can attenuate joint inflammation and pain in experimental OA via α7-nAChRs. α7-nAChR could thus serve as a highly-promising target to manage joint inflammation and pain in OA.

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DOI: 10.1002/eji.201970400

P0415

The mechanism of cigarette smoke-induced collagen deposition

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Background: The pathogenesis of COPD is unknown, which greatly limits clinical treatment. This study aimed to explore the mechanism of collagen deposition in COPD and provide new therapeutic strategies.
Method: C57BL/6 mice were exposed to cigarette smoke for 5 months to establish COPD model. Lung function was detected by Flexivent system, while bronchoalveolar lavage fluid (BALF) was collected for total and differential cell counts. H&E and Masson staining were used for measuring inflammatory cell infiltration, mean linear intercept (MLI) in lung and collagen deposition in heart, liver, spleen, lung and kidney, respectively. CCK8 kit and Western blot were employed for measuring effects of cigarette smoke extract (CSE) on the proliferation and expression of collagen-related proteins (collagen I, fibronectin, α-SMA, CTGF, vimentin) by lung fibroblasts (Mlg and MRC5), respectively.
Results: Increased inspiratory capacity and total cell count in BALF (predominantly by macrophages and neutrophils), inflammatory cell infiltration and MLI in lung were observed in the experimental mice compared with control animals. Furthermore, collagen deposition was noted in lung, spleen and kidney. In addition, CSE promoted proliferation and collagen-related proteins production by lung fibroblasts in vitro.
Conclusion: COPD-like pathological changes and collagen deposition in multiple organs occurred after smoke exposure for 5 months, possibly due to the effects of CSE on lung fibroblasts proliferation, collagen-related proteins expression and differentiation into myofibroblasts.

P0416

Effect of Galectin-9 on TLRs/NF-κB signaling pathway and gut microbiota in experimental colitis

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Background/Aims: T cell Ig domain and mucin domain (Tim) proteins have close relationship with TLRs/NF-κB signaling pathway which plays important role in IBD, and intestinal flora imbalance is the pathogenesis of IBD. Galectin-9 (Gal-9) is the natural ligand of Tim-3, we aimed to clarify the effect of the ligand Gal-9 in experimental colitis.
Methods: Balb/c mice were randomly divided to the control group, TNBS group, and TNBS+Gal-9 group. On the third day after modeling, 10ug Gal-9 was given for consecutive 5 days. The DAI were assessed; Pathological changes were observed by HE; The Tim-3, and TLR4/NF-κB signaling pathway were detected by PCR, IHC and WB; The bacterial DNA of the fresh fecal samples were extracted using the QIAamp DNA kit and then amplify the target 16S rRNA segment.
Results: Tim-3 expression decreased in the TNBS group, and TLR4/NF-κB signaling pathway expressions significantly upregulated. With Gal-9 intervention, DAI decreased, the pathological degree improved, Tim-3 expression increased, and TLR4/NF-κB signaling pathway expressions decreased. At the genus level, *Ruminococcus* and *Coprococcus* increased, while *Bilophila, Butyricimonas* and *Paraprevotella* decreased in the TNBS group. After Gal-9 intervention, the diversity index of intestinal flora significantly improved. Besides, at the genus level, the relative abundance of *Odoribacter* was higher (1.98% vs 0.51%, $p< 0.05$), and the relative abundance of *Prevotella* is lower (1.04% vs 3.27% $p< 0.05$).

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DOI: 10.1002/eji.201970400

Conclusions: Gal-9 can affect the course of colitis by regulating the TLR4/NF-κB signaling pathways, and significantly improve the intestinal flora imbalance.

P0417

Th1 and Th2 cytokines pattern among sickle cell disease patients in Côte d'ivoire, West Africa

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Introduction: Sickle cell disease (SCD) is the most prevalent genetic disease worldwide and particularly reaches its highest prevalence in sub-Saharan African countries. In Côte d'Ivoire, the SCD prevalence rate is 12% and 2% are major patterns. Many evidences show that the immune system plays an important role in this inflammatory condition by secreting inflammatory cytokines.
This work attempts to identify the cytokine pattern displayed by Ivorian patients during the course of disease, as Th1 cytokines, as well as Th2 cytokines.
Patients and Methods: 49 subjects (4 to 55 years) were prospectively enrolled in the study after an informed consent. The patients were assigned in 2 groups, patients in steady state and patients in crisis. Serums were measured in San Diego Biolegend laboratory by using LEGENDplexTM Human Inflammation Panel assays and ELISA.
Results: Evaluation of serum cytokines in SCD crisis patients revealed an increased level for IL-1β, IL-6 and IL-10 cytokines. The IFN-γ(Th1) to IL-4(Th2) ratio was 1.92 in crisis subjects and 3. 55 in steady state subjects indicating a trend toward a Th2 bias in crisis patients.
Conclusion: Accurate data are lacking for people with SCD in Sub-Saharan Africa where the disease is endemic. This study reveals a Th2 bias in SCD patients.

P1003

Analysis of clinical features and prognostic factors in 172 cases renal cell carcinoma in XinJiang

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Objective: To analyze clinical features and prognostic factors of **renal cell carcinoma (RCC).**
Methods: The clinical data of 172 RCC patients treated in the First Affiliated Hospital of XinJiang Medical University. Univariate and multivariate survival with analyses were performed to identify possible prognostic factors.
Results: Univariate analysis showed that **age, pathological type, tumor size, Fuhrman grading, T staging, lymph node metastasis, sarcomatoid, vena cava lumor thrombus,distant metastasis** and **postoperative immunotherapy** had a statistically significant impact on the survival.The multivariate analysis showed that **age≥55years, high Fuhrman grading(III-IV), tumor sizes, tumor staging over T_3, lymph node metastasis, sarcomatoid, vena cava lumor thrombus** and **distant metastasis** were positively associated with improved survival.
Conclusion: Metastasis RCC is rare and associated with an extremely **poor prognosis**. In **non-metastasis RCC age,tumor size, sarcomatoid, vena cava lumor thrombus, grading, staging** and **lymph node metastasis** are **independent factors** for patients with RCC.

DOI: 10.1002/eji.201970400

P1004

TLRs stimulation of human renal mesangial cells in the production of galactose-deficient IgA1

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IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis, and along with an upper respiratory tract infection commonly precedes disease presentation. The diagnostic hallmark of IgAN is the galactose-deficient IgA (Gd-IgA1) deposits in the glomerular mesangium. So far, the IgA was believed to be produced by B cells. However, patients with IgAN did not benefit from B cell depletion with rituximab. Therefore, the precise origin of IgA involved in IgAN and the underlying regulatory mechanisms between IgA production and pathogen infection need further investigation. In this study, we have identified four Ig heavy chain transcripts (Igγ, Igα, Igμ and Igε) with typical V_HDJ_H rearrangements in single mesangial cell of patients with IgAN by single cell-related Ig repertoire analysis. Then, we confirmed that Gd-IgA1 can be produced and secreted by mesangial cell lines under normal culture condition, and significantly increased by staphylococcus aureus (SAC), staphylococcal enterotoxin B (SEB), lipopolysaccharide (LPS) and angiotensin II (Ang II). Evidently, SAC and SEB promoted secretion and extracellular granular deposition of Gd-IgA1 in mesangial cells, consistent with renal pathology. Interestingly, mesangial cells constitutively expressed multiple TLRs, and that engagement of mesangial cell TLRs led to produce of Gd-IgA1 and release of proinflammatory cytokines, which can be inhibited by specific repressor of TLRs. In short, we reported, for the first time, that mesangial cells can produce Gd-IgA1 and regulated by TLRs. This discovery subverted our current understanding for the pathogenesis mechanism of IgAN and hopefully change the current lack of effective treatment for IgAN.

P1005

Normal human epidermal cell-derived IgG plays an important role in immune defense

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The epidermis is a highly specialized epithelium that has evolved to perform multiple essential protective functions, however, so far, no evidence indicate the epidermis can produce immunoglobulin(Ig).In this study, we have reported that the normal human epidermal cells, especially the the cells of stratum spinosum and stratum granulosum can abundantly produce functional IgG by Western blot and epidermal single cell-related RT-PCR. Like our previous reports, the epidermal cell-derived IgG displayed conservative VHDJH rearrangement patterns, which suggested that the epidermal cell-derived IgG serve as conservative function among different individuals. Confocal results shows that IgG showed a filamentous structure in cytoplasm of the epidermal cell line-HaCaT cell, co-localization with keratin 5. When knockdown of the IgG, the HaCaT cells were induced to undergone apoptosis. These results suggested that the IgG serve as a function of cytoskeleton-related proteins, which is involved in cell survival. Immunoprecipitation combined with mass spectrometry shows that the IgG maybe interaction with the desmosome related proteins, such as desmoplakin and plakoglobin, and the keratins. In addition, we also found that the Keratinocyte cell could spontaneously secret IgG, which shows potential antibody activity by binding pathogens such as Staphylococcus aureus.
The above results suggest that normal human epidermal cells can express IgG. The nonsecretory form in the cytoplasm is related to intercellular junctions and cytoskeleton, participates in maintaining the integrity of the skin barrier. The secretory form can bind to pathogenic microorganisms, has natural antibody activity.

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DOI: 10.1002/eji.201970400

P1006

Isolation of mouse CD45 positive leukocytes from tissues

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Immune cell function is often tissue-specific, therefore, isolating cells from tissue microenvironments is necessary to better understand their role in health and disease. This can be challenging in the presence of tissue-derived cells and debris from tissue dissociation. These factors reduce the leukocyte start frequency, which can prolong the isolation process and limit the identification of small but critical leukocyte subsets. To overcome these obstacles, we developed a simple and rapid selection method to enrich CD45+ leukocytes from mouse tissues. Starting with a single cell suspension, cells are labelled with an antibody complex that links CD45+ cells to magnetic particles. Following magnetic separation, the isolated CD45+ cells are ready for use. Using mouse lungs, CD45+ leukocytes were enriched from 63.5 ± 9.4% to 97.1 ± 1.2% purity, and the recovery of viable CD45+ cells was 37.7 ± 14.5% (mean ± SD, n=37). Preliminary testing on subcutaneous mouse 4T1 tumors resulted in efficient enrichment of tumor-infiltrating leukocytes. Importantly, the composition of immune subsets from both lungs and tumor samples was maintained following CD45 selection. To demonstrate functionality, splenic T cells within the CD45 selected population were able to upregulate CD25 and CD69, and proliferate upon stimulation. In as little as 20 minutes, the EasySep™ Mouse CD45 Isolation Kit allows researchers to easily enrich for functional leukocytes from tissue samples. Examining immune cells directly from healthy and tumor tissues will enhance our understanding of how these cells function and aid in developing new approaches to combat disease progression.

P1007

DPP4 controls gut microbiota and mucosal immunity by regulating intestinal CD103+ dendritic cells

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Background: Excessive gut immune activation and dysbiosis have been recognized as important pathogenic factors of inflammatory bowel disease. However, how gut microbiota and enteric immune system are regulated in IBD is not fully understood. We investigated the role of dipeptidyl peptidase-4 (DPP4) in the regulation of gut microbiota and mucosal immunity in this study
Methods and Results: Colon transcriptome analysis and real-time qPCR measurement show that DPP4 expression markedly reduced in the mice with DSS-induced colitis, compared to healthy controls. DPP4 was highly expressed on dendritic cells (DCs, especially on CD103+ DCs) in the gut, but not on the DCs anywhere else. In addition, gut DPP4+CD103+ DCs significantly decreased in mice with colitis. *Dpp4*$^{-/-}$ mice had a more severe colitis phenotype compared to wild-type (WT) mice. RNA transcriptomic analysis of colon tissues show that *Dpp4*$^{-/-}$ mice had a reduced gene expression in TGFβ signaling pathway and an increased expression of multiple inflammatory genes. Cohousing for 4 weeks transmitted the disease phenotype and microbiota profile of *Dpp4*$^{-/-}$ mice to WT cage mates.
Conclusions: DPP4 is involved in inflammatory bowel disease by regulating gut microbiome and mucosal immune balance.

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DOI: 10.1002/eji.201970400

P1604

Characteristic of keratinocytes in pancreatic cancer denotes significant difference in immune status and prognosis

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Backgrounds: Pancreatic cancer is one of the cancers with highest mortality worldwide. The study of microenvironment in pancreatic cancer is significant for treatment, but questions remain due to the limitation of traditional methods. Recently with the development of bio-informatic algorithms, we could explore the cellular components of tumor bulks from genomic level.
Methods: We utilized a novel algorithm xCell which digitally portrays the tissue cellular heterogeneity landscape from transcriptome. The most significant prognostic cell type and a novel molecular subtyping method based on two representative characteristic genes were put forward. Further, we applied GSEA and CIBERSORT methods and investigated the difference of immune pathways and immune cell ratios between two subtypes of pancreatic cancer. Finally, immunohistochemistry analysis were performed.
Results: Among all types of microenvironment cells, the characteristic of keratinocytes is most significant in predicting the survival outcome. We selected two genes DSG3 and GJB4 to perform the molecular subtyping. Significant differences in immunologic signature and immune cell components were found between the subtypes. Moreover, patients received adjuvant chemotherapy had different survival outcomes in different subtypes.
Conclusions: Characteristic of keratinocytes could be used as a negative prognostic factor in pancreatic cancer. Significant differences in immunologic signature and immune cell components were found between molecular subtypes based on keratinocyte-associated genes GJB4 and DSG3 and the subtyping could distinguish the survival outcomes and therapeutic effects of pancreatic patients.

P1605

Integrated analysis of gene expression profiles identifies transcription factors potentially involved in psoriasis pathogenesis

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Psoriasis is a common inflammatory skin disease mediated by cells and molecules in both the innate and adaptive immune systems. Recently, gene expression profiles analysis revealed a large set of immune-related differentially expressed genes (DEGs) in psoriasis. However, the associations between these DEGs and their transcriptional regulation mechanisms have not been fully elucidated. In this study, several psoriasis GEO datasets were systematically analyzed using bioinformatics tools to uncover important transcription factors (TFs) that regulate the expression of immune-related DEGs and further enhance our understanding of psoriasis pathogenesis. Common DEGs encoding chemokines, cytokines, antimicrobial peptides and keratins were identified in psoriasis, and extensive correlations existed among these DEGs. Several common TFs that bind the promoters of the DEGs, including the well-known STAT1, STAT3 and NF-κB, as well as EHF, FOSL1 and FOXC1, which are rarely studied in psoriasis, were also identified. STAT1, EHF, FOSL1, STAT3 and NFKB1 were positively correlated with these DEGs in the psoriasis lesions, while FOXC1 was negatively correlated with most DEGs. The decreased expression of the DEGs was accompanied by the downregulation of

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DOI: 10.1002/eji.201970400

STAT1, EHF, FOSL1, STAT3 and NFKB1 and the upregulation of FOXC1 upon blocking the IL-17 or TNF-α signaling in psoriasis. Additionally, the downregulation of IL37 in psoriasis was negatively correlated with STAT1 and CXCL10, which are associated with Th1 responses. These results suggest that TFs play an important role in the pathogenesis of psoriasis, and interfering with the activity of key TFs may be a promising therapeutic strategy for psoriasis.

P1606

Immune perturbation of B cell compartments in liver cirrhosis impact patients´ humoral immunity

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Liver cirrhosis is associated with defective vaccine response and increased infections. B cell compartments in cirrhotic patients have not been comprehensively characterized. Here, we performed phenotypical, functional and mechanistic study of dysregulated homeostasis of B cell populations in both peripheral blood and spleens from cirrhotic patients. We found that liver cirrhosis led to extensive depletion of marginal zone B cells (MZB, defined as IgD+CD27+), but increased Naïve B cell and plasma cell percentages. The perturbations of B cell compartments correlated with cirrhosis severity and hyperglobulinemia in patients. We found that patients' MZB cells were prone to spontaneous apoptosis and more dependent on extrinsic factor for their survival, including TLR-stimulation, BAFF and IL-21, than the healthy controls. Transcriptomic comparison showed defects of cell cycle and cell death regulatory networks of memory B cells from cirrhotic patients. Finally, BCR repertoire sequencing identified differential usage of IGHV genes and expanded clones among CD27+ memory B cells of cirrhotic patients. In conclusion, liver cirrhosis massively disturbs the B cell compartments. Depletion of MZB cells may contribute to the defective humoral immunity in cirrhosis.

P1607

Silencing of PD-L2 by topical application of small interfering RNA inhibits elicitation of contact hypersensitivity

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PD-L2 is a ligand for the immune checkpoint receptor PD-1, however, its regulatory function is unclear. We previously reported that silencing of CD86 in cutaneous dendritic cells (DCs) by topical siRNA application inhibits the elicitation of DNFB-induced contact hypersensitivity (CH) (2008, Mol Therapy). Here we investigated the effects of topical PD-L2 siRNA application in CH. PD-L2 was induced on *in vitro*and *in vivo*activated DCs concurrently with the elevation of MHC class II and CD86. Topical PD-L2 siRNA application inhibited elicitation of CHS by suppressing expression of early proinflammatory cytokines (IL-1a, IL-1b, TNF-a, and CCL3) and migration of hapten-carrying DCs into lymph nodes. DNFB-challenge induced PD-L2 in the epidermal Langerhans cells (LCs) and the induced expression was suppressed by the PD-L2 siRNA painting. Local injection of neutralizing anti-PD-L2 mAb comparably inhibited CH. PD-L2 siRNA treatment inhibited the CH in PD-1/PD-L1 double-knockout mice and in the sensitized T-cell-transferred skin. These results suggest that the effects of PD-L2 silencing are independent of PD-1 but dependent on local memory T cells. Although most of the

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DOI: 10.1002/eji.201970400

inhibitory effects of PD-L2 and CD86 silencing on CH were comparable, but the effects of PD-L2 silencing we dominant in the local skin. Our results suggest that PD-L2 in local LCs acts as a costimulator rather than a regulator. Local PD-L2 silencing by topical siRNA application represents a new therapeutic approach for contact allergy.

P2195

Alteration of adaptive immunity in a colorectal peritoneal carcinomatosis model

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Colorectal cancer (CRC) usually gives rise to transcoelomic spread and ultimately causes peritoneal carcinomatosis (PC). However, mechanism studies, especially the immunological basis of colorectal PC, are rarely revealed due to lack of a suitable PC model. Here we selected a mouse colorectal cancer cell line MC-38 for intraperitoneal inoculation in the C57BL/6 mice to mimic the development of colorectal PC. We demonstrated that the injected CRC cells preferentially and rapidly migrated and colonized in the visceral fat tissues, but not in other visceral organs. With flow cytometric analysis, we found the proportions of spleen T cells and B cells were not affected by PC progression, while the ratios of blood CD4+ and CD8+ T cells were largely influenced. Especially, the quantity or activity of CD4+ and CD8+ T cells in visceral fats were intimately regulated by PC development. Taken together, we successfully constructed a colorectal PC model in immune-competent mice and revealed the alteration of adaptive immunity in PC development. Our study might potentiate the research and therapy strategies of colorectal PC.

P2196

Urinary nephrin and matrix metalloproteinase-9 in idiopathic membranous nephropathy: potential applications

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Objective: The assessment of Idiopathic membranous nephropathy's severity relies entirely on proteinuria the onset of which occurs after significant renal damage. Early biomarkers are highly desired to facilitate timely interventions so as to reduce the risk of end stage renal disease. The purpose of this study is to assess the potential that urinary Nephrin and Matrix metalloproteinase-9 (MMP-9) hold in evaluating the severity of Idiopathic membranous nephropathy (IMN).
Methods: We quantified urinary Nephrin and MMP-9 in 107 biopsy-proven IMN patients and 70 healthy subjects by ELISA and compared their concentrations between patients and healthy controls, between patients with and without nephrotic syndrome, patients with and without serum Phospholipase A_2 receptor (PLA2R) antibodies and those with estimated glomerular filtration rate (eGFR) above and below the of 90 ml/min/1.73 m^2.
We also determined their correlations with the established parameters of renal function in IMN i.e. proteinuria, serum PLA_2R antibodies and eGFR.
Results: Both biomarkers were higher in IMN patients compared to healthy controls. Only MMP-9 was significantly increased in patients with nephrotic syndrome, in the anti PLA2R-postive patients and in patients with eGFR less than 90 ml/min/1.73 m^2. MMP-9 correlated significantly with both proteinuria and estimated glomerular filtration rate (eGFR) while urinary nephrin correlated significantly with only eGFR. Neither MMP-9 nor nephrin exhibited a significant correlation with serum PLA_2R antibodies.

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DOI: 10.1002/eji.201970400

Conclusion: Our findings suggest that urinary MMP-9 holds a greater potential than urinary Nephrin in evaluating the severity of IMN.

P2969

Inhibitory mechanism of B7H4-Ig fusion protein in imiquimod-induced psoriasis-like skin inflammation

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Background: Psoriasis is a common immune-mediated inflammatory disease characterized by epidermal hyperplasia and inflammation. B7H4 is an immune negative regulatory molecule. It has been reported that high levels of soluble B7H4 were detected in the sera of patients with chronic inflammatory disease, such as SLE and RA. This study was aimed to explore the therapeutic effect and potential mechanism of B7H4-Ig fusion protein on mouse psoriasis-like lesions induced by imiquimod (IMQ).
Methods: The level of B7H4-Ig was detected by ELISA in the sera of patient with psoriasis or sera derived from IMQ-induced mice model. The therapeutic mice were hydrodynamically injected with B7H4-Ig plasmids and control mice were injected with Flag-Ig plasmids following IMQ induction. 5 days after treatment, the skin lesions were determined by hematoxylin-eosin staining and the percentage of immune cells in murine peripheral blood cells was measured by flow cytometric analysis.
Results: Soluble B7H4 was detected in the sera of patients with psoriasis but not in that of healthy individuals. B7H4-Ig hydrodynamic injection alleviated IMQ-induced psoriasis-like skin symptoms, with decreased epidermal inflammation and lower PASI scores. The percentage of neutrophils and TCRγδ-T cells in peripheral blood was reduced significantly in B7H4-Ig-treated groups.
Conclusion: B7H4-Ig fusion protein ameliorated imiquimod-induced psoriasis-like lesions by inhibiting the inflammatory response, indicating the potential therapeutic strategy of B7H4-Ig fusion in psoriasis.

P2970

Roles of immune cells in APAP induced liver injury

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Immune system plays a pivotal role in system homeostasis. However, it also, sometimes, plays a destructive role in organ damage when organism was challenged with stress, such as oxidative stress. APAP is a widely used drug for pain and fever treatment, its overdose usage usually caused hepatocyte oxidative stress and cell death which was amplified by immune system and lead to acute liver injury. In APAP treated mice neutrophils and CD8+ T cells was significantly elevated, CD4+ T lymphocytes was slightly decreased. Percentage of Kupffer cells, NK and NKT cells were also decreased, IFN-gamma, TNF-alpha producing cells were significantly increased, both IFN-gamma and TNF-alpha were considered as the main reason for APAP induced acute injury.
Moreover, relative chemokine mRNA expression was detected, for example CXCL5 expression was elevated which recruited neutrophils to damaged area. CCL5 and CXCL9 mRNA expression were decreased that was correlated with decreased percentage of NK and NKT cells. Our results indicated that damaged tissue release DAMPs to recruited neutrophils and CD8+ T cells to damaged tissue, on the other hand chemokine CXCL5 was released to enhance neutrophil recruitment and amplified inflammation which result in a secondly liver injury.

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DOI: 10.1002/eji.201970400

P2971

IFN-γ$^+$γδ T inhibits fibrogenic activation of hepatic stellate cells and prevents liver fibrosis

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Hepatic fibrosis is a chronic liver disease and is characterized by excessive deposition of extracellular matrix, which is the leading cause of liver cancer and serious threat to the health of human being. IL-17A and IFN-γ produced by γδ T cellsplay a prominent role in maintaining overall liver homeostasis. However the subsets of γδ T cells and the mechanism of the protective role that inhibits the development of liver fibrosis are still not clear. The present study aims to assess the protective role and the mechanism of γδ T subsets in CCl_4induced liver fibrosis. We observed the increased γδ T cells in the progression of live fibrosis and increased the expression of NKG2D and FasL on the surface of infiltrated γδ T cells; And TCRd$^{-/-}$mice were used to prove the protective role of γδ T cell in liver fibrosis; Furthermore, adaptive transfer of IL-17$^{-/-}$and IFN-γ$^{-/-}$γδ T cells to TCRd$^{-/-}$mice γδ T illustrated IFN-γ-producing γδ T cell subset inhibiting Th17 differentiation, which prevents hepatic stellate cells (HSCs) activation. And the co-culture system of γδ T cell and HSCs proved FasL is a potent molecular on against activated HSCs. In conclusion, our data confirmed the protective effects of γδT cells, especially the IFN-γ$^+$γδ T subset, by directly inhibiting HSC activation and killing activated HSCs to mitigate CCl4-induced liver fibrosis, which lay a solid foundation for the application of γδ T cells in immunotherapy against liver fibrosis.

P2972

The frequency of CD19$^+$CD38^{low}CD21^{low}B is decreased in hepatocellular carcinoma

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Objective: To investigate the frequency of CD19$^+$ CD38^{low} CD21^{low} B cells in hepatocellular carcinoma (HCC).
Methods: The frequency of CD19$^+$ CD38^{low} CD21^{low} B cells in hepatocellular carcinoma (HCC) patients was determined by multicolor flow cytometry. And we analyzed its association with the frequencies of CD4$^+$T cells, CD8$^+$T cells and CD4$^+$T cells /CD8$^+$T cells.
Results: The frequency of blood CD19$^+$ CD38^{low} CD21^{low} B cells was significantly lower in the HCC patients than the healthy donors, and these cells were decreased in the tumors (P< 0.001). The frequency of tumor tissue CD19$^+$ CD38^{low} CD21^{low} B cells was negatively associated with CD4$^+$T cells(P< 0.05), CD4$^+$T cell /CD8$^+$T cell ratio (P< 0.05)and positively correlated with CD8$^+$ T cells (P< 0.05)
Conclusion: In HCC, the frequency of CD19$^+$ CD38^{low} CD21^{low} B cells was decreased, which may contribute to development of HCC.
Keywords: CD19$^+$ CD38^{low} CD21^{low} B cells; B cells; multicolor flow cytometry

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DOI: 10.1002/eji.201970400

P2973

Developing a periodontal disease antibody array for the prediction of severe periodontal disease using machine learning classifiers

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Background: The aim of this study was to assess the expression levels of 20 periodontal disease-related proteins simultaneously and quantitatively in gingival crevicular fluid (GCF) from normal controls (NOR) and severe periodontitis (SP) patients with an antibody array.
Methods: Antibodies against 20 periodontal disease-related proteins were spotted onto a glass slide to create a periodontal disease antibody array (PDD). The array was then incubated with GCF samples collected from 25 NOR and 25 SP patients. Differentially-expressed proteins between SP and normal controls were then used to build ROC curves and compare five classification models, including Support Vector Machine, Random Forest, k Nearest Neighbor, Linear Discriminant Analysis and Classification and Regression Tree models.
Results: Seven proteins (CRP, IL-1α, IL-1β, IL-8, MMP-13, Osteoprotegerin, Osteoactivin) were significantly upregulated in SP patients compared with NOR, while RANK was significantly downregulated. The highest diagnostic accuracy using a ROC curve was observed for IL-1β. Five of the proteins (IL-1β, IL-8, MMP-13, Osteoprotegerin, Osteoactivin) were identified as important features for patient classification. The classification model with the highest classification accuracy was the Linear Discriminant Analysis model.
Conclusion: This study highlights the potential of antibody arrays to diagnose periodontal disease.

P2974

Effect and mechanism of epalrestat on renal interstitial fibrosis in unilateral ureteral obstruction rats

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Objective: To evaluate the effects of epalrestat treatment on the improvement of renal interstitial fibrosis induced by unilateral ureteral obstruction (UUO), and whether these effects are related to ameliorate epithelial-mesenchymal transition (EMT) via inhibiting TGF-β1 and aldose reductase (AR) expression in the rat UUO models.
Methods: Rats were randomly divided into four groups, n=8, respectively, sham group, UUO group, UUO + epalrestat (50 or 100 mg/kg). Rats in UUO and UUO + epalrestat group were obstructed left ureter. In the sham group, rats had their left ureters exposed and manipulated without ligation. Renal tissues pathological changes and collagen deposition were observed by HE and Masson´s staining. The AR expression in renal tissues was measured by immunohistochemisty. The expression levels of collagen I, collagen III, alpha-smooth muscle actin (α-SMA), E-cadherin, and fibroblast-specific protein1 (FSP-1), fibronectin (FN), epithelial cadherin (E-cadherin), TGF-β1 and AR from kidney tissues were measured by real-time RT-PCR or Western blot.
Results: Compared with sham group, the renal tissues of the UUO group showed significant fibrosis, including renal tubular epithelial cell atrophy and vacuolated degeneration, collagen deposition, fibroblasts and myofibroblasts proliferation and inflammatory cell infiltration, and concomitantly with the expressions of collagen I, collagen III, TGF-β1, AR, α-SMA, FSP-1 and FN were remarkably up-regulated, but E-cadherin was significantly reduced in UUO group. **Conclusion**: These results

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DOI: 10.1002/eji.201970400

suggested that epalrestat attenuates renal interstitial fibrosis possibly through inhibition of EMT via inhibiting TGF-β1 and aldose reductase (AR) expression.

P2975

The role of tumor infiltrating Tfh-like (CXCR5+PD1+CD4+ T) cells in glioma

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Follicular helper T cell (Tfh), a newly defined CD4+ T cell subset, are specialized in facilitating B cell to generate high-affinity antibodies. Recently, Tfh was identified in several kind of tumors such as breast cancer, colon cancer, as well as lung cancer. However, how tumor infiltrated Tfh-like cells orchestrated anti-tumor immunity remains as an open question. In our study, we found CXCR5+ PD1+ Tfh-like cells also infiltrated in glioma. Notably, the proportion and number of these cell are negatively associated with the patient survival. Sequencing result of sorted tumor infiltrated Tfh-like cell and blood CXCR5+ CD4 T cell revealed different gene expression profiles. Single cell sequencing also reveal the high expression of TIM3 on glioam infiltrated Tfh-like cells, indicating cell exhaustion. By using our Tfh reporter mouse tools (Bcl6-RFP), we show that Tfh cell also infiltrated in mosue glioma. Our study show the tumor promoting feature of tumor infiltrated Tfh cell in glioma, and offer us potential therapeutic implications in human glioma.

P2976

Notch signaling regulates macrophage heterogeneity in liver disease: from mechanism to therapy

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Hepatic macrophages can exert dual function in fibrogenesis by either promoting or abrogating the excessive deposition of extracellular matrix, which have been proposed as potential targets in combating fibrosis. Recent studies reveal the advanced progression on macrophages heterogeneity such as origin and polarization and function. However, the detailed molecular mechanisms on regulating macrophages heterogeneity during liver fibrosis are not well understood.
The Notch-RBP-J (recombination signal-binding protein Jκ) pathway plays critical roles in cell fate specification during embryonic development and cell plasticity in adults. Previous reports including our study have shown that Notch signaling involves in macrophage polarization through several downstream molecules, such as SOCS3, IRF8 and miRNAs. However, the exact role of Notch signaling on regulating macrophage heterogeneity during liver fibrosis remains to be elucidated.
Using macrophage-specific RBP-J deficient mice combined with liver fibrosis model, we find that Notch signaling blockade in macrophages ameliorates hepatic fibrosis by pathological staining. Further studies show that myeloid-specific RBP-J deficiency attenuates NF-κB activation remarkably that can be attributed to the upregulation of CYLD. Our observations are also verified using clinical cirrhosis samples. However, in this study, we do not find obvious macrophage polarization inclination in myeloid-specific RBP-J deficient fibrotic mice, indicating Notch signaling might regulate macrophage subsets and function during liver fibrosis. Moreover, we explore the precision role of different polarized macrophages in liver fibrosis treatment and find that M1-polarized macrophages transplantation can ameliorate murine liver fibrosis by modulating immune environment, suggesting more defined macrophages, more precision therapy for human disease in future.

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DOI: 10.1002/eji.201970400

P2977

Tim-3 aggravates podocyte injury in diabetic nephropathy by promoting macrophage activation via the NF-κB/TNF-α pathway

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Macrophage-mediated inflammation plays a significant role in the development and progression of diabetic nephropathy (DN). However, the underlying mechanisms remain unclear. Accumulated data suggest the critical roles of T cell immunoglobulin domain and mucin domain-3 (Tim-3) in regulating macrophage activation. However, the role of Tim-3 in the dysregulated macrophages in DN. In this work, our study for the first time demonstrated that **Tim-3 expression on renal macrophages was increased in patients with DN and in two diabetic mouse models, streptozotocin (STZ)-induced diabetic mice and db/db mice, and the increased Tim-3 expression positively correlated with renal dysfunction of DN patients**. Moreover, **Tim-3 deficiency ameliorated renal damage in STZ-induced diabetes with concurrent increase in protein levels of Nephrin and WT-1**. Similar effects were observed in mice with kidney-specific Tim-3 knockdown induced by intrarenal injection of Tim-3 shRNA-expressing lentivirus in STZ-treated mice. **Adoptive transfer of Tim-3-expressing macrophages, but not Tim-3 knockout macrophages, accelerated diabetic renal injury in DN mice**, suggesting a key role for Tim-3 on macrophages in the development of DN. Mechanically, Tim-3 activated the NF-κB/TNF-α signaling pathway in renal macrophages, accelerated podocyte injury and promoted proteinuria in DN. Till now, to our knowledge, this is the first report describing the role of Tim-3 on macrophages-promoted renal injury which shed new light on understanding the immunological pathogenesis of diabetic nephropathy.

P2978

Deciphering the mechanism of the anti-tumor function of CD8+ tissue-resident memory T cells in endometrial carcinoma

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Endometrial carcinoma (EC) is a group of gynecological malignancies with highest incidence in China, the morbidity and mortality of which are increasing strikingly. Conventional treatment approaches utilizing surgeries, radio- and chemo-therapies reached far less satisfying efficacy and disease remission rate. In regards to novel therapies, immune checkpoint inhibitors as the representative of cancer immunotherapy with successful treatment experience in other cancers showed poor response rates in EC. Previous studies focusing on the mechanism of immunotherapy in EC was very limited. Research into other types of tumors have shown that tissue-resident memory T cells (T_{RM}) play a key role in mediating tumor killing. Our team found CD103, which is a marker molecule of T_{RM}cells in epithelial sites, is related to prognosis of EC patients. We identified the existence of CD8+CD103+ T_{RM}cells in infiltrating lymphocytes of EC tissues through flow cytometry analysis and single-cell RNA sequencing with further comprehensive bioinformatics analysis. In EC tissues, T_{RM}cells are enriched and their immune-phenotype and transcriptional profile have been confirmed. Human EC T_{RM}cells shared parts of transcriptional profiles with T_{RM}cells in other tissues, and some specific transcription factors and chemokines stood out. We also examined their capacity of cytokine production which showed that EC T_{RM}cells display a stronger cytotoxic function than other CD8+ T cell subsets.

DOI: 10.1002/eji.201970400

Moreover, we explored the regulatory mechanisms of the EC T_{RM}cell function. The keynote of the study is to find the key mechanism of the anti-tumor effect of T_{RM}cells and to explore new immunotherapeutic strategies that benefit EC patients.

Aging and Immunity

O345

Targeting GSK-3β in favor of thymic epithelial cells proliferation and T cells regeneration in aged mice

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Age-dependent atrophy of thymic epithelial cells (TECs) results in the thymic involution and the decreased production of T cells, which is one of the most important factors that cause compromised immunity for aged individuals. Wnt signaling pathway plays a pivotal role in the development of TECs. GSK-3β, one of the main members of Wnt pathway, is in the activated status with age, which leads to the reduced expression of target genes such as *FOXN1*, inhibiting TECs proliferation. However, it is not confirmed whether the targeted inhibition of GSK-3β is able to facilitate T cells regeneration in aged mice by driving TECs proliferation. Here, we show that Targeted inhibition of GSK-3β results in the robust proliferation of TECs and the change of Wnt signaling pathway in TECs from aging mice *in vitro* and *in vivo*. In the fully involuted thymus of aged mice, targeted inhibition of GSK-3β induces obvious thymus regeneration characterized by increased thymopoiesis and enhanced naive T cell output. We verify that the regenerated thymus of aged mice closely resembles the juvenile thymus on the basis of gene expression profile and architecture. Furtherly our results indicate this treatment-mediated regeneration stems from an enlarged TECs compartment, rebuilt from progenitor TECs *in vivo*. Together, our findings prove the targeted inhibition of GSK-3β in aged mice can facilitate T cells regeneration, reverse aged-related thymic involution by driving TECs proliferation, providing the theoretical and experimental basis for improving immune competence of aged individuals and preventing geriatric diseases clinically.

O346

T cell rejuvenation requires multiple steps to restore immune function with aging

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Adaptive immune system is in charge of precise defense against a highly diverse array of microorganisms. For defense against new infections, the organism deploys naïve, previously antigen-unexposed, T and B lymphocytes, whose antigen-specific receptors recognize, and eventually orchestrate the removal of, the invading microorganisms. Naïve B, and even more so T, lymphocytes numerically diminish with aging. However, new data suggests that those that remain appear to have maintained their functional potential, contrary to an earlier dogma. These findings refocused our attention upon cell-extrinsic defects in immunity. Results will be presented showing that thymic rejuvenation is not sufficient to improve/increase naïve T cell pool in the secondary lymphoid organs. Moreover, data will be presented showing that aging of lymph nodes, including alteration in their architecture and stromal cell integrity, as well as changes in circulating, most likely soluble, factors,

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DOI: 10.1002/eji.201970400

critically modulate both homeostasis and function of the aging immune system. Implications for immune rejuvenation will be discussed.

O347

Effects of High Intensity Resistance Training (HIRT) on functional and immunological lung responses in elderly with metabolic syndrome

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The rate of metabolic syndrome (MS) is growing rapidly, especially among elderlies. MS present several comorbidities, also affecting the lung function. Physical exercise is known to attenuates the effects of aging as well the effects of MS, but the effects of high intensity resistance training (HIRT) on the lung function, mechanics, immune response and on respiratory muscle strength is unknown. The present study investigated in a group of 23 elderlies with MS, the effects of HIRT, performed at 80-90% of 1 maximal repetition, 2x/week, during 5 weeks, on classical parameters of lung function: force vital capacity (FVC), forced expiratory volume at first second (FEV1) and on FEV1/FVC value and mechanics total resistance of respiratory system (R5Hz), proximal airways resistance (R20Hz), respiratory reactance (X5Hz), total respiratory impedance (Z5Hz) and on maximal expiratory pressure (MEP) and maximal inspiratory pressure (MIP). The results demonstrated that HIRT did not affect the main parameters of lung function (FVC, FEV1 and FEV1/FVC; $p>0.05$), while improved R5Hz ($p< 0.01$), R20Hz ($p< 0.007$), and mechanics (X5Hz; $p< 0.05$) and Z5Hz ($p< 0.01$). In addition, HIRT also improved MEP ($p< 0.0001$) and MIP ($p< 0.0001$). Furthermore, the levels of IL-1beta, IL-6, IL-8 and TNF-alpha ($p< 0.01$) which are inflammatory mediators centrally involved in the MS, were reduced in the supernatant of induced sputem after 5 weeks of HIRT. We conclude that HIRT performed 2x/week improved the lung mechanics and respiratory muscle strength and reduced lung inflammation, being recommended as complementary therapy for elderlies with metabolic syndrome.

O348

Impulse oscillometry differentiates the lungs of elderly with and without metabolic syndrome: a Functional and immunological approach

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Impulse oscillometry (IOS) is an easy to perform lung function test, presenting great applications for physically or mentally limited populations, such as elderly. Since there is a high incidence of metabolic syndrome (MS) in elderly, which can affect the lung function, the present study investigated for the first time the lung function in elderly with and without metabolic syndrome using traditional spirometry and IOS. In addition, considering that systemic low-grade inflammation observed in MS could also occurs in the lungs driving pulmonary alterations, we also investigated for the first time the levels of inflammatory and fibrotic mediators in breath condensate of 77 elderlies (67,44±6,03 years old) without metabolic syndrome and 75 elderlies (68,52±5,98 years old) with metabolic syndrome. No significant differences in the spirometric values (FVC, FEV1, FEV1/FVC, FEF25-75) comparing non-

DOI: 10.1002/eji.201970400

MS with MS elderly was observed. However, a significant increase in total airway resistance (R 5Hz; p< 0.0091) and in peripheral airway resistance (R 20Hz; p< 0.0011) was observed in MS elderly. Since these changes in R 5Hz and R 20Hz may occurs due to airway inflammation and remodeling, the analysis of pro-inflammatory (IL-1beta, p< 0.0001; IL-8, p< 0.001; TNF-alpha, p< 0.04) and pro-fibrotic (VEGF, p< 0.0001), revealed that MS induces a pulmonary pro-inflammatory and pro-fibrotic response. We conclude that IOS is an important tool to differentiates the lung function of elderly with and without metabolic syndrome and that elderly with MS present increased airway resistance involving increases in pulmonary pro-inflammatory and pro-fibrotic mediators.

O351

Assessment of inflammatory response and fibrosis during muscle degeneration in elderly and patients with muscle dystrophies

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Skeletal muscle is one of the most affected tissues during ageing. It depends on reduced muscle regenerative capacity of stem (satellite) cells and on the deregulation of stromal cells (macrophages/fibroblasts) functions.
In this study we aimed to assess at a molecular and cellular level the key regulators involved in muscle degeneration in aged and in dystrophic muscles and to investigate the impact of anti-inflammatory treatments on inflammation and fibrosis in both groups.
Flow cytometry and cell sorting were conducted to characterize the monocytes subsets in 11 patients with DMD, and 15 elderly. Histological staining as well as gene expression for candidate genes involved in inflammation and fibrosis has been conducted on muscle biopsies.
Analysis of monocytes revealed that elderly and patients with dystrophies have an increased frequency of classical monocytes CD14++CD16+. This was associated with an increase of CD86. We show also that glucocorticoids seem to influence the polarization of macrophages towards an M2a profile. Evaluation of IL33 expression by lymphocytes has shown that this molecule is completely inhibited in patients treated with glucocorticoids, compared to aged muscles. No effect of glucocorticoids was observed on the level of inflammation within muscle tissue. Nevertheless fibrosis diminished. Transcriptomic analysis of relevant inflammatory/fibrosis genes is under investigation.
We showed that in patients with degenerative muscles, there is a dominance of pro-inflammatory monocytes in blood and M2 macrophages in muscle microenvironment; and that the treatment with glucocorticoid drugs doesn't decrease the intensity of inflammation but seem to modulate the secretion of specific profibrotic/inflammatory molecules.

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DOI: 10.1002/eji.201970400

O352

Traditional resistance training improves functional and immunological lung responses in elderly with metabolic syndrome

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Metabolic syndrome adversely affects the lung function. Resistance training (RT) improves metabolic syndrome and lung function, but its effects on functional and immunological lung response are unknown. We investigated the effects of resistance training (RT), 3x/week, for 10 wekks, on lung function in mechanics and pulmonary immune response of the elderly with metabolic syndrome. RT, after ten weeks was able to improve functional capacity ($p< 0.05$), decrease physical activity limitation ($p< 0.05$), pain level ($p< 0.05$), improve overall health status ($p< 0.05$), improve vitality ($p< 0.05$), reduce the level of body fat ($p < 0.05$), reduce the resting blood pressure ($p< 0.05$) and fasting glucose ($p< 0.05$) and glycated hemoglobin levels ($p< 0.05$), reduce total cholesterol ($p< 0.05$), LDL cholesterol ($p< 0.05$), triglycerides ($p< 0.05$) and increase HDL cholesterol ($p< 0.05$, $p< 0.05$). In relation to pulmonary function, there were no significant changes in FVC ($p>0.05$), FEV1 ($p>0.05$) FEV1/FVC ($p>0.05$). In relation to pulmonary mechanics evaluated by impulse oscillometry, there was an improvement of resistance of the distal airways R at 5Hz ($p< 0.001$) and the resistance of the proximal airways R at 20Hz ($p< 0.05$), as well as central resistance ($p< 0.001$). RT resulted in a reduction in IL-1beta, IL-6, TNF-alpha (10 weeks, $p< 0.001$), while increasing IL-1ra levels (< 0.001) and IL-10 ($p< 0.001$) in the supernatant of induced sputum. We conclude that resistance training provided an improvement in cardiometabolic response, pulmonary mechanics and pulmonary immune response of the elderly with metabolic syndrome.

LB100

Selected elements of quality of life in the course of Chronic Obstructive Lung Disease among patients in a residential environment

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Aim: To analyse the quality of life of COPD patients who were provided with outpatient care by the specialist clinic.
Material and methods: The research was performed on 303 patients over 65 years old, diagnosed with COPD and who were treated in specialist clinic in Poland, Swietokrzyskie Voivodeship. The diagnostic poll method with the use of standarized St. George's Hospital Respiratory Questionnaire (SGRQ) was applied in the research.
Results: In Swietokrzyskie Voivodeship, the maximum number of patients with COPD admitted to the hospital was 79 during one day. Approximately 39 patients a day was admitted to the hospital because of the acute exacerbations triggered by respiratory infections. 5,6% of the patients died during 30 days after discharge from the hospital, 7,1% died during 60 days after discharge and 9,1% died during 90 days after discharge. For the general assessment of the quality of life (QoL) of the patients with COPD the mean score was 44,1±14,2 with the median value 41,8. This area included the physical activity level and limitations resulting from dyspnea. The patients had the lowest QoL on the *Symptoms*

DOI: 10.1002/eji.201970400

subscale, where the average of scores was 73,2±12,3 with the median value 74,7.
Conclusions: The crucial symptom of COPD, which decreased considerably the quality of life of the study group was dyspnea. The longer the patient smoked and more often was hospitalized, the lower the quality of life was. Acute exacerbations of COPD entailed high mortality, also in the distant time after the patient was discharged from the hospital.

P0418

Bloom syndrome protein activates AKT and PRAS40 in prostate cancer cells

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Prostate cancer (PC) is a common malignant tumor and a leading cause of cancer-related death in men worldwide. In order to design new therapeutic interventions for PC, an understanding of the molecular events underlying PC tumorigenesis is required. Bloom syndrome protein (BLM) is a RecQ-like helicase, which helps maintain genetic stability. BLM dysfunction has been implicated in tumor development, most recently during PC tumorigenesis. However, the molecular basis for BLM-induced PC progression remains poorly characterized. In this study, we investigated whether BLM modulates the phosphorylation of an array of pro-oncogenic signaling pathways to promote PC progression. We found that silencing BLM in PC-3 cells significantly reduced their proliferative capacity. In addition, BLM downregulation significantly reduced levels of phosphorylated protein kinase B (AKT (Ser473)) and proline-rich AKT substrate of 40 kDa (PRAS40 (Thr246)), and this was accompanied by enhanced ROS (Reactive oxygen species) levels. We found that AKT and PRAS40 inhibition reduced BLM , increased ROS levels, and induced PC cell apoptosis .We demonstrated that BLM activates AKT and PRAS40 to promote PC cell proliferation and survival. We further propose that ROS act in concert with BLM to facilitate PC oncogenesis, potentially via further enhancing AKT signaling and downregulating PTEN expression. Importantly, inhibiting the BLM-AKT-PRAS40 axis induced PC cell apoptosis. Thus we highlight new avenues for novel anti-PC treatments.
Key word: BLM helicase gene; prostate cancer; CRISPR/Cas9 expression vector; iTRAQ; AKT (Ser473); PRAS40 (Thr246);ROS

P0419

Intrinsically altered lung-resident γδT cells prevent melanoma metastasis by producing interleukin-17A in the elderly

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DOI: 10.1002/eji.201970400

Background: Cancer is an age-associated disease, potentially related to the altered immune system of elderly individuals. Lung cancer, as the most common cancer with the leading cause of deaths remains to be elucidated in this population.
Methods: Immune cells were analyzed in the aged mice (20-24 months) compared with the young mice (10-16 weeks). Lung cancer of B16/F10 melanoma metastasis model was used.
Results: The number of lung-resident γδT cells was significantly increased with altered gene expression in aged mice versus young mice. Aged lung γδT cells predominantly produced interleukin-17A (IL-17A), resulting in increased levels in the serum and lungs. This played a critical role in resistance to melanoma metastasis in aged lungs with smaller and reduced number of tumor foci. The much longer survival of aged mice was dependent on IL-17A, since neutralization of IL-17A significantly shortened the survival time of aged mice with B16/F10 melanoma. In contrast, IL-17A supplementation significantly enhanced the survival time of young mice with B16/F10 melanoma. An in vitro cytotoxicity assay showed that aged lung γδT cells were highly cytotoxic to B16/F10 tumor cells with a higher level of CD103 expression. Furthermore, the anti-tumor activity of aged lung γδT17 cells was not affected by alterations in the load and composition of commensal microbiota, demonstrated through co-housing of the aged and young mice.
Conclusions: Intrinsically altered lung γδT17 cells underlying age-dependent changes prevent melanoma metastasis in the lungs, which will help to better understand the lung cancer progression in the aged.

P0420

Age related macular degeneration and innate immunity: molecular crosstalk between the complement system and the long pentraxin 3

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Dysregulation of the complement system is a key event in age-related macular degeneration (AMD), the leading cause of blindness in the developed world. Most of the genetic variation associated with AMD resides in genes encoding complement proteins, with the greatest risk being contributed by factor H (FH), major inhibitor of the alternative pathway of complement (AP). FH regulates complement activation both in solution and on cell surface by mediating the proteolytic cleavage of C3b, activator of the AP. One of the ligands of FH is the long pentraxin 3 (PTX3), a soluble pattern recognition molecule involved in innate immunity, tissue homeostasis and inflammation. PTX3 is locally produced in the retina pigmented epithelium (RPE), where, based on mouse models of AMD, it is believed to modulate complement activation via recruitment of FH. However how this occurs in the human eye is yet to be defined. In the present work we employed an integrated approach using *in vitro* binding assays and a cell model of RPE (ARPE-19) to investigate the PTX3-FH-C3b interactions and their effect on AP activation. In solid phase and surface plasmon resonance binding experiments we found that PTX3 specifically recognizes C3b, and this interaction is strengthened by FH. Importantly, C3b could not trigger AP activation when bound to PTX3. We extended these investigations to ARPE-19 cells under either oxidative or inflammatory conditions, to model the human disease *in vitro*. These findings point to a novel mechanism of complement regulation by PTX3 with potential implications in the etiopathogenesis of AMD.

DOI: 10.1002/eji.201970400

P1008

Differential effects of extracellular vesicles from aging and young mesenchymal stem cells in acute lung injury

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Old age is a known risk factor for mortality in acute respiratory distress syndrome (ARDS, acute lung injury). Mesenchymal stem cells (MSCs) possess potent immunosuppressive and anti-inflammatory properties, while aging MSCs have reduced capacity. Recent studies have demonstrated that MSC-derived extracellular vesicles (MSC-EVs) are able to mimic MSCs in alleviating acute lung injury. The goals of this study were to determine whether EVs from young and aging MSCs had differential effects on lipopolysaccharide (LPS)-induced lung injury in young mice and unravel the underlying mechanisms. Our results showed that both aging and young MSC-EVs had similar physical and phenotypical properties. As their parental cells, young MSC-EVs alleviated LPS-induced acute lung injury, while aging MSC-EVs did not exhibit the protective effects. In contrast to young MSC-EVs, aging MSC-EVs failed to promote the polarization of M2 macrophages in vivo and in vitro. In addition, the internalization of aging MSC-EVs by macrophages was significantly lower compared with that of young MSC-EVs. Aging and young MSC-EVs differed in levels of several miRNAs relating macrophage polarization. In conclusion, aging and young MSC-EVs have differential effects in alleviating acute lung injury, which may be associated with M2 macrophage polarization and their miRNA content.

P1009

Indian hedgehog suppressed senescence in bone marrow-derived mesenchymal stem cell by inhibition of ROS/mTOR pathway

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Premature senescence of bone marrow-derived mesenchymal stem cells (BMSC) was the major concerns for their clinical application and might be associated with aging-related diseases. Hedgehog signaling has been reported to prevent the prominent marker of aging in BMSC. Indian hedgehog (IHH) was one ligand of Hedgehog intracellular pathway, which was considered as an inducer in chondrogenesis of human BMSC. However, the role of IHH in aging of BMSC is still unclear. This study explored the role of IHH in senescence of human BMSC. Isolated BMSC were transfected with IHH siRNA, and aging-related reactive oxygen species (ROS) / mammalian target of rapamycin (mTOR) signaling pathways were investigated. In addition, cellular senescence was evaluated after incubation with exogenous IHH protein. Moreover, the interactions between IHH, mTOR and ROS were evaluated using corresponding inhibitors and antioxidants in the presence of IHH siRNA. We found that BMSC transfected with IHH siRNA showed characteristic senescence-associated features including senescence-associated β-galactosidase activity (SA-β-gal), development of senescence-associated secretory phenotype (SASP), induction of cell cycle inhibitors (p53/p16), activation of ROS and mTOR pathways, as well as promotion of skewed differentiation. Interestingly, BMSC treatment with IHH protein reversed the senescence markers and corrected biased differentiation. Moreover, IHH shortage-induced senescence signs were compromised after treatment by mTOR inhibitor and antioxidants. Our findings presented anti-aging activity for IHH in BMSC through down-regulation of

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DOI: 10.1002/eji.201970400

ROS/mTOR pathways. This discovery might contribute to increasing the therapeutic immunomodulatory and regenerative potency of BMSC and introduce a novel remedy in the management of aging-related diseases.

P1010

Stress induced premature senescence promotes proliferation by activating SENEX and p16^{INK4a}/ Retinoblastoma (Rb) pathway in Diffuse Large B-cell Lymphoma

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Objectives: Cellular senescence has been thought to be an important barrier to tumor formation. Recent studies have shown that stress induced premature senescence (SIPS) can promote partial tumor invasion. But how does SIPS effect in DLBCL, remains inconclusive.
Materials and methods: The immunophenotype of LY8 cell line was measured with Flow Cytometry. SIPS induced by Tert-butyl hydroperoxide (tBHP) was detected by Senescence β-Galactosidase Staining. Cell proliferation was analyzed with CCK8 and levels of ARHGAP18 (SENEX gene encoding protein) and p16/Rb pathway were measured with western-blot. LY8 cell were transfected with SENEX-SiRNA/NC and verified with western-blot.
Results: Our results suggested the immunophenotype of LY8 cell line is CD19, CD20, CD10 positive, and immunoglobulin light chain is kappa type. Cellular senescence model of DLBCL could be successfully induced by 30μM tBHP. ARHGAP18, p21, p16 and RB protein levels were significantly increased but the level of pRB expression were decreased in SIPS group compared with other groups. Meanwhile, the proliferation was increased in SIPS group than other tBHP groups. Further, the expression of p21 and p16 were significantly decreased in SENEX-SiRNA group compared with NC group.
Conclusions: SIPS formation activates ARHGAP18 and p16/Rb pathway and promotes DLBCL cells proliferation. Furthermore, SENEX activates p16 pathway in DLBCL. SIPS promotes proliferation by activating SENEX and p16/Rb pathway in DLBCL. SENEX-related SIPS may serve as an important target for Relapsed/Refractory DLBCL therapy.

P1011

Cardiorespiratory fitness modulates the proportions of monocytes and T helper subsets in lean and obese men

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Background/Aim: Obesity is now recognized as a model of premature immunosenescence and chronic inflammation. Although exercise training induces anti-inflammatory effects without changes in fat mass, the role of Cardiorespiratory Fitness (CRF) on immunity of obese subjects is unknown. We aimed to verify the peripheral frequency of CD4+CD25^{high}CD127^{low} Treg cells, CD39 and CD73 on CD4+CD25-/+ T cells, monocyte subsets and the systemic levels of cytokines in lean and obese men with different levels of CRF.
Methods: Mononuclear cells were obtained from 45 lean and 45 obese men who were assigned into six groups according to their body mass index and CRF (low, moderate or high) to analyze the frequency of monocyte subsets and subpopulations of CD4+ T cells (Treg cells; mTeff, CD4+CD25-CD39+; mTreg, CD4+CD25+CD39+). The systemic levels of interleukin (IL)-6, IL-10, IL-17a, IL-33,

DOI: 10.1002/eji.201970400

leptin and tumor necrosis factor alpha (TNF-α) were also evaluated. Seven obese men with low VO2Peak performed one week of High Intensity Interval Training and blood samples were collected before and 24 h after the last session for CD4+ T cell phenotype.
Results: Higher CRF status modulates the obesity-associated alterations in Treg cells, the frequency of mTeff and mTreg cells and the percentage of intermediate and non-classical monocytes. We observed an imbalance between proinflammatory (IL-6 and TNF-α) and immunoregulatory (IL-10 and IL-33) cytokines according to CRF in all subjects. HIIT increased the proportions of circulating mTreg and Treg cells in sedentary obese individuals.
Conclusion: The aging of immune system of obese individuals may counteract with increasing CRF.

P1608

SENEX gene induced Rb/E2F1 activation promotes proliferation and relapse of diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma with a high proliferation rate. SENEX is a new gene involved in cell senescence, apoptosis and even in cancer. The purpose of this study is to find out the expression, role and related molecular mechanism of SENEX in DLBCL. We found a higher expression level of SENEX in recurrent DLBCL patients. SENEX activated lymphoma cell proliferation at cellular level. Furthermore, SENEX gene promoted the phosphorylation of RB and the activation of E2F1. Together, our study demonstrates that SENEX gene induced Rb/E2F1 activation promotes the proliferation and relapse of DLBCL.

P1609

Aging and diet-induced obesity impairs the innate immune antimicrobial function of dermal fibroblasts

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Staphylococcus aureus is responsible for the majority of skin and soft tissue infections, and aging and the growing obesity epidemic have further increased this risk. Dermal fibroblasts (dFBs) resist infection by locally differentiating into adipocytes and producing antimicrobial peptide cathelicidin (CAMP) in response to S. aureus. We have recently shown that aging leads to loss of this antimicrobial protection from dermal fat and this is mediated by an age-dependent activation of the TGFBR-SMAD2/3 pathway in dFBs. Here we investigated if obesity may also disturb this beneficial immune response from dermal fat. Using diet-induced obesity (DIO) mouse model, we found that obese dWAT was not only characterized by hypertrophic growth of adipocytes and but also by a drastic depletion of adipogenic dermal fibroblasts (dFB), compared to lean controls. As a result, pathogen-triggered adipogenesis responses, including preadipocyte activation and CAMP production, were impaired in obese compared to normal diet mice. In vitro co-culture experiments revealed that that mature adipocytes rapidly suppressed the adipogenic function of dFB and this was mediated by TGFβ secreted from mature adipocytes. Treatments with TGFBR inhibitor (SB) and PPARγ agonist Rosiglitazone (ROSI) not only restored the adipogenic function of dFB the in vitro co-culture, but also improved the reactive adipogenesis response and increasing skin resistance against *S. aureus* in HFD obese mice in vivo.

DOI: 10.1002/eji.201970400

Together, we show aging and diet-induced obesity impairs the innate antimicrobial function of dFB and TGFBR and PPARγ may be potential therapeutic targets to treat *S. aureus* skin infection associated with aging and/or obesity.

P1610

Reduced production of laminin by hepatic stellate cells contributes to impairment in oval cell response to liver injury in aged mice

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Aged liver usually shows insufficient regeneration capacity in response to hepatic injury. Tissue-specific stem cells participate in the repair of tissue injury. However, how oval cells (OCs) respond to liver injury and how the process is regulated by tissue microenvironment in aged mice have not been fully understood. In this study, taking advantage of well established murine OC activation model, we demonstrated that OCs were less activated upon injury in aged mice compared with young mice, and the impairment was mainly attributed to dysfunction in their niche. Through analyzing global gene expression, we found that the genes differentially expressed in damaged young and aged mouse liver tissues were predominantly those required for the formation and remodeling of extracellular matrix. As one of the most important extracellular matrix components in the OC niche, laminin was shown to promote the proliferation of OCs. Not surprisingly, laminin was downregulated with aging. Consistent with the downregulation of genes encoding DNA-dependent protein kinase (DNA-PK) proteins in aged hepatic stellate cells (HSCs), inhibition of DNA-PK also led to reduced expression of laminin in HSCs. Moreover, impairment in OC activation caused by less supporting from DNA-damaged HSCs could be rescued by laminin. This study reveals a new cellular mechanism underlying impaired OCs functionality during aging.

P1611

HMGB1 modulates the balance between senescence and apoptosis in response to genotoxic stress

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HMGB1 is associated with the resistance of many types of human cancers to chemotherapy; however, its role in cancer metastasis remains unexplored. We examined the HMGB1 status of both highly and poorly metastatic cancer cells in response to genotoxic stress. Human and mouse cancer cells were treated with doxorubicin (Dox) and Camptothecin (CPT). While highly metastatic mouse melanoma B16-F10 cells preferred senescence with persistent HMGB1 expression, poorly metastatic B16 cells entered apoptosis with decreasing HMGB1 levels via cleavage under Dox treatment. Similarly, more metastatic human melanoma SK-MEL-24 and human colon cancer LS174T cells underwent senescence, while less metastatic SK-MEL-28 and DLD-1 cells exhibited apoptosis under Dox stimulation. In senescent B16-F10, SK-MEL-24, and LS174T cells induced by Dox, p21 levels were increased by persistent HMGB1 expression. The same effects were observed in these pairs of cancer cells treated with another genotoxic stressor, CPT. Indeed, while WT MEF entered senescence

DOI: 10.1002/eji.201970400

accompanied by p21 increase, HMGB1 KO underwent apoptosis with p21 decrease by Dox treatment. In our cell model system, we demonstrated that highly metastatic cancer cells preferentially enter senescence, whereas apoptosis predominates in weakly metastatic cancer cells under genotoxic stress, which depends on the presence or absence of HMGB1, suggesting that the HMGB1-p21 axis is required for genotoxic stress-induced senescence. These findings suggest that HMGB1 modulation of cancers with different metastatic status could be a strategy for selectively enforcing tumor suppression.

(This work was supported by Korean NRF Grants (2017R1A2B3006704; 2017R1D1A1B03028551)

P1612

Increased monocyte activation as a source of chronic inflammation and driver of biological aging in older adults

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Older adults (≥65 years) are highly heterogeneous in their health status, challenging the ability to provide appropriate care. The concept of biological age (BA) has been used to illustrate this heterogeneity. While many attempts have been made to measure the BA, the underlying mechanisms of biological aging remain unclear. Here, we explore monocytes as a source of chronic inflammation, a potential driver of biological aging.
Two groups of older adults were enrolled in a cross-sectional design: a “high BA”-group with an acute hospital admission four weeks prior to data collection (n=52), and a “low BA”-group consisting of age and sex matched participants without acute hospital admission within the two years preceding data collection (n=52). We assessed the distribution of classical (CD14+CD16-), intermediate (CD14+CD16+), and non-classical (CD14-CD16+) monocyte subsets, as well as NF-κB phosphorylation (Ser529) using flow cytometry.
Participants were on average 76.4 years old, 48% were women. Preliminary results show no differences in monocyte subset distribution between the groups, and no differences in phospho-NF-κB (p-NF-κB) expression between the monocyte subsets. However, monocytes of the low BA group had lower constitutive p-NF-κB compared to the high BA group ($p < 0.0001$), which may indicate a role of monocytes in chronic inflammation.
We will validate these findings by assessing cytokine levels using Luminex. We will also assess p-NF-κB and cytokine production in response to stimulation with lipopolysaccharides, TNF-α, and PBS. Moreover, we will investigate associations of NF-κB phosphorylation with other biomarkers of chronic inflammation as well as measures of physical and cognitive function.

P2199

The association of dietary patterns with knee symptoms and MRI detected structures in patients with knee osteoarthritis (OA)

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DOI: 10.1002/eji.201970400

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Objectives: This study aimed to examine the cross-sectional and longitudinal associations of dietary patterns with knee symptoms and structures in knee OA patients.
Methods: Participants were from a subgroup of an RCT, who had symptomatic knee OA and vitamin D deficiency at baseline and received 50,000IU vitamin D3 (N= 129) or placebo (N= 132) monthly for 24 months in Tasmania, Australia. Dietary was assessed by the Anti-Cancer Council of Victoria food frequency questionnaire. At baseline and 24 months, knee symptoms were assessed using WOMAC index and knee structures using MRI. Factor analysis was used to identify dietary patterns. Each participant received a score for each dietary pattern, with a higher score indicating a great intake of food composing that pattern. Associations between dietary pattern scores and knee OA outcomes were examined using multivariable linear regressions with adjustment for age, sex, BMI, treatment arm, total energy intake and physical activity.
Results: Three dietary patterns were identified. Participants with higher “healthy pattern” or “vegetable and meat pattern” scores had lower baseline WOMAC function scores and also had lower baseline total WOMAC scores. Higher “western pattern” scores were associated with increased total WOMAC scores and WOMAC function scores over 24 months. In multivariable analyses, dietary patterns were not significantly associated with any knee structure cross-sectionally or longitudinally.
Conclusion: These results suggest that maintaining a healthy dietary pattern may have beneficial effects on knee function, whereas maintaining a western pattern may contribute to increased functional disability over time in knee OA patients.

P2200

Immune age derived from T-cell receptor features as an indicator of individual health status

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Human immunity gradually declines with age, however, there is currently no good way to monitor the recession of individual immunity. With the rise of high throughput sequencing technics, it is possible for us to scrutinize each individual's T cell receptor(TCR) repertoire which is essential to the adaptive immunity. To this end, we recruited thousands of healthy individuals from 0 to 80 years of age to perform peripheral blood TCR repertoire sequencing. We found that features vary with age, for example, TCR ´s diversity indicators and diseases associated unique TCR clone number decrease significantly with age, however, the sum of diseases related TCR proportion correlates positively with age. Based on these discoveries, we were able to map the baselines for several immune indicators significantly correlates with age, and build up an immune age evaluation model. The immune age index calculated through this model is in correlation with many non-immune health indicators including nutrients, metabolism, skin, etc. Compared with the chronological age, immune age derived from our model can better characterize the immune health status of individuals, thus support researches and therapies that fight against immune aging.

DOI: 10.1002/eji.201970400

P2201

Vitamin D supplementation improves depressive symptoms in knee osteoarthritis patients over 24 months

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Objectives: To determine the effect of vitamin D supplementation and maintaining sufficient serum vitamin D on depressive symptoms in patients with knee osteoarthritis (OA) and vitamin D deficiency.
Methods: Participants with symptomatic knee OA and vitamin D deficiency were enrolled in a multicentre, randomized, double-blind, placebo-controlled trial and received 50,000IU vitamin D3 (N= 209) or placebo (N= 204) monthly for 24 months. Serum 25-hydroxyvitamin D [25(OH)D] was measured at baseline, 3 and 24 months. Depressive symptoms were measured using the patient health questionnaire (PHQ-9, 0- 27) at baseline, 3, 6, 12 and 24 months. Multilevel mixed-effect models were used to compare differences of change in PHQ-9 scores between groups.
Results: Over 24 months, 340 participants (82.3%) completed the study. The baseline prevalence of depression (PHQ-9 score ≥5) was 25.4%. Depressive symptoms improved more in the vitamin D supplementation group (β: -0.45, 95% CI: -0.84 to -0.07) compared to the placebo group (β: 0.21, 95% CI: -0.19 to 0.61) (P for difference = 0.02) and in the participants who maintained vitamin D sufficiency (β: -0.44, 95% CI: -0.88 to -0.00) compared to those who did not (β: 0.40, 95% CI: -0.18 to 0.97) (P for difference = 0.02) over 24 months.
Conclusions: These findings suggest that vitamin D supplementation and maintaining adequate vitamin D levels over 24 months may be beneficial for depressive symptoms in patients with knee OA.

P2202

Epidermal dysfunction leads to an age-associated increase in levels of serum inflammatory cytokines

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Even though elderly populations lack visible or other clinical signs of inflammation, their serum cytokine and C-reactive protein levels typically are elevated. However, the origin of age-associated systemic inflammation is unknown. Our previous studies showed that abnormalities in epidermal function provoke cutaneous inflammation, and because intrinsically aged skin displays compromised permeability barrier homeostasis and reduced stratum corneum hydration, we hypothesized here that epidermal dysfunction could contribute to the elevations in serum cytokines in the elderly. Our results show first that acute disruption of the epidermal permeability barrier in young mice leads to a rapid increase in cutaneous cytokine mRNA expression and serum cytokine levels. Second, cytokine levels in both the skin and serum increase in otherwise normal, aged mice. Third, expression of TNFα and amyloid A mRNA levels increased in the epidermis, but not in the liver, in parallel with a significant elevation in serum levels of cytokines. Fourth, disruption of the permeability barrier induced similar

DOI: 10.1002/eji.201970400

elevations in epidermal and serum cytokine levels in normal and athymic mice, suggesting that T cells play a negligible role in the elevations in cutaneous and serum inflammatory cytokines induced by epidermal dysfunction. Fifth, correction of epidermal function significantly reduced cytokine levels not only in the skin but also in the serum of aged mice. Together, these results indicate that the sustained abnormalities in epidermal function in chronologically aged skin contribute to the elevated serum levels of inflammatory cytokines, potentially predisposing the elderly to the subsequent development or exacerbation of chronic inflammatory disorders.

P2203

The effects of traditional Chinese medicine KSLP on adipose-derived stem cells in aged mice

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Aging is considered as a major risk factor for the growth and multilineage differentiation potential of Adipose-derived stem cells (ADSCs). In current study, the effects of an anti-aging tablets (KSLP) on ADSCs derived from aged mice were evaluated in the aspects of cell cycle, apoptosis as well as the adipogenesis and osteogenesis abilities of ADSCs. RNA-Seq technology was applied to define differentially expressed genes (DEGs) for biological informatics analysis. Results showed that with the increasing of donor age, percentage of ADSCs in G0/G1 phase rose, in the meantime, their proliferation index decreased. KSLP decreased cells percentage in G0/G1 phase and improved the proliferation index. There was no obviously difference in apoptosis between the groups. In addition, weaken of adipogenesis and osteogenesis of ADSCs appeared in aging mice. KSLP effectively increased the osteogenesis ability of ADSCs. RNA-Seq results showed that there were a total of 146 DEGs between ADSCs derived from 6-8 weeks and 6-8 months mice, and 175 DEGs between ADSCs derived from 6-8 months mice with or without KSLP treatment. GO and KEGG analyses indicated that KSLP may participate in the negative regulation of apoptosis, protein metabolism and modification, osteoblast differentiation and phagocytosis. In summary, our study provided evidences indicating that KSLP can promote the cell proliferation and osteogenesis ability of aged ADSCs, the role of which is related to the negative regulation process of apoptosis, protein metabolism and modification, osteogenesis differentiation and phagocytosis.

P2979

PIM1 induces cellular senescence through phosphorylation of UHRF1 at Ser311

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PIM1 is a proto-oncogene, encoding a serine/threonine protein kinase that regulates cell proliferation, survival, differentiation, apoptosis, and drug resistance. The expression of PIM1 has been shown to be tightly controlled at multiple levels: transcriptional, post-transcriptional, translational, and post-translational. Expression in normal tissues is nearly undetectable. However, in hematopoietic malignancies and in a variety of solid tumors, increased PIM1 expression has been shown to correlate with the stage of disease. Recent evidence suggests that PIM1 play a role in immune regulation and inflammation. Previous reports suggest that the expression of PIM1 is increased in senescent cells and the overexpression of PIM1 can induce cellular senescence. However, the molecular mechanism underlying this process is not fully understood. Here we report that UHRF1 is a novel substrate of PIM1 kinase, which could be phosphorylated at Ser311and therefore promoted to degradation. Our data demonstrates that PIM1 destabilizes UHRF1, leading to DNA hypomethylation, which

DOI: 10.1002/eji.201970400

consequently results in genomic instability, increased p16 expression, and subsequent induction of cellular senescence.Taken together, our results suggest that down-regulation of UHRF1 is an important mechanism of PIM1-mediated cellular senescence.

P2980

Application of eHSP70 and CRP in auxiliary diagnosis and risk monitoring of H type hypertension

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Objective: To explore the application of eHSP70 and CRP in the auxiliary diagnosis and risk monitoring of H-type hypertension.
Methods: Serum samples of healthy people, normal hypertension and H-type hypertension were collected. The levels of eHSP70 in serum were detected by ELISA. Serum CRP and blood lipid were detected by automatic biochemical analyzer. Whole blood white blood cell count (WBC) was detected by automatic blood analyzer.
Results: Compared with healthy control group, eHSP70, CRP, WBC and triglyceride were significantly increased in normal hypertension and H-type hypertension group ($P < 0.05$), and cholesterol was not significantly different. The levels of eHSP70 and CRP in H-type hypertension group were significantly higher than those in normal hypertension group (P < O.05), and those in high-risk group (Hcy > 20) and low-risk group (10 < Hcy < 20) were also significantly higher (P < O.05).
Conclusion: Serum eHSP70 and CRP in patients with H-type hypertension were significantly elevated and positively correlated with the degree of risk, which could be used as laboratory indicators for clinical auxiliary diagnosis and risk monitoring of H-type hypertension.

P2981

T-cell immunosenescence promotes bone resorption by facilitating osteoclasts proliferation

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Immunosenescence is characterized by a progressive functional decline of cells, tissues and organs, including bone and T lymphocytes (naïve, effector and memory T-cell subsets), yet whether T-cell immunosenescence is closely related to bone resorption remains unclear. To address this issue, we established a mouse model of T-cell immunosenescence induced by dexamethasone. We found apoptosis of thymocytes and reduced percentage of naïve T cells in aging model mice, which impaired the renewability of T lymphocytes. Another major aspect of T-cell immunosenescence was the imbalance of T cell subsets, as well as the decreased ratio of Th1/Th2 and increased ratio of Th17/Treg in bone marrow and in spleen. However, the serum concentrations of TRACP5b which is specific for osteoclasts activation were seriously elevated along with the increase of RANKL-production by T lymphocytes in model mice. In addition, the rate of bone lacuna was significantly increased, consistent with the smaller amount of trabecular bones in model mice. Furthermore, NFAT2, the key transcriptional factor for osteoclastogenesis was markedly upregulated in mRNA levels, and the expressions of upstream target genes TRAF6, p38, and MEK1/2 signaling associated with osteoclast differentiation also elevated in aging model mice. Hence, our study has revealed that abnormal activation of osteoclast was accompanied with T cell subsets imbalance in aging model mice.

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DOI: 10.1002/eji.201970400

P2982

RECQ4 restart stalled replication fork at telomere though the interaction with CST

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In the previous study, we have reported that the newly identified telomere binding protein complex CTC1-STN1-TEN1 could reinitiate the stalled telomere replication forks via dominate origins firing. However, the CST complex doesn´t exhibit the properties of DNA polymerase, topoisomerase or helicase, which is required for the DNA replication re-initiation, suggesting other replication factors might participate in this process. To further investigate the mechanism of replication re-initiation at telomere, we started to identify the CST interacting proteins. RECQ4 turned out to be one of these factors. RECQ4 is a member of the RECQ helicase family, which could unwind double strand DNA and initiate the replication. Mutations in RECQ4 lead to specific features of Rothmans-Thomson syndrome, including skeletal abnormalities, short life span and increased risk of cancer. Here, we identified that RECQ4 knockout could induce telomere replication disorders as well as stalled telomere replication forks. At the meanwhile, we found that RECQ4 was recruited to the stalled forks through the interaction with CST since the mutation disrupts the interaction between RECQ4 and CST lead to MTSs and other telomere replication defectives. Moreover, our results showed that RECQ4 could initiate the Pre-initiation complex formation with MCM10 and fired the dominate origin to restart the stalled replication forks at the telomere. In summary, our result showed that RECQ4 could restart the stalled replication forks though the interaction with CST.

P2983

Anti-aging effect of hPMSCs via upregulating the Nrf2/ARE pathway and attenuating oxidative Stress of $CD4^+$T cells

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Aging is accompanied with increased levels of reactive oxygen species (ROS) and oxidative stress, which contribute to the initiation and progression of various diseases in the elderly. The transcription factor Nrf2 (nuclear factor [erythroid-derived 2]-like 2) plays crucial roles in the defense mechanisms against oxidative stress and mediates anti-inflammatory actions under various pathological conditions. The aim of this study is to evaluate the protective effect of human placenta-derived mesenchymal stromal cells (hPMSCs) on ROS-mediated $CD4^+$T cells oxidative damage through Nrf2/ARE pathway during aging. Aging mice model was induced by subcutaneous injection of D-gal (200 mg kg-1 d-1 for 8 weeks). hPMSCs intervention significantly increased the levels of antioxidantenzymes SOD, CAT and GSH, decreased the levels of oxidative damage and apoptosis of activated $CD4^+$T cells in peripheral blood of aging mice. Further in vitro results showed that Nrf2 mRNA and protein levels significantly increased in activated $CD4^+$T cells after hPMSCs treatment. In addition, the antioxidant effect of hPMSCs was significantly inhibited after the treatment of Nrf2 inhibitor ML385 in activated $CD4^+$T cells. The results suggest that the exogenous administration of hPMSCs could protect $CD4^+$T cells against oxidative stress mediated apoptosis via Nrf2/ARE signaling pathway. The results of this study may provide a novel idea for application of hPMSCs in immunomodulatory and anti-aging.

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DOI: 10.1002/eji.201970400

P2984

Th1 biased progressive autoimmunity in aged *Aire*-deficient mice accelerated thymic epithelial cell senescence

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Although autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, are frequently associated with premature aging of the thymus, a direct link is missing between autoimmunity and thymic atrophy. Here we monitored the progression of thymic involution in *Aire*-deficient mice, in which defective negative selection causes spontaneous and progressive development of autoimmunity. In young and middle-aged mice, *Aire* deficiency appeared to be protective as supported by the reduced β-gal$^+$ epithelial cells and the enhanced thymic output. However, once the autoimmune phenotype was fully developed in aged *Aire*-deficient mice, their thymuses underwent accelerated involution. In comparison to the age-matched wildtype littermates, old *Aire*-deficient mice showed lower numbers of total thymocytes and recent thymic emigrants but more β-gal$^+$ thymic epithelial cells. This phenomenon may partly be attributable to the increased number of activated Th1 cells homing to the thymus. This speculation was further supported by the enhanced thymic aging following repeated challenges with complete Freund's adjuvant immunization. Taken together, the present study highlights a unique mechanism by which autoimmunity facilitates the senescence of thymic epithelial cells through returning Th1 cells.

Neuroimmunology

O153

Neuroimmunological changes during pulmonary Tuberculosis in association with behavioural abnormalities

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis,* and it induces chronic inflammation. During the development of chronic inflammation, there is neuronal activation and changes in the synthesis and production of neurotransmitters and cytokines that affect functions such as learning ability, memory and mood.
Patients with TB develop a myriad of mental illness like depression and anxiety. Thus, we investigated the effect of lung infection with *M. tuberculosis* on the immune response in the Central Nervous System and its relationship with behavioural changes in the absence of culturable mycobacteria in the brain in a murine model of TB.
The results showed an increase in TNFa, IFNg and IL-12, IDO and iNOS enzymes and IL10, IL4 and TGFb in the hypothalamus, hippocampus and cerebellum. There were significant changes in the production of neurotransmitters noradrenaline, adrenaline, dopamine and serotonin.
Neurodegeneration occurred in the hippocampus and cortex at an early stage of the disease, and it led to neuronal death in the progressive phase of the disease, with an increase of p38 and JNK, BDNF

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DOI: 10.1002/eji.201970400

levels decreased and rose in the permeability of the blood-brain barrier. All these changes were related to the existence of depressive-like behaviour, sickness behaviour, anxiety-like behaviour, neurological damage and impairment not only in short-term memory but also in long-term memory. Our results suggest for the first time that cytokines' lung production and local inflammation during pulmonary Tuberculosis affects the CNS and causes Neuroinflammation in the absence of brain infection.

O154

Microglia regulate cardiovascular function via modulation of the activities of hypothalamic sympathetic neurons

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Microglia, the primary immune cells in the central nervous system, are critical in the maintenance of brain homeostasis. Our previous work showed that microglia were activated in hypertensive mice, and depletion of microglia had profound effects on blood pressure, possibly through autonomic neurons. In this study, we assessed the sympathetic neuronal activity in the hypothalamic paraventricular nucleus (PVN), a nucleus orchestrating sympathetic outflow. PVN sympathetic neurons were pre-labeled with retrograde fluorescent beads from rostral ventrolateral medulla in the brain stem. By intracerebroventricular infusion of diphtheria toxin into CD11b-DTR mice, we specifically depleted microglial cells in the brain. Using a whole-cell patch clamp technique, we found that there were decreased frequencies of both miniature excitatory and inhibitory synaptic inputs to the sympathetic neurons in the absence of microglia. Meanwhile, depletion of microglia inhibited outward potassium currents in the sympathetic neurons. These results suggest that microglia regulate both synaptic inputs and intrinsic activities of the PVN neurons. To further examine the role of microglia in the regulation of cardiovascular function, we investigated the baroreflex sensitivity in the absence of microglia. It showed that loss of microglia attenuated baroreflex responses induced by sodium nitroprusside or phenylalanine compared to PBS-infused CD11b-DTR control mice. Together, our results strongly suggest that resident microglia are important in the central regulation of autonomic neuronal activity and cardiovascular function.

O155

All trans-retinoic acid protects against acute ischemic stroke by modulating neutrophil functions through STAT1 signaling

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Background and Purpose: Regulation of post-stroke neural inflammation is considered as a vital therapeutic target in ischemic stroke. All trans-retinoic acid (atRA), a potent immune modulator, has raised interest in the field of stroke therapy. However, the immunological mechanisms for atRA-

DOI: 10.1002/eji.201970400

mediated neuroprotection remain elusive. The current study evaluated the impact of atRA on post-stroke neural inflammation and elucidated the mechanisms involved in regulation of related neutrophil functions.
Methods: atRA was prophylactically administered to mice 1d before transient middle cerebral artery occlusion (tMCAO, 1h) and repeated daily immediately after reperfusion. Stroke outcomes, neutrophil polarization, and neutrophil extracellular traps (NETs) formation in stroke lesions were assessed. Neutrophil depletion was induced with anti-Ly6G antibodies. Primary neutrophil cultures were used to explore the mechanisms of atRA treatment.
Results: Prophylactic atRA treatment reduced infarct volumes and neurological deficits at 1d after tMCAO. Post-stroke neural inflammation was attenuated and neutrophil accumulation in ischemic lesion was down-regulated. atRA treatment skewed neutrophil towards N2 phenotype which facilitated its clearance by macrophage and inhibited NETs formation. The functions of neutrophil were vital in the protective effects of atRA and were associated with suppression to STAT1 signaling by atRA. Post-stroke systemic administration of atRA still provided efficient protection to cerebral ischemia.
Conclusion: atRA displays potent therapeutic efficacy in ischemic stroke by attenuating neural inflammation. Treatment of atRA impeded neutrophil accumulation, favored N2 polarization and forbade NETs formation in ischemic lesion. STAT1 signaling played a decisive role in the mechanisms of atRA-afforded regulation to neutrophil.

O156

Neuroprotective effects of A151 in mice stroke model in relation to cytoplasm dsDNA sensing induced inflammation

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Background and Purpose: Cytosolic DNA is a very dangerous signal for cells, which can be recognized by dsDNA sensors such as cyclic GMP-AMP (cGAMP) synthase (cGAS) and absent in melanoma 2 (AIM2) inflammasome, and subsequently promote neuroinflammation. Here, we examined the role by using their inhibitor, A151, a suppressive oligodeoxynucleotides containing TTAGGG motifs, on brain injury and inflammation in a mouse model of experimental stroke.
Methods: Here, in middle cerebral artery occlusion (MCAO) mice, we study the inflammatory response related to cytoplasm dsDNA in the context of brain tissue, especially in the microglia by therapeutically inhibiting cGAS and AIM2. And we determined their roles on neuroinflammation. All animal experiments were approved by the local ethics board.
Results: A151 administration dramatically reduced infarct volume compared with that in vehicle group after MCAO surgery. A151 treatment attenuated neurodeficits, improved clinical outcomes and diminished cell death in neurobehavioral tests. The protection of A151 was associated with inhibition of AIM2 inflammasome and cGAS activation, both in vitro and in vivo. Additionally, mice treated with A151 showed a dampened immune response to stroke. A151 administration reduced the number of Iba-1 cells and microglial production of IL-6 and TNF-α. Notably, the protective effect of A151 was abolished in mice depleted of microglia cGAS.
Conclusions: These findings indicate that A151 treatment attenuates brain injury and inflammation after MCAO. A151 is a potential therapeutic option for MCAO that warrants further investigation.

DOI: 10.1002/eji.201970400

O157

Suppressors of cytokine signalling SOCS4 and SOCS5 as controllers of neuroinflammation during viral encephalitis

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CNS viral infections are one of the major causes of morbidity and mortality worldwide and a significant global public health concern. Viral encephalitis is being reported in 1 in 10,000 people with neurologic complications, particularly in children, being a major health issue. There is a body of evidence indicating that neuroinflammation, despite its protective role, can be harmful to the brain and is considered to be a risk factor in neurogenerative disorders. Controlling the brain inflammation and its collateral damage is critical to reducing the mortality and morbidity associated with viral encephalomyelitis. The suppressor of cytokine signalling (SOCS) proteins are one of the mechanisms that can modulate brain inflammatory and anti-viral responses. The SOCS proteins are an important family of small intracellular proteins which act to limit the duration of signalling responses. While the biochemical and biological functions of SOCS1-3 and CIS are well characterised, comparatively little is known regarding the biological role of SOCS4 and SOCS5. We demonstrate that lack of either SOCS4 or SOCS5 results in changes in susceptibility to neurotropic infection with Semliki Forest virus (SFV). SOCS4-deficeint mice show accelerated viral clearance in the brain and reduction in persisting viral RNA accompanied by increased inflammatory infiltration and more severe histopathology. SOCS5 knockout mice display exacerbated weight loss upon SFV infection, overall dysregulation in cytokine production followed by a significantly greater inflammatory infiltrate composed mainly by CD11b+ cells. These results demonstrate that SOCS4 and SOCS5 are critical regulators of anti-viral immunity in the CNS.

O158

Recruited brain conventional dendritic cells and T cells promote protection against CNS-TB

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Tuberculosis is a primarily a pulmonary infection caused by *Mycobacterium tuberculosis* (Mtb) and approximately 1% of extra-pulmonary TB cases are attributed to central nervous system tuberculosis (CNS-TB). CNS-TB is a severe form of TB that primarily develops in children and immunocompromised adults and its associated with high morbidity and mortality. Dendritic cells (DCs) are antigen presenting cells and get recruited to the brain during inflammation. We phenotypically and functionally characterized DCs and T cells during CNS-TB using flow cytometric analysis. Female C57BL/6 mice were intracerebrally infected with H37Rv (Mtb) or mock-infected with saline, then sacrificed at 2, 4, 6 weeks and organs harvested. Bacilli were detected in the brain and bacterial burdens significantly decreased 4 weeks post infection. Inflammatory responses resulted from both mock and Mtb infection and were significantly higher in Mtb infected brains and cervical lymph nodes (CLNs). Conventional dendritic cells were preferentially recruited to the brain and drained into CLNs, these DCs showed increasing brain kinetics at week 4. Activated Mtb DCs highly expressed CD86 and produced significantly high levels of IL-1β, IFNγ and IL-12. Brain Infiltrating Mtb specific T cells were

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DOI: 10.1002/eji.201970400

dominantly CD4+ T cells than CD8+ T cells and had a highly activated phenotype. Mtb infected mice expressed high Tbet levels during CNS-TB in the brain and CLNs. Pro-inflammatory cytokines produced by mature DCs contributed to protection against CNS-TB. DCs contributed to inducing a regulated Th1 immune response during CNS-TB, suggesting DCs are potential candidates for therapeutic intervention.

O159

AM580, a retinoic acid receptor-α-selective agonist, is not effective for CNS autoimmunity

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Supplementation with all-trans retinoic acid, a metabolite of vitamin A, ameliorates inflammation in multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). AM580, a stable benzoic derivative of retinoic acid and a selective retinoic acid receptor (RAR)-α agonist, has been recently shown to have dual effects, i.e., anti-inflammation and neuroprotection. We thus hypothesized that AM580 could be highly effective in suppressing EAE development. Unexpectedly, intraperitoneal (i.p.) injections of the optimal dose of AM580 was not effective in suppressing EAE severity and CNS leukocyte infiltration and demyelination. No change was observed in $CD4^+$ T cells in the periphery and the CNS for their production of IL-17, IFN-γ, and IL-10, as well as Foxp3 expression. Overall, RAR-α specific agonist AM580 failed to suppress clinical development of EAE, even though it seemed a strong candidate for treatment of MS/EAE based on its previously reported dual effects, i.e., anti-inflammation in vitro and neuroprotection in vivo. Attempting to find more effective and safe RAR-specific agonist(s) as MS/EAE therapy is still required.

This work was supported by NIH grants NS099594 and AI135601. Q.W. and C.C.M were supported by grants from the National Natural Science Foundation of China (No. 81371414 and 81473577).

O160

Neuronal MHC class I expression is regulated by Mir146a in hippocampus development

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Classical major histocompatibility complex (MHC) class I, first identified in the immune system, is also expressed in the developing and adult central nervous system (CNS). It is proven that neuronal MHC-I molecules play important roles in synaptic formation, remodeling and plasticity, which are significantly different from their immunological functions. Our previous study shows that the expression patterns of MHC class I molecules is temporally and spatially regulated during mouse and human CNS development. MHC class I proteins continuously decrease in the hippocampus of C57BL/6 J mice after P15 despite a sustained increase at the mRNA level, suggesting a post-transcriptional regulation mechanism of MHC class I expression. Here, we employ computational algorithms to search for the microRNA that targets H-$2D^b$ and H-$2K^b$ and *Mir146a* is predicted. The expression of *Mir146a* is upregulated in hippocampus of C57BL/6 J mice after P15. Intriguingly, cotransfecting *Mir146a* mimic and the pmiR-GLO plasmid inserted with H-$2D^b$ /H-$2K^b$ 3′-UTR results in the downregulation of luciferase activity in HEK293 cells and this effect is reversed when transfected with a mutated H-$2D^b$ /H-$2K^b$ 3′-UTR that cannot bind to *Mir146a*. In cultured mouse hippocampal neurons, silencing

DOI: 10.1002/eji.201970400

Mir146a via an anti-miR-146a lentivirus significantly increases MHC-I expression at protein level, whereas the mRNA level is unchanged. These results suggest that *Mir146a* might modulate the translation of MHC-I mRNA without attenuating its stability. In conclusion, our findings unveil the new function of microRNA in their ability to regulate neuronal MHC class I molecules expression at post-transcriptional level during the development of hippocampus.
Funding: The work was supported by National Nature Science Foundation of China, 81571615

P0421

Soluble CD146, a cerebrospinal fluid marker for neuroinflammation, promotes blood brain barrier dysfunction

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The blood-brain barrier (BBB) dysfunction is an initial event of various neuroinflammatory diseases. Soluble CD146 (sCD146) is mainly derived from vascular endothelial cells (ECs) and highly elevated in inflammatory settings. Nevertheless, whether sCD146 monitors and regulates the BBB dysfunction remains unknown.
Between July 20, 2011, and February 31, 2017, we collected coupled serum and CSF samples from 823 patients, of which 562 (68.3%) had neuroinflammatory diseases, 44 (5.3%) had remitting MS, and 217 (26.4%) had non-inflammatory neurological diseases (NIND). We found that sCD146 in CSF, but not in serum, is abnormally elevated in neuroinflammatory diseases (37.3±13.3 ng/mL) compared with NIND (4.7±2.9 ng/mL) and remitting MS (4.6±3.5 ng/mL). Abnormally elevated CSF sCD146 is significantly correlated with the hyperpermeability-related clinical parameters of BBB and neuroinflammation-related factors. Moreover, CSF sCD146 shows higher sensitivity for evaluating BBB damage than other related molecules. Using an *in vitro*BBB model, we found that sCD146 impairs BBB function by promoting BBB permeability via an association with integrin αvβ1. Blocking integrin αvβ1 significantly attenuates sCD146-induced hyperpermeability of the BBB.
Conclusion: Our study provides convincing evidence that CSF sCD146 is a sensitive marker of BBB damage and neuroinflammation.Furthermore, sCD146 is actively involved in BBB dysfunction.

P0422

The small molecule ISRIB rescues cognitive functions in rat with Alzheimer´s disease via PERK-eIF2α-ATF4-CHOP pathway

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Emerging evidence suggests that the integrated stress response (ISR) may be involved in the pathogenesis of Alzheimer´s disease (AD). In this study, we intracerebroventricular injected amyloid-β oligomers (Aβo) to create AD model in rats. Two weeks later, a small molecule inhibitor of ISR (ISRIB) was used to modulate the eukaryotic translation initiation factor-2 (eIF2α) pathway via intraperitoneal injection. As results, Aβo causes cognitive deficits (abnormal scores of Morris water maze) and neuroinflammation (accumulations of glial cells in focal hippocampal zone and disorder of

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DOI: 10.1002/eji.201970400

inflammatory factors). ISRIB corrected Aβo induced memory injury which showed the improvement of maze scores and ameliorate neuroinflammation which showed reduction of glial cells and inflammatory factors including IL-6, TNF-a and IL-18. Moreover, ISRIB was founded to downregulate the protein expression of PERK-eIF2α-ATF4-CHOP pathway by interacting with PERK protein. Physiological mechanically, Aβo induced suppression of long-term potentiation (LTP) in the hippocampus was fully restored by ISRIB treatment. These results indicate that activation of integrated stress response and neuroinflammation in the hippocampus via PERK-eIF2α-ATF4-CHOP pathway play an important role in the pathophysiological processions of Aβo induced memory dysfunction. ISRIB might inhibited integrated stress response and neuroinflammation and might be a novel promising therapeutic drug to reverse AD related cognitive deficits.

P0423

TSPO knockout promotes amyloid pathology and neuroinflammation by affecting microglia phagocytosis

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Increasing evidence has pushed inflammation and immune dysfunction to the center of the pathogenesis of Alzheimer's Disease (AD). The mitochondrial translocator protein (18 kDa) (TSPO) is involved in neuroinflammation and neurodegenerative diseases such as AD and Parkinson's disease. However, the biological role of TSPO in the pathogenesis of AD remains elusive. Here, we show that TSPO is upregulated in the brain tissue of AD patients and AD mice. APPswe/PSEN1dE9 (APP/PS1) mice lacking TSPO generate significantly more $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides and more β-amyloid plaques in the cortex and hippocampus than control mice. The brain tissue of the TSPO knockout APP/PS1 mice, especially the area around the Aβ plaques, also exhibited an increased level of microglial activation. Furthermore, the TSPO-deficient microglia cultured *in vitro* showed a significant decrease in the phagocytosis of latex beads and $A\beta_{1-42}$ peptides. Taken together, these results indicate that TSPO has potential protective functions against neuroinflammation and beta-amyloid pathology and is involved in the phagocytosis of microglia.

P0424

Exogenous neuritin promotes nerve regeneration after acute spinal cord injury in rats

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Insufficient local levels of neurotrophic factor after spinal cord injury (SCI) are the leading cause of secondary injury and limited axonal regeneration. Neuritin belongs to a family of neurotrophic factors that promote neurite outgrowth, maintain neuronal survival, and provide a favorable microenvironment for the regeneration and repair of nerve cells after injury. However, it is not known whether the exogenously applied neuritin protein has a positive effect on nerve repair after SCI. This was investigated in the present study using purified human recombinant neuritin expressed in and purified from Pichia pastoris, which was tested in a rat SCI model. A recombinant neuritin concentration of 60 lg/ml induced the recovery of hind limb motor function and stimulated nerve regeneration in rats with SCI. Continuous administration of neuritin at this dose at an early stage after SCI inhibited poly ADP ribose polymerase (PARP) protein degradation and decreased neuronal apoptosis. In addition, during the critical postinjury period of axonal regeneration, exogenous neuritin treatment increased the

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DOI: 10.1002/eji.201970400

expression of neurofilament 200 and growth-associated protein 43 in the damaged tissue, which was associated with the restoration of hind limb movement. These results suggest that neuritin creates an environment that promotes nerve cell survival and neurite regeneration after SCI, which contribute to nerve regeneration and the recovery of motor function.

P0425

Epac1-SOCS3 signaling in CRH neurons of the hypothalamic paraventricular nucleus modulates visceral sensitization induced by neonatal colorectal distension in rats

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Visceral pain is widespread but effective therapies for it remain elusive, whose increasingly importance has been attached to the link between the gut and brain inflammation. We previously demonstrated that an increase in CRH neuron sensitization in PVN accompanied by HPA hyper-activation is responsible for adult visceral pain sensitization by neonatal colorectal distension (CRD). The suppressor of cytokine signaling 3 (SOCS3) signaling is known to exert its anti-inflammation influences as immunomodulators but the role of such signaling in neonatal CRD-induced visceral sensitization has not previously been studied. This study suggested that neonatal CRD downregulated the Epac1-SOCS3 pathway in PVN, decreased SOCS3 could not effectively inhibit IL-6 receptor signaling, and amplified IL-6 inflammatory cascade further perpetuated the neonatal CRD-induced visceral sensitization. Then, overexpressed SOCS3 in PVN in neonatal CRD group rats increased the nociceptive threshold and alleviated the visceral sensitization. Furthermore, localized interference through siRNA Epac1 and SOCS3 microinjection in PVN in control rats decreased the nociceptive visceral threshold and facilitated the activation of PVN CRH neurons and HPA axis, which demonstrated that downregulation of Epac1-SOCS3 pathway in PVN in control rats mimicked neonatal CRD-induced visceral sensitization. Finally, we used electrophysiology to verify the discharge of specific CRH neurons in PVN after Epac administration. Together, downregulation of the Epac1-SOCS3 pathway in CRH neurons of hypothalamic PVN facilitates the neonatal CRD-induced visceral sensitization, which partly resulted from inadequate IL-6 intracellular inhibitory effects. Overlapping mechanisms involving the Epac1-SOCS3 pathway and IL-6 signaling offer potential therapeutic targets for the treatment of chronic visceral.

P0426

Peripheral immune activation and gut microflora dysbiosis in ASD

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Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders in children. While the potential etiologic role for immune dysfunction in ASD has been suggested, it is still unclear what kinds of T cell subsets or their effector mechanism are involved. In the present study, peripheral blood T cells subsets and serum cytokines from 40 children diagnosed with ASD and 50 age and sex-matched typically developing controls were measured through flow cytometry and bead-based immunoassay respectively. Our data showed that the percentage of TH1, TH2, Tc1 were increased in ASD (p=0.000); however, percent of Tregs were dramatically decreased (p=0.000). Meanwhile the serum concentration of IL-2, IL-4, IL-5, IL-6, IL-10, IL-17A, TNF-α, IFN-γ were elevated

DOI: 10.1002/eji.201970400

($p < 0.03$) in ASD group. Partial correlation analysis showed that serum concentrations of IL-5, IL-10, TNF-α and IFN-γ as well as frequencies of TH1, TH2 and Tc1 subsets were positively correlated with the behavioral impairments with Tregs negatively correlating with impairment. Since accumulating evidence suggests an important role of microbiota in immune activation and brain function, we evaluated changes of fecal microbiota in children with ASD compared with controls. The results showed high bacterial diversity were observed in ASD group accompanied by decreased abundance of the dominant Firmicutes and Bacteroidetes. The PCA and PCoA of bacterial beta diversity analysis suggest that the community composition of ASD significantly distinguished from that of healthy controls. Taken together, this study suggested a potential correlation between peripheral immune activation and gut microflora dysbiosis in ASD.

P0427

B cells are recruited through the blood-CSF barrier and, dependent on their activation state, traffic more easily in multiple sclerosis

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Background: The role of B-cells in MS immunopathogenesis is increasingly recognized. B-cells undergo compartmentalized redistribution in blood and cerebrospinal fluid (CSF) during active MS, whereby antigen-experienced memory B-cells accumulate in the CSF. While trafficking of B-cells across the blood-brain barrier has been intensively investigated, cellular diapedesis through the blood-CSF barrier (BCSFB) is incompletely understood.
Objectives: To investigate how B-cells interact with the choroid plexus to transmigrate into the CSF.
Methods: We isolated B-cells from blood samples of healthy donors (HC) and MS patients, then utilized an inverted transwell culture system of human choroid plexus papilloma (HIBCPP) cells to determine transmigration rates of distinct B-cell subsets and immunofluorescence microscopy to analyze their migration route through the epithelial barrier, cytokine assays and RT-PCR were used to determine the cytokines/chemokines mediating B-cell transmigration.
Results: Spontaneous transmigration of both HC- and MS-derived B-cells across HIBCPP cells was scant, yet increased significantly in response to B-cell specific chemokines (including CXCL-12, -13) and was further boosted upon pre-activation. Migrating cells were characterized by upregulation of several genes involved in B-cell activation and an enhanced expression of chemokine receptors CXCR4 and CXCR5, predominantly exhibited a $CD27^+$ memory phenotype, and chemotactic activities of this subset were more pronounced in B-cells from MS than in those from HC.
Conclusions: Our findings provide new information on how antigen-experienced B-cell phenotypes and the BCSFB act together to facilitate aberrant B-cell accumulation in the CSF of MS patients.

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DOI: 10.1002/eji.201970400

P0428

Understanding host immune responses in astrocytes during CNS-TB infection

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Central nervous system TB (CNS-TB) is classified as one of the severest clinical extra-pulmonary manifestations of the tuberculosis disease. Although microglia are the prime immune-effector cells involved in CNS immunity, the immune functions of astrocytes are starting to be acknowledged as active players in cerebral immunity. We first established *Bacillus Calmette-Guérin* (BCG) infection in primary murine astrocytes and found bacterial loads to be increasing in time and infection dose dependent manner, thus indicating the viability of the bacilli and confirming replication of BCG within astrocytes. By the use of immuncytochemistry, we labeled the astrocytes with GFAP antibodies and quantified the infectivity of GFP-expressing BCG in astrocytes to be 6,52% at 2hr and 18.96% at 24hr. Internalization of intracellular bacilli by astrocytes was further demonstrated by confocal microscopy and shown their encapsulation within astrocyte cytoskeletal filaments. It has been indicated that astrocytes are important for modulation of immune cell responses. BCG infection of astrocytes was capable of inducing the upregulation of pro-inflammatory cytokines and CD86 and MHC class II molecules necessary for antigen presentation. Furthermore, our observations indicate the activation of naïve T cells following co-culture with BCG infected murine astrocytes *in vitro.* Characterizing the role of astrocyte in mycobacterial infection and investigating their contribution to the development of neuroinflammation witnessed in CNS-TB survivors may is of clinical significance as it may offer an unexplored territory for therapeutic strategies.

P0429

The regulation of neuroinflammation in brains of 5xFAD mice is underlying mechanisms of prevention of onset of Alzheimer's diseases by mature silkworm powders

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Alzheimer's disease (AD) is the most common dementia in the world without cure yet. Recent studies suggested that neuroinflammation in brains play pivotal roles in the onset and progression of AD. We found that the onsets of AD in several AD models were prevented if they were fed with mature silkworm powders (MSP). By investigating the mechanisms underlying prevention of onset of AD using AD model animals, we found that mitochondria complex 1 ~ 4 in AD model brains fed with MSP were significantly enhanced compared with those of control AD model brains. In addition, we also found that neuroinflammation in AD model brains treated with MSP were significantly reduced compared with those of control AD model brains. Our results suggested that MSP may prevent the onset of AD by enhancing mitochondria activities and inhibiting neuroinflammation in AD mouse model brains. Thus, it will be very intriguing to test whether MSP also has similar effects in human AD patients.

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DOI: 10.1002/eji.201970400

P0430

Angiotensin AT2-receptor induced interleukin-10 attenuates neuromyelitis optica spectrum disorder-like pathology

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Neuromyelitis optica spectrum disorder (NMOSD) is a relapsing inflammatory CNS disease for which there is no cure. Immunoglobulin-G autoantibodies specific for the water channel aquaporin-4 are a serum biomarker, believed to induce complement-dependent astrocyte damage with secondary demyelination. To investigate the effect of angiotensin AT2-receptor (AT2R) stimulation on NMOSD-like pathology and its underlying mechanism, NMOSD-like pathology was induced in mice by intracerebral injection of Immunoglobulin-G isolated from NMOSD patient serum, with complement. This mouse model produces the characteristic histological features of NMOSD. A specific AT2R agonist, Compound 21 (C21), was given intracerebrally at day 0 and by intrathecal injection at day 2. Loss of aquaporin-4 and glial fibrillary acidic protein was attenuated by treatment with C21. Administration of C21 induced mRNA for interleukin-10 in the brain. NMOSD-like pathology was exacerbated in interleukin-10-deficient mice, suggesting a protective role. C21 treatment did not attenuate NMOSD-like pathology in interleukin-10-deficient mice, indicating the protective effect of AT2R stimulation was dependent on interleukin-10. Our findings identify AT2R as a novel potential therapeutic target for the treatment of NMOSD. Interleukin-10 signaling is an essential part of the protective mechanism counteracting NMOSD pathology.

P0431

IL-23-IL-23R signaling in the central nervous system drives the apoptosis of neurons during experimental autoimmune encephalomyelitis

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Pathogenic Th1 and Th17 cells play a crucial role in neuroinflammation and neuronal autoimmunity. How does the expression of transcription factors T-bet and RORγt regulate the entry of the pathogenic Th1 and Th17 cells in the central nervous system (CNS) and causes neuronal damage are not known. In the present work, we showed that expression T-bet regulates the entry of both Th1 and Th17 cells into the CNS whereas expression of RORgt promotes the apoptosis of neuronal cells leading to experimental autoimmune encephalomyelitis (EAE). We showed that during EAE, apoptosis of neuron in the CNS caused by RORγt^{+}CD4^{+} T cells was independent of induced nitric oxide synthase (iNOS). We identified that neurons in the brain and spinal cord express IL-23 receptor during EAE, and IL-23-IL-23R signaling in neurons promote apoptosis. The apoptosis of CNS neurons was dependent on expression of RORgt in CD4^{+} T cells, its mobilization and secretion of IL-23 in the brain and spinal cord. Deficiency of RORγt in CD4^{+} T cells or neutralization of IL-23 prevents the neuronal apoptosis whereas intrathecal injection of IL-23 in the RORγt$^{-/-}$ mice cause apoptosis of neurons. Thus, we provided a mechanism of neuroimmune communication in the CNS which can be further explored to develop strategies to prevent neuroinflammation and CNS autoimmunity.

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DOI: 10.1002/eji.201970400

P1012

The homocysteine-induced immune responses in H type hypertensive rats

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Purpose: This study was to investigate the immune response mechanisms induced by homocysteine (Hcy) in H-hypertensive.
Methods: WKY and SHR rats were divided into the control, methionine or treatment groups (WKY-C/M/T and SHR-C/M/T groups). The plasma levels of IL-17, IL-10, TGF-β and IL-6 were detected by ELISA at 8, 12 and 16 weeks. The mRNA levels of IL-10, IL-17, Foxp3 and RORγt were detected in rat brain tissues using quantitative PCR (q-PCR) at 8, 12 and 16 weeks. Additionally, Foxp3 and RORγt protein expression was detected in rat brain tissues via western blotting at 16 weeks. Flow cytometry was used to analyze the proportion of Th17 and Treg cells among peripheral blood lymphocytes from the WKY-C/M/T and SHR-C/M/T groups at 0, 4, 8, 12 and 16 weeks.
Results: Compared with WKY groups, plasma cytokines, related-gene mRNA and protein levels and Th17/Treg lymphocyte numbers were significantly changed in SHR groups. Compared with WKY-C and SHR-C groups, plasma Hcy levels, cytokine levels and Th17/Treg proportions were significantly changed in WKY-M and SHR-M groups, which were partially restored after treatment. High methionine levels caused significant alterations to plasma Hcy levels, cytokine expression profiles and Th17/Treg proportions in rat peripheral blood cells.
Conclusion: Weakening Hcy effect via folate and vitamin B intervention could be a novel strategy for the treatment of immune response in H-type hypertension.

P1013

Anti-citrullinated protein antibodies are associated with neutrophil extracellular trap formation in rheumatoid arthritis

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Objectives: We evaluated the associations of anti-citrullinated protein antibodies (ACPAs) from patients with rheumatoid arthritis (RA) and serological and cytological levels of neutrophil extracellular trap (NET) formation.
Methods: Serum levels of myeloperoxidase-DNA and elastase-DNA complexes (NET remnants) were examined in 51 patients with RA and 40 healthy controls using a modified enzyme-linked immunosorbent assay. The associations between the levels of these complexes and indicators of RA were statistically evaluated. Neutrophils were isolated by density gradient centrifugation. IgG antibodies were purified by affinity chromatography. NET formation in RA and control neutrophils was assessed by microscopy *in vitro*. NETs were purified and co-incubated with fibroblast-like synoviocyte (FLS) cells. Interleukin (*IL*)-*6* and *IL-8* expression in FLS cells was determined by real-time polymerase chain reaction and protein levels were evaluated by enzyme-linked immunosorbent assay.
Results: NET remnant levels were higher in patients with RA with high ACPA titers. And NET formation of neutrophils was associated with the concentrations of IgG antibodies containing ACPAs. Furthermore, significantly higher mRNA and protein levels of IL-6 and IL-8 were detected after FLS cells interacted with NETs which derived from neutrophils stimulated with ACPA-containing IgG antibodies. Moreover, NET remnant levels were correlated with IL-8 in the peripheral circulation.
Conclusions: ACPA may enhance NET formation and contribute to inflammation development in RA

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DOI: 10.1002/eji.201970400

by stimulating NET formation, such as by subsequent activation of FLS cells by NETs.
Keywords: anti-citrullinated protein antibodies, neutrophil extracellular traps, rheumatoid arthritis, inflammation

P1014

Elevated resistin levels may regulate high mobility group box 1 expression in Guillain-Barré syndrome

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Interactions among cytokines have important roles in the inflammatory processes underlying Guillain-Barré syndrome (GBS). Resistin and high mobility group box 1 (HMGB1) are involved in many inflammatory processes. This study examined 51 GBS patients, and found that serum resistin levels were elevated in 51 patients with GBS and correlated with HMGB1 levels. In vitro, resistin induced the release of HMGB1, interleukin (IL)-1β, and IL-6 in THP-1 macrophages. This process was dependent on activation of p38 mitogen-activated protein kinase and NF-κB signaling pathways. These results suggest that signaling between resistin and HMGB1 might be a potential therapeutic target in GBS.

P1015

Ursolic acid ameliorates experimental autoimmune neuritis by regulating the Th1/Th17/Treg balance and suppressing the lymphocyte proliferation

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Background: Guillain-Barre syndrome (GBS) is an autoimmune disease characterized by myelin sheath or axon damage in peripheral nervous system, and EAN is a ideal animal model for it. Studies showed that Th1, Th17 cells are dominant in the development of GBS. Ursolic acid (UA), a transcription factor inhibitor of Th17 cells, has the anti-inflammatory effects, so we speculated that UA may has the therapeutic potential on EAN.
Methods: To determine whether UA protected against the development of EAN, UA (at doses of 20 and 100 mg/kg/day in a volume of 100 µl DMSO, respectively) was administered to immunized rats by intraperitoneal injection. Treg cells, cytokines, and lymphocyte proliferation were carried out on flow cytometry, and the number of $Foxp3^+$ cells in thymuses was also examined using immunocytochemistry to evaluate the effect of UA on EAN rats.
Results: UA could alleviate the clinical symptoms of EAN, which was associated with the increase of Treg cells in peripheral lymph nodes and $Foxp3^+$ cells in thymus, the decrease of Th1/Th17 cytokines in peripheral lymph nodes, and the inhibition of lymphocyte proliferation.
Conclusion: Our data suggest that UA is involved in inflammatory reaction mediated by T cells in EAN, and our study provides a potential drug for treatment of GBS and other autoimmune diseases.
This work was supported by the National Natural Science Foundation of China (81501037) and the Medical Science and Technology Planning Project of Shandong Province (2013WS0060).

DOI: 10.1002/eji.201970400

P1016

Glycyrrhizin protects mice against experimental autoimmune encephalomyelitis by inhibiting HMGB1 expression and neuronal HMGB1 release

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The inflammatory mediator high-mobility group box 1 (HMGB1) plays a critical role in the pathogenesis of human multiple sclerosis (MS) and mouse experimental autoimmune encephalomyelitis (EAE). Glycyrrhizin (GL), a glycoconjugated triterpene extracted from licorice root, has the ability to inhibit the functions of HMGB1; however, GL's function against EAE has not been thoroughly characterized to date. To determine the benefit of GL as a modulator of neuroinflammation, we used an *in vivo* study to examine GL's effect on EAE along with primary cultured cortical neurons to study the GL effect on HMGB1 release. Treatment of EAE mice with GL from onset to the peak stage of disease resulted in marked attenuation of EAE severity, reduced inflammatory cell infiltration and demyelination, decreased TNF-α, IFN-γ, IL-17A, IL-6, and TGF-β1 and increased IL-4 both in serum and spinal cord homogenate. Moreover, HMGB1 levels in different body fluids were reduced, accompanied by a decrease in neuronal damage, activated astrocytes and microglia, as well as HMGB1-positive astrocytes and microglia. GL significantly reversed HMGB1 release into the medium induced by TNF-α stimulation in primary cultured cortical neurons. Taken together, the results indicate that GL has a strong neuroprotective effect on EAE mice by reducing HMGB1 expression and release and thus can be used to treat central nervous system inflammatory diseases, such as MS.

P1017

Maternal nicotine exposure modulate immune responses of hippocampal microenvironment in C57/BL6 mouse offspring

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Maternal smoking or perinatal Nicotine Replacement Therapy (NRT) may affect hippocampal development in her offspring during a variety of perinatal conditions, which cause neurogenesis impairment and behavior problem. To further understand the mechanism underlying nicotine-induced hippocampal deficits, the gene expression profiles of hippocampus after nicotine exposure was studied. C57BL/6 female mice were treated with nicotine in drinking water (200 µg/ml in 1% saccharin from 2-weeks premating until her offspring was weaned at postnatal day 20), while vehicle female mice were received 1% saccharin. Open field test revealed that NRT significantly reduced locomotor activity and exploration time in the center at postnatal day 20 in offspring. Hippocampal RNA was analyzed using Arraystar RNA Flash Labeling Kit. Only genes with a signal-to-noise ratio of above 1.5 were included in the statistical analysis. The differentially expressed genes and enrichment pathways in hippocampus of the offspring were processed using the GeneSpringGX v. 12.1 software. 295 genes (132 up-regulation and 163 down-regulation) involved in varieties of biological functions were expressed differently after NRT, as compared to the vehicle control. We further identified 14 biochemical pathways that are enriched in NRT, including cytokine-cytokine receptor interaction, signaling of chemokine, TNF, neurotrophic and MAPK. Finally, we confirmed the microarray results using real-time reverse transcriptase polymerase chain reaction for several representative genes (BDNF, CXCL10, JUNB, CCL12, IL-4, et al.). In short, this study first reported nicotine-induced

DOI: 10.1002/eji.201970400

disregulation of immune-related gene expression, and nicotine-mediated suppression of immune-based signaling in juvenile hippocampus, which may finally cause neurogenesis impairment and abnormal behavior.

P1018

The spinal NR2BR/ERK2 pathway as a target for the central sensitization of collagen-induced arthritis pain

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Objective: Pain management is a huge challenge in the treatment of rheumatoid arthritis (RA), and central sensitization is reportedly involved in the development of pain. The current study was undertaken to explore the possible role of N-methyl-D-aspartate receptors (NMDARs) in the spinal mechanism of central sensitization in RA using a collagen-induced arthritis (CIA) model.
Methods: Mechanical hypersensitivity was assessed in C57BL/6 mice, before and after the induction of CIA via administration of chick type II collagen. Analgesic drugs, receptor antagonist, and kinase inhibitor were administrated intrathecally in the spinal cord. Protein expression and phosphorylation changes were detected via immunoblotting.
Results: CIA mice developed significant mechanical hypersensitivity, and spinal administration of the NMDAR antagonist D-2-amino-5-phosphonovaleric acid (D-APV) effectively attenuated peripheral pain hypersensitivity. There was specific enhancement of synaptic NR2B-containing NMDAR (NR2BR) expression in the spinal dorsal horns of the mice. Both the increased total protein expression of NR2B subunit and the enhanced total phosphorylation level of NR2B subunit at 1472 tyrosine promoted the synaptic expression of NMDAR in the mice. Intrathecal injection of tramadol suppressed synaptic NMDAR expression mainly by changing the synaptic phosphorylation state of NR2B subunit at Tyr1472. Extracellular signal-regulated protein kinases 2 (ERK2) activity synchronized with the synaptic expression of NR2BR, which was downregulated by the action of tramadol.
Conclusion: Specific enhancement of NR2BR in the spinal dorsal horn may be vital for central sensitization in the CIA model of RA. The NR2BR/ERK2 pathway may be a promising target for pain management in RA patients.

P1020

Spatiotemporal profile of NG2 glia and their association with microglial activation in the CA1 region of the rat hippocampus after transient forebrain ischemia

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The present study was designed to investigate the nature and the time course of reactive NG2 glia and their associations with microglial activation in the CA1 hippocampus after transient forebrain ischemia. In control hippocampus, NG2 immunoreactivity was restricted to resting NG2 glia with thin processes. In the ischemic CA1 hippocampus, the numbers of NG2 glia in both the pyramidal cell layer and the stratum radiatum were significantly increased at 3 days after ischemia, and remained

DOI: 10.1002/eji.201970400

elevated until 28 days, and this increase was due to new cells generated via proliferation. The morphologies of reactive NG2 glia in the stratum radiatum were altered with time: the thick and multiple processes of these cells at 3 days were transformed into shorter and highly ramified processes forming a dense network on days 7-14 days. By contrast, NG2 glia in the pyramidal cell layer transformed into irregular cell bodies with thick and short processes and this transformation was maintained during the post-ischemic period. In addition, reactive NG2 glia were localized in association with activated microglia/macrophages expressing NG2. These observations demonstrate that in the ischemic hippocampus, NG glia undergo proliferation and dynamic structural changes, suggesting that activation of NG2 glia may be involved in the degeneration and subsequent tissue repair and reorganization after ischemia. In addition, our data revealed that activation of NG2 glia correlate temporally and spatially with microglial activation/recruitment, indicating the presence of the functional and complex interactions between NG2 glia and activated microglia/macrophages in response to brain insults. Number of grant: NRF-2017R1A2B4002922.

P1021

The effects of optimism on cell-mediated immunity in rats

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It has been shown that optimistic expectancies are associated with psychosocial outcomes and may also predict immune system changes and health, but the nature and mechanisms of any such physiological effects have not been identified. Most studies has focused on the determination of impact of negative emotion on disease susceptibility and course of illness. In contrast, the effects of dispositional optimism, as defined by generalized positive expectation for the future, on the immune system is poorly recognised. We used a recently developed ambiguous-cue interpretation (ACI) paradigm to investigate whether optimism and pessimism as behavioural traits may be interrelated with immune functions in rodents. The behavioral tasks were performed in computer-controlled Skinner boxes as an ACI test, a task that can be used to measure the natural propensity of rats to interpret environmental stimuli in a positive or negative manner. We found significant differences in immune biomarkers between “optimistic” and “pessimistic” animals. “Pessimism” was associated with significantly lower relative weight of spleen and thymus and significantly decreased proliferative activity of splenocytes. The level of spleen cytokines was measured by RayBio Rat Cytokine Antibody Array - the protein array system. “Optimistic” animals reveals decrease secretion of spleen proinflammatory cytokines in comparison to “pessimistic” animals. Pessimism was associated with an increased production of interleukin-(IL)1β and IL-4, activin A, l-selectin, interferon (IFN)-γ and some chemokines and receptors for advanded glycation endoproducts. The findings indicate an inflammatory profile in “pessimistic” animals.
This work was supported by and the statutory funds of the IP PAS.

DOI: 10.1002/eji.201970400

P1022

Apical splenic nerve electrical stimulation discloses an anti-inflammatory pathway relying on adrenergic and nicotinic receptors in myeloid cells

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The autonomic nervous system innervates all lymphoid tissues including the spleen therefore providing a link between the central nervous system and the immune system. The only known mechanism of neural inhibition of inflammation in the spleen relies on the production of norepinephrine by splenic catecholaminergic fibers which binds to b2-adrenergic receptors (b2-ARs) of $CD4^+$ T cells. These $CD4^+$ T cells trigger the release of acetylcholine that inhibits the secretion of inflammatory cytokines by macrophages through a7 nicotinic acetylcholine receptor (α7nAchRs) signaling. While the vagal anti-inflammatory pathway has been extensively studied in rodents, it remains to be determined whether it coexists with other neural pathways. Here, we have found that three nerve branches project to the spleen in mice. While two of these nerves are associated with an artery and contain catecholaminergic fibers, the third is located at the apex of the spleen and contain both catecholaminergic and cholinergic fibers. We found that electrical stimulation of the apical nerve, but not the arterial nerves, inhibited inflammation independently of lymphocytes. In striking contrast to the anti-inflammatory pathway mechanism described so far, we also found that the inhibition of inflammation by apical nerve electrical stimulation relied on signaling by both b2-ARs and α7nAchRs in myeloid cells, with these two signaling pathways acting in parallel. Most importantly, apical splenic nerve electrical stimulation mitigated clinical symptoms in a mouse model of rheumatoid arthritis further providing the proof-of-concept that such an approach could be beneficial in patients with Immune-mediated inflammatory diseases.

P1023

Therapeutic interferon-gamma in experimental autoimmune encephalomyelitis is dependent on regulatory T cells Induction by CD11b+ cells

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Experimental autoimmune encephalomyelitis (EAE) is a model of multiple sclerosis, an autoimmune disease affecting the central nervous system (SNC). Our previous results have shown that interferon (IFN)-γ-treatment induced a significant amelioration of EAE symptoms associated with a significant increase of regulatory T cells (Tregs) in the SNC.
Here, we show that IFN-γ did not exert a direct effect on the ex vivo induction of CD4+CD25- T lymphocytes obtained from EAE and that, on the contrary, this cytokine inhibited FOXP3 expression in conditions of iTregs differentiation. The *in vivo* combined administration of IFN-γ and neutralizing antibodies against IL-10, TGF-beta or PD-1, showed that the protective effect of IFN-γ in the chronic phase of EAE depended on TGF-beta and programmed cell death (PD)-1 and its ligands (PD-Ls), but not on IL-10. The analysis of regulatory molecules expression in different cell subpopulations obtained from EAE mice determined that IFN-γ induced an increased expression of TGF-beta (LAP) and PD-L1 on different subgroups of CD11b+ cells (antigen presenting cells). In addition, it was found that both *in vivo* and *in vitro*, IFN-γ-stimulated CD11b+ cells were able to induce an increase of Tregs frequency in a TGF-beta- and PD-L1-dependent manner starting from naïve T CD4+ cells obtained from healthy (without EAE) wild type or 2D2 (expressing a specific TCR for myelin peptide) mice.
Overall, therapeutic IFN-γ in EAE was dependent on regulatory T cells induction and associated with

DOI: 10.1002/eji.201970400

an enhanced expression of TGF-beta and PD-L1 on antigen presenting cells (CD11b+).
Supported by FONDECYT 1191874.

P1613

Anti-inflammatory and neuroprotective effect of Bilobalide on Parkinson´s disease model

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Background: Parkinson's disease (PD) is one of the most widespread neurodegenerative disorders. Bilobalide exhibits neuroprotective, anti-inflammatory and anti-apoptotic effects. However, the role of Bilobalide in the treatment of PD is not reported.
Objective: To observe the therapeutic potential of Bilobalide in MPTP-induced PD model, and explore its possible mechanisms of action.
Material and methods: Mice were randomly divided into three groups: healthy group, MPTP group and MPTP+Bilobalide group. PD model was induced by intraperitoneal injection of MPTP into male C57BL/6 mice. Then, mice received intraperitoneal injection of Bilobalide. The injection of saline was set up as control in a similar manner.
Results: Bilobalide effectively relieved motor symptoms and declined the loss of Th+ neurons in the substantia nigra pars compacta (SNpc) of the brain as compared with MPTP-PD mice. Bilobalide induced the transformation of inflammatory M1 into anti-inflammatory M2 microglia. Bilobalide promoted the expression of neurotrophic factors BDNF and GDNF in GFAP astrocytes in the brain, and reduced CD4+IFN-γ+ and CD4+IL-17+ cells. In addition, MPTP injection resulted in the formation of anti-α-synuclein (Syn) specific antibodies, which was inhibited by Bilobalide treatment.
Conclusion: Bilobalide exhibited therapeutic potential in MPTP-induced PD model, which might be related to the inhibition of microglia-associated neuroinflammation, upregulation of neurotrophic factors BDNF and GDNF as well as the inhibition of CD4+IFN-γ+ /CD4+IL-17+ and α-Syn antibodies.

P1614

Induced relapsing-remitting course at EAE inC57BL/6 mice with MOG35-55

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Background: EAE is an animal model helping research MS, immunized animals with antigens, such as MBP, MOG, PLP or brain and spinal homogenate. Commonly, the manifestation is chronic or chronically progressing course, in C57BL/6 mice immunized with MOG35-55, without relapse and remission.
Aim: To explore inducing the relapsing-remitting course of EAE using C57BL/6 mice and MOG35-55.
Method: We immunized 18 C57BL/6 mice, which were all female and 6-8 weeks with weight during 18-22g. The immunization process was implemented according to the commonly international used way. These mice, every day, were recorded weight and quantity of food intaking, meanwhile evaluating the NDS by 15-point score.
Result: 1. Four mice showed the recurrent and remission course of disease, accounting for 16.66%. 2. The first onset presented averagely, for the four mice with RR-EAE manifestation, on the 16th day after immunized, with 2-6 scores in NDS lasting 3-5 days; after then, decreasing NDS at 1-4 scores

DOI: 10.1002/eji.201970400

lasting 4-13 days; observing the relapse by rising 1-7 scores in NDS on the 28.25th day medially after immunized.
Conclusion: These results showed that immunizing C57BL/6 mice with MOG35-55 could establish relapsing-remitting EAE model, which may show analogous presentations and courses as what MS possessing.

P1615

Oral effect of FSD on antioxidant mechanisms in Parkinson's disease mice

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Background: FSD has a potential therapeutic effect on Parkinson´s Disease(PD) Mice, but the mechanism is not clear.
Objective: To explore the therapeutic effect and mechanism of FSD on PD mice.
Material and methods: The male C57BL/6 mice were randomly divided into control group, model group and FSD group. The mice in model group were established by MPTP. The mice in FSD group were administrated with FSD and MPTP from 0 day to 14 days. At the 15th day, Gait, pole and adhesive removal tests were used to evaluate the behavioral performance. The contents of GSH and MDA in brain were detected by microenzyme labelling and TBA. The expression of SOD and CAT in brain were detected by Western Blot.
Results: The gait contact area, pole climbing and adhesive removal time significantly were decreased in the mice of FSD group compared with the model group. FSD increased the content of GSH and the expressions of CAT and SOD. FSD reduced the content of MDA.
Conclusion: The results clearly show that the oral treatment of FSD improves motor capacity of PD mice, and FSD may have antioxidant activity.
(Shanxi International Science and Technology cooperation project 201703D421016, NNSF of China 81703978 and 81102552 and 81473577, Shanxi Scholarship Council project 2017-129 and 2017-19, Scientific and technological innovation team of integrated Chinese and western medicine for the prevention and treatment on nervous system diseases of Shanxi university of Chinese medicine 2018TD-012, Shanxi Key Laboratory project SXIDL-2018-04, Shanxi Key project 201803D31209.).

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DOI: 10.1002/eji.201970400

P1616

Comparative evaluation of modified-dose of rituximab and other initial treatment choices for Myasthenia Gravis

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Background: Comparative real-world effectiveness studies of agents as monotherapeutic choices to stabilize myasthenia gravis (MG) are lacking. We aim to assess the effectiveness and drug discontinuation rates of modified-dose of rituximab among patients with myasthenia gravis compared with oral corticosteroids, azathioprine, or mycophenolate mofetil.
Methods: This retrospective cohort study examined specialized care of patients in 2 Neuroimmune Centers in China from Jun 2015 to Oct 2018. All reasons for drug discontinuation of monotherapy choice (main outcome) and specific reasons for switching (secondary outcomes) were analyzed with multivariable Cox regression, including propensity scores.
Findings: Among 523 patients, 134 received lower-dose of prednisone (< 15 mg/d) (25.6%); 123 (23.5%), higher-dose of prednisone (>=15 mg/d); 140 (26.8%), azathioprine; 81 (15.5%), mycophenolate mofetil; 45 (8.6%), modified-dose of rituximab. After 3-year follow-up, the proportion of disease progression free for rituximab, higher dose of prednisone, mycophenolate mofetil, azathioprine, lower-dose of prednisone were 88.9%, 76.7%, 73.5%, 66.3%, 45.8%, respectively. The annual discontinuation rates for the agents were 0.09, 0.26, 0.15,0.27, and 0.19 respectively. Disease progression was significantly lower for rituximab compared with azathioprine and lower-dose of prednisone, with a tendency for lower progression rate also compared with mycophenolate mofetil and higher-dose of prednisone. For treatment-refractory patients, tocilizumab help prevent disease activity with good tolerance.
Conclusion and relevance: Rituximab was superior to all other agents as monotherapy in terms of prevention of disease progression and drug discontinuation. New drugs targeting antibody secreting cells may be appropriate choice for refractory patients.

P1617

Pertussis toxin induced inflammatory response exacerbates intracerebral hemorrhage in mice

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Intracerebral hemorrhage (ICH) is a devastating type of stroke. After ICH, the development of perihematomal edema induces secondary injury by impaired blood-brain-barrier (BBB) integrity and adjacent tissue destruction. Emerging evidence indicates that inflammation contributes to PHE expansion and neurological deterioration. The features and direct effect of brain inflammation following ICH injury is still unknown. We use collagenase inducing ICH. Mice were treated with saline or 2.5ug/g or 5ug/g Pertussis Toxin(PT) intraperitoneally after ICH. Infarct volume was assessed on days 1, 3, 7 after ICH. Evaluating Neurological deficits everyday until 7 days. The permeability of the BBB was evaluated by immunofluorescent staining (CD31 and ZO-1/Claudin-5/MMP9) and the infiltration of immune cells in the brain was detected by FACS (CD4 T, CD8 T , CD19 B, NK, neutrophils, microglia). Brain inflammation was evaluated using Optical in Vivo Imaging. Compared with the sham group, hematoma and edema increased during 7 days with PT. The experimental groups have worse

DOI: 10.1002/eji.201970400

outcomes compared to sham control. Brain inflammation was much stronger in living mice injected with PT, as shown by Optical in Vivo Imaging. Immunofluorescent staining showed increased permeability of the BBB in PT group. Neutrophils, NK, CD4+ T and CD8+ T cells significantly increased in PT group from day 1 to day 3. Microglia and CD19+B cells decreased significantly until 7 days. PT increased inflammatory response and exacerbated ICH in mouse model. This study will advance our understanding of brain inflammatory features after ICH, also pave the way for immune intervention to benefit ICH patients.

P1618

Functional dynamics of neutrophils after ischemic stroke

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Background and Purpose: Neutrophils are forerunners to brain lesions after ischemic stroke and exert elaborate functions. However, temporal alterations of cell count, polarity, extra cellular trap formation and clearance of neutrophils remain elusive. The current study was aimed at providing basic information of neutrophil functional dynamics in stroke.
Methods: Stroke was induced with transient middle cerebral artery occlusion (tMCAO, 1h) in mice. Temporal and spatial dynamics of neutrophil were assessed. Correlation of neutrophil counts and stroke outcomes was evaluated. Clearance of neutrophil by macrophages, neutrophil polarization, formation of extracellular traps and its impact on neuronal survival after ischemia were explored.
Results: Neutrophil constitution in peripheral blood increased soon after stroke onset of patients. Higher neutrophil count indicated detrimental stroke outcomes. Neutrophil count in peripheral blood of stroke mice peaked at 12h, followed by a 1-2d spike in brain lesion. In stroke lesion, clearance of neutrophils peaked at 2d and extracellular traps were mostly detected at 2-3d. In neutrophil infiltrated into stroke lesion, expression of N2 marker CD206 was relatively stable. N2 phenotype facilitated neutrophil clearance by macrophage and did not further induce neuronal death after stroke compared with N0 or N1 neutrophils. Conditioned medium of ischemic neurons drove neutrophils away from N2 phenotype and increased formation of extracellular traps.
Conclusion: Neutrophil frequency in the peripheral blood could be an early indicator of stroke outcomes. N2 neutrophils facilitate macrophage phagocytosis and are less harmful to ischemic neurons. Directing neutrophils towards N2 phenotype could be a promising therapeutic approach for ischemic stroke.

P1619

Topoisomerase 1 inhibition mitigates microglia-mediated neuroinflammation

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Effective treatments for neurological conditions, such as multiple sclerosis (MS) and amyotrophic lateral sclerosis, represent a currently unmet medical need. Inflammation within the central nervous system, usually mediated by dysfunctional microglial cells, is a determinant pathogenic factor. Based

DOI: 10.1002/eji.201970400

on a public GEO database (GSE76731) of gene expression of polarized human microglia, in which isolated human microglia are polarized with either IFN-g/LPS (inflammatory phenotype) or IL-4/IL-10/IL-13/TGF-beta (immuno-regulatory phenotype), we identified the differential gene expression pattern of microglia from inflammatory phenotype to immune-regulatory phenotype. The expression pattern was analyzed with Connectivity Map and a batch of drugs with the potential to induce similar gene expression change on microglia were revealed. Camptothecin (CPT), which is a topoisomerase 1 inhibitor, together with its derivatives, appear as promising candidates to modulate microglia function. We isolated primary microglia from adult mice, followed by stimulation with LPS/IFN-g. CPT-incubated microglia release less nitric oxide and inflammatory cytokines, and surface markers of microglia are also significantly altered after CPT incubation. For in vivo experiments, topotecan (TPT), water-soluble derivative of CPT, was used to replace CPT. In a LPS-induced lethal inflammatory mice model, TPT rescued 43% mice from death. In a MOG-induced experimental autoimmune encephalomyelitis (EAE) model, which is a classic animal model mimicking the disease progression of MS, preventative TPT treatment suppressed the disease development and TPT treatment from disease onset also significantly inhibited the disease progression. Our current data suggest that topoisomerase 1 inhibition is a potential pattern to prevent neuro-inflammatory disorders.

P1620

Association between interleukin 1 beta -511 T/C polymorphism and multiple sclerosis in Algerian population

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IL-1β is pro-inflammatory cytokine, released by monocytes, microglialcells, brain endothelial cells and seems to play an important mediator role in immunological and pathological responses including inflammatory reactions of the CNS, neurodegeneration and neuroregeneration. Two base-exchange polymorphisms with functional properties have been described for IL-1B gene, at position -511 in the promoter region and at position +3,953 in fifth exon.
We investigated the association between IL1β -511 T>C (rs 16944) polymorphism and susceptibility to multiple sclerosis development, also, the association between this polymorphism, intrathecal secretion of IgG and HLA-DRB1*15 allele.
The study included 56 Algerian patients with defined MS, who were recruited to the neurology department of Mustapha Pacha University Hospital (Algiers). The control population was composed of 34 healthy subjects; all these subjects are free from any inflammatory or autoimmune pathology. Single Nucleotide Polymorphism (SNP) analysis was performed using a real-time PCR (taqman technology).
Comparison of genotypic, allelic and phenotypic frequencies between patients and controls found no statistically significant difference. Our results remain statistically insignificant after stratification of patients according to: sex, age of onset of illness, clinical form of MS and HLA DRB1*15 allele. However, our study showed a significant difference between patients with intrathecal IgG synthesis (oligoclonal bands OB+) and patients without intrathecal IgG synthesis (OB-): the frequency of 511 TT genotype was significantly higher in OB+ patients (19% vs 0%, P = 0.04).
In conclusion, our present finding suggests that gene encoding IL-1B make no major contribution to susceptibility or disease severity of MS patients in Algeria.

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DOI: 10.1002/eji.201970400

P1621

CSF level of CXCL13 chemokine is high and correlated with the intrathecal humoral immune response in multiple sclerosis disease

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The CXCL13 chemokine plays an important role in B cell recruitment within the central nervous system (CNS) during inflammatory disorders. Our aim was to evaluate CXCL13 level in cerebral spinal fluid (CSF) of patients with multiple sclerosis (MS) compared to other inflammatory disorders of the CNS and to study its correlation with other features of patients.
In this study, 88 patients were included: 60 with definite diagnosis of MS according to McDonald criteria and 38 non-MS patients with other inflammatory conditions of the CNS. CSF Levels of CXCL13 were determined using ELISA kit. Couples of serum/CSF were analyzed for oligoclonal bands (OCB) using CSF isofocusing. Levels of IgG and albumin were determined for Total IgG Index calculation. For MRZ reaction testing, Measles, Rubella and Zoster viruses specific-IgG levels were measured in CSF and serum. Statistical analysis was performed using SPSS.20 software.
Our results showed that the mean level of CXCL13 in CSF were higher in MS patients (23 pg/ml) in comparison with control group (10.7pg/ml)(p=0.065). CSF levels of CXCL13 were correlated with total IgG Index and CSF levels of IgG in the 2 groups. There was also a correlation with CSF levels of anti-VZV specific IgG. Levels of the studied chemokine were higher in OCB-positive (19.7pg/ml) than in OCB-negative MS patients (16, 2pg/ml) (p=0.7).
In conclusion, CXCL13 seems to be an important mediator in the inflammatory cascade associated with the polyspecific intrathecal B cell response revealed by IgG Index, OCB and /or MRZ Reaction.

P1622

Role of the innate immune response in the progression of Alzheimer's disease

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Alzheimer´s disease (AD) is a progressive irreversible neurological brain disorder. Inflammation and immune alterations have been linked to AD, suggesting that the peripheral immune system plays a role during the asymptomatic period of AD. NK cells and neutrophils (PMN) participate in innate immune surveillance against intracellular pathogens and malignancy but their role in AD remains controversial. We have investigated changes in peripheral NK cell and PMN phenotypes and functions in amnestic mild cognitive impairment (aMCI, n = 10), patients with mild AD (mAD, n = 11), and healthy elderly controls (n = 10). Patients selected according to NINCDS-ADRDA criteria were classified using neuropsychological assessment tests. Phenotype analysis revealed differences in expression of CD16 (increased in mAD), NKG2A (decreased in aMCI), and TLR2 and TLR9 (both decreased in mAD) for NK and CD14 (increased in MCI). Functional assays revealed that NK cell killing activity and degranulation (CD107 expression) were unchanged in the three groups. In contrast, expression of the CD95 receptor was increased in aMCI and mAD. Granzyme B expression and cytokine production (TNFα, IFNγ) were increased in aMCI but not in mAD. Similarly, PMN phagocytosis and free radical production were differentially modulated in MCI and mAD patients. Our data suggest that the number of alterations observed in peripheral NK cells and neutrophils in aMCI represent an activation state compared to mAD patients and that may reflect an active immune response against a still to be defined aggression, but most probably of infectious origin.

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DOI: 10.1002/eji.201970400

P1623

Analysis of immune alterations and their relationship to bacterial infection after stroke

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Stroke blocks the blood that enters the brain, thereby inducing the death of brain cells. At the same time, stroke also causes systemic immunosuppression, resulting in secondary infections such as pneumonia or urinary infection. Thus, the interaction between brain and systemic immune system is becoming more apparent in many researches. Therefore, we investigated to analyze the pathological phenotype of systemic immune alterations, especially T lymphocytes from early to late phase after stroke including the results of bacterial infections at the following time points: 1-5 days, 1-2 weeks, 2-6 months by flow cytometry analysis. When the stroke occurred, the number of total splenocytes and thymocytes were significantly decreased in tMCAO mice at 1 week. And activated T lymphocytes were significantly increased, but decreased in spleen. In addition, when lymphocytes were stimulated with α-CD3 and α-CD28 antibodies, expression of IL-2, 4, 13, and IFN-γ were decreased at 5 days. CD25 expression was also decreased in tMCAO mice. Next, we analyzed thymus. Numbers of DP (CD4+CD8+) cells were significantly decreased at 1 week after tMCAO. Development stages of thymocytes from DN1 to DN2 were also blocked. Intriguingly, the percentages of TCR-β+ cells were increased in thymocytes from tMCAO mice compared to Sham mice. Finally, we could detect bacterial infections in blood and lungs which were identified as Enterococcus faecalis, an intestinal microorganism. This infection was correlated with decreased peripheral lymphocytes numbers. Taken together, tMCAO decreased number of thymocytes and peripheral lymphocytes which may result in the increased infection of commensal bacteria.

P2205

Anti-inflammatory effects of alpha 7 nicotinic AChR through interacting with adenylyl cyclase 6

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Alpha 7 nicotinic acetylcholine receptor (α7 nAChR) has been shown to suppress inflammation through diverse pathways in various immune cells, which is potentially involved in a bunch of inflammatory diseases. In the present study, we found that activation of α7 nAChR with agonist such as PNU282987 leads to accumulation of cyclic AMP in a murine macrophage cell line (RAW 264.7) and bone marrow-derived macrophages (BMDM). We further showed that activation of α7 nAChR stimulates adenylyl cyclase 6 (AC6), which plays a key role in negative regulation of TLR4 signaling by shifting its clathrin-mediated endocytosis to lipid raft-dependent endocytic pathway, leading to accelerated protein degradation of TLR4, so to dampen inflammation in macrophages, while knockdown of AC6 considerably abnegated anti-inflammatory effects upon activation of α7 nAChR. Moreover, administration of α7 nAChR agonist PNU282987 attenuates pathological and inflammation changes, putatively in alveolar macrophages, in chronic obstructive pulmonary disease (COPD)-like mice model induced with porcine pancreatic elastase combined with lipopolysaccharide (LPS). Thus, our work reveals a novel mechanism of negative regulation of inflammation by α7 nAChR through interacting with AC6, which might provide a novel therapeutic approach for limiting inflammatory diseases.

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DOI: 10.1002/eji.201970400

P2206

Microglia as immune cells in CNS can be activated by bacterial endotoxin or aβ and downregulating neuroinflammation by natural products

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Microglia are immune cells of the brain that display a range of functions. They can clean the debrils and maintain homeostasis as well as respond to toxic molecules such as bacterial endotoxin LPS or amyloid-β peptide$_{1-42}$ (Aβ$_{1-42}$) to lead to inflammatory response. Based on the recent findings that the human papillomavirus, herpes simplex virus-1or Epstein-Barr virus and related antibodies have been found in the brains of Alzheimer's patients, further studies on microglia are toward extremely necessary and important . To explore the onset mechanisms of neurodegenerative diseases such as Alzheimer's disease (AD) and find out drug candidates, our a series of previous studies have tested how microglia respond to LPS at 100ng/ml and two conformations of Aβ$_{1-42}$ included oligomeric or fibrillar Aβ$_{1-42}$ at 100uM in the paralleled experiments. Furtherore, to sreen some drugs to modulate microglial activation induced by LPS or Aβ$_{1-42}$, a couple of natural products have been tested in time-course and concentration-course experiments. Proinflammatory cytokines such as IL-1β, TNF-α and oxidative-stress molecules like NO, iNOS and ROS, as targeted endpoints, were measured via Griess assay, NBT assay, Western Blot analysis or immunofluorescence staining. Our studies have shown that the primary microglia, derived from rats, monkey or postmortem human brains, induced by bacterial endotoxin LPS or Aβ$_{1-42}$, lead to neuroinflammation and oxidative stress, and can be down-regulated by the natural products. Also, these observations demonstrate substantially greater ability of human microglia to phagocytose oAβ when compared to fAβ.

P2207

Tocilizumab versus azathioprine in highly relapsing neuromyelitis optica spectrum disorders (TANGO): a head-to-head comparative study

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Background: Tocilizumab, anti-IL-6R mAb, reduced disease progression of neuromyelitis optica spectrum disorders (NMOSD) in previous non-randomised case series studies. We aim to compare the safety and efficacy of tocilizumab and azathioprine in patients with highly relapsing NMOSD.
Methods: In the ongoing, investigator-initiated, randomised, open-label, parallel-group study

DOI: 10.1002/eji.201970400

(TANGO), NMOSD patients aged 18-65 years with at least two attacks in the preceding year or three attacks in the previous 2 years were enrolled. Patients were randomly assigned 1:1 to receive 8 mg/kg intravenous tocilizumab monthly or 2-3 mg/kg oral azathioprine daily. The primary endpoint was set at the first relapse following beginning of study participation. Secondary endpoints were determined through disability measurements and new lesions imaged by MRI. Adverse events were recorded upon follow-up visits. This trial is registered on ClinicalTrials.gov, number NCT03350633.
Findings: Between Oct, 2017 and Aug, 2018, 118 patients across six centers throughout China were enrolled and randomly assigned to receive tocilizumab or azathioprine (each group n=59). After a mean observation period of 48 weeks (in the interim), the proportion of relapse-free was 91.5% in the tocilizumab group and 67.8% in the azathioprine group (hazard ratio [HR]=0.32, 95%CI 0.14-0.70, p=0.004). Sustained reduction in disability was more likely among patients treated with tocilizumab than patients with azathioprine (HR=0.34, 95% CI 0.13-0.90, p=0.03). Fewer and milder treatment-associated adverse events were observed in tocilizumab group patients.
Interpretation: TANGO interim results show that tocilizumab had a significant risk reduction and a favorable safety profile as compared with azathioprine for highly relapsing NMOSD.

P2208

Molecular mechanisms, temporal and spatial dynamics of inflammasome formation in ischemic stroke model of mice

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Background and Purpose: The sensors of NLRP3, NLRC4 and AIM2, the executors of Caspase-1/8/11 and the products of IL-1β, IL-18 and GSDMD are key factors in inflammasome activation. Inflammasome is found in macrophage, microglia and dendritic cell (DC) in mice after ischemic stroke. However, the molecular mechanisms and the dynamics of inflammasome formation in stroke remain elusive. The current study explored the molecular mechanisms and dynamics of inflammasome activation after stroke.
Methods: Stroke was induced with transient middle cerebral artery occlusion (tMCAO, 1h) in mice. Expression dynamics of sensors, executors and products of inflammasome, inflammasome activation in brain cells were evaluated. Correlation of inflammasome activation and stroke outcomes was explored. Therapeutic effects of inhibiting inflammasome on stroke outcomes were evaluated.
Results: As sensors of inflammasome, NLRP3 and NLRC4 showed expression up-regulation at 1-3d after stroke, followed by a peak at 3-5d. Similar dynamic pattern was observed in the executor of cleaved Caspase-11, as well as the products of IL-18 and GSDMD. Expression of Caspase-1 and cleaved Caspase-1 increased at 1d and steadily up-regulated until 7d. Production of IL-1β peaked at 1d then decreased but remained at high level until 7d. Macrophage was the main cell in which inflammasome was formed which peaked at 3-5d. Increased inflammasome activation was correlated with worse stroke outcomes. Inhibiting NLRP3 and NLRC4 inflammasome activation in macrophages showed therapeutic efficacy in ischemic stroke.
Conclusion: The impact of inflammasome activation is detrimental to ischemic stroke. Inhibiting NLRP3 and NLRC4 inflammasome is a promising therapeutic strategy for stroke.

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DOI: 10.1002/eji.201970400

P2209

Transcriptome profile of rat genes in injured spinal cords after adoptive transfer of M2 macrophages by RNA-sequencing

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Our previous studies showed that M2 macrophages adoptive immunity can improve the proportion of local M2 macrophages and play the role of neuroprotective effect after spinal cord injury (SCI). However, the detailed mechanism is not entirely clear. This study intends to use RNA-sequencing (RNA-Seq) to analyze the influence of adoptive transfer of M2 macrophages on the local expression of gene transcription in the SCI.
The results showed that compared with the sham group, there were 5812 different genes in SCI group, 3196 of which were up-regulated and 2616 down-regulated. In total, there were 3348 differentially expressed genes in M2 adoptive immunizated SCI group compared with the sham group, of which 2270 were up-regulated and 1078 were down-regulated. Totally, there were 311 differentially expressed genes in M2 adoptive immunizated SCI group compared with SCI group. 88 of which were up-regulated and 223 down-regulated. GO enrichment analysis showed the significant differentially expressed genes were included immune system process, defense response, regulation of response to stimulus. KEGG pathway analysis found that the most enriched pathways included Toll−like receptor signaling pathway, RIG-I-like receptor signaling pathway, Natural killer cell mediated cytotoxicity, etc.
In summary, the results of RNA-Seq showed that adoptive immunization of M2 macrophages can lead to the change in local gene expression of the injured spinal cord. These genes may be related to Toll−like receptor signaling pathway, RIG-I-like receptor signaling pathway, Natural killer cell mediated cytotoxicity, etc.

This study was supported by grants from the National Natural Science Foundation of China (Nos. 81571194, 81772321).

P2210

Procyanidin B2 ameliorate experimental autoimmune encephalomyelitis via suppression of TH17 differentiation by inhibiting NOX2 pathway

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Background: In the central nervous system (CNS), chronic inflammatory activity may lead to oxidative stress which induce neurotoxicity, suggesting that therapeutic approaches aimed at restoring oxygen metabolic balance may be useful. Procyanidin B2 (PC-B2), an excellent antioxidant, have anti-inflflammatory activities. We thus hypothesized that PC-B2 could be highly effective in suppressing experimental autoimmune encephalomyelitis (EAE) development.
Methods: C57BL/6 mice were immunized with MOG and equally divided into EAE and EAE+PC-B2 groups. 12 days after induction of EAE, the mice were treated with PC-B2 at the doses of 1 mg/mouse/day for 9 days subcutaneously. The 9 days postimmunization splenocyte and Lymph node cells of EAE mice and purity T cells stimulated with anti-CD3CD28 were treated with/without PC-B2 in vitro to determine its mechanisms.
Results: In the present study, we define mechanisms by which adaptation to PC-B2 functionally ameliorates the clinical symptoms of EAE. PC-B2 treatment prevents the demyelination and inflammation caused by EAE via the suppression the activity of NOX2. Also, decreased numbers of cluster of differentiation of Th17 cells were found in the PC-B2 treatment group *in vivo* and *in vitro*,

DOI: 10.1002/eji.201970400

which mechanism is related to the reduction of hydrogen peroxide production by inhibiting NOX2. **Conclusion:** PC-B2 treatment effectively prevents immunological and histological damage caused by EAE due to the effect of inhibiting NOX2 pathway. It was thought that the beneficial effects of PC-B2 are likely a result of its strong antioxidant properties. Further understanding of these mechanisms system may pinpoint possible new therapeutic targets to treat neurodegenerative disease.

P2211

Using repopulated microglia to treat radiation induced side effects on mice

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Radiotherapy is still one of the most efficient ways to kill tumor cells in the Brain both on Children and adults. However meantime it can also lead to severe side effects, one of the most important reasons is CNS inflammation initiated mainly by microglia. Repopulated microglia after microglia depletion has been shown anti-inflammation effects. But whether repopulated microglia can suppress inflammation and protect the brain after radiation is still unknown. We used Cx3cr1$^{CreER/+}$R26$^{DTA/+}$ mice to erase microglia and get repopulated microglia in the brain, and then irradiated the brain in 3 different repopulate stages. Stage1, up to 90% microglia were depleted and monocyte infiltrated in to the brain. Stage 2, all the microglia repopulated and 90% of these repopulated microglia originated from infiltrated monocyte. Stage 3, all the microglia repopulated, half of them originated from monocyte, another half originated from origin local microglia. After we did the irradiation on Stage1, we detected higher caspase3 activity and more dead cells in the SGZ at 24 hours after IR but no difference at 6 hours after IR, this means, microglia are needed to remove the dead cells. But no difference was found on the delayed outcomes(stem cell pool, new born neurons, DG volume) after IR. Futher study will be performed on stage2 and stage3.

P2212

Valine catabolism modulates autoimmune neuroinflammation

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Autoimmune diseases arise through a combination of genetic predisposition and environmental factors, but very few studies have directly investigated how genetic variation together with environmental cues leads to autoimmune activation. Through experiments in rats using the Multiple Sclerosis animal model Experimental Autoimmune Encephalomyelitis (EAE) we have found that genetic variation in a locus on chromosome 4 conferred a strong disease phenotype. This locus codes for 3-hydroxyisobutyrate dehydrogenase (Hibadh), a mitochondrial enzyme that participates in valine degradation. Using a novel mouse strain for the conditional deletion of Hibadh we show that knock out animals have smaller secondary lymphoid tissue and weaker initial inflammatory cell recruitment. However, once established, the inflammation progressively exacerbates leading to increased infiltration of both brain and spinal cord by immune cells. Using bone marrow chimeras we show that this effect mostly stems from non-immune system tissues. Through Mass Spectrometry, we show that the substrate for Hibadh, 3-hydroxyisobutyrate (3-HIB), accumulates in knock out animals and that 3-HIB is able to directly affect inflammatory phenotypes in immune cells. Finally, we also show that 3-HIB is increased in cerebrospinal fluid of MS patient during relapses, supporting a role for our observations in rodents to MS disease in humans.

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DOI: 10.1002/eji.201970400

P2213

Role of CD137L in lumbar spinal cord microglial cytokine responses following sciatic nerve crush

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Previously, we showed that CD137 ligand (CD137L; AKA 4-1BBL) contributes to the development of behavioral hypersensitivity and functional recovery in mice following peripheral nerve injury-induced neuropathic pain. Mice that lack CD137L showed reduced sensory sensitivity and faster functional recovery. Here, we sought to further investigate the potential involvement of lumbar spinal cord microglial responses in CD137L-mediated behavioral changes following sciatic nerve crush. Lumbar spinal cord microglia were isolated from both wild type B6 and CD137L knockout (KO)_B6 mice via positive selection based on CD11b expression using magnetic beads at days 0 (naïve), 3, 7, 14, 28 and 56 following sciatic nerve crush or sham surgery. Following RNA extraction, quantitative real-time PCRs (qRT-PCRs) were performed to examine cytokine/chemokine expression in isolated microglia. Despite that many cytokines are below the detection levels, we were able to observe increased expression of several chemokines, CCL2, CCL3, CCL4, CCL5, and CXCL10 in isolated lumbar spinal cord microglia. Our preliminary results showed differential expression patterns of these chemokines between the CD137L KO mice and the WT mice, suggesting differential contributions of these chemokines to CD137L-mediated behavioral changes. Future investigation will further determine the involvement of microglial chemokine responses in CD137L-mediated sensory hypersensitivity and delayed functional recovery following peripheral nerve injury. Sex-dependent differences in microglial chemokine responses will also be examined in future studies.

P2214

Evaluation of the GM-CSF blocking on the phenotype and function of human monocytes

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multipotent cytokine that prompts the proliferation of bone marrow-derived macrophages and granulocytes. GM-CSF is one of the most important cytokines involved in the pathogenesis of autoimmune disorders such as multiple sclerosis and rheumatoid arthritis. Some reports have indicated that innate immune cells like monocytes, with the cooperation of GM-CSF, have a significant role in the induction of inflammation. Elucidating the cellular and molecular mechanisms of GM-CSF blocking can result in the identification of possible targets and help to provide more options for treatment and therapy. Here, we examined whether GM-CSF blocking affects both phenotype and function of human monocytes. We found that GM-CSF induced an inflammatory phenotype in monocytes, while GM-CSF blocking resulted in an immunomodulatory phenotype, characterized by the production of CXCL11. We found that CXCL11 suppressed T cell proliferation induced by GM-CSF-treated monocytes. In addition, it inhibited GM-CSF-induced pro-inflammatory effects of monocytes on $CD4^+$ T cells. Our findings suggest that blockage of GM-CSF induces a CXCL-11-dependent immunoregulatory phenotype in monocytes.

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DOI: 10.1002/eji.201970400

P2215

Features of food hypersensitivity in children with autism spectrum disorder

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According to world statistics, the incidence of autism spectrum disorders (ASD) continues to increase in the children´s population. The pathogenesis of autism remains controversial.
We evaluated the effect of the elimination diet for the food hypersensitivity (FHS), the balance of interleukins and physiological parameters in children with ASD.
This analysis included 89 children (7±2 years): healthy (n=20) and diagnosed with ASD (n=69). The levels of specific IgG (sIgG) (111 food antigens) and interleukins were measured by ELISA, psychophysiological parameters were evaluated in the ATEC test.
It was established that children with ASD were significantly more likely to have FHS to antigens of dairy and grain products. A direct correlation was found between the concentration of INFγ, sIgG concentrations to antigens of dairy and grain products, IgG to antigens of *C.albicans*. High ratios of the INFγ/IL4 and INFγ/IL10 ratios were revealed. Each child for a period of 6 months was assigned an individual elimination diet. As a result, that children was noted significant decrease in the concentration of sIgG to food antigens, sIgG to *C.albicans*, the average score before the diet was 76.8±3.2 disorders, after a diet - 57.9±5. With a high adherence to diet, the ATEC indicators decreased by 41.3±4.5, the average score was 35±3.4 (mild degree).
The data obtained revealed the unexplored effects the influence of FHS on the process of initiation and maintenance of immune inflammation. A personalized elimination diet can improve the immunorehabilitation and the quality of life of children with autism.

P2985

IL-21R deficiency leads to depressive-like behaviors in mice

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Major depressive disorder (MDD), which affects about 16% of the world´s population, has become mental disorder in the world. Major depressive disorder (MDD) is characterized by depression, lack of interest in the outside world, lack of concentration,increased suicidal propensity , and the high incidence of the disease. At present, depression has become the first major mental illness in the world, so it is very important to explore the specific pathogenesis of depression.We aim to explore the mechanism of IL-21R signaling pathway in the formation of depressive behavior in mice, and to provide an effective therapeutic target for depression.Research methods such as immunology, neurology and ethology are all used in this study.The results show us that IL-21R knockout mice showed depressive behavior, and the main pathological system was the nervous system.The conclusion of this study is that the blockage of IL-21 signaling pathway leads to depressive behavior in mice.

P2986

Structural insight into the serotonin (5-HT) receptor family by molecular docking, molecular dynamics simulation and systems pharmacology analysis

DOI: 10.1002/eji.201970400

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Serotonin (5-HT) receptors are proteins involved in various neurological and biological processes, such as aggression, anxiety, appetite, cognition, learning, memory, mood, sleep, and thermoregulation. They are commonly associated with drug abuse and addiction due to their importance as targets for various pharmaceutical and recreational drugs. However, due to a high sequence similarity/identity among 5-HT receptors and the unavailability of the 3D structure of the different 5-HT receptor, no report was available so far regarding the systematical comparison of the key and selective residues involved in the binding pocket, making it difficult to design subtype-selective serotonergic drugs. In this work, we first built and validated three-dimensional models for all 5-HT receptors based on the existing crystal structures of 5-HT1B, 5-HT2B, and 5-HT2C. Then, we performed molecular docking studies between 5-HT receptors agonists/inhibitors and our 3D models. The results from docking were consistent with the known binding affinities of each model. Sequentially, we compared the binding pose and selective residues among 5-HT receptors. Our results showed that the affinity variation could be potentially attributed to the selective residues located in the binding pockets. Moreover, we performed MD simulations for 12 5-HT receptors complexed with ligands; the results were consistent with our docking results and the reported data.

P2987

Intercellular A2AR-mGluR5 interaction is involved in neutrophil-neuron direct contact and nerve recovery in traumatic brain injury

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G-protein coupling receptors (GPCRs) form a large protein family of receptors that detect molecules outside the cell and induce cellular responses by activating intended signal pathways. Despite external signals like specific ligands or mediators that activate GPCRs, the formation of homo- or hetero-dimmers between GPCRs could lead to conformational changes that affect intracellular signal pathways as well. In this study, we found an elevated hetero-complex formation of A2AR-mGluR5 (belong to Gs and Gq GPCR respectively) in mouse brain tissue after traumatic brain injury (TBI), characterized with onrushing neutrophil infiltration. This complex can be formed of A2AR and mGluR5 not only on neutrophils or neurons themselves, but also between neutrophils and neurons. Based on the results of Z-dock (protein molecular docking software) and FRET, we identified several amino acid binding sites being responsible for the intracellular (A2AR intracellular C3 part) and intercellular (A2AR extrecellular N2 & N3 part; mGluR5 extracellular N1 part) A2AR-mGluR5 interaction. Further function detections showed that:1. A2AR-mGluR5 complex on neutrophils promoted excessive release of inflammatory mediators; 2. A2AR-mGluR5 complex on neurons aggravated neuronal damage; 3.Intercellular A2AR-mGluR5 complex between neutrophils and neurons downregulated neuropilin-1 (NRP1) and protein phosphates regulatory subunit 12A (PPP1R12), damping nerve recovery mediated by neutrophils. Taken together, our study for the first time detect the intercellular GPCR interaction between neutrophils and neurons, disclosing a novel mechanism exerted by peripheral innate immune cells in brain under pathological conditions.

(This work is supported by National Natural Science Foundation of China (No. 81771693, No. 81322044).

DOI: 10.1002/eji.201970400

P2988

Nicotine inhibits MeHg induced M1 microglia polarize via activation ofα7nAChR/NFκB signaling

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Aim: Methyl Mercury, MeHg can directly activated Microglia. but its neuroinflammation mechanism remain elusive. Recent studies have revealed that α7nAChR, as a key role in the cholinergic anti-inflammatory pathway(CAP), regulates activation of microglial and inhibits inflammation. Nicotine increases the expression of α7nAChR. Here, we investigated the effects of nicotine on MeHg stimulated microgia and α7nAChR signaling.
Methods: Methylmercuric chloride, MeHgCl(5mg/kg) expossed rat hippocampus and cultured BV-2 cells supernatants were collected for cytokine using ELISA. The expression of M1/M2 microglia phenotypes with immunofluorescence and flow cytometry. The levels of α7nAChR and nuclear factor (NF) κB (p65) were determined by Western-Blot and qRT-PCR.
Results: MeHg exposure remarkably promoted M1 microglia polarization by increase in expression of CD16/32^{+}cells and the level of IL-1β, IL-6 in hippocampus, and down-regulated M2 microglia by decrease in CD206^{+}cells with no significant difference in IL-10 level, whileas nicotine enhanced the expression of α7nAChR in dendate gyrus region and reversed M1/M2 polarizes induced by MeHg. *In vitro,* significantly down-regulating mRNA expression of α7nAChR and up-regulating level of p65 were found in MeHg treated cells, While nicotine dose dependently reduced protein of p65, limited the production of IL-6, elevated the M2 Polarization of microglia even after 24h and 48h with MeHg treatment.
Conclusion: Nicotine suppresses MeHg-induced neuroimmune is associated with attenuation of ratio of M1/M2 phenotypes of microglia through activation ofα7 nAChR /NFκB pathway.
Keywords: methylmercury, microglia, α7nAChR

P2989

Organ- and cell-specific immune responses are associated with the outcomes of intracerebral hemorrhage

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Background: Severe brain injury significantly influences immune responses. However, the levels at which such influence occur and the involvement of neurogenic pathways are not well defined.
Methods: patients with supratentorial ICH within 24 hours of onset and 20 matched healthy controls were enrolled. Hematoma sizes at admission were calculated via computed tomography. Perihematomal edema (PHE) and spleen volume at day 3 and 14 were measured via T2-weighted magnetic resonance imaging, respectively. Peripheral lymphocyte subsets were quantified by flow cytometry.
Results: Average spleen shrinkage of 37ml accompanied by splenic capillary perfusion increase occurred in ICH patients at day 3 after disease onset. The magnitude of spleen shrinkage was associated with hematoma size upon admission. Concurrently, patients with severe spleen shrinkage (> 37ml) had less progression of PHE. Lymphopenia was observed in ICH patients after ictus and persisted up to 14 day, which was not parallel with spleen alteration. Patients with infections exhibited poorer functional outcome and significant T and NK cell deficiency. In ICH models, signals derived from adrenergic and hypothalamus-pituitary-adrenal (HPA) axis activation contributed to loss of white blood cells in the white pulp.
Conclusions: Spleen shrinkage and lymphopenia reflect the impact of ICH on the immune system at

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DOI: 10.1002/eji.201970400

the organ and cellular levels, such impacts are derived from coordinated action of sympathetic innervation and HPA axis. The magnitude of spleen shrinkage might be associative with the progression of PHE and clinical outcome of ICH patients. Additionally specific cellular immunity deficiency was associated with increased infection risk in patients.

P2990

Astrocytic interleukin-15 reduces pathology of neuromyelitis optica in mice

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Astrocyte loss induced by NMO-IgG and complement-dependent cytotoxicity (CDC) is the hallmark of NMO pathology. The survival of astrocytes is thought to reflect astrocyte exposure to environmental factors in the CNS and the response of astrocytes to these factors. However, still unclear are how astrocytes respond to NMO-IgG and CDC, and what CNS environmental factors may impact the survival of astrocytes. In a murine model of NMO induced by intracerebral injection of NMO-IgG and human complement, we found dramatic upregulation of IL-15 in astrocytes. To study the role of astrocytic IL-15 in NMO, we generated a transgenic mouse line with targeted expression of IL-15 in astrocytes, in which the expression of IL-15 is controlled by a glial fibrillary acidic protein (GFAP) promoter. We showed that astrocyte-targeted expression of IL-15 attenuates astrocytes and aquaporin-4 loss in the brain. Reduced BBB leakage and immune cell infiltration are also found in the lesion of IL-15^{tg} mice subjected to NMO induction. *In vitro* data indicates that IL-15^{tg} astrocytes are less susceptible to NMO-IgG-mediated CDC when compared to their WT littermates. Importantly, IL-15 enhances the ability of astrocytes to resist cytotoxicity and cell death via NF-κB signaling pathway. Our findings suggest that IL-15 reduces astrocyte loss and NMO pathology.

P2991

Inhibition of fibrin formation reduces neuroinflammation and improves long-term outcome after intracerebral hemorrhage

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Coagulation and inflammation are two interactive processes that are involved in ICH injury. The conversion of fibrinogen to fibrin is an essential step of coagulation processes, however, its influences on brain inflammation and ICH injury remain incompletely understood. Here we show that fibrinogen levels were increased in the plasma of patients with ICH. Hirudin binds to thrombin and inhibits the conversion of fibrinogen to fibrin. In a mouse model of ICH, blockade of fibrin formation using hirudin reduced neuroinflammation and improved the long-term outcome. Hirudin treatment reduced leukocyte accumulation in the brain and shifted microglia toward an anti-inflammatory phenotype. In addition, depletion of microglia in ICH mice diminished the benefit of hirudin in ICH mice. Collectively, our results suggest that ablation of fibrin formation may be beneficial for ICH treatment.

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DOI: 10.1002/eji.201970400

P2992

Inhibition of activator protein-1 attenuates neuroinflammation and brain injury after experimental intracerebral hemorrhage

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Aims: Intracerebral hemorrhage (ICH) is a devastating type of stroke without specific treatment. Activator protein 1 (AP-1) is key transcription factor that switches on cytokine expression in response to environmental stimulation. In this study, we investigated the role of AP-1 in neuroinflammation and brain injury following ICH.
Methods: ICH is induced in mice by injection of autologous blood or collagenase. We measured the expression of AP-1 in brain tissues. The effects of an AP-1 inhibitor SR11302 on brain injury and neuroinflammaiton and underlying mechanisms were investigated using ICH mice and cell culture.
Results: AP-1 was significantly upregulated in mouse brain tissues as early as at 6 h after ICH induction, accompanied by dramatic upregulation of proinflammatory factors, including interleukin 6 (IL-6), interleukin 1 beta (IL-1β) and Tumor necrosis factor α (TNF-α). Inhibition of AP-1 using SR11302 reduced neurodeficits and brain edema at day 3 after ICH. SR11302 ablated the production of IL-6 and TNF-α by microglia and brain infiltration of leukocytes in ICH mice. In addition, SR11302 treatment diminished thrombin-induced production of IL-6 and TNF-α in cultured microglia.
Conclusions: AP-1 induced by ICH curbs neuroinflammation and reduces brain injury following ICH.

P2993

Upregulation of PGC-1α in neurons protects against experimental autoimmune encephalomyelitis

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Background/Aims: Increasing evidence have associated mitochondrial dysfunction and reactive oxygen species (ROS) generation to neuron loss in multiple sclerosis (MS). PGC-1α regulates mitochondrial biogenesis and respiration and plays a pivotal role in modulating ROS levels. Here, we investigated the potential function of peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) in experimental autoimmune encephalomyelitis (EAE).
Methods: The transgenic mice with neuron-specific overexpression of PGC-1α and a neuronal cell line transfected with lentivirus were applied to explore the anti-apoptotic effect of neuronal PGC1 in EAE .
Results: We observed that PGC-1α were decreased at 13d, continuously decreased at 20d, then mildly increased at 30d in wild-type (WT) EAE mice, accompanied with alterations of mitochondrial antioxidants SOD2, Prx3, Trx2 and UCP4,5. Our study revealed that PGC-1α$^{f/f}$ Eno2-Cre mice improved EAE clinical symptoms, ameliorated inflammation and demyelination in spinal cords, increased mitochondrial antioxidants SOD2, Prx3, Trx2 and UCPs, reduced the production of ROS and inhibited the apoptotic pathways. In addition, PGC-1α$^{f/f}$ Eno2-Cre mice showed decreased apoptotic neurons compared with the WT EAE mice. In vitro, overexpressing PGC-1α by lentivirus transfection revealed similar results with that of in vivo by using NSC-34 cells treated with 1mg/ml lipopolysaccharide. Furthermore, RNA-seq analysis showed that apoptotic processes were significantly enriched in the top 10 significant GO terms of differentially expressed (DE) gene and apotosis pathway was significantly enriched in KEGG pathway analysis.

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DOI: 10.1002/eji.201970400

Conclusion: Taken together, our findings indicate that upregulation of neuronal PGC-1α protected neuron apotosis in EAE and in vitro.

P2994

Exposure to cigarette smoke augments post-ischemic brain injury and inflammation via mobilization of neutrophils and monocytes

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Cigarette smoke is a major risk factor for stroke and linked to stroke severity in these patients. Although evidence has demonstrated an increase of circulating leukocytes in smokers, it remains unclear whether and how smoke priming influences the responses to stroke onset. In this study, we investigated the impact of cigarette smoke on brain injury and inflammation after brain ischemia in mice. We found that cigarette smoke exacerbated post-ischemic neurodeficits and brain infarction, accompanied by augmentation of pro-inflammatory factors and brain infiltration of neutrophils and monocytes. Further, exposure to cigarette smoke induced mobilization of peripheral neutrophils and monocytes prior to brain ischemia. In addition, the deleterious effect of cigarette smoke on ischemic brain injury was abolished either by inhibition of the homing of neutrophils and monocytes to brain, or by blockade of inflammasome, an effector protein of neutrophils and monocytes. Our findings suggest that cigarette smoke-induced priming of peripheral neutrophils and monocytes augments ischemic brain injury.

P2995

A mouse allergic contact dermatitis model induced by DNFB and associated up-regulation of GRP, substance P and TRPV-1 in DRGs

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Objectives: To establish an allergic contact dermatitis model in C57BL/6 mice induced by DNFB and analysis GRP, Substance P and TRPV-1 expression in the cervical DRGs and their possible role in pathogenesis of chronic itch associated with ACD.
Methods: DNFB was topically applied to induce ACD in C57BL/6 mice, later the dermatitis symptoms, skin pathology and total IgE and IL-4 levels in serum were evaluated by ELISA. In addition, mRNA levels of GRP, Substance P and TRPV-1 in DRGs were detected by quantitative RT-PCR.
Results: Compared to the controls, DNFB model mice showed more severe inflammatory wounds on the nape of neck, significantly increased the count of mast cells among much thicker stratum spinosum (indicated by dermatological pathology) as well as elevated IgE and IL-4 levels in serum. mRNA levels of GRP, Substance P and TRPV-1 in cervical DRGs were significantly elevated in DNFB model mice.
Conclusion: Topically applied DNFB on the nape of neck skin of C57BL/6 mice could build a useful way to establish atopic dermatitis in mice. GRP, Substance P and TRPV-1 might be implicated in the pathogenesis of chronic itch in DNFB induced ACD.

DOI: 10.1002/eji.201970400

P2996

Expression changes of pro- and anti-inflammatory cytokines and VEGFs in spinal cord of mice with dry skin pruritus induced by AEW

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Objective: To investigate the expression of pro- and anti-inflammatory cytokines and Vascular endothelial growth factor (VEGFs) in the cervical spinal cord of dry skin disease model (AEW model) mice and the possible role in the pathogenesis of chronic itch of pro- and anti-inflammatory cytokines and Vascular endothelial growth factor (VEGFs) in the cervical spinal cord of C57BL/6 mice with chronic itch caused by an established acetone/diethyl ether solution and water method induced dry skin disease model (AEW model).
Methods: AEW model of C57BL/6 mice were established by using acetone/diethyl ether mixture(1:1) and water for 7 days. The behavior of mice were observed. Quantitative PCR was used to analyze the expression of proinflammatory cytokine including Il-1β, Il-6, Il-12a and Tnf-α, anti-inflammatory cytokines including Il-4, Il-11 and Il-13, and three kinds of vascular growth factor Vegf-a, Vegf-b and Vegf-c from cervical spinal cord tissues.
Results: Compared with the control group, the number of spontaneous scratching in the AEW mice was significantly higherincreased. In the cervical spinal cord of AEW mice, the Il-1β, Il-6, Il-12a, Il-18 and Tnf-α mRNA levels were significantly increased, the Il-11 and Il-13 mRNA levels were significantly decreased, and among the three VEGFs only the Vegf-a mRNA level was significantly increased.
Conclusions: A variety of inflammatory cytokines and growth factors are associated with central (spinal) mechanisms of chronic itch, and the interventions for these factors and/or their receptors would provide a new approach for clinical treatment.

P2997

The role of cerebrospinal fluid pentraxin-3 in patients with hemorrhagic stroke

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Objective: To investigate brain tissue inflammation and endothelial function damage in stroke.
Methods: We detected serum and cerebrospinal fluid (CSF) levels of PTX3, HSP70, VEGF, IL-6 and CRP in 85 patients with stroke during 2015 and 2016, 52 cases for ischemic stroke (IS) and 33 cases for hemorrhagic stroke (HS), additional 20 cases for control without inflammatory and neurological disease. The levels of PTX3, HSP70, VEGF, IL-6 and CRP in serum on the 1th, 5th and 14th day after admission were measured and compared with those in CSF, NIHSS and Barthel score were performed for assessing patients' status.
Results: The serum levels of PTX3, HSP70, VEGF, IL-6, CRP with HS were higher than those with IS patients (P< 0.001, P=0.013, P=0.009, P=0.013, P< 0.001, respectively), and PTX3 level began to fall after 14th day. The CSF PTX3, HSP70 and IL-6 levels were higher than those in the serum (P=0.026, P< 0.001, P< 0.001, respectively) in patients with HS , while VEGF, CRP instead (P=0.011, P< 0.001, respectively). The level of PTX3 in CSF was positively correlated with IL-6 in HS patients, and both were positively correlated with patients´ NIHSS score, while negatively correlated with Barthel index score.
Conclusion: Inflammation and endothelial damage were more severe in patients with HS. The levels

DOI: 10.1002/eji.201970400

of PTX3 and IL-6 in CSF could estimate the severity and prognosis of HS. PTX3 may be a potential biomarker in HS, it could provide potent basis for doctors to select appropriate treatment measures.
Keywords: stroke, PTX3, Inflammation, prognosis

P2998

The roles of C3d and C3d-reactive memory T cells in Guillain-Barré syndrome

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Complement activation plays critical roles in the pathogenesis of Guillain-Barré syndrome (GBS). C3d, the product of C3 activation, can serve as the adjuvant to promote the $CD4^+CD45RO^+$memory T cells to differentiate into helper T (Th) subsets. There were increased Th1 and reduced Th2 cells in patients with GBS. We hereby aimed to investigate if C3d can account for the abnormalities of Th subsets in GBS. 30 patients with GBS within 2 weeks after onset and 30 matched healthy controls were included to prepare the peripheral blood mononuclear cells (PBMCs) and blood plasma. The levels of C3d in blood plasma were detected using ELISA. In addition to the common stimulus of IL-2 and IL-7, synthesized C3d was used to promote the differentiation of memory T cells in culture of PBMCs. Flow cytometry was used to detect the frequency of memory T cells, naïve T cells and Th subsets. There were significant higher levels of C3d in patients with GBS than the healthy controls. There was no difference in frequency of both memory T cells and naïve T cells (both, $p>0.05$) in patients with GBS and the controls before and after the stimulus. After stimulation, there was significant higher frequency of Th2 cells in culture of PBMCs from health controls but no change in culture of PBMCs from patients with GBS ($p=0.036$, $p=0.11$, respectively). A failed differentiation of memory T cell into Th2 cells induced by C3d in patients with GBS may contribute to the development and process of this disease.

P2999

Chronic psychological stress leads to apoptotic loss of plasmacytoid dendritic cells in mice

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Recent studies have shown that chronic psychological stress leads to compromised antiviral immune responses. However, the underlying mechanisms remain elusive. As the type I interferon-producing cells, plasmacytoid dendritic cells (pDCs) play a vital role on antiviral immune responses. Thus, it is of interest to explore the relationship between pDCs and chronic psychological stress. In this study, we report that chronic psychological stress results in reduced number of pDCs with slight decreased expression of CD317, but not other surface markers, in the bone marrow and spleen of C57 BL/6 mice. beta-adrenergic receptor inhibitor propranolol significantly reverses the reduction of pDCs in stressed mice. Blockade of glucocorticoid peripheral action shows the same tendency. Moreover, beta-agonist and glucocorticoid directly induces the apoptosis of bone marrow-derived pDCs. Consistent with the reduced number of pDCs in stressed mice, the plasma level of IFN-b upon vesicular stomatitis virus (VSV) challenge was lower in stressed mice than that in control mice. Infusion of splenic pDCs significantly reversed the reduction of IFN-b production in stressed mice. Furthermore, the replication of VSV in lung was higher in stressed mice than that in control mice with enhanced lung inflammation. Infusion of purified pDCs reversed the increase of VSV replication and enhanced lung inflammation in stressed mice. Together, our data suggest that the apoptotic loss of pDCs contributes to the impaired antiviral immune responses during chronic psychological stress.

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DOI: 10.1002/eji.201970400

P3000

Elevated plasma BDNF levels are correlated with NK cells activation in patients with traumatic spinal cord injury

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The precise role of innate immune responses, especially those mediated by natural killer (NK) cells, involved in the early stage of traumatic spinal cord injury (SCI) is poorly understood. Here we report that NK cells displayed a significant increase in frequency and activated phenotype reflected by enhanced expression of CD69, HLA-DR, NKG2D, and NKp30 in traumatic SCI patients within 24h in comparison with healthy controls. Meanwhile, a higher level of the brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, was observed in plasma and the elevated level of BDNF was strongly and positively correlated with the percentage of NK cells during the early period after traumatic SCI. Furthermore, BDNF facilitated expression of CD69, and NKp30 after NK cells were stimulated for 24h in vitro, which is consistent with the in vivo observation in SCI patients. Our findings demonstrate the NK cells activation within 24h after traumatic SCI and reveal a novel role of BDNF in regulating NK cells activation.

P3001

Different sensitivity of B cells at different development stages and different subsets to restraint stress

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Chronic psychological stress is characteristic of apoptotic loss of conventional T lymphocytes and B lymphocytes, which may explain the compromised host defenses against bacterial and viral infections. Here, we report that B cells at different development stages and different subsets showed different sensitivity to restraint stress, which is associated with elevated levels of plasma glucocorticoids or catecholamines. The percentages and absolute numbers of B cells decreased in all the tissues tested. Simultaneous knockout of all beta-adrenergic receptors partially reversed the reduction. Blockade of glucocorticoid peripheral action in wild type mice significantly reversed the reduction. Blockade of glucocorticoid peripheral action in all beta-adrenergic receptor knockout mice fully reversed the reduction. B cells showed increased apoptosis in stressed mice. Thus, glucocorticoids and catecholamines collaborate to kill B cells, especially immature B cells and certain subsets of splenic B cells.

P3002

Liuwei Dihuang-active fraction combination, LW-AFC improved spatial learning and memory impairment induced by TNF-α in mice

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DOI: 10.1002/eji.201970400

Objective: Tumor necrosis factor-α (TNF-α) plays a vital role in cognitive dysfunction caused by stress. In our previous study, Liuwei Dihuang-active fraction combination (LW-AFC) could attenuate the effects of mental stress and non-psychotic stress in mice. The aim of this study is to investigate the effects of LW-AFC on cognitive dysfunction caused by TNF-α in mice.
Methods: 40 male BALB/c mice were divided into 4 groups according to their body weight, including control, TNF-α model, LW-AFC treatment and Etanercept (TNF-α antagonist) treatment groups. LW-AFC (1.6 or 3.2 g/kg/day) were orally administrated for 7 consecutive days before TNF-α administration. Etanercept was injected subcutaneously at 30 mg/kg the day before TNF-α administration. One hour before the behavioral test, TNF-α were injected intraperitoneally at 0.2 mg/kg to mice.
Results: Compared with control group, the time of mice stayed in the target quadrant and the number of mice crossing the plate significantly decreased after TNF-α injection, suggested that the spatial learning and memory ability of the mice were impaired. LW-AFC administration could increase the time of mice stayed in the target quadrant and the number of mice crossing the plate significantly at 1.6 g/kg, indicated that LW-AFC could improve spatial learning and memory in TNF-α treated mice.
Conclusion: LW-AFC can improve spatial learning and memory impairment induced by TNF-α in mice, the further mechanism still need to be clarified.
Keywords: LW-AFC ; stress ; learning and memory; TNF-α

P3003

LW-AFC alleviated the cognitive function impairment and glial cells activation in hippocampus of mice by modulating immune responses and downregulating the TLR4 expression

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Neuroinflammation is known as a typical feature associated with many neurodegenerative diseases including Alzheimer's disease (AD), and TLR4 is one of the key receptors triggering neuroinflammatory response. LW-AFC is an active fraction combination extracting from Liuwei Dihuang decoction, a classic traditional Chinese medicine prescription. This study investigated the anti-inflammatory activity of LW-AFC and explore the potential target. The results showed that the administration of LPS caused fever and long-term potentiation (LTP) impairment in mice. The pretreatment with LW-AFC had an antipyretic effect on fever and improved the impaired LTP induced by LPS injection, alleviated the microglia and astrocytes activation in hippocampus, reversed the decrease of T lymphocyte subpopulation $CD3^+CD4^+$ in spleen, $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD25^+$ in blood caused by LPS, reduced the aberrant secreted cytokines IL-1β, IL-6 and MCP-1 in hippocampus, TNFα, IL-6, IL-12 and INF-γ in plasma. Furthermore, LW-AFC administration did not improve the spatial learning and memory function and fear condition memory impairment in TLR4 knockout mice. Overexpression of TLR4 in the brain of mice caused the excessive spontaneous activity, increased the context fear memory in mice, LW-AFC administration reduced the excessive spontaneous activity and fear memory in TLR4-overexpressed mice, and significantly reduced the mRNA and protein level of TLR4 in cortex and hippocampus. **The data suggest that LW-AFC improves cognitive function might be associated with alleviates the glial cells activation and modulating inflammatory responses, and TLR4 is one of the targets of LW-AFC.**
Keywords: LW-AFC; traditional Chinese medicine; cognitive function; lipopolysaccharide; lymphocyte subpopulation; cytokine; TLR4

DOI: 10.1002/eji.201970400

P3004

LW-AFC, a new formula derived from Liuwei Dihuang decoction, ameliorates behavioral and pathological deterioration via modulating the neuroendocrine-immune system in Alzheimer`s disease mouse models

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Objective: Alzheimer's disease (AD), the most common cause of dementia among older people, could not be prevented, halted, or reversed up till now. A large body of pharmacological study has revealed that Liuwei Dihuang (LW) possesses potential therapeutic effects on AD. LW-AFC is key fractions from LW. In the present study, we investigated the effect of LW-AFC on AD mouse models.
Methods: PrP-hAβPPswe/PS1$^{\Delta E9}$ (APP/PS1) mice and senescence-accelerated mouse prone 8 strain (SAMP8), classic AD animal models, were employed. After the treatment of LW-AFC, mice were cognitively evaluated in behavioral experiments.
Results: LW-AFC ameliorated the cognitive impairment observed in APP/PS1 and SAMP8 mice, including the impairment of object recognition memory, spatial learning and memory, and active and passive avoidance. In addition, LW-AFC alleviated the neuron loss in the hippocampus, suppressed amyloid-β (Aβ) deposition in the brain, and reduced the concentration of $A\beta_{1-42}$ in the hippocampus and plasma of APP/PS1 mice. LW-AFC treatment also significantly restored the imbalance of hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axis, enhanced the proliferation of splenocytes, corrected the disorder of lymphocyte subsets, and regulated the abnormal production of cytokine in APP/PS1 and SAMP8 mice. Effects of LW-AFC on pharmacodynamics and neuroendocrine immunomodulation network in APP/PS1 and SAMP8 mice were better than memantine and donepezil.
Conclusions: LW-AFC ameliorated the behavioral and pathological deterioration of AD mouse models via the restoration of the NIM network, which supports the use of LW-AFC as a potential agent for AD therapy.
Keywords: LW-AFC; Alzheimer's disease; learning and memory; neuroendocrine; lymphocyte subset

P3005

Pathological changes in Alzheimer's disease analyzed using induced pluripotent stem cell-derived human microglia-like cells

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Microglia constitute the majority of innate immune cells in the brain, and their dysfunction is associated various central nervous system diseases. Human microglia are extremely difficult to obtain experimentally, thereby limiting studies on their role in complex diseases. Microglia derived from human stem cells provide new tools to assess the pathogenesis of complex diseases and to develop effective treatment methods. This study aimed to develop a reliable method to derive human microglial-like cells (iMGLs) from induced pluripotent stem cells (iPSCs) expressing microglia-specific markers IBA1 and TMEM119 and respond to lipopolysaccharide (LPS) stimulation. Thereafter, we compared iMGL functions from Alzheimer´s disease (AD) patients and cognitive normal controls (CNCs). AD-iMGLs displayed stronger phagocytic ability with or without stimulation. High LPS concentrations (>2 µg/ml) caused death in CNC-iMGLs, while AD-iMGLs did not display significant cell death. Cytokine analysis revealed that TNF-α, IL-6, and IL-10 secreted by AD-iMGLs were significantly increased upon LPS stimulation compared to those in CNC-iMGLs. **The present results indicate that AD-iMGLs exhibit significant inflammatory characteristics and can reflect some pathological changes in microglia in AD, thereby providing new valuable tools to screen candidate drugs for AD and to elucidate the mechanisms underlying AD pathogenesis.**

DOI: 10.1002/eji.201970400

P3006

Effects of indole alkaloids 297, 307, 315 and 327 on LTP damage induced by LPS

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Objective: In this study, we evaluated the neuroprotective effects of 4 plant-derived alkaloids on long-term potentiation (LTP) deficit in neuroinflammation animal model induced by lipopolysaccharide (LPS).
Methods: 18-22g male BALB/c mice were divided into control group, model group and compound treatment group (1μg/per mouse, icv); each group contained 5 animals.model group and compound treatment group were intraperitoneally injected LPS (50μg·kg^{-1}, ip) 4 hours before LTP induction. LTP of perforant path-dentate gyrus pathway in hippocampus was induced by high frequency stimulation and used to evaluate the effects on synaptic plasticity.
Results: Compared with control group, the LTP of the model group was significantly impaired. Compound 297, 327, and 307 could significantly improve LPS-induced LTP impairment. 315 had no significant effect on LPS-induced LTP impairment.
Conclusion: LPS has significant damage to hippocampal synaptic plasticity. Compounds 297, 327 and 307 have protective effects against LPS induced LTP impairment, and 315 have little effect on LPS induced LTP impairment. **Theses results suggested that 297, 327 and 307 might have potential effects on neuroinflammation induced memory deficits.**
Keywords: LPS; hippocampal; LTP

P3007

Total salvianolic Acids attenuate AD-like pathology in the hippocampus of middle-aged APP/PS1 mice involved in free fatty acids modulation

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Purpose: We investigated the effect of TSA on AD-like pathology attenuation including amyloid plaques, activated microglia, ultrastructure of neurons and capillary endothelial cell in hippocampal CA1 region, afterwards the pharmacological mechanisms of TSA on free fatty acids and autophagy modulations were discussed.
Methods: TSA were put to be used to treat 3.5-month-old APPswe/PS1dE9 mice. HE and Nissl's staining were performed to analyze pathology in the hippocampus of mice. Abeta plaques burdens were assessed by congo red fluorescence staining. Iba1 positive cells were then observed and analyzed. The ultrastructural morphology of neurons and the capillary endothelial cells were scrutinized by TEM. Finally the changes of free fatty acids and autophagy markers were detected by the methods of GC-TOF-MS and Westernblot.
Results: TSA treatment reduced the pathological vesicular degeneration in APP/PS1 mice.The plaque burdens stained with congo red fluorescence were alleviated in the APP/PS1 mice after TSA treatment. Amyloid β plaque burdens in the hippocampal sections revealed that TSA treatment relieved their corresponding plaque area ratios in APP/PS1 mice.The treatment with TSA reversed the impairments in Iba1 positive microglia density ($P< 0.01$). TSA treatments improved the impairment in the ultrastructure of mitochondrial in the neurons and capillary endothelial cell in the hippocampal CA1

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DOI: 10.1002/eji.201970400

region of APP/PS1 mice. TSA modulate free fatty acids such as palmitoleic acid but not autophagy markers including Beclin 1 and LC3B/MAP1LC3B antibodies.
Conclusions: Treatment with TSA attenuated AD-like pathological changes of middle-aged APP/PS1 mice involved in free fatty acids modulation but not autophagic effect.

P3008

Neuropsychiatric involvement in lupus is associated with the Nogo-a/NgR1 pathway

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Neuroinflammation- and neurodegeneration-induced nerve injury may represent important components of neuropsychiatric lupus (NPSLE). Myelin-associated neurite outgrowth inhibitor (Nogo)-a and its receptor, NgR1, limit recovery of the adult central nervous system after injury. We detected a soluble Nogo-a product in the cerebral spinal fluid of patients with NPSLE. In a mouse model of lupus, aging was associated with an increase in Nogo-a positive neurons, diminished myelin sheaths, enhanced pro-inflammatory cytokines, and impaired cognition and memory. Treatment with the Nogo-66 antagonist promoted myelin repair, improved cognition and memory, and downregulated pro-inflammatory factors. Our data imply the Nogo-a/NgR1 pathway is involved in NPSLE.

P3009

Potential role of miR-7 in the negative neuroinflammation loop of GnRH and c-FOS

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The mechanisms by which neuroinflammation damaging neuronal functions is not clear. It is reported that Leukemia inhibitory factor (LIF) due to neuroinflammation induces FBJ osteosarcoma oncogene (c-FOS) and represses gonadotropin-releasing hormone (GnRH) gene. And c-FOS belongs to AP-1 transcription factor subunits regulating follicle-stimulating hormone (FSH), one of gonadotropins which can negatively regulate GnRH through hypothalamus-pituitary-gonadal axis. In our present research, we found that miRNA-7 (miR-7) is highly expressed in mice hypothalamus and pituitaries. And miR-7 expressing cells are co-localized with gonadotropes by in situ hybridization and immunohistochemistry staining. GnRH was added to mice pituitary primary cells with time gradient. Real-time PCR was used for gene detection and RIA was used for hormone detection. Results showed that GnRH inhibits miR-7 while increases expression, synthesis and secretion of gonadotropins. Further investigation proved that miR-7 mediates the regulation of GnRH on gonadotropin expression. Signal pathway inhibitors proved that in mouse pituitaries, GnRH inhibits miR-7 expression through PKC signal pathway. Dual-luciferase experiments and western blot demonstrated that miR-7 can directly target c-FOS and further inhibit gonadotropins. Therefore, we hypothesis that miR-7 may function in the negative regulation loop of c-FOS and GnRH. In the next, we will focus on the role of miR-7 in neuroinflammation in GT1-7 cells. The relationship of miR-7, c-FOS and GnRH needs more experiments to elucidate.

P3010

Translocator protein (TSPO) regulates microgila-drived ASC specks release

DOI: 10.1002/eji.201970400

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Translocator protein (TSPO) is an outer mitochondrial membrane protein. The expression level of TSPO in central nervous system is very low and it only expresses in astrocytes and microglia. However, the level of TSPO expression is significantly increased in these cells in a neuroinflammatory environment in the central nervous system. Apoptosis-associated speck-like protein containing a CARD (ASC), a key protein of inflammation, recruits caspase-1 and activates NLRP3, resulting in pro-inflammatory IL-1βrelease and activation. Here we assessed ASC specks release of microglia cells isolated from wild type (WT) mice, TSPO knockouts and TSPO transgenic mice after treatment with ATP and lipopolysaccharide (LPS). The results showed that the level of ASC specks release was significantly increased in microglia from both TSPO knockout and transgenic mice compared with those in WT mice. We also observed similar results when LPS was intraperitoneally injected in to TSPO knockout and transgenic mice. Together, both TSPO deletion and overexpression could increase ASC specks release and promote inflammatory reaction in microglia. These data provide us a better understanding the function of TSPO in neuroinflammation.

P3011

Monocarboxylate transporter 1 promotes classical microglial activation and pro-inflammatory effect via 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3

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Microglia, the resident macrophages of central nervous system, have been initially categorized into two opposite phenotypes: classical activation related to pro-inflammatory responses and alternative activation corresponding with anti-inflammatory reactions and tissue remodeling. The correlation between metabolic conversion and microglial activation has been identified, however, little is known about the mechanism of metabolic alteration regulating microglia polarization and neuroinflammation. In this study, glycolysis-related regulators, monocarboxylate transporter 1 (MCT1), MCT4 and pro-glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3), were specifically increased in LPS-stimulated primary microglia and microglia cell line BV2. Knockdown of MCT1 suppressed glycolysis rate and decreased LPS-induced expression of iNOS, interleukin-1b (IL-1β), IL-6 and phosphorylation of STAT1 in BV2 cells. Importantly, **overexpression of PFKFB3 restored the classical activation suppressed by MCT1 silence**. All above strongly suggested that MCT1/PFKFB3 might promote glycolysis and accelerate LPS-induced classical polarization of microglia. Interestingly, additional administration of moderate lactate, which may block MCT1 transport function, decreased LPS-induced classical activation and expression of PFKFB3 in BV2 cells. **Intracerebroventricular injection of lactate ameliorated LPS-induced sickness behavior and classical polarization of microglia in mice.** Together, our results demonstrate the key role of MCT1 and lactate in microglial classical activation and neuroinflammation in pathological conditions. In addition, lactate administration may be a potential therapy to suppress neuroinflammation by altering microglial polarization.

P3012

Restraint stress leads to reduced number of gdT cells in mice

DOI: 10.1002/eji.201970400

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Objective: To explore how chronic psychological stress might affect gdT cells.
Methods: Five 16-h restraint sessions were used to induce chronic psychological stress. The organ weight and cell numbers of the mouse thymus and spleen were measured. The cell numbers of mesenteric lymph nodes were also measured. Then single-cell suspensions were subjected to flow cytometry analysis of the percentages of CD3+gdTCR+ cells. Glucocorticoid receptor inhibitor and beta-adrenergic receptor inhibitor were used to explore the possible reversal. Whether glucocorticoid directly induces apoptosis of purified gdT cells *in vitro* was also analyzed.
Results: Here, we report that the reduction of gdT cells upon chronic psychological stress occurs in the thymus, spleen, and mesenteric lymph nodes, which can be reversed by blockade of glucocorticoid peripheral action. Furthermore, glucocorticoid directly induces apoptosis of purified gdT cells *in vitro,* which is associated with augmented apoptosis of gdT cells in the spleen of stressed mice.
Conclusion: Chronic psychological stress leads to apoptotic loss of gdT cells through glucocorticoid.

P3013

Alterations of iNKT cells and γδT cells in peripheral blood of Parkinson´s disease

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Objective: To investigate the alterations of iNKT cells and γδT cells in peripheral blood of Parkinson´s disease.
Methods: Peripheral blood samples from 26Parkinson's disease patients (PD group) and 24 healthy control subjects (HC group) matched on age were collected, frequencies and cell counts were analyzed by flow cytometry.
Results: The percentage and cell count of iNKT cells in PD group (0.036 (0.03)% and 275(283)/mL respectively) were both significantly less than HC group (0.131 (0.09)% and 1387 (1176) /mL respectively) (both P=0); The percentage and cell count of γδT cells in PD group (3.62 (2.01)% and 30 (26)/mL respectively) were both significantly less than HC group (7.04 (4.31)% and 63 (58) /mL respectively) (both P=0). Correlation analysis showed that the peripheral iNKT cells and γδT cell percentage and cell counts are not associated with PD severity (P>0.05).
Conclusions: Frequencies and cell counts of iNKT cells and γδT cells in peripheral blood of Parkinson´s disease were significantly reduced, which may contribute to the development of Parkinson's disease.

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DOI: 10.1002/eji.201970400

P3014

Interferon regulatory factor 5 regulates the neuro-inflammatory response by the MyD88/TRAF6 signaling pathway and affects the early outcomes of experimental ischemic stroke in mice

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Microglia-mediated inflammatory response is one of the pathophysiological mechanisms of ischemic stroke. Interferon regulatory factor 5 (IRF5) and IRF4 regulate the activation of microglia M1-type and M2-type, respectively. The effect of IRF5 intervention on the early outcome of ischemic stroke is not clear. C57BL6 mice suffered from middle cerebral artery occlusion (MCAO) for 120 minutes and reperfusion for 24 hours to simulate acute ischemic stroke. IRF5 recombinant protein (rIRF5) and IRF5 siRNA loaded with nanoliposomes were injected into the lateral ventricle to intervene in the IRF5 pathway. Neurological deficit score (NDS) and infarct volume were used to evaluate the early prognosis. Western blot and ELISA were used to detect the content of inflammatory molecules, and immunofluorescence was used to observe the changes of microglia phenotype after stroke. IRF5 recombinant protein increases the content of MyD88, TRAF6, IL-6 and TNFα; and reduces the levels of anti-inflammatory cytokines IL-4 and IL-10 in post-stroke brain tissue of mice; increases the activation of M1-type microglia; increases cerebral infarction volume; aggravate the symptoms of neurological deficits. IRF5 siRNA reduced the content of pro-inflammatory molecules; increased the content of anti-inflammatory cytokines in the post-stroke brain tissue of mice; decreased the activation of M1-type microglia, increased the activation of M2-type microglia; and improved the symptoms of neurological deficits. The results demonstrate that IRF5-mediated inflammatory response was an important component of the post-stroke inflammatory response. Intervention of IRF5 signaling pathway affects the early prognosis of ischemic stroke, which is a potential target for the treatment of ischemic stroke.

Transplantation

O241

Combined use of ruxolitinib and PD-1 inhibitor ameliorates acute graft-versus-host disease without impairing graft-versus-leukemia response

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Donor-T-cell-mediated eradication of residual tumor cells, known as graft-versus-leukemia (GVL) response, is a curative action of allogeneic hematopoietic cell transplantation (allo-HCT). Immune escape of tumor cells impairs the effectiveness of GVL. Boosting T-cell alloreactivity enhances GVL but increases acute graft-versus-host disease (aGVHD), a fatal complication of allo-HCT. In this study, we applied murine aGVHD model and challenged recipients with tumor cells to induce GVL response. Donor T cells expressed high level of programmed death-1 (PD-1), a hallmark of exhausted T cells, after long-term exposure to recipient antigens and/or tumor cells. Blockade of PD-1 with PD-1 inhibitor enhanced GVL, indicated by less tumor cells *in vivo*. However, PD-1 inhibitor failed to improve survival of recipients in GVL model which associated with aggravated aGVHD. Ruxolitinib, a *JAK1/JAK2* inhibitor, was reported to ameliorate aGVHD. We showed that ruxolitinib decreased aGVHD of allotransplant recipients but failed to improve survival in the presence of tumor cell challenge, which was in association with increased burden of tumor cells. Interestingly, combined use of ruxolitinib and

DOI: 10.1002/eji.201970400

PD-1 inhibitor suppressed aGVHD without impairing GVL, and therefore improved survival of allotransplant recipients challenged by tumor cells. PD-1 inhibitor increased levels of pro-inflammatory cytokines and blocked apoptosis of T-cell in allotransplant recipients, while ruxolitinib possessing the opposite effect. Combined use of ruxolitinib and PD-1 inhibitor not only reduced levels of pro-inflammatory cytokines but also blocked apoptosis of T-cell. Our findings indicate that pro-inflammatory cytokines is important in induce aGVHD, while blockade of T-cell apoptosis is important in enhancing GVL response.

O242

Rorc restrains the potency of ST2+ regulatory T cells in ameliorating intestinal graft-versus-host disease

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Soluble stimulation-2 (ST2) is increased during graft-versus-host disease (GVHD), while Tregs that express ST2 prevent GVHD through unknown mechanisms. Transplantation of Foxp3- T cells and Tregs that were collected and sorted from different Foxp3 reporter mice indicated that in mice that developed GVHD, ST2+ Tregs were thymus derived and predominantly localized to the intestine. ST2-/- Treg transplantation was associated with reduced total intestinal Treg frequency and activation. ST2-/- versus WT intestinal Treg transcriptomes showed decreased Treg functional markers and, reciprocally, increased Rorc expression. Rorc-/- T cells transplantation enhanced the frequency and function of intestinal ST2+ Tregs and reduced GVHD through decreased gut-infiltrating soluble ST2-producing type 1 and increased IL-4/IL-10-producing type 2 T cells. Cotransfer of ST2+ Tregs sorted from Rorc-/- mice with WT CD25-depleted T cells decreased GVHD severity and mortality, increased intestinal ST2+KLRG1+ Tregs, and decreased type 1 T cells after transplantation, indicating an intrinsic mechanism. Ex vivo IL-33-stimulated Tregs (TregIL-33) expressed higher amphiregulin and displayed better immunosuppression, and adoptive transfer prevented GVHD better than control Tregs or TregIL-33 cultured with IL-23/IL-17. Amphiregulin blockade by neutralizing antibody in vivo abolished the protective effect of TregIL-33. Our data show that inverse expression of ST2 and RORγt in intestinal Tregs determines GVHD and that TregIL-33 has potential as a cellular therapy avenue for preventing GVHD.

O243

Mesenchymal stem cells alleviate diabetic nephropathy by inhibiting CD103$^+$DCs maturation

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Background: Diabetic nephropathy (DN) caused by the consistent high levels of blood glucose often leads to end-stage renal disease (ESRD). Studies have shown that CD103$^+$ dendritic cells (DCs) display a pathogenic role in murine CKD. Mesenchymal stem cells (MSCs) were reported to be able to improve DN and affect maturation of DCs. Here we examined the effects of MSCs on DN and explored the underlying mechanism though CD103$^+$ DCs in rat DN.
Methods: Rats were randomly assigned into normal control group (NC, healthy rats); diabetic

DOI: 10.1002/eji.201970400

nephropathy group (DN+Saline, diabetic rats receiving injection of saline); BMSCs treatment group (DN+MSC, diabetic rats receiving injection of BMSCs).
Result: After six times BMSCs transplantation, urea, BUN and u-ACR were significantly lower than in rats with DN alone. DN rats showed increased renal injury. BMSCs treatment markedly suppressed pro-inflammatory cytokine and chemokine production as shown by reduced mRNA expression of interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α) and MCP-1. Masson's trichrome staining revealed that renal interstitial fibrosis was alleviated markedly in DN rats treated with BMSCs compared with untreated DN rats. Immunostaining for markers associated with fibrosis indicated that MSC-treated DN rats had less deposition of a-SMA in kidney than untreated DN rats.
Treatment with BMSCs reduced $CD11c^+$ $CD103^+$ DCs and CD8 T cells accumulation in kidney of DN rats.
Conclusion: This study demonstrated that BMSCs transplantation attenuates renal inflammation, fibrosis and the kidney injury of DN rats, and was able to inhibit $CD103^+$ DCs maturation to decline CD8 T cell responses.

O244

Chemokine receptor signaling induced CD8+ effector T cell migration into bone marrow and the GVL effect in Allo-BMT recipients

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The graft-versus-leukemia (GVL) effect, mediated by alloreactive T cells, reduces the risk of leukemia relapse after allogeneic bone marrow transplantation. However less attention has been paid to how activated T cells find leukemia. We tracked the migration of pertussis toxin (PTX) treated or untreated $CD8^+RAG1^{-/-}$OT-1 effectors by two-photon intravital microscopy and flow cytometry. PTX^+ and PTX^- OT-1 were transferred together into unmanipulated B6 or irradiated CB6F1mice transplanted with WT or IFN-$\gamma R^{-/-}$ B6 bone marrow, with or without T cells. Some F1 also received WT or IFN-$\gamma R^{-/-}$ chronic myeloid leukemia (CML). PTX treatment reduced OT-1 migration into the BM of unmanipulated B6 and similarly in F1 recipients without T cells. However, the presence of a GVHD reduced the recruitment of OT-1 into the marrow and diminished the reliance on chemokine receptor signaling. Enumeration of OT-1 by flow confirmed these results, meanwhile OT-1 migration to GVHD target organs was PTX^- sensitive. Transplantation with IFN-$\gamma R^{-/-}$ BM did not reduce OT-1 recruitment to marrow in the GVH setting. The presence of WT leukemia in F1 GVH recipients increased recruitment of PTX^- OT-1 into the BM, both relative to PTX^+ OT-1 and in total numbers compared to experiments without leukemia. However, IFN$\gamma R^{-/-}$ and WT CML similarly increased PTX^- OT-1 recruitment. Infusion of antibodies against IFNAR1 and IFNγ also did not affect OT-1 recruitment. Surprisingly, CXCR3 on OT-1 was higher in the IFN-blocking group, suggesting that IFNs may promote $CXCR3^{high}$ T egress from blood. Importantly, PTX treatment did not impair GVL in an antigen specific way.

DOI: 10.1002/eji.201970400

O245

mTOR inhibitor rapamycin induce PMN-MDSCs mobilization and function in protecting against acute graft-versus-host disease after bone marrow transplantation

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The mammalian target of rapamycin (mTOR) inhibitor rapamycin (RAPA) has been shown to be an effective immunosuppressor in the management of acute graft-versus-host disease (aGVHD) after bone marrow transplantation. Myeloid-derived suppressor cells (MDSCs) also have a protective effect in aGVHD regulation. However, the relationship between RAPA and MDSCs in aGVHD models is unclear. Meanwhile, the effect of RAPA on different subgroups of MDSCs is also less well described. In our study, we demonstrate that in vivo administration of RAPA results in the expansion and functional enhancement of polymorphonuclear MDSCs (PMN-MDSCs) in murine model of aGVHD. RAPA treatment can enhance the suppressive function of PMN-MDSCs via up-regulation of arginase1 (Arg1) and induced nitric oxide synthase (iNOS) at later time points. Moreover, RAPA can also induce a strong immunosuppressive function in PMN-MDSCs from murine bone marrow in vitro, but has a contrary effect on monocytic MDSCs (M-MDSCs). We find that RAPA-treated PMN-MDSCs can restrain the differentiation of Th1/Th2 cells and promote induction of regulatory T cells in in vitro studies.

O246

In vivo characterization of human immune response against neonatal pig islet xenografts

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Neonatal Pigs are being considered as an alternative source of islets for transplantation. However, the mechanism on how human immune cells recognize and reject pig islets is not well understood. We investigated the immune response of individuals with (T1D) or without (NT1D) type 1 diabetes to neonatal pig islets (NPI) *in vivo*.
NPI were isolated and cultured for 7 days, and 2,000 IEQ were transplanted under kidney capsule of streptozotocin-induced diabetic NOD.SCID gamma mice. Blood glucose level (BGL) was monitored weekly post-transplantation. After achieving normoglycemia, mice were reconstituted with 15 million human PBMCs from T1D or NT1D donors. Kidneys bearing islet xenografts were harvested at 1-, 2- or 3-week(s) post-reconstitution. Islet grafts were analyzed for the presence of endocrine cells and immune cells by immunohistochemistry.
All mice achieved normoglycemia and had stable response to glucose challenge at 6-27 weeks post-transplantation. Increased BGL was seen in 1 of 11 mice at 3 weeks post-reconstitution with T1D PBMCs, while 3 of 15 mice at 2 to 3 weeks post-reconstitution with NT1D PBMCs.
Immunohistochemistry revealed massive insulin-positive cells in islet grafts at 1-week post-reconstitution, which became sporadic and less at 2- and at least 3-weeks post-reconstitution. Human immune cells such as CD45, M2 macrophage, $CD4^+$ T cell, $CD8^+$ T cells were identified in grafts of reconstituted mice.
NPI rejection by human immune cells started at 2-3 weeks post-reconstitution and was related to macrophage, $CD4^+$ and $CD8^+$ T cells.

DOI: 10.1002/eji.201970400

O247

Interleukin-22 accelerates thymus regeneration *via* Stat3/Mcl-1 and decreases chronic graft-versus-host disease in mice after allotransplants

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High-dose chemotherapy and/or radiation given pretransplant severely damage thymic epithelial cells (TECs) resulting in poor immune recovery following allogeneic hematopoietic cell transplantation. IL-22 mediates recovery of TECs *via* a pro-regenerative effect but the precise mechanism is unknown. In this study, we found IL-22 improved thymus recovery in allograft recipients in association with increased number of TECs. This effect was blocked by ruxolitinib, a *JAK1*/*JAK2*-inhibitor. In the mTEC1 murine thymic epithelial cell line, IL-22 stimulated proliferation of TECs *via* Stat3-dependent signaling. This, in turn, regulated transcription of myeloid cell leukemia sequence 1 (*Mcl1*) resulting in TEC proliferation. Similar effects were seen in mice given IL-22. Defects in IL-22 resulted in delayed thymus recovery and had an impact on levels of thymus function related genes such as *Foxn1*, *Aire* and *Kgf*. Posttransplant use of IL-22 in mice improved repair of TECs, increased numbers of thymus T cells, increased intra-thymic levels of Aire and increased proportion of natural regulatory T cells resulting in decreased severity of chronic graft-versus-host disease (GVHD). Our data highlight the critical role of the IL-22/Stat3/Mcl-1 pathway in regeneration of TECs after allotransplants in mice and highlight circumstances where normalizing thymus T-cell function with IL-22 decreases GVHD.

O248

Stem cell and novel neurotrophic factors to promote functional outcomes in limb transplantation

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Introduction: Recently, transplantation of hand and face has become a new clinical specialty. The objective of this study was to determine whether adult Mesenchymal Stem Cells (MSCs), Granulocyte-Colony Stimulating Factor (G-CSF) and/or Dihexa can promote limb transplant function.
Methods: We used rat sciatic nerve transection-repair model. There were 10 experimental groups (n=6/group). Bone marrow derived syngeneic MSCs (2 million), G-CSF (50-100µg/kg), (Dihexa 2-4mg/kg) and/or Vehicle were administered locally via hydrogel at the site of nerve repair, i.v./i.p., and to gastrocnemius muscle.
Results: Total sensory function was ~1.4, 1.7, 2.7 and 2.9 at 2, 4, 8 and 16 weeks post-nerve repair, respectively, on a scale of Grade 0-3 (0=No function; 3= Normal function) in all groups combined; peroneal nerve function recovered quickly by one week (~2.0) and sural nerve function recovered slowly by 4 weeks (~1.0). Motor function at 16 weeks post-nerve repair as determined by walking foot print grades 0-4 (0=no print; 4=heel plus 4-5 toe prints) was 3.0±0.9, 3.0±0.8, and 2.0±0.6 in MSC+G-CSF, MSC+Dihexa and MSC+vehicle groups with gastrocnemius injections, respectively; however, without gastrocnemius injection it was ~1.6. G-CSF or Dihexa injections to gastrocnemius significantly ($P< 0.05$) improved motor function, mitigated muscle atrophy and reduced flexion contractures. MSCs expanded *ex vivo* were CD29+, CD90+, CD34-, CD31-, MHC Class I+, Class II- and multipotent. In a parallel study with tibial nerve repair we observed significant nerve regeneration/myelination with MSC therapy (n≥6).
Conclusion: It appears, MSC, G-CSF and Dihexa are promising candidates for adjunct therapies to promote limb transplant function.

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DOI: 10.1002/eji.201970400

LB064

Lyp expression in human renal transplant biopsy is associated with rejection episode

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Background: Tyrosine phosphatase non-receptor 22 protein (PTPN22; Lyp) regulates B and T cells in innate immune signaling. However, Lyp implication in immune-mediated diseases, such as kidney allograft rejection, has not been explored.
Objective: To evaluate the expression of Lyp in human renal transplant biopsy, focusing on its association with type rejection episode.
Methods: Renal Lyp expression level was analyzed from patients with humoral rejection (n=11), mixed rejection (n=4), stable allograft function (n=5) and three native kidney control specimens from nephrectomies meeting normal histopathology. Samples were scored according to the percentage of cells with positive Lyp expression.
Results: Lyp expression in renal proximal and distal tubule epithelial cells, from stable allograft function, was significantly higher than humoral rejection, mixed rejection and native kidney control groups ($p< 0.05$). Lyp expression was significantly augmented in inflammatory infiltrates and mesangial cells in transplant kidney tissue with mixed rejection than in patients with stable allograft function biopsy, humoral rejection and native kidney control groups ($p< 0.05$).
Conclusion: Lyp expression in epithelial tubular cells, inflammatory infiltrates and mesangial cells is associated to the pathogenesis of kidney allograft rejection episode.

LB101

Gene expression analysis of GvHD induced by glucocorticoid-resistant allogeneic T cells

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Graft-versus-host disease (GvHD) is a complication that frequently occurs after hematopoietic stem cell transplantation and contributes to the high transplant-related morbidity and mortality. Glucocorticoids (GCs) are widely used to treat GvHD, but their mechanisms of action remain partially unclear. In our study, we used an acute GvHD mouse model based on the transplantation of GC-resistant donor T cells derived from cell type-specific GC receptor (GR) knock-out mice. We found that mice transferred with GC-resistant T cells developed a more severe GvHD that those receiving wild type T cells. We analyzed the expression of 54 candidate genes in the inflamed small intestine, an organ which is strongly affected in this disease, using a high-throughput gene chip technology. The majority of the genes were significantly up-regulated in mice transplanted with GC-resistant T cells. Next, we performed RNA-sequencing to identify additional target genes linked to GC-regulation of immune and epithelial cells in the small intestine. The identified differential gene expression was confirmed again by high-throughput quantitative PCR. Our findings show an altered gene expression

DOI: 10.1002/eji.201970400

profile caused by GC resistance of transplanted allogeneic T cells, which might be helpful to use as biomarker or develop therapeutic intervention for GvHD.

P0432

Age-related characterization of human adipose tissue-derived mesenchymal stromal cells during short and long-term *in vitro* culture

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Aim: We explore whether age affect the biological characteristics of human adipose-derived mesenchymal stromal cells (ADSCs) during short and long-term *in vitro* culture.
Methods: The experiments were divided into three groups according to donors age, “infant” ADSCs group, “adult” ADSCs group, and “aged” ADSCs group. The morphologies, growth, proliferation, migration, antiapoptosis, immunophenotypes, multi-lineage differentiation, senescence, chromosome karyotype, telomerase activity, and paracrine activity of ADSCs in three age groups were compared between early and late passages.
Results: The results were showed that adipose tissue from different age donors all gave rise to ADSCs. Although ADSCs from three age groups have similar levels of antigenic phenotype and multi-lineage differentiation ability, the proliferative, migration, and antiapoptosis abilities of the infant ADSCs were better than the adult and aged ADSCs. At late passages, although karyotype alterations and telomerase activity did not observe significant abnormal with age, the infant ADSCs had significantly lower expression of p53 and p21 compared with the adult and aged ADSCs. Co-cultures of ADSCs and mitogen-activated T cells significantly suppressed T-cell proliferation, down-regulated the secretion of inflammatory cytokines, and increased the percentage of regulatory T cells, with infant ADSCs being most effective.
Conclusion: Our results demonstrate that infant ADSCs have biological advantages in comparison to adult sources. There data provide support to the concept that the biological properties of human ADSCs after *ex vivo* expansion remain suitable for use in cell therapy approaches.

P0433

Agonist c-Met monoclonal antibody augments the proliferation of hiPSC-derived hepatocyte-like cells and improves cell transplantation therapy for liver failure in mice

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Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) have been developed to address the shortage of primary human hepatocytes (PHHs) for therapeutic applications. However, the *in vivo* repopulation capacity of HLCs remains limited. This study investigated the roles of agonist antibody activating the c-Met receptor in promoting the *in vivo* proliferation and repopulation of engrafted PHHs and/or HLCs in mice with liver injuries due to different causes. An agonist c-Met receptor antibody (5D5) was used to treat PHHs and hiPSC-HLCs in both cell culture and hepatocyte-engrafted immunodeficient mice mimicking various inherited and acquired liver diseases. The promoting roles and potential influence on the hepatic phenotype of the 5D5 regimen in cell transplantation-based therapeutic applications were systematically evaluated. In hiPSC-HLC cell

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DOI: 10.1002/eji.201970400

cultures, 5D5 treatment significantly stimulated c-Met receptor downstream signalling pathways and accelerated cell proliferation in dose-dependent and reversible manners. In contrast, only slight but nonsignificant promotion was observed in 5D5-treated PHHs. *In vivo* administration of 5D5 greatly promoted the expansion of implanted hiPSC-HLCs in fumarylacetoacetate hydrolase deficient mice, resulting in significantly increased human albumin levels and high human liver chimerism. More importantly, transplantation of hiPSC-HLCs in combination with 5D5 significantly prolonged animal survival and ameliorated liver pathological changes in mice with acute and/or chronic liver injuries caused by Fas agonistic antibody treatment, carbon tetrachloride treatment and/or tyrosinemic stress. Our results demonstrated that the proliferation of hiPSC-HLCs can be enhanced by antibody-mediated modulation of c-Met signalling and facilitate hiPSC-HLC-based therapeutic applications for life-threatening liver diseases.

P0434

Urinary neutrophil gelatinase associated lipocalin (NGAL) reduced quickly in the first week kidney post-transplant

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Background: Recipient delayed graft function is one of the most common early stage complications kidney transplantation. This study aimed to evaluate the daily changes of renal function related biomarkers in the first week post-transplant.
Methods: A retrospective study was taken. A total of 72 kidney transplant recipients were included in this study. Clinical and laboratory test data were collected daily, including urinary concentrations of NGAL, serum concentrations of NGAL, creatine, urea, uric acid, β_2-microglobulin (β2-MG), cystatin and glomerular filtration rate (GFR) in the first week post-transplant.
Results: Urinary NGAL ($p = 0.000$), serum NGAL ($p = 0.000$), creatinine ($p = 0.000$), β_2-microglobulin ($p = 0.000$) and GFR ($p = 0.000$) were statistically significant in the first week post-transplant. The decrease ratio comparison of urinary NGAL, serum NGAL, creatinine and β_2-microglobulin between the first day and the second day after kidney transplantation was 45.4%, 20.7%, 38.5% and 25.8% respectively with the increase ratio of GFR 38.9%. The decrease ratio comparison of urinary NGAL, serum NGAL, creatinine and β_2-microglobulin between the first day and the seventh day after kidney transplantation was 77.6%, 59.0%, 59.3% and 64.5% respectively with the increase ratio of GFR 72.6%. In comparison with serum creatinine ($p = 0.000$), β_2-microglobulin ($p = 0.001$) and GFR ($p = 0.001$), Urinary NGAL was the fastest variations.
Conclusions: Urinary NGAL was a sensitive renal function marker. The detection combined with other markers was more helpful in the evaluation of renal function in the first week kidney post-transplant.

P0435

Are tolerogenic dendritic cells effective on inducing immune tolerance after kidney transplantation?

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Dendritic cells (DCs) are powerful antigen-presenting cells, playing immunogenic or tolerogenic roles depending on their status. For autoimmune diseases and transplantation, tolerogenic DCs (tDCs) show great clinical value and attract many researchers. Our group also focuses on study of tDCs and

DOI: 10.1002/eji.201970400

kidney transplantation for the appropriate and effective tDCs. We obtained tDCs pulsed by liver X receptor agonist-T0901317 derived from mice bone marrow. And we found that these tDCs can secret high levels of TGF-β1, IL-10, and induction of Treg in the local environment and protect skin allografts; these tDCs can migrate to liver, spleen, lymph node, peripheral blood, but not the kidney. Now some tDCs-based clinical trials have been ongoing or completed in several research centers, including an immune tolerance trial in kidney transplantation, named "The One Study", in which autogenic peripheral blood mononuclear cells were isolated and stimulated by low quantities of GM-CSF or immunosuppressive agents. Maybe different induction methods or different cell sources of tDCs could function in different ways to different organs, although the "The One Study" research report has not been published. Kidney transplantation is the optimum therapy method for the end-stage renal diseases. Kidney allografts should become the focus of immune response attacks and form specific immune microenvironment. Are tDCs effective on inducing immune tolerance in kidney transplantation recipients? What kinds of tDCs may be suitable for inhibiting kidney allograft rejection? The manuscript will analyze the probability of tDCs from the point of the regional immunity of kidney.

P0436

Detection of anti-MICB allo-antibodies in recipients with kidney and heart allografts using MICB single antigen array

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Major histocompatibility complex class I related chain B(MICB) is a type of ligand for the activating NKG2D receptor, which is expressed in natural killer (NK) cells, NK T cells, γδT cells, and αβ $CD8^+$T cells. MICB polymorphisms and the role of MICA antibodies in renal transplant rejection have been demonstrated and reported. But detection of anti-MICB antibodies in transplant recipients have not reported yet. The assay of anti-MICB antibody defection is developed in our lab using Luminex plate format. MICB antibodies were detected in 10.6% patients awaiting kidney transplantation and in 7.8% patients with heart transplantation. Most of the positive sera had antibodies against either MICB*002 and MICB*008, or MICB*004 and MICB*005. **An association between antibodies to MICA/MICB and development of Transplant Coronary Artery Disease (TCAD) was observed.** The results suggested that **MICB antibodies, as well as anti-MICA antibodies, may contribute to development of TCAD in heart transplant patients**.

P0437

Higher post-transplant CXCL13 correlates with the occurrence of infection within 1 year after kidney transplantation

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Background: Chemokine is a subtype of cytokines that involved in the process of inflammation and immune responses, and play an important role in both kidney transplantation (KT) and infection. We aimed to explore the predictive abilities of post-transplant 30 days (POD 30) CXCL9, CXCL10, CXCL13 and CCL2 levels on the occurrence of infection after KT.
Methods: Serum levels of POD 30 CXCL9, CXCL10, CXCL13 and CCL2 were measured with Bio-Plex® suspension array system in 82 recipients undergoing KT at our hospital between July 2014 to

DOI: 10.1002/eji.201970400

June 2016. Receiver-operating characteristic curves (ROC) and Cox regression model were applied to assess the predictive performances and the impact of these biomarkers on the incident of post-transplant infection. Logistic regression analysis was conducted to calculate the combined predictor values for the combination of the potential markers.
Results: POD 30 CXCL9 and CXCL13 levels were significantly higher in KTRs with infection complication compared to non-infected KTRs (P=0.021 and P=0.002), while no significant differences were observed in CCL2 and CXCL10. The areas under the curve (AUCs) for CXCL9 and CXCL13 to predict 1- year post-transplantation infection were 0.651 (95%CI, 0.520-0.782) and 0.709 (95%CI, 0.584-0.833) respectively. By combining CXCL9 and CXCL13, the AUC value was improved to 0.721 (0.591-0.852). Multivariate Cox regression analyses confirmed that CXCL13 and the combined predictor were independent predictors of post-transplant infection.
Conclusions: Higher serum POD 30 CXCL13 level was independently associated to an increased risk of infection after KT, suggesting a role for CXCL13 in the KTRs' susceptibility to post-transplant infection.

P0438

Plasmacytoid dendritic cells promote hepatocellular carcinoma recurrence after liver transplantation

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Background: Post-transplant tumor recurrence affects the long-term survival of the recipients with hepatocellular carcinoma. We reported that early phase liver graft injury mediates post-transplant tumor recurrence via CXCL10/CXCR3 signaling. Plasmacytoid dendritic cell (pDC) is a unique DC subset with an immunosuppressive property in the tumor microenvironment. In this study, we aim to investigate the role and mechanism of pDC on tumor recurrence after liver transplantation.
Methods: The correlation between pDC and tumor recurrence was investigated in patients underwent curative HCC resection. The phenotype of pDC was studied in liver transplant recipients with HCC and rat orthotopic liver transplantation models. The direct role of CXCL10/CXCR3 signaling on pDC migration was investigated in CXCL10-/- and CXCR3-/- mice. The role of pDC on tumor progression was further investigated in mouse orthotopic liver tumor model.
Results: Clinically, pDC infiltration was negatively correlated with disease-free survival in HCC patients (5.65±2.30 vs. 50.53±8.89 months, P< 0.001), more circulating pDC was detected in recipients with GWR < 60% graft at day 30 after liver transplantation. In rats OLT model, there were more pDC both in liver graft (P=0.015) and draining lymph nodes(P=0.076) of the recipient with small-for-size liver graft. Both of the intragraft infiltration of pDC and in situ migration of splenic pDC was significantly reduced after liver injury both in CXCR3-/- and CXCL10-/- mice. The depletion of pDC attenuated orthotopic liver tumor growth in mice.
Conclusion: pDC recruited through CXCL10/CXCR3 signaling at early phase liver graft injury may promote late phase tumor recurrence.

DOI: 10.1002/eji.201970400

P0439

Qa-1/PD-L1 peptides artificial liposome protects mouse islet allografts from rejection

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NKG2A and PD1 as important immune checkpoint molecules conteract fine-tune immune responses by negatively regulating T cell functions. If extracellular domain of the NKG2A/PD1 ligands Qa-1 / PD-L1 loaded artificial liposome could prolong their lifespan *in vivo* and protects islet allograft rejection remained to be investigated. In the present study, **t**he extracellular domain of Qa-1 and PD-L1 peptides was loaded on liposome surface by streptavidin-biotin system. The loading efficiency and half-life *in vivo* was measured by fluorescence or FACS. The mixed lymphocyte reaction was performed to measure Qa-1/PD-L1 liposome biological function. Then the liposome was co-transplanted with allo-islets via portal vein in T1D mice. The blood glucose and C-peptide was detected every day after transplantation. The hepatic lymphocytes after transplantation were also isolated to determine proportion of activated cells as well as signaling pathway changes. The loading efficiency with biotinylated peptide was more than 95%, and liposome diameter was between 50nm to 200nm. Qa-1/PD-L1 peptides liposome could significantly inhibit lymphocyte proliferation, activation and secretion of IFN-γin MLR by activation of SHP1/2 and then inhibition of Syk pathway. Compared to the control group, Qa-1/PD-L1 peptides liposomes protect islet allografts from rejection, maintaining normal blood glucose level of recipients more than 14 days. Therefore, Qa-1/PD-L1 peptides loaded liposome could effectively inhibit islet allografts rejection and may be developed a promising approach to improve the outcomes of islet transplantation.

P0440

The significance of chimerism monitor in lineage-specific immune cell populations after allogeneic hematopoietic stem cell transplantation

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Background: Donor chimerism (DC) in immune cell populations is a significant immunological predictor after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: We analysed 68 patients with hematologic diseases, underwent allo-HSCT between 2016 and 2018, to determine the prognostic relevance of chimerism with engraftment, relapse and virus infection after allo-HSCT.
Results: Among 55/68 cases of stable full donor chimerism (SFDC) group, 3 cases (5.54%) occurred relapse, overall survival (OS) was 80%(44/55); 4/68 cases of increased mixed chimerism (IMC) group, 3 cases (75%) occurred relapse, OS was 50%(2/4); 8/68 cases of decreased mixed chimerism (DMC) group, no patient occurred relapse, OS was 87.5%(7/8); 1/68 case of never full donor chimerism (NFDC) group, this patient died of relapse 8 months after transplantation. Relapse rate (RR) was higher in IMC and NFDC groups than SFDC and DMC groups ($P< 0.001$).
28 days after transplantation, T cell chimerism of CMV infection group and non-CMV infection group were 97.64±4.32%, 93±9.58%($P< 0.05$). T cell chimerism of EBV infection group and non-EBV infection group were 99.24±1.03%, 94.62±7.64%($P< 0.05$). Otherwise, the cumulative probability of CMV/EBV infection was higher in T cell FDC group than MC group($P< 0.05$).
Conclusions: Chimerism monitoring can provide strong evidence for estimating disease relapse post allo-HSCT. And the high chimeric rate of T cell early post-transplantation has predictive value for CMV

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DOI: 10.1002/eji.201970400

and EBV infection.
Keywords: Allogeneic hematopoietic stem cell transplantation, chimerism, engraftment, relapse, infection

P0441

Increased ratio of circulating Tfh for Tfr cells in chronic renal allograft dysfunction

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Background: T follicular helper (Tfh) cells play a control role in contribution of B cell differentiation and antibody production. T follicular regulatory (Tfr) cells inhibit Tfh-B cell interaction.
Methods: To identify whether circulating Tfh (cTfh) and Tfr (cTfr) cells contribute to chronic renal allograft dysfunction (CAD), 82 kidney transplant recipients (45 recipients with CAD, 37 recipients with stable fucntion) were enrolled. The frequency of cTfh and cTfr cells, the level of serum CXCL13 were measured.
Results: The frequency of cTfh or cTfr cells in CAD group was significantly lower than that in stable group (16.7% vs 21.2%, P=0.015, Figure 1(A); 0.31% vs 0.68%, P=0.002, Figure 1(F), respectively). The ratio of cTfh for cTfr cells in CAD group was significantly higher than that in stable group (55.4 vs 25.3, P=0.013, Figure 1(G)). Serum CXCL13 in CAD group was significantly higher than stable group (30.4 vs 21.3 ng/ml, P=0.025, Figure 1(H)). After linear regression analysis, the ratio of cTfh for cTfr cells was an independent risk factor for eGFR in recipients (Standardized coefficient=-0.420, P=0.012). After logistic regression analysis, the ratio of cTfh for cTfr cells was an independent risk factor for CAD (OR=1.043, 95%CI=1.004-1.085, P=0.031).
Conclusion: The imbalance of cTfh and cTfr cells contribute to the development of CAD.

P0442

Correlation of FK506 and bone metabolism in male kidney recipients

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Background: Bone disorder is a common clinical problem after kidney transplantation. High cumulative dosage of corticosteroids has a main role in the pathogenesis of alterations of bone metabolism after renal transplantation. However, some steroids-free studies indicate that unwanted changes of bone metabolism persist beyond the initial period after renal transplantation, indicating that other immunosuppressive agents may have an impact on bone status.
Methods: This cross sectional study included 54 male kidney allograft recipients who received a triple immunosuppressive therapy (steroids plus FK506 plus Mycophenolate Mofetil). The exclusion criteria were as follows: age< 18 years, thyroid or parathyroid disorders, diabetes mellitus, gastrointestinal disease, and other diseases that affect bone mass(Cushing's syndrome, acromegaly). To avoid the possible affect of hormones on bone metabolism in women and the well-described effects of the initial using of high-dose steroid on bone metabolism, only male patients whose time since transplantation more than 5 months were included.
FK506 blood concentration and allied bone biochemical markers were measured simultaneously, such as osteoclast-specific tartrate-resistant acid phosphatase-5b(TRAP-5b), bone-specific alkaline phosphatase(B-ALP), total calcium (Ca), inorganic phosphate (PO4), 25-hydroxyvitamin D [25(OH)D].
Results: The correlation analysis showed that FK506 concentrations were correlated with serum Ca (r=0.349, P＜0.05) and TRAP-5b (r=0.307, P＜0.05). No correlations were found in FK506 concentrations with PO4, 25(OH)D, B-ALP, PTH, CrossL (P > 0.05).

DOI: 10.1002/eji.201970400

Conclusion: FK506 may cause bone disorders involved in accelerated bone resorption. More studies should be performed to investigate the optimal targeted therapeutic concentrations and achieve optimal balance between effective concentrations and toxic concentration.

P0443

Recipient APC dependent tissue-resident memory T cells recall response mediated allograft rejection

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Tissue-resident memory T cells (T_{RM}) play an important role in local immunosurveillance to defense against pathogen infection and other diseases. Mounting reports have revealed the effectiveness of T_{RM} to suppress the development of diseases including tumor, autoimmune disease and virus infection. However, the role of T_{RM} in transplantation is poorly understood. Here we established an allo-skin transplant model in which alloantigen sensitized skin was grafted on T/B cell deficient severe combined deficiency (SCID) mice. After recovery, the SCID can reject a second challenging allo-skin directly. Notably, the skin T_{RM} can also reject a distal allogeneic transplanted heart. Further investigation revealed that both $CD4^+$ and $CD8^+$ T_{RM} cells can mediate allograft rejection and $CD4^+$ T_{RM} mediate allo-skin rejection more efficiently than $CD8^+$ T_{RM}. Furthermore, recipient derived isogeneic antigen presenting cells were indispensable for T_{RM} recall and subsequent rejection. Thus, using a novel transplantation model, we demonstrated T_{RM} can mediate allogeneic rejection which indicate the significant role of T_{RM} in this process. Furthermore, the vital functions of T_{RM} in clinical treatment need to be considered seriously.

P0444

Non-genotoxic conditioning facilitates tolerance induction through stable mixed hematopoietic chimerism of gene modified bone marrow

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Transplanting genetically-engineered haematopoietic stem cells (HSC) encoding antigen provides an efficient means to induce antigen-specific tolerance. Low-level, but stable HSC engraftment is sufficient for T-cell tolerance and prevention of autoimmune disease in animal models. Therefore, highly-toxic myeloablative and/or immunoablative pre-transfer recipient conditioning regimens that are typically used to achieve high engraftment levels for engineered HSC in many settings may not be necessary. One alternative that has been reported is the use of a CD45-targeted immunotoxin (CD45-SAP) that has been shown to deplete >90% of HSC when used at high doses in mice. However, at high doses CD45-SAP is immunodepleting. To interrogate clinical applicability that might be achieved by avoiding immunodepletion, and as low-levels of engraftment are suitable to generate T-cell tolerance, we tested the suitability of lower doses of CD45-SAP. Titrating the dose of CD45-SAP led to a dose-dependent depletion of phenotypic long-term repopulating HSC in bone marrow. Dose-dependent HSC depletion by CD45-SAP was verified by competitive bone marrow transplantation. A single low-dose administration of CD45-SAP (0.5 mg/kg) depleted ~45% of HSC and facilitated a moderate level of donor HSC engraftment (10-12%). In contrast to high dose CD45-SAP, a lower dose (0.5 mg/kg) preferentially targeted HSC and hematopoietic progenitors whereas peripheral leukocytes

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DOI: 10.1002/eji.201970400

were largely preserved (~80% retained). Using this regime, transfer of genetically-engineered bone marrow encoding a nominal antigen induced T-cell tolerance in recipients. Thus low doses of CD45-SAP could provide a useful alternative to toxic conditioning approaches for tolerance induction with gene-engineered bone marrow.

P0445

Monocyte subpopulations and serum cytokines after kidney allograft transplantation

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Mononuclear phagocytes play a key role in the induction of effector mechanisms but also in downregulation of immune responses by anti-inflammatory cytokines. The aim of our study was to characterize subpopulations of monocytes together with serum levels of selected pro-inflammatory (TNF alpha, IL-1alpha, IL-1beta and IL-18) and anti-inflammatory cytokines (IL-10, IL-1RA and IL-18BP) in kidney allograft recipients.
The study population of 129 patients after kidney allograft transplantation from deceased donors was enrolled. The expression of CD14, CD16, CD163, CD206, and CD209 was measured by flow cytometry. Serum concentrations of cytokines were evaluated by multiplex immunoassay Luminex. During the first week after the transplantation, the expression of CD163 (a marker of tissue M2 macrophages) was markedly upregulated together with a trend to downregulation of pro-inflammatory CD14+ CD16++ monocytes. When evaluating pro-inflammatory cytokines, serum levels of TNF alpha, IL-1 alpha and IL-1 beta did not change significantly after the transplantation and concentrations of IL-18 were downregulated ($p< 0.001$). Regarding anti-inflammatory cytokines, serum levels of IL-10 increased during the first week after the transplantation ($p< 0.002$) while concentrations of IL-1Ra ($p< 0.0001$) and IL-18BP ($p< 0.005$) were decreased.
Our data suggest that CD163+ blood monocytes might be involved in downregulation of effector mechanisms early after kidney transplantation together with upregulation of IL-10 production. Low serum levels of IL-1Ra and IL-18BP may reflect their higher consumption due to the local induction of IL-1 and IL-18 in transplanted kidney.
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P1024

A murine model reveals a critical role for reactive oxygen species in hyperacute graft-versus-host disease

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Hyperacute graft-versus-host disease (GVHD) is a severe and rapidly fatal disease and is clinical defined as a GVHD occurring within 14 days after allogeneic haematopoietic stem-cell transplantation (allo-HSCT). Despite numerous studies, the pathological process and the crucial factors driving this disease remain undefined. Myeloid derived suppressor cells (MDSCs) have a beneficial role in

DOI: 10.1002/eji.201970400

transplantation and can suppress alloreactive T-cell responses with the help of reactive oxygen species (ROS). In this study, we established a murine model of hyperacute GVHD, allowing us to discover that NADPH oxidase deficiency causes the mice to rapidly die of hyperacute GVHD developed before engraftment and depending only on allogeneic cell infusion. Furthermore, the donor splenic T cells are critical for the development of hyperacute GVHD, while alloreactive T cells are activated massively, proliferate and obtain strong killing ability during hyperacute GVHD. We find that NADPH oxidase-dependent ROS production by MDSCs is essential for preventing alloreactive T cells from over-activation. Moreover, treatment with ROS agonist rescues the defects of hyperacute GVHD in NADPH oxidase-deficient mice. Together, our findings suggest that NADPH deletion impairs the ability of MDSCs to produce ROS, leading to the activation and proliferation of allo-reactive T cells and subsequently hyperacute GVHD. Thus, we provide new insights into the pathogenesis of GVHD, which may improve the clinical management of this fatal complication.

P1025

Low ratio of pDC after hematopoietic stem cell transplantation can predict the occurrence of severe aGVHD

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Dendritic cells are the strongest antigen presenting cells in human body. Recent studies have shown that the normal reconstruction of dendritic cells after allogeneic hematopoietic stem cell transplantation plays an important role in the success of hematopoietic stem cell transplantation. We randomly selected patients who underwent hematopoietic stem cell transplantation (HSCT) in our center in 2018. The ratio and quantity of DC (LIN-HLADR+), pDC (CD123+) and CD1c+ DC (CD1c+) in peripheral blood were measured by flow cytometry every two weeks after engraftment of neutrophil after hematopoietic stem cell transplantation (HSCT). After 100 days of follow-up, the patients were divided into 0-1GVHD group(n=18) and II-IVGVHD group(n=27) according to the occurrence of aGVHD, we found that the reconstruction of DC, especially pDC, was slow in II-IVGVHD group, and there was significant difference when neutrophil engraftment(p=0.0008). According to the ratio of pDC to DC at the time of neutrophil engraftment, 45 patients were divided into two groups: high pDC group(n=22) and low pDC group(n=23). We found that the low pDC group was higher than the high pDC group in the incidence(61% to 19%) and mortality(18% to 9%) of aGVHD. However, there was no significant difference in leukemia relapse after transplantation.(13% to 13%) In conclusion, the ratio and quantity of pDC may be used as indicators for regular detection after hematopoietic stem cell transplantation in the future, indicating aGVHD and prognosis for clinicians.

P1026

High stearic acid diet aggravates aGVHD by akkermansia muciniphila on the balance of Th1/Treg cells

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T-cell metabolism is a promising target for controlling aGVHD. By using GC-MS technique, we previously identified that serum stearic acid/palmitic acid ratio on day 7 was an excellent biomarker to predict aGVHD after allo-HSCT, suggesting an important role of saturated fatty acid intake in the pathogenesis of aGVHD. However, the mechanism remains unknown. By using mouse aGVHD

DOI: 10.1002/eji.201970400

model, here we found that high stearic acid diet (HSAD), but not palmitic acid diet, significantly aggravated aGVHD, and its promoting role was mediated by gut microbiota. 16S rRNA sequencing identified *Akkermansia* mu*ciniphila (Akk)* was obviously increased in HSAD recipient. Add back *Akk* in WT mice ameliorated aGVHD-related mortality. Furthermore, depleting CD4 T cells significantly rescued HSAD induced aGVHD mortality, whereas depleting CD8 T cells could not. In addition, HSAD recipients of IFN-$\gamma^{-/-}$ allografts had improved survival compared with recipients of WT allografts, indicating that Th1 cells were involved in HSAD induced aGVHD. Meanwhile, we found that Treg cells were significantly decreased in aGVHD target tissues in HSAD recipients. Functional studies showed that stearic acid could promote *Akk* growth in vitro, and extracts from *Akk* promoted Th1 cells differentiation and inhibited Treg cells differentiation in vitro, suggesting that *Akk* changed the balance of Th1/Treg cells, thereby exacerbating aGVHD.

P1027

Evaluation of the effect of active screening on bloodstream infection of carbapenem-resistant Enterobacteriaceae in patients with hematopoietic stem cell transplantation

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Objective: To investigate the effect of Carbapenem-resistant Enterobacteriaceae (CRE) on the prevention and control of CRE bloodstream infection in patients with hematopoietic stem cell transplantation（HSCT）in the early stage of transplantation.
Methods: Retrospectively analyzed those patients who underwent HSCT in the First Hospital Affiliated to Zhejiang University School of Medicine during Jan 2016 to Feb 2019, a total number of 719 cases. The changes in CRE infection rate before and after CRE active screening in the early stage of transplantation, the outcome of intestinal CRE colonization and the antibiotic resistance rate of CRE were analyzed.
Results: Continuous CRE screening significantly improved screening positive rate compared with single screening. After screening measures and intervention management of colonized patients, the CRE infection rate decreased from 1.6% before screening to 1.2% after screening. The mortality rate decreased from 66.7% before screening to 50% after screening, and there was no CRE bloodstream infection during the continuous screening stage. Among the CREs detected, Klebsiella pneumoniae and Escherichia coli were the main strains, and the resistance to aminoglycosides was low, and tigecycline-resistant strains had not been detected.
Conclusion: Regular continuous CRE screening can effectively improve the CRE detection rate of hematopoietic stem cell transplantation patients, thus playing an early warning role for late CRE infection. Strengthening the management of CRE colonizers can improve the prognosis of these transplant patients.

DOI: 10.1002/eji.201970400

P1028

Induced IL-17-producing γδT cells play a protective role in intestinal graft-versus-host disease

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Acute graft-versus-host disease (aGVHD) is one of the lethal complications after allogeneic stem cell transplantation (allo-SCT). Intestine, as the major target organ of aGVHD, has a significant morbidity and mortality. T-cell-derived interleukin-17A (IL-17A) can mediate immunopathology in GVHD. According to a recent study in murine SCT models, the recipients who lack IL-17 would develop intestinal hyper-acute GVHD. Our previous study demonstrated that γδT17 cells were the main source of IL-17 in intestinal aGVHD. These findings suggest that γδT17 cells may have the capacity to attenuate intestinal aGVHD and have the potential for prognosis prediction. To explore the function of γδT17 cells in aGVHD, we improved an in vitro induction protocol to generate large numbers of purified activated γδT17 cells. Then we performed survival studies in a murine allo-SCT model. Co-infusion of γδT17 cells largely improved survival, with 40% of recipients surviving 60 days after transplant, while the aGVHD group all died. The median survival time of aGVHD group is 40.5 days, which is obviously lower than aGVHD+γδT17 group. There was also a significant reduction in the clinical score in mice co-infused γδT17 cells, with a nonsignificant decrease in the weight loss. On day 28, there was a decrease in pathology score in the colon.

P1029

A prospective study of microtransplantation in acute myeloid leukemia and myelodysplastic syndrome

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Objective: To observe the efficacy and safety of chemotherapy combined with microtransplantation in the treatment of elderly patients with AML and intermedium/high-risk group MDS.
Materials and methods: 16 patients were included from Oct. 2017 to Dec. 2018, 9 of whom were elderly AML (≥60 years) and 7 were intermedium/high-risk group MDS. Induction chemotherapy mainly consisted of decitabine and CAG, followed by an infusion of HLA-mismatched donor cell 24 hours after the completion of chemotherapy. Patients received 3 courses of microtransplantation as postremission therapy.
Results: The OR of AML patients was 85.7% and that of MDS patients was 71.4%. The median PFS of AML patients and MDS patients were 10 months and 3.7 months, respectively. And the median OS of AML patients was 12 months. After the stem cell transfusion, patients showed a T cell rebound, which had a positive linear correlation with the change of cytokines (INF-γ, IL-2 and IL-6). More than half of the stem cell infusion had fever accompanied by cytokines as INF-γ, IL-2, IL-6 and IL-10 increasing. 1 patient developed grade 3 aGVHD and died, 1 patient died of heart failure, and the rest of the patients did not have serious adverse reactions.
Conclusion: The treatment of microtransplantation in elderly AML and intermedium/high-risk MDS has high overall responses and favorable security. The presence of cytokine related fever may be associated with the increasing of T cells, which may be related to the improvement of therapeutic response rate, however needed to be further studied.

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DOI: 10.1002/eji.201970400

P1030

Loss of NLRP3 function alleviates murine hepatic graft-versus-host disease

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The aim of this study is to explore the effects and mechanisms of the absence of NLRP3 function on hepatic graft-versus-host-disease. We established an allogeneic hematopoietic cell transplantation mice model by infusing bone marrow mononuclear cells and spleno-T cells of the BALB/c mouse into either NLRP3 knockout (NLRP3$^{-/-}$) or wild-type C57BL/6 mice. Elevated inflammatory cell infiltration, liver fibrosis, and secretions of alanine amino-transferase and aspartate transaminase, together with weight loss, were observed in C57BL/6 recipients after transplantation compared with NLRP3$^{-/-}$ recipients. Likewise, proinflammatory cytokine IL-1β, a downstream effecter of NLRP3 inflammasome activation, showed significantly lower expression in the liver of NLRP3$^{-/-}$ recipients relative to C57BL/6 recipients at day 7 and day 21. Interestingly, NLRP1a was expressed at a significantly reduced level in the liver of NLRP3$^{-/-}$ recipients. Furthermore, the concentration of serum IL-1b achieved a maximum at day 14, then decreased in NLRP3$^{-/-}$ recipients, which was gradually increased from day 7 to day 28 in C57BL/6 recipients. Serum adenosine triphosphate (ATP) levels reduced from day 7 to day 28 in NLRP3$^{-/-}$ recipients, but were extremely high in C57BL/6 recipients from day 14 to day 28. The decreased levels of P2X7R were connected to less ATP in NLRP3$^{-/-}$ recipients at day 21 and day 28. In conclusion, NLRP3 knockout in recipients could significantly relieve liver injury after transplantation and block the NLRP3 inflammasome pathway, thus providing a promising strategy for the treatment of graft-versus-host disease prophylaxis.

P1031

NLRP3 knock-out in macrophages ameliorate murine gut graft-versus-host disease by reducing inflammasome-dependent cytokine IL-1β/IL-18 and chemotactic factor

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NLRP3 inflammasome, predominantly expressed in macrophages, has been shown to be important in the regulation of graft-versus-host disease (GVHD). Our previous study indicated increased macrophages infiltration in gut after hematopoietic cell transplantation (HCT). However, it is not well understood whether macrophages affect GVHD through NLRP3 inflammasome. To explore the effects and mechanisms of the absence of NLRP3 function on gut GVHD, we established an allogeneic HCT mice model with either NLRP3 knockout (NLRP3-/-) or wild-type C57BL/6 mice as recipients. NLRP3-/- recipients showed reduced gut inflammation infiltration, and decreased protein levels of IL-1β, caspase-1, CD68 and CD11b (macrophage markers). Moreover, we prepared and cultured bone marrow derived macrophages and established NLRP3 inflammasome activation in vitro model. After induced activation, NLRP3-/- macrophage exhibited decreased mRNA levels of inflammasome-dependent IL-1βand IL-18, as well as pro-inflammatory factor TNF-α and IL-6, concomitant with chemotactic factor CCL3/CCL4. In conclusion, this study identifies macrophages were involved in GVHD depending on NLRP3 inflammasome, suggesting that therapies targeting NLRP3 or macrophages might be beneficial for treating GVHD.

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DOI: 10.1002/eji.201970400

P1032

The research of regulation of Tim-3$^+$CD4$^+$ and Tim-3$^+$CD8$^+$T cells on aGVHD after related HLA-haploidentical peripheral blood hematopoietic stem cell transplantation

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Objective: The T cell immunoglobulin and mucin domain-3 protein (TIM-3) is expressed on many fully activated effector T cells, and interaction with its ligand galectin-9, leads to selective death of Tim-3$^+$ cells. A large number of papers suggested that the expression of Tim-3 in human organ transplantat rejection was significantly increased, but little is known on the role of Tim-3/Galectin-9 signaling pathway in aGVHD after related HLA-haploidentical non T cell-depleted in vitro peripheral blood hematopoietic stem cell transplantation (RH-PBSCT).
Methods: Using flow cytometry and ELISA assay, we measured the expression levels of Tim-3, Gal-9, IL-17, IL-10 and percentages of Th17, Treg and CD8+T cells in aGVHD, non-aGVHD patients and Healthy Control.
Results: In our study, the number of Tim-3+CD4+IL-17+ and Tim-3+CD8+T cells were increased, but the level of Gal-9 was very low in aGVHD patients. Th17 cells and IL-17 maintained at high levels whereas Treg cells and IL-10 decreased significantly compared to non-aGVHD patients and Healthy Control, Tim-3/Galectin-9 signaling pathway was not activated during aGVHD stage.
Conclusion: Our data suggest that Tim-3+CD4+IL-17+ and Tim-3+CD8+T cells were up-regulated especially in aGVHD after RH-PBSCT, Treg and Th17 subsets may be involved in regulating immune tolerance and tissue inflammation after RH-PBSCT. There were Treg/Th17 imbalance in aGVHD and that the Treg/Th17 imbalance may be regulated by Tim-3/Galectin-9 signaling pathway.
Key words: RH-PBSCT; acute graft-versus-host disease (aGVHD); Treg/Th17; Tim-3/Galectin-9 signaling pathway

P1033

Induction of hematopoietic cells from human embryonic stem cells using self-assembling peptide hydrogel

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Hematopoietic stem cells (HSCs) have been utilized to treat various hematological diseases. Embryonic stem cells (ESCs) are a potential source of HSCs. However, functional HSCs are difficult to obtain from ESCs using approaches being used. Here we address the effects of self-assembling peptide hydrogel on regulation the hematopoietic specification of human ESCs.
Self-assembling peptide hydrogel was composed of nanofibers, the pore diameter of which ranged from 50 to 200 nm. The hydrogel formed a three-dimensional (3D) scaffold for cell culture. Cell aggregates became progressively larger during differentiation, and many round cells appeared around the cell aggregate after differentiating 6 to 8 days. A number of CD34+ cells could be produced in 3D culture system. Compared to embryoid body (EB) culture system, ESCs in 3D culture system differentiated more potently. The expression of pluripotent genes were decreased more quickly. However, the expression of mesodermal genes and hematopoietic genes, such as GATA2 and HOXB4, were higher in 3D culture system. In addition, the expression of KDR, CD31, CD144, CD34 and CD45 were also higher in 3D culture system. The engraftment was not obtained in differentiated cells from EB culture system. However, when the differentiated cells from 3D culture system were injected into the NOG mice, myeloid cells, B cells and T cells could be detected in peripheral blood

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DOI: 10.1002/eji.201970400

three weeks after transplantation.
3D hydrogel culture system facilitates hematopoietic specification of ESCs, and the differentiated cells possess the short-term engraftment potential.

P1034

The reconstitution of alveolar macrophages is crucial for lung-tissue homeostasis after hematopoietic stem cell transplantation

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Infectious pneumonia is one of the major complications after hematopoietic stem cell transplantation (HSCT), however, why recipients of HSCT are more susceptible to pulmonary bacterial infection than healthy people and the underlying immunological mechanisms remain poorly understood. By utilizing a haplo-identical mouse model of HSCT we found that neutrophils, monocytes and interstitial macrophages in lung tissue could reconstitute rapidly while alveolar macrophages (AMs), whose reconstitution was relatively delayed after HSCT. Irradiation and donor T cell-mediated GVHD contributed to the initial elimination of host-derived AMs, while the complete reconstitution of donor-derived AMs happened 4 weeks after HSCT. We therefore evaluated the alveolar proteinosis in HSCT recipients and their ability against intranasally infected bacteria and found that recipients with weak AM reconstitution had increased protein level in alveolar lavage fluid and decreased resistance to pulmonary infection. The development of alveolar proteinosis could be partially reversed by adoptive transfer of AMs from wild type donors. Studying cytokine expression levels of lung tissue by quantitative RT-PCR, we found that GM-CSF and M-CSF expression increased after HSCT and correlated with the reconstitution of AMs. To elucidate the potential functions of these cytokines on AM development, we found that GM-CSF could effectively expand AMs and maintain their mature phenotype in vitro, while M-CSF treatment could not stimulate AM proliferation but drive the transformation of mature AMs into immature AMs.
Our study provided evidences of AM reconstitution in maintaining lung-tissue homeostasis after HSCT and the findings suggest potential intervention targets for improvement of AM reconstitution in vivo.

P1035

Adipose-derived mesenchymal stem cells alleviate aGVHD by affecting the balance of interferon-γ and interleukin-4

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Background: Acute graft-versus-host disease (aGVHD) is the most serious complication after hematopoietic stem cell transplantation, and many patients are not sensitive to drug treatment. We investigate whether adipose-derived mesenchymal stem cells (ADCSs) can alleviate aGVHD and explore its mechanisms.
Method: We established a rat model of aGVHD by infusing a mixture of allogeneic HSCs and spleen lymphocytes after receiving a total of 6Gy body irradiation. ADSCs were infused at a dose of $1\times10^{7/}$kg. Occurrence of aGVHD was characterized by manifestations such as weight loss, abnormal hunched posture, decreased motion, hair loss and skin ulcers. Livers and small intestines were obtained from sacrificed rats for pathological examinations, and the expression of serum interferon-γ interleukin-4 was detected by ELISA after transplantation.

DOI: 10.1002/eji.201970400

Result: After transplanting HSCs and spleen lymphocytes, the rats showed typical aGVHD manifestations, including hunched arched posture, hair loss and decreased movement. ADSCs significantly reduced the weight loss of the rats .Moreover, pathological changes in liver and intestinal track such as sinusoidal dilation and congestion, periportal inflammation, intestinal epithelial necrosis and submucosal congestion and edema in aGVHD rats were remarkably ameliorated after receiving ADSCs infusion. The concentration of interleukin-4 increased gradually after receiving ADSCs infusion, simultaneously ,INF-γ decreased gradually compared with the aGVHD model group ($P < 0.05$).
Conclusion: ADSCs can alleviate aGVHD, and the mechanism may be by regulating INF-γ and IL-4 levels, thereby affecting the balance of Th1 and Th2 cells.
Keywords: aGVHD, ADSCs

P1036

The pathogenic role of IL-35 in murine chronic graft-versus-host disease

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective way of treating hematopoietic malignant diseases, but chronic graft-versus-host-disease (cGVHD) remains a major barrier to successful transplant outcome. Interleukin (IL)-35 is a recently identified member of IL-12 cytokine family and has been shown to be associated with the pathogenesis of autoimmune disease such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), *etc*. Except for a few reports showing its pro-inflammatory effect, most studies have described an attractive immunosuppressive role of IL-35 in autoimmune disease, which is predominantly through its essential effect on the immune regulatory activity of regulatory T (Treg) and B (Breg) cells. We hypothesize that IL-35 may also have the potential to alleviate cGVHD. In the current study, we observed that IL-35 overexpression significantly exacerbated cGVHD in both autoantibody-mediated and sclerodermatous mouse models. Additionally, although IL-35 overexpression reduced tissue damages in some target organs in both autoantibody-mediated and scleroderma models, it also dramatically aggravated the pathology of small intestines and resulted in shortened survival of recipients. Furthermore, we found that IL-35 exacerbated cGVHD mainly through their effects on facilitating the expansion of Th17/Tc17, Th2, tumor necrosis factor (TNF)-α producing T cells and the promotion of pathogenic B-cell maturation. Collectively, our results demonstrate that IL-35 overexpression aggravates the severity of murine cGVHD, providing insights in its role of exacerbating clinical cGVHD during allo-HSCT.

P1037

Immunomodulation by HLA-G: variability of expression impacts renal transplant outcome

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HLA-G is an immunomodulatory molecule known to play crucial role in allogenic situations like pregnancy and allo-transplantation and HLA-G expression impacts transplant outcome. The study aimed to investigate the role of HLA-G in North Indian patients undergoing renal transplantation. A

DOI: 10.1002/eji.201970400

total of 39 recipient-donor pairs and 50 healthy controls were enrolled. Of the enrolled cases, 11 recipients experienced graft rejection episodes. HLA-G exon8-14bp INDEL polymorphism was evaluated by PCR-SSP and sequencing. Soluble HLA-G levels (pre-transplant, day 15, 30, 60, and 90) were measured using ELISA. The distribution of HLA-G exon8-14bp genotypes among controls was, ins/del-46%, del/del-24% and ins/ins-30%. The genotype distribution among recipients was evaluated on segregation into rejection and non-rejection groups. Higher percentage of non-rejection group carried ins/ins genotype as compared to the rejection group (32.1% vs 20% respectively). Distribution of del/del genotype was similar in both groups. The median sHLA-G level was 31.7 U/ml in healthy controls. sHLA-G levels were higher in the individuals with ins/ins genotype in comparison to del/del or ins/del genotype. The sHLA-G levels were significantly higher in transplant recipients than controls. Among recipient experiencing rejections, sHLA-G levels were lower (median-105.4 U/ml) as compared to recipients maintaining well-functioning grafts (WFG) (median-260.4 U/ml). In the WFG group, sHLA-G levels were better maintained throughout the post-transplant period. In the rejection group, the sHLA-G levels improved only after anti-rejection therapy, however it remained lower than WFG. We can speculate that HLA-G 3'UTR genotyping and serial monitoring of sHLA-G levels in serum/plasma may be useful for predicting rejection.

P1624

HMGB1 blockade differentially impacts pulmonary inflammation and defense responses in poly(I:C)/LPS-exposed heart transplant mice

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A large number of recipients are in a compromised immune defense condition because of the routine application of immunosuppressive regimens after heart transplantation. Our previous work demonstrated that blockade of high-mobility group box 1 (HMGB1) prolongs the graft survival. Whether and how HMGB1 blockade impacts respiratory responses against pathogen-like challenge in organ transplant recipients when it improves cardiac graft are not elucidated. At the present study, after abdominal heterotopic heart transplantation, the recipient mice were treated with HMGB1 mAb, and then challenged with poly(I:C) or LPS intratracheally on day 7 post transplantation. We found that the level of bronchoalveolar lavage (BAL) HMGB1 was elevated after heart transplantation, and aggravated responses to respiratory tract poly(I:C)/LPS challenge were observed. HMGB1 neutralizing mAb treatment in poly(I:C)-challenged recipient mice alleviated pulmonary histopathological changes, neutrophil infiltration and inflammatory cytokine release, but unaffected the level of IFN-g, the distribution of CD11b+CD27+/CD11b+CD27− NK cell subsets, and CD8+ T cell responses. In LPS-exposed recipient mice, HMGB1 mAb treatment ameliorated pulmonary inflammatory damage and enhanced the phagocytosis of phagocytic cells. Thus, this study may establish a basis for the application of HMGB1 blockade to improve the outcomes of heart transplant recipients because HMGB1 inhibition ameliorates pulmonary inflammation, but maintains defense-associated responses.

DOI: 10.1002/eji.201970400

P1625

Cellular rejection barriers unvascularized transplantation of embryonic rat metanephros anlages

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Low immunogenicity advantages in unvascularized embryo anlages or cells (Stem Cells, SC) transplantation have been previously reported and SC allografts even been applied in clinical trials without immunosuppression. This study aims to evaluate whether such grafts lack immunogenicity and identify whether their development is obstructed by rejection or immunosuppressive agents. Metanephros rudiments of rats were transplanted to the omenta of syn-, allo-, and xenogeneic recipients. Wherever it was possible, the processes of histopathology, electron microscopy, and biochemistry were used to assess the grafts and their development. Similarly, graft survival rate and recipient survival time were measured. Primordia of Lewis ($RT1^n$) rats were grouped (each group n>15) and allgrafted or xenografted into untreated adult Brown Norway (BN)($RT1^n$) rats or C57BL/6(H-2b) mice that failed to grow. However, in CsA-treated BN rats and syngeneic Lewis recipients or untreated xenogeneic nude Balb/c mice (athymia mice), metanephric anlages were vascularized, subsequently formed mature tubules and glomeruli, and finally developed an excretory function. After discontinuation of CsA, BN developed metanephroi were acutely rejected. Syngeneic metanephroi had significantly lower values of wet weight, higher values of graft survival rate ($P < 0.01$) than CsA-treated BN transplants and similar graft survival rate to nude mice metanephroi ($P>0.05$). The unvascularized transplantation of rat metanephros anlages may prevent humoral immune attack; however, cellular immunogenic factors still remain the major barriers in the development of such fetal structures.

This work was also financially supported by Hainan provincial Key Cooperation Program (ZDYF2017162).

P1626

Rabbit antithymocyte globulin induced complement-independent apoptosis and lymphocyte activation

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Over the past three decades, rabbit antithymocyte globulin (rATG) is one of most clinically-used induction immunosuppressive drugs in solid organ transplantation and haematology. Compared to IL-2R antibody, rATG shows stronger potential in preventing acute rejection, thereby making it becomes the first choice in induction therapy among pre-sensitized patients. After infusion, ATG induces immediate immune cell depletion, particularly T lymphocyte depletion, mainly through complement-dependent cell cytotoxicity. However, anti-complement therapy is currently being used in transplantation for several indications, especially in the prevention of antibody-mediated rejection for pre-sensitized patients. Given that complement system roles in induction therapy and immunological

rejection remain incompatible, several concerns should be addressed regarding lymphocytic clearance by rATG in patients in complement suppressive condition. Herein, we demonstrate that rATG directly induces human peripheral blood mononuclear cell apoptosis without complement existing *in vitro*. The cytotoxicity of rATG shows time and dose dependence. T cells are more sensitive to rATG induced apoptosis when compared to other lymphocyte subsets. However, pan-caspase inhibitor is incapable of reducing rATG induced lymphocyte apoptosis, suggesting the caspase-independent cell apoptosis pathway is involved. Meanwhile, the rapid cell activcation as well as large amounts of Th1/2/17 cytokine production are detected after rATG treatment. This work illustrates rATG is able to induce lymphocytic clearance through a complement-independent manner. In addition, the followed lymphocytes activation and cytokine secretion reminds a closer monitor on possible side effects for rATG usage under situation of complement suppression.

P1627

The effects of dendritic cells overexpressed IL-10 and FasL on liver transplantation tolerance in a heterotopic liver transplantation rat model

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Acute rejection is the major determinant for the long-term survival of donor liver after liver transplantation (LT). The aim of this study was to examine the therapeutic potential of IL-10-FasL-overexpressing immature dendritic cells (imDCs) to induce local immunosuppression in liver grafts. ImDCs derived from donors were transfected by lentiviral vectors expressing human (h)IL-10 and/or hFasL gene, and the expressions of surface molecules and the ability to induce T-cell proliferation were measured. ImDCs were intraperitoneally injected to recipient rats as a model of LT to examine the rejection grade (Banff rejection activity index, RAI), liver functions (ALT, AST and TBIL) and post-transplant survival. IL-10 and FasL cotransfection of imDCs induced a greater reduction of CD80, CD86 and MHC-II expressions, as well as T-cell proliferation, but increased levels of IL-10 and FasL in culture supernatants compared with monotransfected or untransfected imDCs ($P < 0.05$). The infusion of cotransfected imDCs in LT recipients reduced RAI scores, decreased plasma AST and TBIL, and prolonged survival compared with monotransfected or untransfected imDC treated liver allografts. These findings demonstrated that the transfusion of IL-10-FasL/imDCs enhanced immune tolerance and prolonged the survival of liver allografts after LT. The immunomodulatory activity of IL-10 and FasL modified imDCs might be a new therapeutic approach to preventing organ rejection in clinical transplantation.

P1628

Mesenchymal stem cells-derived exosomes ameliorate acute kidney injury (AKI)-chronic kidney disease (CKD) transition in mice

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DOI: 10.1002/eji.201970400

Background: Acute kidney injury (AKI) is a worldwide health issue associated with high morbidity and mortality, and poor repair of AKI will trigger renal fibrosis and further lead to chronic kidney disease (AKI-CKD transition) and renal failure. Mesenchymal stem cells (MSCs)-derived exosomes (MSC-Exos) has shown benefits for AKI, but their effects in AKI-CKD transition are unclear. This study aimed to evaluate the protective role and underlying mechanisms of MSC-Exos on renal function in a mouse model of AKI-CKD transition.
Methods: MSC-Exos were isolated from the culture medium of primary mouse bone marrow-derived MSCs by ultracentrifugation method. AKI mice were induced by renal ischemia-reperfusion injury (IRI). AKI mice were injected with MSC-Exos, and the changes of renal function, inflammation, apoptosis, and fibrosis were evaluated.
Results: Our results showed that MSC-Exos effectively improved renal function parameters (reduced levels of blood urea nitrogen and serum creatinine), and decreased the levels of pro-apoptotic factors (Fas and Bax), renal injury maker NGAL expression, TUNEL-positive apoptotic cells and inflammatory factors (TNF-α, ICAM-1, MCP-1) in the kidney of AKI mice at 3 days after surgery. Furthermore, MSC-Exos attenuated chronic renal fibrosis, as demonstrated by decreased renal collagen, FN deposition and α-SMA-positive fibrotic areas at 14 days after AKI.
Conclusion: This study indicated that MSC-Exos could ameliorate renal inflammation, apoptosis and fibrosis after AKI, and it is a promising cell-free therapeutic tool to prevent AKI-CKD transition.

P1629

Trefoil factor 3 relieves acute graft-versus-host disease through inhibiting mast cell tryptase

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Trefoil factor 3 (TFF3) plays a key role in colonic epithelial homeostasis. Whether TFF3 can relieve acute graft-versus-host disease (aGVHD) remains unknown. Here, we established an aGVHD model by transferring bone marrow (BM) cells and equal amounts of spleen cells from donor mice C57BL/6 (H-2b) to host mice BALB/c (H-2d). The aGVHD+TFF3 mice were aGVHD mice with prophylaxis use of TFF3. We found that the aGVHD clinical symptoms, especially intestinal aGVHD, became more severe from 10 days after transplantation. As expected, aGVHD mice had more serious symptoms compared to the mice given BM cells alone. In the aGVHD group, first dead event occurred on day 6 after transplantation, and the median survival time was 16.5 days, and all mice died within 35 days. The aGVHD+TFF3 mice had a longer survival time than aGVHD mice. Immunohistochemical staining showed that aGVHD mice had higher expression of tryptase and activity of tryptase compared with BMT mice on day 7 and day 14. The activity of tryptase in aGVHD+TFF3 mice was lower than that in aGVHD mice on day 7 and 14. Thus, our results suggested that TFF3 can delay the occurrence and reduce the severity of aGVHD, which may be related to the content and activity of tryptase.

P1630

Glycogen synthase kinase 3alpha serine 21 phosphorylation inhibits hepatocyte autophagy to facilitate liver ischemia reperfusion injury

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Glycogen synthase kinase 3 (Gsk3) is a unique protein kinase that both a and b isoforms are constitutively active and inhibited upon stimulations by N-terminal serine phosphorylation. Although

DOI: 10.1002/eji.201970400

roles of active Gsk3 in ischemia reperfusion injury (IRI) have been well appreciated, the clinical significance of its inhibitory phosphorylation has not been fully understood. In a murine liver partial warm ischemia model, we compared wild-type (WT) and phosphorylation-resistant Gsk3aS21A/bS9A single or double mutant knock-in (KI) mice to study whether and how Gsk3 inhibitory phosphorylation regulated liver IRI. Our results showed that Gsk3aS21A, but not Gsk3bS9A, mutant KI protected mice from liver IRI. Bone marrow chimera experiments showed that a mutant KI recipients of WT BMs were protected but WT recipients of a mutant KI BMs suffered more severe liver IRI, indicating that Gsk3 inhibitory phosphorylation plays distinctive roles in liver parenchymal vs. non-parenchymal cells. To study how Gsk3 inhibitory phosphorylation regulate liver parenchymal responses against IR, we focused on hepatocyte autophagy. The Gsk3 mutant KI hepatocytes had increased activation of TIP60 and AMPK, but inhibition of mammalian target of rapamycin complex 1, leading to enhanced autophagy induction and decreased cell death in response to inflammation and stress. Gsk3a mutant KI also resulted in higher levels of liver autophagy in response to IR. The inhibition of autophagy diminished hepatocyte resistance to cell death in vitro and abrogated the liver protection in the mutant KI mice in vivo. Thus, Gsk3 inhibitory phosphorylation suppresses hepatocyte autophagy to facilitate the pathogenesis of liver IRI.

P1631

The IL-17-neutrophil-CD8$^+$ T cell axis plays a critical role in airway epithelial injury after transplantation

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Airway epithelium is the primary target of trachea and lung transplant rejection, and the degree of epithelial injury is closely correlated with the development of obliterative bronchiolitis, the major obstacle limiting long-term allograft survival of lung transplantation. Th17 cytokines and signals play important roles during inflammatory diseases and transplant graft rejection, their roles in airway epithelial injury and inflammatory responses after transplantation has not been fully elucidated. In this study, we showed that elevated levels of IL-17 were associated with airway epithelial injury and the accumulation of neutrophils and CD8$^+$ T cells after orthotopic allogeneic trachea transplantations, while neutralization of IL-17 or devoid of IL-17 production by RORγt deficiency improved airway epithelial integrity of the trachea allografts. Depletion of CD8$^+$ T cells prevented airway epithelial damage, and neutralization of IL-17 or inhibition of neutrophil migration impaired the recruitment of CD8$^+$ T cells into the trachea allografts, further demonstrating that airway epithelial injury was largely due to neutrophil-mediated CD8$^+$ T cell accumulation. Together, our results reveal **the critical role of the IL-17-neutrophil-CD8$^+$ T cell axis in airway epithelial injury after transplantation,** and further highlight that **IL-17 is the key therapeutic target for airway epithelial protection and allograft survival.**

DOI: 10.1002/eji.201970400

P1632

Legumain is upregulated along with the enhanced immunogenicity of tubular epithelium during ischemic acute kidney injury

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Legumain is a well-recognized lysosomal cysteine protease and plays a crucial role in class II MHC antigen processing. Also, Legumain is abundant in renal proximal tubular epithelium (PTE) and is essential for tubular homeostasis. Here, we evaluate the role of Legumain-mediated antigen presentation during ischemic acute kidney injury (AKI) associated allograft T cell mediated rejection (TCMR).
Zero-hour biopsy samples during Oct 2018 to Jan 2019 from Union Hospital, Tongji Medical School, Huazhong University of Science and Technology were enrolled. The intensity of Legumain was measured by immunostaining as well as the score of AKI was calculated. In vitro, mPTE and T cell line (C10) coculture system was adopted to evaluate the immunogenicity of PTE and T cell activation during hypoxia. Pretreatment with chemical inhibitor of Legumain, lysosomal enzyme inhibitor as well as genetic intervention of Legumain in PTE were introduced.
The intensity of Legumain is upscaled in donor biopsy samples with AKI, which is positively correlated with AKI scores. Also, the predominant Legumain staining is in PTE. In vitro, chemically or genetically inhibition of Legumain reduces the immunogenicity of PTE presenting by diminished CD40, MHC Ⅱ and HMGB1 expression with less T cell activation supporting by subsided CD40L level and IL-2 secretion. Whereas overexpression Legumain in PTE enhances its immunogenicity with ensuing T cell activation.
In conclusion, Legumain is upregulated in AKI donor which contributes to the enhanced immunogenicity of PTE and allograft TCMR. Add Legumain inhibitor into the storage solution of allograft is beneficial for its survival.

P1633

Unrelated donor peripheral blood stem cell transplantation for patients with β-thalassaemia major based on a novel conditioning regimen

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Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only available curative treatment for patients with β-thalassemia major (β-TM). However, the problem of finding a suitable sibling donor (SD) with well-matched human leukocyte antigens (HLAs) is still a major obstacle to curing these patients. With the progress in high-resolution HLA typing technology and supportive care, outcomes after allo-HSCT from a HLA-well-matched unrelated donor (UD) now approach those of well-matched SDs. However, UD-HSCT is hampered by an increased risk of graft-versus-host disease (GVHD) and transplant-related mortality (TRM). Here we report the outcome of transplantation in β-TM patients using a novel WZ-14-TM transplant protocol, based on cyclophosphamide, intravenous busulfan, fludarabine, and antithymocyte globulin, in our center. Forty-eight patients between 2 and 11 years of age with β-TM received HLA-well-matched UD peripheral blood stem cell transplantation (PBSCT) following the WZ-14-TM protocol. All of the transplanted patients achieved donor engraftment. The incidences of grade II-IV acute and chronic GVHD were 8.3% and 8.3%, respectively. The overall survival (OS) and thalassaemia-free survival (TFS) rates were both 100%. This encouraging result

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DOI: 10.1002/eji.201970400

suggests that the WZ-14-TM protocol is a feasible and safe conditioning regime for β-TM patients undergoing UD-HSCT.

P1634

IL-27/IL27Rα promotes acute allorejection through STAT5 pathway

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Background and Aim: A recent report demonstrates that IL-27 and its receptor play an important role in inflammation and liver transplantation. Blockade of IL-27 alleviates GVHD (graft-versus-host disease) in mice. However, the specific role of IL-27 receptor (IL-27Rα) on T cells has not been well characterized. Our preliminary study found that IL-27Rα up-regulated in allogeneic $CD4^+$T cells, however, the effect of IL-27 and IL-27Rα in acute allorejection remains unclear. In this study, we investigated the expression of IL-27/IL-27Rα and its effect on allorejection with allogeneic mice model.
Methods: The syngeneic and allogeneic graft mouse model were established, recipient spleen and graft was separated to detect expression of IL-27Rα and IL-27. CD4, IFN- γ and IL-10 immunofluorescent staining for isolated graft tissues were done. Mix lymphocyte culture with stimulation of mIL-27 and phosphorylation of the STAT pathway was performed.
Results: IL-27Rα expression was apparently increased in allotransplanted skin graft and splenic $CD4^+$T cell and highest IL-27Rα and IL-27 up-regulation was found in allograft on day 10 (top point of rejection) post-transplantation. At same time point, allograft exhibited higher IFN-γ and lower IL-10 level. IL-27 could promote allogeneic spleen cell proliferation and allogeneic spleen cell phosphorylation of STAT1/STAT3/ STAT5 were all increased, especially higher for STAT5 in mix spleen cells culture treated with mIL-27.
Conclusion: IL-27 and IL-27Rα expression on allograft was up-regulated during acute rejection co-related with higher IFN-γ and lower IL-10. IL-27 may be as proinflammatory cytokine promoting allograft rejection mainly through STAT5 pathway.

P1635

NK cell deletion facilitates tumor recurrence after liver transplantation via TLR4 pathway

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Background: Impaired host immunity is one of the reason that promotes tumor recurrence after liver transplantation. We aim to investigate the role of NK cells in tumor recurrence after liver transplantation and the underlying mechanism.
Methods: To investigate the clinical relevance of circulating NK cells, 50 HCC patients who underwent liver transplantation were recruited. To explore the association between NK cells and HCC development, a rat orthotopic liver transplantation model with tumor development was established.
Results: Decreased peripheral blood NK cells and higher intragraft TLR4 mRNA levels were observed in LDLT recipients and significantly associated with higher tumor recurrence compared to DDLT after liver transplantation. These findings were further validated in a rat transplantation model. Besides, the expression of TLR4 in tumor was higher in small-for-size graft compared to whole graft.
Conclusions: TLR4 signaling upregulated during liver graft injury directly induced dysfunction of NK cells, which further promoted HCC recurrence after transplantation.

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DOI: 10.1002/eji.201970400

P1636

Combinations of superagonistic anti-CD28 and anti-GITR antibodies induced mouse cardiac allograft tolerance via Type 1 regulatory T cells induction

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Operational tolerance after transplantation avoids the adverse effects of life-long immunosuppressive therapy. Type 1 regulatory T (Tr1) cells, which represent a subset of $CD4^+Foxp3^-$ T cells able to secrete high amounts of IL-10 with potent immunosuppressive properties, are considered an emerging approach to improve transplant tolerance and enhance allogeneic graft survival. However, as with $Foxp3^+$ Treg cells, no effective pharmacological approaches could expand antigen-specific or disease-specific Tr1 cells *in vivo*. The purpose of this study was to investigate an effective Tr1 cells induction approach *in vivo* and its role in transplant tolerance. By employing a mouse model of heart heterotopic transplantation, we shown that combinations of superagonistic anti-CD28 antibody (Clone: D665) and anti-GITR antibody (Clone: G3c) treatments resulted in permanent allograft acceptance in fully MHC mismatched mice combinations (Donor: BALB/c, $H\text{-}2K^d$; Recipient: C57BL/6, $H\text{-}2K^b$). Large amounts of IL-10/IFNγ co-producing $CD4^+Foxp3^-$ Tr1 cells were generated both in graft and spleen after D665 and G3c treatments. $CD8^+$ effector T cells were significantly suppressed. Superagonistic anti-CD28 antibody potently expanded Treg cells over Teff cells. Anti-GITR antibody played an important role in the induction of the Tr1 cell on the basis of superagonistic anti-CD28 antibody treatment. Although $Foxp3^+$ Treg cells were also induced, the allograft tolerance was in an IL-10 dependent but Treg independent manner. We conclude that combinations of superagonistic anti-CD28 and anti-GITR antibodies mediated allograft tolerance via *in vivo* Tr1 cell induction in an IL-10 dependent manner. *In vivo* Tr1 cells induction opens a new avenue to therapeutic manipulation of transplant tolerance.

P1637

Interleukin-21, 23 and 27 gene expression and protein level in patients with liver transplant rejection

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Cytokines have prominent roles in activating of different T cells and shifting the immune response, in this study the role of three cytokines (IL-21, IL-23 and IL-27) is investigated in the liver transplant rejection. Three EDTA-treated blood samples were collected from each liver transplanted patient in 1st, 4th and 7th day of post-transplantation. The expression level of the mentioned cytokines was determined using real-time PCR for all samples. Also, the serum levels of cytokines were determined using ELISA tests. In acute rejection (AR) group (51 patients), mRNA expression pattern of IL-21and IL-23 showed a steady increase, but this pattern was converse for IL-27. Our results in non-acute rejection (non-AR) group (54 patients) showed an elevation in day 4 and then a decrease in day 7 for IL-21 and IL-23 genes. This pattern was converse again for IL-27 gene. In comparison between the two groups, in all 3 sampling times the mean of mRNA expression level of IL-21 and IL-23, showed an increase in AR group which this increase was significant for IL-21 in the 3rd ($p=0.007$) and for IL-23 in 2nd ($p=0.048$) and 3rd ($p=0.049$) sampling time, but the pattern of mRNA expression for IL-27 was contrary to the results of IL-21 and IL-23. Furthermore, ELISA technique also, showed the serum level changes the same as cytokines. In this study IL-21 and IL-23 showed pro-inflammatory properties in

DOI: 10.1002/eji.201970400

the liver transplant rejected patients. Also, IL-27 having different expression pattern, showed anti-inflammatory behavior which needs more considerations in future.

P2216

Pre-engraftment syndrome is mediated by inflammatory monocytes

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Unrelated cord blood transplantation (UCBT) is an effective treatment for hematopoietic diseases, especially advanced or refractory leukemia. However, this compelling approach is frequently accompanied by pre-engraftment syndrome (PES), which is characterized by high-grade fever, skin rash and diarrhea. Severe cases of PES can be life-threatening, and may even lead to death. To date, there is no effective therapy for the prevention of PES, and the only available treatment is corticosteroids. Preventive treatments and improved therapeutic require a better understanding of PES physiopathology, which has so far remained unclear. In this study, we show that GM-CSF produced by cord blood-derived inflammatory monocytes drives PES pathology, monocytes are the main source of IL-6 during PES. Accordingly, blocking IL-6 receptor with tocilizumab is efficient to abate the syndrome. These findings offer a new therapeutic strategy to tackle PES and improve overall survival.

P2217

Relationship between NLRP6 and liver injury after allogeneic hematopoietic stem cell transplantation

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Aims: To investigate the expression and function of NLRP6 in injured liver tissue after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: C57BL/6 and NLRP6$^{-/-}$ mice received total body irradiation followed by infusion of BABL/c-derived bone marrow cells. Mice was sacrificed on days 7, 14, 21, and 28after allo-HSCT to analyze the changes of liver function indexes, liver histopathological changes by H&E staining, the expressions of NLRP6,phosphorylated p38-MAPK, phosphorylation IκBα, Pro caspase-1, p20 and NLRP3 by Western Blot as well as inflammatory cytokines by ELISA.
Results: The protein expression of NLRP6 in liver tissue of WT mice increased from day 7 after transplantation, peaked on day 14, and then gradually decreased. Compared with WT mice, higher AST, ALT and TBIL, more severe liver cell edema, inflammatory cells infiltration, thrombosis and more fibrin deposition were showed in the liver tissue of NLRP6$^{-/-}$ mice after transplantation. More MPO, TGF- β, phosphorylated p38-MAPK, phosphorylated IκBα, pro caspase-1, p20,inflammatory cytokinesIL-6, TNF-α, IL-1 and IL-18 were expressed in liver of NLRP6$^{-/-}$mice than WT mice on day 14. To explore the reason why Pro caspase-1 p20 increased in NLRP6$^{-/-}$ mice, we also tested the expression of NLRP3 and found that the expression of NLRP3 was higher in NLRP6$^{-/-}$ mice than in WT mice on day 14 after transplantation.

DOI: 10.1002/eji.201970400

Conclusion: NLRP6 expression in liver tissue can relieve liver damage after allo-HSCT through inhibiting inflammatory cell signaling pathways p38-MAPK and NF-κB.

P2218

The distinguishing features of HMGB1 blockade and tacrolimus treatment in infection-like transplant recipient mice

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Immunosuppressive drugs, such as tacrolimus, are routinely used to treat recipients with heart transplantation. However, a significant number of recipients suffered from the respiratory tract infection. Our previous work has demonstrated that HMGB1 blockade obviously prolongs the graft survival. This study will compare the effect of HMGB1 blockade and tacrolimus treatment in infection-like transplant recipient mice, recipient mice challenged with poly(I:C) or LPS intratracheally were used to mimic the immunosuppressive medicine treated recipients suffered from respiratory tract infection. It indicated that treatment with HMGB1 neutralizing monoclonal antibody (mAb) in poly(I:C)-exposed recipients alleviated pulmonary inflammatory injury, but unaffected host defense against virus-like infection. In LPS-exposed recipients, HMGB1 mAb treatment ameliorated pulmonary inflammatory damage and enhanced the phagocytosis of phagocytic cells. Comparing with tacrolimus, which alleviated allograft rejection and infection-like immune response completely, HMGB1 mAb treatment inhibited the infection-associated pulmonary inflammatory injury, but was harmless to the recipients defense against pathogen-like stimulation. HMGB1 blockade controls allograft rejection and pulmonary infection-like inflammatory injury simultaneously, so this study establish a basis for the application of HMGB1 blockade to improve the outcomes of heart transplant recipients.

P2219

Mesenchymal stem cells (MSCs) mediates acute tubular injury through effects on mtDNA transcription and mitochondrial biogenesis

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Background: Acute kidney injury (AKI) is a serious worldwide health issue with high mortality and morbidity. Kidney is a high energy-demanding organ that requires well-functioning mitochondria. MSCs have shown multiple benefits to AKI, but their specific role on renal mitochondria is not clear. In this study, we evaluated the effects of MSCs on renal function and mitochondrial injury in AKI mice.
Methods: MSCs isolated from C57/BL6 mice were infused into ischemic-reperfusion (I/R)-induced AKI mice via tail vein injection. Mice were sacrificed at 5 days after surgery, and blood/kidney samples were collected for clinical biochemistry, real-time PCR, TEM and histological examination.
Results: MSCs significantly improved renal function with decreased levels of serum BUN/CREA and renal necrotic tubules, and reduced KIM-1 (renal injury marker) and inflammatory cytokines (IL-1β/ICAM-1) in AKI mice. Moreover, BMSCs significantly rescued renal mitochondrial lesions with reduced mtROS, increased levels of ATP, mtDNA copy number, TFAM (master mediator of mtDNA

DOI: 10.1002/eji.201970400

transcription), mitochondrial biogenesis related genes (PGC1-α, ATP5a-1, NDUFS8), and elevated mitochondrial cristae density and length/width ratio in AKI mice.
Conclusion: This study indicated that MSCs therapy could ameliorate renal dysfunction after AKI, and their renal protective effects were through enhancing mtDNA transcription and mitochondrial biogenesis.

This work was supported by grants from National Natural Science Foundation of China (31200754, 81571808, 31871001).

P2220

Extracellular high-mobility group box 1 exacerbates autoimmune progression and recurrence of type 1 diabetes by impairing regulatory T cell stability

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Previous studies indicate that the high-mobility group box 1 (HMGB1) contributes to the induction of autoimmune diabetes, but the mechanisms involved remain to be fully characterized. In the current study, we used NOD mice as a model to dissect the impact of blocking HMGB1 on the prevention and treatment of type 1 diabetes. Neutralizing HMGB1 both delayed diabetes onset and, of particular relevance, reverted diabetes in 8 out of 12 treated NOD mice. Furthermore, blocking HMGB1 prolonged function and survival of islet isografts into diabetic NOD mice. Using a transgenic reporter mouse that carries a Foxp3 lineage reporter construct, we found that administration of HMGB1 impairs Treg stability and function. Mechanistic studies revealed that HMGB1 activates RAGE and TLR4 to enhance phosphatidylinositol 3-kinase (PI3K)-Akt-mechanistic target of rapamycin (mTOR) signaling, thereby impairing Treg stability and functionality. The present data indicate that HMGB1 is an interesting therapeutic target to preserve/stimulate Tregs and thus prevent β cell destruction in type 1 diabetes.

P2221

NKT cell predicts the severity of acute graft-versus-host disease in patients after allogeneic stem cell transplantation using antithymocyte globulin(ATG) in pretreatment scheme

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Graft-versus-host disease (GVHD) is one of the most complex complications after allogeneic stem cell transplantation. Current standard of grading system is based on clinical symptoms in skin, liver and intestinal. However, sometimes its not easy to identify GVHD through clinical manifestations. Here our retrospectively analyzed cell immune function in 51 patients who implemented allogeneic stem cell transplantation in Ningbo first Hospital from Jan 2013 to Jan 2018, studied lymphocyte subsets at the time of GVHD after stem cell transplantation. The median NKT cell percentage in severe GVHD (Grade III-IV) was 39.31%, in mild GVHD (Grade I - II) was 16.98%, while in No GVHD group was 21.15%, statistical analysis showed difference among each grade. Further analysis were implemented in Antithymocyte globulin (ATG) used group and ATG unused group, the result showed NKT Cell percentage were sharply different in ATG used group: in severe GVHD is 47.34%, in mild GVHD group was11.98%, while in no GVHD group is 18.3%. However, in ATG unused group, data indicated

DOI: 10.1002/eji.201970400

the median NKT cell percentage in severe GVHD is 23.27%, in mild GVHD group was 23.22%, while in no GVHD group is 21.13%. **Conclusion: ATG can prevent GVHD by increasing NKT cell percentage. NKT cell seemed to be a useful probe to indicate the severity of GVHD in allogeneic stem cell transplantation patients using ATG in pretreatment. By monitoring NKT cells change after allogeneic stem cell transplantation can indicate severe GVHD in ATG used group.**

P2222

Study on the efficacy of pro-lymphangiogenic therapy in the treatment of acute rejection after lung transplantation

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Background: Lung transplantation is the only viable option for patients with end-stage pulmonary diseases. Acute lung rejection occurs in transplant recipients despite aggressive immunosuppression. Therefore, exploration of novel therapeutic options is imperative. Anastomosis of severed donor lymphatic vessels to those of the recipient is not performed in transplant surgery, and the exact roles of lymphatic vessels in lung allograft survival remain enigmatic.
Method: Orthotopic mouse left lung transplantations were performed in syngeneic （C57BL/6 donor to C57BL/6 recipient） and allogeneic （BALB/c donor to C57BL/6 recipient） strain combinations. Mice receiving allogeneic lung grafts were treated with PBS or VEGF-C156S through intravenous injection from day 20 after transplantation until sacrifice on day 30. VEGF-C156S, a VEGF-C mutant with exclusive binding affinity for VEGFR-3, induces lymphangiogenesis but not angiogenesis. Lung grafts were functionally and histologically evaluated. Presence of pulmonary lymphatic vessels and their draining function were examined.
Results: Compared with control lungs and isografts, allograft lungs displayed histopathological features of severe acute rejection and a marked decline in LYVE-1-positive lymphatic vessels. VEGF-C156S resulted in the induction of lymphangiogenesis and recovery of lymphatic function in allograft lungs. The treatment also led to amelioration of graft rejection, indicated by a significant reduction in the acute cellular rejection score as well as improvement in radiographic and pulmonary function parameters.
Conclusion: Acute lung rejection leads to loss of pulmonary lymphatic vessels. Induction of therapeutic lymphangiogenesis mitigates established allograft rejection. These findings underscore the importance of lymphatic vessels in lung transplantation.

P2223

Construction of a gene set of hematopoiesis and immunologic process in xenotransplantation and the cross-species compatibility analysis between human and the Inbred Wuzhishan Pig

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Xenotransplantation of porcine donor organs has been considered as a potential alternative to ease the shortage of donor organs. With the development of genetic engineering and cloning technologies, the modification of the chimeric environment has been a breakthrough to make an organ a consistent life-supporting survival possible. However, only a few kinds of organ xenotransplantation have

DOI: 10.1002/eji.201970400

progressed to clinical trials. For most kinds of organs, the proper gene modification strategies to improve the effects of xenotransplantation are still in exploration and remain unsystematical. The survival and functional maintenance of organs are mainly dependent on the heterogeneous compatibilities of important cellular functional molecules including extracellular matrices, signaling molecules, etc. in heterogeneous chimeric environments. To focus on the genes which may play critical roles in xenotransplantation, we constructed a gene set of hematopoiesis and immunologic process named XenoTransplantome. To screen for genes which are necessary to be modified in WZSP (the Inbred Wuzhishan Pig), the widely-used miniature pig in the field of xenotransplantation, we annotated XenoTransplantome genes of WZSP and analyzed the cross-species compatibilities of XenoTransplantome between human and WZSP. We found key incompatible processes between human and WZSP are mainly in hematopoiesis, blood coagulation and cell adhesion. In each incompatible process, key genes or gene interacting pairs showed obviously low similarities as well as different domain distributions between human and WZSP. Our work systematically revealed the key incompatible molecules between human and WZSP during xenotransplantation, indicating which genes to be modified in the next step.

P2224

Ex vivo fucosylation enhances NK cell function in graft-versus-leukemia

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Recent studies demonstrated that ex vivo fucosylation can increase the persistence and anti-GVHD potency of Treg cells. Fucosylation also improves human cord blood engraftment. However, the role of fucosylation in graft-versus-leukemia remains unknown.
Here, we established a murine GVL model by intravenously injecting luciferase (lus+)/yfp-expressing B-cell lymphoma (A20) cells and founded that adoptive transfer of fucosylated NK cells markedly prolonged the survival than control NK cells. Ex vivo fucosylation significantly upregulated IFN-γ production in NK cells and enhanced their cytotoxic activity. Flow cytometry analysis revealed that fucosylation promoted the infiltration of NK cells in spleen, which may be due to the elevated binding to CD62E. Further studies are needed to confirm the contribution of IFN-γ by using IFN-γ KO mice and investigate the detailed mechanisms of fucosylation in NK cells mediated GVL effect.

P2225

IL-27Rα may be as a new marker for in vivo allorejection monitoring

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Purpose: Predictive diagnosis of allograft rejection is urgently needed for monitoring therapeutic response and prognosis. However, high selected targeting marker on allograft was limited in clinic until now. In our primary research, Interleukin-27 Receptor α (IL-27Rα) was found up-regulated in allogeneic $CD4^+$T cell. Here, we aimed to evaluate the possibility of IL-27Rα as a non-invasive allorejection monitoring target in vivo.
Methods: The syngeneic and allogeneic transplantation mice models were established and graft were isolated to detect IL-27Rα expression. Anti-IL-27Rα Ab, labeled with radioisotope iodine 125, as a tracer, was injected into mice model through tail vein, biodistribution ex vivo and Autoradio-Phosphor Screen scanning was performed.
Results: IL-27Rα was expressed both on recipient's allograft, the highest expression was detected in

DOI: 10.1002/eji.201970400

allograft on the top rejection period (day 10 post transplantation). Autoradio-Phosphor Screen scanning showed that radioactivity was remarkably increased in allograft compared with syngraft at 24h, 48h and 72h post injection. Allograft image was obtained early at 24h, high-contrast allograft visualization was obtained at 48h. Biodistribution study showed that ^{125}I-anti-IL-27Rα uptake of allograft was higher than syngraft at 48h ($p< 0.05$). T/NT (graft/ Opposite side skin) ratio of allograft was higher than syngraft (6.178±0.7267 vs 3.580±0.5194, $p< 0.01$). The ^{125}I-anti-IL-27Rα uptake exhibited high positive correlation with IL-27Rα expression within graft tissue ($r>0.92$, $p< 0.05$).
Conclusion: IL-27Rα is higher expressed on rejected allograft. It may be as a target for non-invasively visualize and quantify allograft rejection.

P2226

Association of TLRs and Dectin-1 Polymorphism with viral and fungal infections in liver transplant patients

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Innate immunity play a crucial role in Liver transplantation (LT) by activating Toll-like receptors (TLRs) and non-Toll-like Dectin-1 receptors, which are widely expressed on neutrophil, macrophages or dendritic cells. In present case control study, LT recipients (n=30), their respective donors (n=30) and healthy volunteers (n=60) were incorporated to analyze the SNPs in TLR2 (A2258G) and TLR4 (A896G and C1196T) and Dectin-1 (714T>G) genes *via* DNA sequencing. Cytokine levels (IL-1β, IL-6, TNF-α, IFN-ϒ, IL-17A and IL-10) were analyzed in Pam3Cys/LPS/β-glucan -stimulated polymorphonuclear cells to quantify the functional alteration in TLR2, TLR4 and Dectin-1 receptors, respectively. Furthermore, surface expressions of receptors were also evaluated using flow cytometry and correlated with SNPs and fungal/viral infections including Candida colonization, Invasive Aspergillosis (IA) and cytomegalovirus (CMV).
The polymorphisms of TLR2, TLR4 and Dectin-1 in LT recipients (overall; 13% heterozygous & 5% homozygous) and donor (overall; 4% heterozygous & 3% homozygous) have been reported for the first time in Pakistani population. In LT recipients, heterozygous Dectin-1 714T>G phenotype augmented the aspergillosis and candidiasis infection during post-transplant period. Whereas, homozygous TLR2 (A2258G) speculates the CMV infection risk, while heterozygosity for TLR4 (A896G and C1196T) were found more frequently in IA and CMV cases. SNPs in TLRs and Dectin-1 were significantly attributed with decline in both cytokines (55-70%) and receptors expressions (30-50%).
The present findings urge the critical role of TLRs and Dectin-1 SNPs and their corresponding protein expressions in organ transplantation that might be accountable for the deleterious decline in immune response and to susceptibility of infections.

P2227

Tale of HCV in renal transplant recipients; infection and rejection episodes in HCV positive compared to non-HCV

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DOI: 10.1002/eji.201970400

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Introduction: Hepatitis C virus (HCV) infection impairs both innate and adaptive immune systems and is associated with other infections and autoimmunity. There prevalence of HCV infection is very high in patients with end-stage-renal-disease, who underwent renal transplantation.
Objective: We analyzed the frequencies of various infections and rejections in anti-HCV positive renal transplant recipients (RTX) and compared them with anti-HCV negative RTX.
Materials and methods: A retrospective analysis (from December 2008 to January 2009) for infections and rejections episodes was done for 103 RTX. Of these 58 were anti-HCV positive. Follow-up period was six months to one year. Data was analyzed using SPSS software version 16.
Results: Mean age of the patients was 31.4 ±12.3 years with 72 males. There were more number of infection episodes in anti-HCV positive compared to anti-HCV negative RTX (3.1 ± 2.4 vs. 2 ± 1.9; P = 0.03). Rejection episodes were seen in 11 (19%) anti-HCV positive and 4 (8.9%) anti-HCV negative RTX ($P > 0.05$).
Eight patients had developed chronic allograft nephropathy (CAN) with seven had more than one infection episode and 6/7 were positive for anti-HCV antibodies. Rejection episodes were not seen in these eight patients.
Conclusions: HCV infection is associated with a higher number of infection episodes leading to rejections and CAN. However there is a need to analyze a large cohort of patients with long-term post-transplant follow-up to determine the effect of HCV infections on graft outcome. Nevertheless anti-HCV positive RTX require vigilant surveillance and prompt treatment of infections.

P2228

Non-inherited maternal antigen-related tolerance in cord blood: therapeutic potential in hematopoietic stem cell transplantation

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Allogeneic haematopoietic stem cell transplantation (HSCT) is considered as the ultimate therapeutic modality for many haematologic malignancies and immunodeficiency disorders in paediatric patients. However, better outcome in HSCT requires optimal matching between donors and recipients at HLA loci. In comparison with unrelated volunteer bone marrow (BM) donors, cord blood (CB) is an important source for HSCT, with less restriction on HLA matching and ease of availability, contributing approximately 22% of all unrelated transplantations worldwide. Notably, non-inherited maternal antigens (NIMA), the HLA haplotypes from the mother not inherited by the offspring, played a critical role in the alloimmune tolerance during HSCT. In the present study, we demonstrated that NIMA may promote the function of regulatory T cells (Tregs) in controlling effector T cell responses. In co-cultures, mononuclear cells (MNCs) from CB suppressed effector T cell proliferation with significantly increased IL-10 and TGF-beta, decreased IFN-gamma in response to stimulus of maternal PBMNC, when compared with paternal or control PBMNC. However, the immunosuppressive effect was abolished upon removal of CD25+Treg cells. Since NIMA-containing virtual match has shown significantly reduced transplant-related mortality in studies, we further performed retrospective studies (n=520) of cohort in Hong Kong, with 120 pediatric and 400 adult patients. Using maternal HLA genotypes of NIMA CB file from Hong Kong Cord Blood Bank (n=3892), we have found that NIMA-containing CB units markedly increased the virtual 6/6 matches by 33.4% and 25.8% for adult and pediatric patients respectively, strongly suggesting the clinical extension of CB in HSCT donor selection.

DOI: 10.1002/eji.201970400

P2229

Interplay between cytomegalovirus infection and graft-versus-leukaemia response

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Introduction: Human cytomegalovirus (HCMV) is an important pathogen after allogeneic bone marrow transplantation (allo-BMT). Post-transplant HCMV reactivation can lead to target organ damage and increased transplant-related mortality. However, HCMV reactivation has also been associated with a significantly reduced risk of leukaemia relapse after allo-BMT. The aim of this study is to investigate the effect that MCMV has on graft-versus-leukaemia (GvL) response and to identify the mechanism determining this effect.
Materials and methods: Mice were infected with murine cytomegalovirus (MCMV) and following viraemia clearance, used as either donors or recipients in allo-BMT. Lethally irradiated recipient mice were transplanted with donor BM and T cells, and challenged with GFP^+ primary murine myeloid leukaemia cells (derived by insertion of MLL-AF9 oncogene) on the day of transplant. Recipients were monitored for graft-versus-host diseases (GvHD), leukaemia burden and MCMV reactivation by clinical scoring, flow cytometry and qPCR, respectively. Two models of allo-BMT were used: MHC-mismatched transplant using Balb/c (H-$2D^d$) donors and C57Bl/6 (H-$2D^b$) recipients and MHC-haploidentical transplant with C57Bl/6 (H-$2D^b$) donors and B6D2F1 (H-$2D^{bd}$) recipients.
Results and Conclusions: Leukaemia survival was significantly improved in recipients of MCMV-seropositive donors versus MCMV-seronegative donors in MHC-mismatched model (median survival: 41 vs 31 days, $p < 0.05$) but not in MHC-haploidentical allo-BMT. We demonstrated that the stronger GvL effect was independent of NK cells but was associated with distinct transcriptomic profiles in CD4 and CD8 T cells post-transplant, the validation of which is ongoing.

LB121

Preformed natural/non-HLA antibodies are associated with early rejection in kidney transplant

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Introduction: In the absence of anti-HLA donor specific IgG antibodies (DSA), pathogenic natural/non-HLA antibodies are increasingly being recognized as one of the major cause of early graft rejection. We have analyzed the pre and post-transplant antibody profile in serum of renal transplant recipients negative for anti-HLA DSA but had early antibody mediated rejection (AMR).
Study Group: ABO matched renal transplant recipients with early AMR and no pre-transplant HLA DSA (n=21) and those with pre-transplant DSA (n=18). Recipients with DSA were subjected to plasamapheresis and rituximab therapy and had no DSA prior to transplant.
Results: Patients who had biopsy proven AMR with no evidence of anti-HLA DSA but had IgG antibodies to endothelial antigens (MICA) assessed on solid-phase multiplex platform. 30% of the recipients with early AMR showed the presence of preformed anti-MICA antibodies (12/39) out of which one recipient had a hyperacute rejection and four cases that had accelerated acute rejection (< 10 days post-transplant).

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DOI: 10.1002/eji.201970400

Conclusion: Absence of pre-transplant DSA, even subsequent to rituximab therapy does not imply clearance of natural / non-HLA antibodies in the recipient. These antibodies may still persist and have the potential to cause early graft rejection in transplant recipients and may also be responsible for poor graft prognosis at later stages. Detection methods for some of these antibodies like MICA exist and further assays need to be developed to effectively screen for such antibodies.

P3015

Huc-MSCs protect porcine islets from hypoxia-induced cell death via alleviating oxidative stress and enhancing the ERK pathway

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Islet transplantation is a promising treatment for selected patients with type 1 diabetes mellitus (T1DM). Immue-related mechanisms are considered as one of the central players in the process of islet graft death. Oxidative stress is involved in immue-responses and can affect the the outcome of graft function. Mesenchymal stem cells (MSCs) have been shown to enhance cell survival mainly through paracrine secretion. However, mechanisms of action underlying the protective effects of MSCs on islets have not been fully elucidated. In this study, we investigated whether human umbilical cord-derived MSCs (huc-MSCs) could inhibit hypoxia and ROS-related cell death of neonatal porcine islet cell clusters (NICCs) and further determined the underlying molecular mechanisms. NICCs were cultured in vitro under normoxic and hypoxic (1% O_2) conditions with or without MSC-conditioned medium (MSC-CM). Apoptosis of NICCs was evaluated by the AO/EB staining and Annexin V/PI flow cytometry analysis. Total and mitochondrial ROS production was detected by fluorometric assays. Western blot and the ERK pathway inhibitor, PD98059, were used to assess the possible pathways involved. The results showed that MSC-CM suppressed hypoxia-induced oxidative stress and cell death of NICCs. MSC-CM also activated several pro-survival pathways in NICCs under hypoxic conditions. Furthermore, MSC-secreted exosomes and IL-6 partially recapitulated the multifunctional benefits of MSC-CM. This study showed that huc-MSCs protected NICCs from hypoxia-induced cell death by regulating the cell redox state and cell signaling pathways. This increased understanding may enable MSCs to become a more promising adjuvant cell therapy for islet transplantation.

P3016

Dual roles of IL-22 at ischemia-reperfusion injury and acute rejection stages of rat allograft liver transplantation

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Interleukin-22 (IL-22) is a recently identified regulator of inflammation, but little is known about its role in liver transplantation. Therefore, in this study, we explored the roles and the underlying mechanisms of IL-22 in acute allograft rejection by using a rat allogeneic liver transplantation model. Results showed that allograft liver transplantation led to damage of the parent liver and to significantly increased IL-22 expression in the allograft liver and plasma of the recipient rats compared with the rats who received isografts. Moreover, the significantly increased IL-22 expression was accompanied by markedly increased level of phospho-STAT3 in the allogeneic liver tissues after transplantation. Of note, neutralization of the IL-22 protein in recipient rats significantly worsened the function of the allograft liver at 1 day post-transplantation (ischemia-reperfusion injury, IRI) but improved the function at 7 days post-transplantation (acute rejection, AR). At IRI stage, IL-22 protected liver function through

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DOI: 10.1002/eji.201970400

the increase of anti-apoptosis and pro-regeneration cytokines. However, IL-22 led to the increase of pro-inflammation factors at AR stage, accompanied by the marked increase of the Th17 and the marked decrease of Treg cells in allograft recipient rats through modulating the expression of chemokines for different cell types, which however were reversed by in vivo IL-22 neutralization. Results indicate the dual roles of IL-22 and suggest the differential potential clinical application of IL-22 at different stage of allograft liver transplantation.

P3017

Immune reconstitution by syngeneic hematopoietic stem cell transplantation in sensitized recipients to prevent antibody-mediated rejection

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Antibody mediated rejection of sensitized renal transplant recipients is difficulty in transplantation field because of the lack of effective prevent and treatment method. Successful renal transplantation in skin sensitized rats with lethal dose irradiation and fludarabine combined with syngeneic hematopoietic stem cell transplantation (SHSCT) treatment performed by our research group. The DSA level of sensitized recipients were significantly degraded, prevent DSA rebound and AMR, significantly prolonged the survival time of sensitized recipient kidney transplantation in animals with strong antibody reactivity to the donor MHC. Further study on the effect of fludarabine on the humoral immune system of sensitized recipients, found that the percentage of CD27+ memory B cells and long-lived CD138+ plasma cells in the recipient rats was significantly reduced after the SHSCT combined with fludarabine, while fludarabine alone had no effect. Our finding proved that irradiation and chemical treatment depleted CD27+ memory B cells and long-lived CD138+ plasma cells which maintaining the long-term humoral immunological memory and humoral immunology was reset after SHSCT. Alloantibody decreased and antibody mediated rejection was prevented in this model. This study provides an important theoretical basis and therapeutic strategy for the prevention and treatment of antibody-mediated rejection in sensitized recipients.

P3018

miR-5119 inhibitor prolonging allograft survival in heart transplantation by regulation of inhibitory receptor ligands in DCs

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Introduction: T cells are critical to the graft versus host immune engagement regulating acute rejection. T cell exhaustion is an immune modulatory event, favorable for graft acceptance. that reduces T cell activity, However, the mechanism underlining T cell exhaustion in transplant tolerance remains unknown. We hypothesized that miRNA regulation of inhibitory receptor (iR) ligands in DCs can induce T cell exhaustion and increase heart allograft survival.
Materials and Methods: We evaluated the expressions of iR ligands in DCs from mice that tolerated allogeneic heart transplants for long periods (>100 days post-surgery). A miRNA microarray was used

DOI: 10.1002/eji.201970400

to screen up/downregulated miRNAs in PD-L1+ tolerogenic DCs from the allograft-tolerant mice. Mimics or inhibitors of miR-5119 were transfected into DCs for in vitro and in vivo experiments, followed by treatment of allogeneic (C57BL/6 to BALB/c) heart transplantation.
Results: We found that miRNA-5119 was significantly downregulated in rapamycin-treated mice tolerant to heart transplantation, by miRNA array analysis and RT-qPCR validation. *In silico* analysis indicated miR-5119 targets immune regulatory molecules, such as PD-L1 and IDO2. Treatment of heart recipients with miRNA-5119 inhibited DCs significantly prolonged allograft survival. The tolerance is associated with an upregulation of PD-L1 and IDO2 expression, promotion of T cell exhaustion and the generation of T_{reg} cells in vivo.
Conclusion: microRNAs such as miR-5119 can target multiple immune molecules in DCs and generate immune tolerance, thus prevent allograft rejection after heart transplantation

P3019

Human mesenchymal stem cell-derived exosomes enhances porcine islets resistance to hypoxia via suppression of NLRP3 inflammasome activation

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Hypoxia-induced damage is one of the key factors associated with islet graft dysfunction. In prior study, we found that human mesenchymal stem cell-derived exosomes could protect neonatal porcine islet cell clusters (NICCs) from hypoxia induced dysfunction. Here, we investigated whether human mesenchymal stem cell-derived exosomes could effect the survival and function of anoxic NICCs via NLRP3 inflammasome pathway.The NICCs were cultured with or without human mesenchymal stem cell-derived exosomes(control group) under 20% O_2 or 1% O_2 conditions. The viability and function of NICCs in different group were studied by fluorescence activated cell sorter (FACS) and the extra-cellular flux assay, respectively. The expression of hypoxia survival and NLRP3 inflammasome activation related proteins was detected by Western-blot. Compared with the control groups, the results showed that the survival ratio, viability, and function were improved significantly in NICCs cultured with exosomes ($P< 0.05$). The expression of hypoxia-inducible factor 1α（HIF-1α）was up-regulated, meanwhile, the expression of NLRP3 inflammasome pathway and apoptosis associated proteins decreased in Western-blot assay. Our results demonstrate that human mesenchymal stem cell-derived exosomes can protect NICCs from hypoxia-induced dysfunction by suppression of inflammation in reactive oxygen species (ROS)-NLRP3-the thioredoxin-interacting protein(TXNIP) axis, which was characterized by pretreating with the ROS scavenger N-acetylcysteine (NAC) and performing TXNIP knockdown. It provides new insight into the relationship between mesenchymal stem cell-derived exosomes and hypoxia induce inflammation in islet transplantation.

P3020

Treatment of Zhimaqianzi capsule combined with autologous peripheral blood stem cell transplantation long-term efficacy of refractory myasthenia gravis

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Objective: To investigate the long-term efficacy of Zhimaqianzi capsule combined with autologous peripheral blood stem cell transplantation in the treatment of refractory myasthenia gravis.
Methods: Autologous hematopoietic stem cell transplantation was performed in 3 patients with

DOI: 10.1002/eji.201970400

refractory myasthenia gravis. FAC protocol was used for pretreatment and oral administration of Zhimaqianzi capsule.
Results: Of the 3 patients, 2 were followed up for more than 3 years and stopped taking all drugs. The long-term effect was better.
Conclusion: Maqianzi capsule combined with autologous peripheral blood stem cell transplantation can reduce the dose and adverse reactions of Western medicine, promote immune recovery, and have a good long-term effect.

P3021

The relationship between indoleamine 2,3-dioxygenase activity and acute rejection of heart transplantation in rat immunosuppression with CsA

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Objective To study the relationship between indoleamine 2,3-dioxygenase activity and acute rejection of heart transplantation in rat immunosuppression with CsA. Methods Using SD and Wistar rats respectively as the donors and recipients with heart transplantation model . All the recipients were randomly divided into 3 groups:control group,low-dose and high-dose CsA group. Blood samples and heart transplants were harvested at the seventh day after transplantation. Results The level of heart acute rejection in low-dose and high-dose CsA group was obviously weaker than the control group（P＜0.01）， and acute heart allograft rejection level in high-dose CsA group was also significantly weaker than low-dose CsA group（P＜0.01）. Serum concentration of IFN-γ in low-dose and high-dose CsA group was obviously lower than the control group（P＜0.05）. The concentration of Kyn in low-dose and high-dose CsA group was significantly lower than that of control group（P＜0.01），and concentration of Kyn in High-dose CsA group was lower than that of low-dose CsA group（P＜0.05）. IDO activity in peripheral blood of low-dose CsA group and high-dose CsA group was significantly lower than control group, and the IDO activity in high-dose CsA group was lower than low-dose CsA group（P＜0.05）.Conclusion In the period of acute rejection in rat cardiac transplantation immunosuppression with CsA，IDO activity is close related with the degree of allograft acute rejection. Detection of serum IDO activity may be a non-invasive method for prognostic and diagnosis of heart acute rejection in rat.

P3022

The effects of 3,4-DAA on improving mouse renal ischemic reperfusion injury and its mechanism

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Objective: Analyzes the effects of 3,4-DAA (named Tranilast in commodity) on mice renal ischemic reperfusion injury(RIRI), detects the expression and activity of indoleamine 2,3-dioxygenase(IDO), and investigates its mechanism.
Methods: Erects a RIRI model with C57BL/6 mice, clamps bilateral renal pedicles for 30 minutes, and lavages for three days before the operation. The mice divided into four groups(n=40): sham, positive control, lowdose drug (Tranilast 300mg/kg)and highdose drug (Tranilast 600mg/kg) . Mice were

DOI: 10.1002/eji.201970400

sacrificed 24h after ischemia and blood was obtained from the fossa orbitalis vascular plexus to determine serum creatinine and urea nitrogen; Flowcytometry was applied to evaluate the mice spleen T lymphocytes; Kidneys were removed either for Western blot to determine IDO expression or for RT-PCR to analyze IDO mRNA transcription; serum kynurenine ,tryphtophan concentration, and the IDO activity were assessed.
Results: Renal function and tissue damage improved in Tranilast intervention groups($p<0.01$); Mice after drug intervention Th1/Th2 ratio was lower than those in positive control group($p<0.01$); Compared to positive control group, kideys from Tranilast treated groups displayed significant improvement in renal function ($p<0.01$) and increase in renal IDO expression, IDO mRNA and IDO activity($p<0.01$ or $p<0.05$). Dose dependence exists in drug intervention.
Conclusion: Mice RIRI pretreated with Tranilast had significant amelioration, expression of renal IDO and serum IDO activity increased significantly, moreover this effect had drug dose dependence. It is illustrated that 3,4-DAA protects mouse from RIRI and had a promising application in perioperation of renal transplantation.

P3023

The effect and mechanism of N-(3,4-dimethoxycinnamonyl) anthranilic acid (3,4-DAA) mediating negative immune regulation in mice skin transplantation

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Objective: The aim of this research project is to investigate the effect of N-(30,40-dimethoxycinnamonyl) anthranilic acid (3,4-DAA) in mice skin transplantation,and then explore the mechanisms.
Methods: To design a mice skin transplantation model,and T lymphocytes were isolated from mice splenocytes, collection serum; MTT was applied to measurement the splenic T lymphocyte proliferation inhibition rate; Western blot was applied to analyze the expression of IDO protein on the culture supernates of mice splenic lymphocytes and mice spleen; high performance liquid chromatography (HPLC) was applied to determinate the kynurenine and tryptophan concentration in cell culture supernates, the ratio of Kyn/Try was used to determinate IDO activity.
Results: T lymphocyte proliferation was significantly decreased by 3,4-DAA; the damage of the transplantation skin was minor in 3,4-DAA disposal group compared with the control group;IDO protein expression and IDO activity were found in 3,4-DAA disposal group compared with the control group.
Conclusion: 3,4-DAA suppresses clinical and histological severity of mice skin transplantation,and perform their immunosuppressive function by IDO activity. Therefore, 3,4-DAA might be a novel strategy to induce posttransplant immune tolerance.

DOI: 10.1002/eji.201970400

P3024

Dexamethasone delayed graft rejection through inducing myeloid-derived suppressor cells

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Objective: To investigate the role of myeloid-derived suppressor cells, which induced by dexamethasone in the immunoregulation of skin transplantation.
Methods: The recipient BALB/c mice (n=40) receiving transplantation of skin allograft from the tail of C57BL/6 mice were randomly divided into experimental group (receiving an intraperitoneal injection of dexamethasone 5mg/(kg·day)) and control group (receiving an equal volume of saline). On the seventh day after the mice were transplanted, pathological examination (HE staining) of the transplanted skin was did in some recipients. Sacrificed some recipients on the ninth day and use flow cytometry to detect the spleen and draining lymph nodes.
Results: The median survival time of allograft skin in the experimental group was 24±3.062 d, significantly longer than the control group (9±0.816 d) ($P< 0.05$). HE staining showed that the rejection of skin graft in the experimental group was significantly relieved than the controls. In spleen, the number of $CD11b^+GR1^+$ cells was significantly increased in the experimental group, and the expression of proinflammatory cytokine TNF-αwas up-regulated, IL-10 was down-regulated. Accompanied by the increased expression of chemotactic factor receptor CXCR2 and adhesion molecules CD44, CD62L. In the draining lymph nodes, the number of $CD11b^+GR1^+$cells was also increased, while the proportion of $CD4^+$T cells and the expression of IFN-γwere decreased.
Conclusion: Dexamethasone may induce the MDSCs and promote MDSCs migrate to the allograft region. By inhibiting the function of $CD4^+$T cells in the graft region to achieve the delay of graft rejection and extend the graft survival.

P3025

MNPseq as a highly sensitive method for detecting chimerism after allogeneic hematopoietic stem cell transplantation

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Introduction: STR analysis is the most popular method for monitoring chimerism following Allo-HSCT. However, this method suffers from a large number of technical and quality issues such as low detection sensitivity, stutter interference, et al. Multiple Nucleotide Polymorphism (MNP) refers to the combination of multiple SNPs within < 200bp DNA, and the polymorphisms of different SNPs are MNP. In this study, we carried out a clinical validation study of using MNPseq to detect chimerism based on next-generation sequencing that enable us to analyze MNPseq's sensitivity and accuracy.
Materials and methods: A total of 106 peripheral blood or bone marrow samples were collected from donors and patients undergoing Allo-HSCT. MNPseq was detected based on next-generation sequencing, which containing 100 amplicons and 646 SNPs.
Results: Chimerism measured by MNPseq showed an excellent correlation and detection sensitivity with that measured by STR analysis. Among 57 samples with no recipient chimerism detected by STR, 32 samples were positive (0.04~2.13%) by MNPseq analysis. When separate genetic information was availiable or none, MNPseq could not only be used for chimerism detection, but also for detection sensitivity could reach 0.01% or 1%. Furthermore, MNPseq results were 2.13~1.21% in 13 of 57 samples with no recipient chimerism when genetic information of donor and recipient was absent.

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DOI: 10.1002/eji.201970400

Conclusions: MNPseq is not only a highly sensitive chimerism detection method, but also suitable for chimerism detection without genetic information of donor and recipient.

P3026

Role of berberine in expansion ex vivo of CD133+ hematopoietic stem cells

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Umbilical cord blood (UCB) is an ideal source for hematopoietic stem cells (HSCs) transplantation, but the limited number of HSCs in a single UCB unit limits its application in cell transplantation. Therefore, expansion of HSCs ex vivo always attracted attentions. We investigated the role of berberine, an effective anti-diabetic drug from traditional Chinese medicine, in expansion ex vivo of CD133+HSCs.
Method: CD133+HSCs were isolated from fresh UCB and cultured in serum-free medium supplemented with SCF, Flt3-L, TPO and different concentrations of berberine. The CD34+, CD133+ biomarker of stem cells were analysed by flow cytomerty.
Results: After 8 days culture, both the concentration of berberine at 10uM and 20uM increased the positive rate of CD34+CD133+ cells to 33.9±1.7% and 40.0±0.7% respectively compared with the control group (27.9±0.6%), At 10 days, the positive rate of CD34+CD133+ cells showed a little decrease in the control group (22.55±1.8%). Meanwhile, berberine still maintained a higher positive rate of CD34+CD133+ cells at 37.6%±2.1% and 46.05±0.1% respectively. However, compared with the control group, berberine at 20uM inhibits HSCs proliferation ($p< 0.05$), but that didn't occurred at 10 uM.
Conclusion: Berberine could maintain the phenotype of HSCs, but showed a bidirectional effect on the proliferation of stem cells. This suggest that berberine at low concentration (< 10uM) may be an effective reagent to apply to expansion ex vivo of HSCs.
Keywords: Berberine, CD133+ hematopoietic stem cells, ex vivo expansion,

P3027

Sirtinol synergizes with tacrolimus to prevent cardiac allograft rejection

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Current immunosuppressive medications used after transplantation induce significant toxicity, and a new medication regimen is needed. Sirtinol is a sirt1 inhibitor, but its impact on allograft rejection and its molecular mechanisms have not yet been reported. In this study, we demonstrated the synergistic effects of sirtinol and non-therapeutic dose of tacrolimus (FK506) in prolonging the allograft survival in a murine cardiac transplant model. Based on an examination of the allograft, allografts from sirtinol-treated recipients show significantly lower levels of IL-17A expression and higher levels of Foxp3 expression. *In vivo*, sirtinol reduces the proportion of Th17 cells and increases the proportion of Treg cells in splenocytes from recipients. In vitro, sirtinol reduces the proportion of Th17 cells and decreases the expression of IL-17A and RORγt in an isolated CD4+ T cell population. Moreover, we identified synergistic effects of sirtinol and FK506 on prolonging allograft survival, and sirtinol synergizes with FK506 to promote Foxp3 expression. Our findings indicate that sirtinol, a sirt1

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DOI: 10.1002/eji.201970400

inhibitor, may be a promising immunosuppressive drug to prevent the rejection reaction in combination with FK506.

P3028

Jak2 regulates B cells function to produce alloantibodies and its effects on humoral rejection

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The antibody-mediated rejection (AMR) seriously affects on the long-term survival of kidney, liver and other solid organ transplantation. Janus kinase 2 (Jak2) is preferentially expressed in antigen-presenting cells including B cells, and is involved in the regulation of cell proliferation, activation and other functions, but whether it affects the post-transplantation AMR remains to be clarified. In this study, Jak2 conditional knockout mice were immunized with alloantigen, and the splenic B220+ cells, plasma cells, and germinal center formation as well as the level of allogenic IgG in peripheral blood were analyzed to explore the role of Jak2 in regulating B cell function. With the AMR model, the effect of Jak2 knockdown on antibody mediated rejection and the therapeutic effect of Jak2 inhibitor on AMR were studied. After 2 weeks of alloantigen immunization, the spleen size of Jak2 knockout mice was significantly smaller than that of control mice.The number of B220+ cells, plasma cells and germinal centers were significantly reduced compared with control mice. IgG level in peripheral blood was significantly lower than that in control mice. Jak2 knockout mice or administration of JAK2 inhibitor can significantly prolong allograft survival in AMR model. Therefore, our results suggested that JAK2 is a key target for the regulation of antibody production in B cells. Inhibition of jak2 can effective protects antibody-mediated graft rejection, exhibiting a promising approach for treatment AMR.

P3029

Characteristics of distribution of ethnic minorities and retrospective analysis of treatment of thalassemia

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Thalassemia is a single gene genetic disease. As a gathering place of ethnic minorities, Yunnan province has certain national characteristics in its occurrence and distribution. In addition to iron overload, the common clinical manifestations also show symptoms such as splenomegaly and infection which in turn increases the load on the spleen. How to relieve the symptoms of anemia without removing the spleen remains to be studied. By analyzing the epidemiological characteristics of inpatients from January 2014 to December 2017 in our hospital, in order to describe the occurrence in various ethnic groups in Yunnan province in recent years, which may providing scientific basis for preventing birth defects and prevention and management of thalassemia. In the past four years, 172 patients were admitted to our hospital, the prevalence of α-thalassemia was 22.7%, and β-thalassemia was 77.3%, of which Han nationality accounted for 68% and ethnic minorities were 32%. The male minorities account for 29.6% and female minorities is 38.3%. The morbidity of minority patients was led by 41.8% of the Dai, followed by the Zhuang 18.2% and the Yi 14.5%, with statistically significant difference (P< 0.01).We found that the minority female patients were more than the male, while the

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DOI: 10.1002/eji.201970400

han female patients were less than the male patients, it may be related to the national characteristics. We hope that in the future hematopoietic stem cell transplantation and molecular genetics level breakthrough, as soon as possible for the clinical treatment of thalassaemia patients, improving the quality of life.

P3030

Loss of Lkb1 impairs Treg function and stability to aggravate graft-versus-host diseases after bone marrow transplantation

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Accumulating evidence suggests that reduced number of Foxp3$^+$ regulatory T (Treg) cells contributes to the pathogenesis of acute graft-versus-host disease (aGVHD), a major adverse effect after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the precise features and mechanism underlying Treg defects remain largely unknown. In this study, we demonstrated that Treg frequencies were more dramatically decreased in bone marrow compared with peripheral blood from aGVHD patients, and bone marrow Treg defects were negatively associated with hematopoietic reconstitution. Tregs from aGVHD patients exhibited multiple defects including instability of Foxp3 expression especially in response to IL-12, impaired suppressor function, decreased migratory capacity, and increased apoptosis. Transcriptional profiling identified the down-regulation of Lkb1, a previously identified critical regulator of murine Treg identity and metabolism, and murine Lkb1-regulated genes in Tregs from aGVHD patients. Knockdown and overexpression of the Lkb1 gene could respectively decrease and increase Foxp3 expression in human Tregs. Furthermore, loss-of-function assay on an aGVHD murine model confirmed that Lkb1 deficiency could impair Tregs and aggravate disease severity. These findings uncover multiple defects of Tregs in human aGVHD, contributed by Lkb1 down-regulation, and highlight Lkb1-related pathways as potentially manipulable therapeutic targets to mitigate aGVHD.

Tumor Antigens and Escape Mechanisms

LB027

Proteogenomics uncovers a vast repertoire of shared tumor-specific antigens in ovarian cancer

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High-grade serous carcinoma (HGSC), the most common histologic subtype of ovarian cancer, remains as the principal cause of death from gynecological malignancies in the world. While HGSC infiltration by lymphocytes correlates with superior survival, patients have not significantly benefited

DOI: 10.1002/eji.201970400

from the recent progress in cancer immunotherapy. Due to their exclusive presentation on the major histocompatibility complex of cancer cells, tumor-specific antigens (TSAs) are ideal targets to trigger specific anti-tumor activity by T cells. However, the nature of HGSC TSAs is unknown. Applying a mass spectrometry pipeline that interrogates all reading frames of all genomic regions, we characterized a global TSA landscape of HGSC in 23 primary tumors. Our study identified in a total of 103 TSAs, only three of which would have been uncovered by classic TSA discovery approaches focusing strictly on exonic mutations. The majority of our TSAs resulted from noncoding sequences and out-of-frame exonic translation. Interestingly, 91 TSAs derived from unmutated genomic sequences which have no expression in normal tissues. These aberrantly expressed TSAs (aeTSAs) derived primarily from non-exonic sequences, regulated at the transcriptional level by alterations in gene copy number and DNA methylation. Notably, many aeTSAs are encoded by transcripts shared by a large proportion of HGSCs. Taking into account the frequency of aeTSA expression and HLA allele frequencies, a median number of five aeTSAs per tumor would be expected. Considering their number and frequent occurence, aeTSAs are attractive targets for HGSC immunotherapy.

LB028

Differing forms of PD-L1 are found in serum-derived exosome fractions from patients with cervical cancer and precursor lesions

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Among the most novel therapeutic strategies for the treatment of several cancers is the blockade of important immune checkpoint molecules expressed by T cells. One of these is the receptor PD-1, which negatively regulates T cell activation upon interaction with its ligands, such as PD-L1. PD-L1 can be secreted by tumors via exosomes, promoting tumor immune escape. Cervical cancer, one of the most common malignant tumors in women worldwide, is characterized by serious defects in T cell-mediated immunity, which might be explained, in part, by overexpression of PD-1. Soluble PD-L1 has been described in patients with cervical cancer; however, whether PD-L1 is mostly confined to serum exosome fractions is not well described yet. Therefore, our main objective was to address this question. Serum exosomes were prepared from patients with cervical cancer, precancerous lesions or from healthy donors. PD-L1 was detected by Western blot. Further characterization of PD-L1 was performed using an immunoproteomic approach based on 2DE-Western blot. Our results showed different molecular weights of PD-L1 with the most abundant PD-L1 band between 25-30 kDa, followed by a 100 kDa band, and in some samples a 50 kDa band was also present. The 100 kDa band was absent in the exosome-enriched fraction, though the other bands were present. This pattern was practically the same in all groups; however, it is possible that the presence of the 25-30 kDa bands, which were confined to exosomes, might be a biomarker to identify patients most likely to respond well to anti-PD-1 or anti-PD-L1 immunotherapy.

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DOI: 10.1002/eji.201970400

LB065

DHX34 may be involved in regulating the immunogenicity of Hepatocellular carcinoma

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Tumor immunogenicity can affect the sensitivity and response rate of tumor immunotherapy(PD-1 or CTLA-4 antibody therapy). Key genes mutation will improve tumor mutation (TMB) and play key roles in enhancing immunogenicity. DHX34 is an RNA helicase involved in RNA editing, rRNA processing, mRNA transcription, transport and degradation. In our study, we sequenced HCC tissue samples(not response to PD-1 therapy) and their matched Blood samples by whole exome sequencing (WES). We predicted somatic mutations by neoantigen prediction algorithm. TCGA data was excavated to analyze the relationship between somatic mutations and HCC patient survival. The results showed top 9 Somatic mutations such as DHX34, DLAT, PCBP2, AIMP2, A1BG NUDCD1, PCDH1 RTCA, ALDH1L1 showed higher MHC binding affinities and neoantigen characters. DHX34 mutation showed the more postive correlation in DC, CD4+T, CD8+T immune cells infiltration and Granzyme, Perforin A, IFN-γ, TFN-a, CCL2, CCL5 cytokines or chemokines in HCC microenvironment. DHX34 neoepitope peptide has the strong ability to active T cell *in vitro*. In conclusion, we inferred that DHX34 could regulate the immunogenicity of HCC based on our data.

LB102

The evaluation of the frequency and function of MDSCs in breast cancer patients undergoing chemotherapy

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Introduction: Myeloid-derived suppressor cells (MDSC) with immunosuppressive activities are one of the immune escape mechanisms of tumors in different kinds of cancers, such as breast cancer.
Methods: The frequency and phenotypes of MDSCs in blood and tissue samples of patients with breast cancer and healthy donor were determined by flow cytometry. MDSCs were purified via MACS technique, and then using BrdU incorporation assay, suppressive function of these cells on T CD3+ cells was established. Moreover, we investigated the frequency and function of MDSCs in breast cancer patients undergoing chemotherapy.
Results: Our study demonstrated that CD33+HLA-DR-Lin- myeloid cells have a very low percentage in blood circulation of healthy people, but this percentage is significantly increased in patients with breast cancer. It is also observed that the presence of MDSCs in patients with breast cancer is not limited to immature myeloid cells; it includes monocytic and granulocytic MDSCs as well. Our results showed that however after chemotherapy the circulating MDSCs frequencies were decreased, but the MDSCs of patients with breast cancer after chemotherapy still had considerably more T cell-suppressive activity than MDSCs isolated from healthy individuals.
Conclusion: Our findings pave the way to develop novel diagnostic and immunotherapeutic strategies, aimed at detecting and inhibiting MDSCs in patients with BC.

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DOI: 10.1002/eji.201970400

O009

Cytokine-mediated crosstalk between HER2-positive breast cancer and NK cells promotes resistance to trastuzumab via HLA-G/KIR2DL4 signaling

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Despite the successful use of the humanized monoclonal antibody, trastuzumab, in clinical treatment of HER2-overexpressing breast cancer, the low patient response rate and frequently occurring drug resistance have greatly compromised its therapeutic efficacy. The regulatory mechanisms of trastuzumab-elicited immune response against breast cancer remain largely uncharacterized. The non-classical histocompatibility antigen HLA-G is a known unique ligand for the natural killer (NK) cell receptor KIR2DL4. Here, **we found that clinical breast cancers which express high levels of HLA-G exhibited a poorer prognosis and a higher probability of recurrence** and the interaction between HLA-G and KIR2DL4 suppresses trastuzumab-mediated antibody-dependent cellular cytotoxicity (ADCC) and affects the prognosis of breast cancer patients. Mechanistically, IFN-γ produced by NK cells promotes KIR2DL4 expression via JAK2/STAT1 signaling. **KIR2DL4 synergizes with the Fcγ receptor to activate trastuzumab-induced NK cells unless engaged by HLA-G which indicated that KIR2DL4 can promote NK cell activation and the binding with HLA-G leads to the switch of its function**. However, the autocrine TGF-β upregulates HLA-G in neoplastic cells and contributes to a remarkable suppression of trastuzumab-triggered ADCC. Blockade of HLA-G/KIR2DL4 signaling improved the vulnerability of HER2-positive breast cancer to trastuzumab both *in vitro* and *in vivo*. **We also found an elevated expression of HLA-G and co-expression of KIR2DL4 on infiltrating CD56$^+$ NK cells in breast cancer tissues of trastuzumab resistant patients**. These findings provide novel insights into the mechanisms underlying trastuzumab resistance, and demonstrate the applicability of combined HLA-G and HER2 targeting in the treatment of breast cancer.

O010

The macrophage tetraspan MS4A4A enhances Dectin-1-dependent NK cell-mediated resistance to metastasis

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DOI: 10.1002/eji.201970400

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The membrane spanning 4-domains A4A (MS4A4A) is a tetraspan-like molecule that belongs to the MS4A family. Although it has been reported that MS4A4A is part of the transcriptional signature of alternatively-activated macrophages (M2), the function of MS4A4A is totally unknown. We demonstrated that MS4A4A expression is restricted to murine and human mononuclear phagocytes and is induced during monocyte-to-macrophage differentiation. We showed that MS4A4A is coexpressed with M2/M2-like molecules in subsets of human normal tissue resident macrophages, infiltrating macrophages from inflamed synovium, and tumor-associated macrophages. We identified Dectin-1 as a receptor partner for MS4A4A. By split ubiquitin yeast two-hybrid analysis, FLIM-FRET and STED, we showed that MS4A4A interacted and co-localized with Dectin-1 in lipid rafts. MS4A4A also impacts on signalling downstream Dectin-1 by promoting the Syk-dependent pathway in macrophages and thus their production of pro-inflammatory cytokines and ROS. While MS4A4A deficiency in macrophages did not affect primary tumor growth, MS4A4A is essential for Dectin-1-mediated activation of macrophages associated to metastasis and it is required for the efficient activation of NK cell anti-metastatic functions. Therefore, we demonstrated that MS4A4A is a tetraspan molecule selectively expressed in macrophages during differentiation and polarization. We showed MS4A4A is essential for Dectin-1-dependent activation of macrophages and for their ability to induce NK cell-mediated resistance to metastasis. These results identified for the first time a functional role of MS4A4A on macrophages and its importance in preventing cancer dissemination.

O011

MEK1/2 inhibitor Trametinib reverses the down-regulation of MHC class II in glioma-associated microglia

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Gliomas, the most notorious primary tumor in the brain, are known for its immune evasive hallmarks. There is significant microglial infiltration in gliomas. However, the expression of MHC class II molecules in microglia is compromised, our previous study revealed that several kinds of endogenous TLR2 ligands, such as the heat shock proteins, in glioma tissues lead to activation of MAPK/ERK1/2 signaling pathway, and the decrease of histone H3 acetylation at promoters of Ciita (the master regulator of MHC class II molecules transcription), which synergistically led to the inhibition of Ciita and MHC class II expression. In the present study, a MEK1/2 inhibitor, Trametinib, was used to test the possibility for reversing microglial MHC class II down-regulation in glioma. We found that MHC class II are mainly distributed at the invasive margin of glioma. In the parenchymal region of the tumor, especially in the necrotic region, microglia and mononuclear macrophages hardly express MHC class II. Trametinib could significantly improve the microglial expression of MHC class II in a primary culture system and orthotopic murine glioma model. These results suggest that the MEK1/2 pathway plays a key role in regulating MHC expression in microglia. Trametinib is expected to promote numerous T cell-mediated immunotherapies by up-regulating MHC II expression of tumor-infiltrating antigen-presenting cells.

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DOI: 10.1002/eji.201970400

O012

Enhanced immunogenicity of mitochondrial localised proteins in cancer cells

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T cell responses elicited by mutated proteins in cancer cells contribute to the control of tumour growth. However, it remains unclear whether the sub-cellular localisation of mutated proteins influences the efficiency of T cell priming. To address this question we compared the immunogenicity of NY-ESO-1 and OVA localised either in the cytosol or in mitochondria and showed that tumours expressing mitochondrial localised NY-ESO-1 and OVA proteins elicit significantly higher frequencies of antigen specific $CD8^+$ T cells in vivo. Enhanced T cell responses against mitochondrial OVA and NY-ESO-1 are dictated by intrinsic factors related to their mitochondrial location, including but not limited to their higher steady state amount. Cross-presentation events following uptake of mitochondria by $CD103^+$ and $CD11b^+$ DC are dependent on STING activation. Despite the more efficient direct presentation of the rapidly degraded cytoplasmic OVA compared to mitochondrial OVA, mitochondrial ovalbumin encoding tumours are controlled better by the host, due to the stronger immune response generated. In line with these findings we show that immunization with mitochondria from wild type B16 can specifically protect mice against a challenge with B16 tumours. Finally, we extend these findings to patients by demonstrating the presence of T cell responses specific for mutated mitochondrial localised proteins. These findings highlight the importance of prioritising epitopes derived from mitochondrial localised mutated proteins as targets for cancer vaccination strategies.

O013

Breast cancer cell-derived IL-35 promotes tumor progression via induction of IL-35-producing induced regulatory T cells

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Interleukin 35 (IL-35) is a potent immunosuppressive cytokine, consisting of an Epstein-Barr virus-induced gene 3 (EBI3) subunit and a p35 subunit. IL-35 is mainly produced by regulatory T and regulatory B cells, and plays a crucial role in the development and prevention of infectious and autoimmune diseases. However, the effect of IL-35 in malignant disease is not well understood. In this study, we demonstrated that breast cancer cells (BCCs) also expressed and secreted IL-35 and higher level of IL-35 in BCCs was closely associated with poor prognosis of patients and was an independent unfavorable prognostic factor for breast cancer. Subsequent study revealed that BCC-derived IL-35 inhibited conventional T (T_{conv}) cell proliferation and further induced suppressed T_{conv} cells into IL-35-producing induced regulatory T (iTr35) cells. Furthermore, BCC-derived IL-35 promoted the secretion of inhibitory cytokine IL-10 and obviously decreased the secretion of Th1-type cytokine IFN-γ and Th17-type cytokine IL-17 in T_{conv} cells. Meanwhile, the expression of inhibitory receptor CD73 was also elevated on the surface of T_{conv} cells following the BCCs' supernatant treatment. Mechanistically,

DOI: 10.1002/eji.201970400

BCC-derived IL-35 exhausted T_{conv} cells and induced iTr35 by activating transcription factor STAT1/STAT3. Hence, our results indicate functions of BCC-derived IL-35 in promoting tumor progression through proliferation inhibition of tumor-infiltrating T_{conv} cells and induction of iTr35 cells in tumor microenvironment. This study highlights that IL-35 produced by BCCs are a potential therapeutic target for breast cancer.

O014

Activin-A protects against lung cancer progression by potentiating anti-tumor immunity

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Background: Activin-A is a cytokine that exerts diverse effects on immune responses depending on the spatiotemporal context. While it is overexpressed in lung cancer patients and associated with metastasis, its effects on T cell-mediated anti-tumor responses remains unexplored.
Methods: In order to evaluate whether activin-A directs effective, T cell-mediated anti-tumor responses during lung cancer development, we utilized a syngeneic mouse lung cancer model induced by Lewis Lung Carcinoma (LLC) cells and administered activin-A therapeutically. In a complementary approach, we disrupted activin-A's signaling on $CD4^+$ T cells using an inducible model of $CD4^+$ T cell-specific knockout of activin's A type I receptor, ALK4.
Results: Our findings demonstrate that activin-A administration, induced a marked regression in lung cancer progression, evidenced by macroscopic, PET/CT imaging and histological studies, concomitant with a greatly extended overall survival. Activin-A's anti-tumor effects were associated with increased infiltration of $CD4^+$ T effector cells in lung tumors and decreased frequencies of $Foxp3^+$ Treg and myeloid derived suppressor cells. Moreover, activin-A treatment significantly decreased the expression of the immune checkpoint inhibitors (e.g. PD-1, CTLA-4, Lag3, Tim3) among lung TILs. Mechanistic studies demonstrated that $CD4^+$ T cells were essential in mediating the anti-cancer effects of activin-A, as disruption of its signaling in these cells, resulted in enhanced tumor progression accompanied by diminished effector responses and heightened percentages of $Foxp3^+$ Treg cells and immune checkpoint inhibitors.
Conclusions: Our studies reveal a novel role for activin-A in enhancing anti-tumor T cell-mediated responses that may be beneficial in the combat of lung cancer progression.

O015

Role of circulating miR-182 and miR-150 as biomarkers for cirrhosis and hepatocellular carcinoma post HCV infection in Egyptian patients

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In Egypt, liver diseases are exceptionally high, maintaining the highest prevalence of hepatitis C virus (HCV) worldwide, and increasing rates of hepatocellular carcinoma (HCC). Available diagnostic methods show poor performance in early diagnosis of HCC. Definite factors contributing in the development of HCV are still lacking. MicroRNAs have been reported as promising biomarkers for diagnosis and in virus-host interaction.
This study was conducted to detect the role of miR-182 and miR-150 as biomarkers for development of cirrhosis and malignant transformation in HCV infected patients.
The expression of miR-182 and miR-150 was evaluated using real-time quantitative PCR (qRT-PCR)

DOI: 10.1002/eji.201970400

in 120 subjects:40 HCC patients,40 hepatitis C patients (20 cirrhotic and 20 non-cirrhotic HCV genotype 4) and 40 healthy controls.
In HCC, statistically significant decrease of miR-182 and miR-150 compared to non-cirrhotic HCV patients (p=0.015, p=0.006 respectively) and of miR-150 compared to controls (p=0.039). In cirrhotic HCV patients,significant down regulation of miR-182 and miR-150 compared to non-cirrhotic HCV (p=0.003, p=0.024 respectively).On the other hand, significant upregulation of miR-182 was observed in non-cirrhotic HCV compared to controls (p=0.036).Alpha-fetoprotein (AFP) showed sensitivity 15% for HCC diagnosis at the cut-off value of 400 ng/ml, while combining AFP with miR-182 and miR-150,resulted in improving sensitivity to (90%) and diagnostic accuracy to (80%).
miR-182 and miR-150 can be used as non invasive biomarkers for HCC and combination of these miRNAs and AFP markedly improve the diagnosis of HCC.Both miR-182 and miR 150 can also be used as predictive markers for detection of cirrhosis progression in HCV infected patients.

O016

Intratumoural immune heterogeneity is linked to genomic mutation and clinical outcome

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Intratumoural genomic heterogeneity and its implication on tumour evolution and clinical prospect were previously described in various cancers. The degree of intratumoural heterogeneity (ITH) in immune landscapes, the co-evolution between tumour and its immune microenvironment, was however not previously explored. Here, we revealed significant level of immune-ITH, its relationship with tumour evolution and its clinical impact by simultaneous single-cell cytometry by time-of-flight (CyTOF), whole genome sequencing (WGS) and RNA sequencing on multiple tumour sectors from resected hepatocellular carcinoma (HCC) (total 95 sectors from 28 patients). Immune-ITH was observed on major immune lineages as well as specific subsets, including regulatory T cells (Treg) and exhausted CD8+ T cells. Remarkably, we found that patients with high immune-ITH had advanced stages of disease, bigger tumour size and a higher risk of recurrence than those with low immune-ITH. High immune-ITH was also characterised by enhanced immunosuppression, exhaustion and reduced infiltration by cytotoxic or activated immune cells characteristics of a reduced immunoselective pressure. Consistently, we found the enhanced neoantigen loads to be linked to high immune-ITH indicating the tumour-immune co-evolution process. In tumours with high immune-ITH, We discovered a unique transcriptomic signature and tumour-immune network associated to disease progression. This transcriptomic signature also correlated to poor overall survival profiles in public HCC cohorts. The in-depth investigation of immune-ITH along with tumour genomics provided a holistic view on the complexity and co-evolution process of tumour-immune interface.

P0446

Topical application of temperature sensitive Caerin 1.1 and 1.9 gel inhibits TC-1 tumour growth in mice

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DOI: 10.1002/eji.201970400

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Genital warts are one of the most common sexually transmitted diseases (STDs), resulting from persistent infection with human papillomavirus 6 or 11. Topical application of 5% imiquimod cream (Aldara) is currently recommended as first line treatment choice for genital warts, but warts clearance rate and patient compliance rate remain to be increased.Caerin peptides from the skin secretion of tree frog inhibit HPV16 E6/E7 transformed TC-1 growth both in vitro and *in vivo*. In the current study, we developed a temperature sensitive gel containing caerin 1.1 and 1.9 peptides. Growth of HPV16 E6/E7 transformed TC-1 cells was inhibited *in vitro* and *in vivo* when the gel was intra-tumour injected. Furthermore, when the gel was topically applied, it can inhibit TC-1 growth, and the inhibitory effect was comparable to 5% imiquimod cream. In addition, the gel maintained a similar level of bioactivity after incubation at room temperature for 30 days. Our results suggest that the gel, with further optimization, may provide an alternative method for the management of genital warts.

P0447

Inhibitory effect of miR-200c on EMT in triple negative breast cancer and its mechanism

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Objective: To study the effect of miR-200c on the migration and proliferation abilities of MDA-MB-231 and BT-549 cells, and to clarify the mechanism of miR-200c inhibition the epithelial-mensenchymal transition (EMT) of triple negative breast cancer (TNBC).
Methods: Human TNBC cell lines (MDA-MB-231 and BT-549) were chosen in this study. The cells were transiently transfected with miR-200c mimics and Lipo2000(experimental group), miR-200c negative control and Lipo2000(negative control, C1), Lipo2000 alone (reagent control, C2) and blank control group (C3). The expression levels of vimentin and β-catenin mRNA and protein were detected by RT-PCR and Western-blot. The proliferation rates and migration abilities of MDA-MB-231 cells and BT-549 cells after transfection of miR-200c were analyzed by CCK8 assay and wound healing assay.
Results: The RT-PCR and Western-blot showed that the expression of vimentin and β-catenin mRNA and protein in experimental group were decreased, and the differences were statistically significant compared with C1-3 group ($P< 0.05$). The CCK8 results showed that the proliferation rates of the cells in experimental group were lower than C1-2 group ($P< 0.05$). The wound healing assay results showed that the recovery rate of scratch width in experimental group was lower than C1-2 group ($P< 0.05$).
Conclusion: miR-200c might inhibit EMT in TNBC by regulating the expression of β-catenin and vimentin mRNA and protein in MDA-MB-231 cells and BT-549 cells and decreasing the abilities of migration and proliferation of TNBC.
Keywords: miR-200c; β-catenin; vimentin; EMT (epithelial-mensenchymal transition); TNBC (triple negative breast cancer)

DOI: 10.1002/eji.201970400

P0448

Analysis of VISTA expression and function in renal cell carcinoma highlights VISTA as a potential target for immunotherapy

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The checkpoint blockade therapy elicits strong immune-mediated tumor control in a wide spectrum of cancer types with variable degrees of optimal responses. It is therefore urgently needed to understand why most patients fail in responding to such therapy. The response rate to anti-PD-1/PD-L1 in patients with renal cell carcinoma (RCC) is around 25%. We thus in this study explored the expression and function of other B7 family checkpoint molecules in RCC. We found that VISTA mRNA and protein was selectively highly expressed in human clear cell RCC (ccRCC) tumors, mostly by tumor-associated macrophages. VISTA expression strongly correlated with poor $CD8^+$ T cell responses. VISTA blockade significantly reduced the growth of murine RENCA RCC model, and elicits TNFa production in human ccRCC samples. Taken together, these findings indicate that VISTA functions in suppressing tumor immunity and could serve as a target for immunotherapy in ccRCC.

P0449

Spi-B-mediated silencing of claudin-2 promotes early dissemination of lung cancer cells from primary tumors

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Dissociation from epithelial sheets and invasion through the surrounding stroma are critical early events during epithelial cancer metastasis. Here we find that a lymphocyte lineage-restricted transcription factor, Spi-B, is frequently expressed in human lung cancer tissues. The Spi-B-expressing cancer cells coexpressed vimentin but repressed E-cadherin and exhibited invasive behavior. Increased Spi-B expression was associated with tumor grade, lymphatic metastasis, and short overall survival. Mechanistically, Spi-B disrupted intercellular junctions and enhanced invasiveness by reconfiguring the chromatin structure of the tight junction gene claudin-2 (CLDN2) and repressing its transcription. These data suggest that Spi-B participates in mesenchymal invasion, linking epithelial cancer metastasis with a lymphatic transcriptional program.

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DOI: 10.1002/eji.201970400

P0450

Myeloid-specific inactivation of PDK1-MTOR1 signaling promotes NK cell-mediated antitumor immunity and inflammation by reprograming macrophage polarization

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The project was supported by Beijing Natural Science Foundation (7184218).
Classically-activated (M1) and alternatively-activated (M2) macrophage phenotypes, which generally mediate pro-inflammatory and anti-inflammatory functions, respectively, represent the polarization of immunoregulatory plasticity in this population. Tumor-associated macrophages (TAMs) mostly exhibit immunosuppressive M2 phenotype. Here we initially found that the M2 polarized macrophages were abundant within PyMT-induced mammary tumors, and they showed increased energy consumption. The number of M2 macrophage largely correlated with tumor progression and defective NK cell function. In parallel, M1 macrophage polarization primed by LPS promoted NK cell activation, which contributed to LPS-induced sepsis. We then showed that PDK1 deletion in macrophage exacerbated LPS-induced M1 macrophage polarization, which worsened sepsis syndromes. Remarkably, **PDK1 inactivation could reprogram the M2-polarized TAMs to M1 status in the PyMT-driven tumors.** Thus, this treatment suppressed the tumor progression and metastasis to lungs, probably through improving NK cell infiltration and functions. Finally, **PDK1 deficiency in macrophages reduced glucose assumption by dampening Akt-mTORC1 activation, so that the disassociation of mTORC1 via knocking-down Raptor, a scaffold protein of mTORC1, gave rise to the severer phenotype of LPS-induced shock.** Therefore, myeloid-specific inactivation of PDK1 reprograms TAMs towards immunostimulatory M1 phenotype, which overcomes tumor-associated immunosuppression and refreshes anti-tumor immunity with potential therapeutic implications. Moreover, sepsis risk and inflammatory status should be carefully considered when tumor immunity is improved.

P0451

Expression and research of the pyroptotic gene GSDME in paclitaxel resistance of breast cancer

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Objective: To establish a paclitaxel (Taxol)-resistance human breast cancer cell lines MCF-7/Taxol and identify the establishment ofMCF-7/Taxol. By detecting its expression level of the pyroptotic gene GSDME, it provides an experimental basis for the drug resistance mechanism and its reversal of drug resistance.
Methods: Drug sensitivity was revealed by CCK-8 assay. Cell morphology was observed by transmission electron microscope. Cell cycle distribution was measured by flow cytometry. The relative mRNA expression levels of MDR1 and pyroptotic gene GSDME were identified by qRT-PCR. Western Blot was used to detect the P-gP and GSDME protein expression.
Results: 1. Compared to MCF-7, the IC_{50} value of MCF-7/Taxol increased, the nuclear to cytoplasmic ratio and nucleolus increased under transmission electron microscopy. It was in the cell cycle of prolonged G1 phase and shortened S phase. The relative expression of MDR1 mRNA and P-gP protein were increased (all $P< 0.01$). 2. Compared with MCF-7, the relative expression of GSDME mRNA and protein in MCF-7/Taxol both increased (all $P< 0.01$).

DOI: 10.1002/eji.201970400

Conclusion: The successful development of breast cancer resistance to paclitaxel MCF-7/Taxol cell lines, the increase of the classical drug resistance gene MDR1 in drug-resistant cells, and the decrease of pyroptotic gene GSDME, providing a theoretical basis for further study of multidrug resistance and its reversal mechanism in breast cancer.
Keywords: breast cancer cells MCF-7; MCF-7/Taxol; paclitaxel; multidrug resistance; pyroptosis; GSDME

P0452

Exosomal IL-35-regulated DSCAM-AS1 facilitates breast cancer progression via polarizing M2 macrophages

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Exosomes released by cells can serve as vehicles for delivery of biological materials. Long non-coding RNAs (lncRNA) have been shown to play vital roles in tumor progression, however the function of exosomal LncRNAs in breast cancer remains unclear. Interleukin 35 (IL-35), a potent inhibitory cytokine, is highly expressed by tumor-infiltrating regulatory T cells and breast cancer cells. Here we showed that exosomes from IL-35-stimulated breast cancer cells (IL-35-BCC exo) were taken up by macrophages and enhanced M2 polarization from human crod blood monocytes *in vitro*, characterized by an increase of Arg-1 and CD206. We further revealed that pretreatment of IL-35-BCC exo promoted inhibitory microenvironment via inducing more $CD68^+CD163^+$ M2 macrophages, which accelerated tumor growth *in vivo*. Mechanismly, IL-35-BCC exo mediated these effects by the activation of STAT3 (transducer and activator of transcription 3), JNK and p38. Moreover, we identified exosomal lncRNA (DSCAM-AS1) down-regulated by IL-35 through lncRNA microarray screening and examined its expression in exosomes from breast cancer cells. Knockdown of DSCAM-AS1 in breast cancer cells-derived exosomes promoted the expression of M2 markers Arg-1 and CD206. Collectively, the IL-35-regulated DSCAM-AS1 involved in bresat cancer exosomes contributed to immune dysregulation and tumor progression via promoting polarization of M2 macrophages.

P0453

TMEM106B promotes proliferation and angiogenesis of malignant meningiomas

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The TMEM106B gene located on chromosome 7p21.3 , is a transmembrane protein expressed in lysosomes, which is associated with central nervous system diseases such as Alzheimer´s disease, frontotemporal deformity lesions,Amyotrophic lateral sclerosis etc. However,the relationship between TMEM106B expression and malignant meningiomas remains unclear.Here we have found for the first time that the expression of TMEM106B genes are associated with malignant meningiomas through extensive analyses of gene chip and high-throughput screening. Further validation methods for gene expression by immunohistochemistry analysis proved that TMEM106B is significantly upregulated in meningioma tissues, as compared with normal meningeal tissues. Furthermore,we showed that TMEM106B knockdown can inhibit the proliferation and angiogenic ability of malignant meningioma cells in vivo and vitro. Taken together,TMEM106B gene dysregulation in human malignant meningiomas might accumulate the proliferation and angiogenesis of meningiomas,and suggest that

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DOI: 10.1002/eji.201970400

TMEM106B may represent a promising candidate tumor suppressor gene that contributes to malignant meningiomas.

P0454

Tumor-repopulating cells induce PD-1 expression in CD8$^+$ T cells by transferring kynurenine and AhR activation

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Despite the clinical successes fostered by immune checkpoint inhibitors, mechanisms underlying PD-1 upregulation in tumor-infiltrating T cells remain an enigma. Here, we show that tumor-repopulating cells (TRCs) drive PD-1 upregulation in CD8+ T cells through a transcellular kynurenine (Kyn)-aryl hydrocarbon receptor (AhR) pathway. Interferon-g produced by CD8+ T cells stimulates release of high levels of Kyn produced by TRCs, which is transferred into adjacent CD8+ T cells via the transporters SLC7A8 and PAT4.Kyn induces and activates AhR and thereby upregulates PD-1 expression. This Kyn-AhR pathway is confirmed in both tumor-bearing mice and cancer patients and its blockade enhances antitumor adoptive T cell therapy efficacy. Thus, we uncovered a mechanism of PD-1 upregulation with potential tumor immunotherapeutic applications.

P0455

Tumor suppressor ZHX2 inhibits HCC progression through functioning in hepatoma cells and macrophages

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Abundant genes in hepatoma cells and immune cells in the tumor microenvironment are dysregulated, which is specially evident in HBV-associated HCC. ZHX2 is a novel tumor suppressor in HCC identified in our lab and acts as a transcriptional factor in macrophages and B cells. Our recent data demonstrated that **HBV promoted miR-155 level in hepatoma cells, which further downregulated expression of ZHX2 through targeting the seed sites in 3'UTR, invoking deprivation of tumor-suppression properties of ZHX2**. Interestingly, another study in our lab illustrated that **ZHX2 in turn suppressed expression of HBV antigens and replication of HBV via inhibiting activity of HBV promoters and restricting cccDNA in the transcriptional or epigenetic manner**. The above studies showed that ZHX2 in hepatoma cells involved in the progression of HBV-promoting HCC, suggesting the regulation loop between HBV and ZHX2 in liver cancer. Moreover, our data determined that **decreased ZHX2 expression in tumor-associated macrophages (TAMs) induced the secretion of cytokines**, which led to further investigation about function of ZHX2 in immune cells in tumor microenvironment. Taken together, low expression of ZHX2 in both liver cancer cells and TAMs are positively correlated with tumor progression and its overexpression potentiates a novel therapy for HCC.

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DOI: 10.1002/eji.201970400

P0456

Identification of HSPA4 as tumor antigen and relative autoantibody for predicting breast cancer progression

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Antibody-mediated humoral immunity plays important roles in eliminating foreign pathogens and malignant cells. However, clinical studies have revealed that high antibody levels in serum are correlated with tumor grade, metastasis and poor survival in certain human cancers. **Using mass spectrometry after immunocoprecipitation by serum antibodies, we identify heat shock protein 4 (HSPA4) as tumor antigen to induce related autoantibody production in tumor-bearing mice. And the autoantibodies can recognize the glycosylation sites of HSPA4, which are variable in different tumors. Mice bearing with HSPA4 knock-out tumor cells have lower concentrations of serum antibodies and much less LN metastasis than wide-type clone bearing mice. In clinical breast cancers, high expression of HSPA4 in invasive ductal carcinoma (IDC) is strongly associated with axillary LN metastasis and molecular subtype of tumors. And patients with upregulation of tumor-intrinsic HSPA4 show a reduced survival time. Furthermore, we find that the increased levels of serum anti-HSPA4 antibody are consistent with higher HSPA4 expression in cancers. And cases with LN metastasis show higher concentrations of serum anti-HSPA4 antibody compared with non-metastatic ones.** Thus, identification of tumor antigens and their related autoantibodies suggests new potential cancer diagnostic and therapeutic targets, which may result in substantial survival benefit.

P0457

Cancer/testis antigen FATE/BJ-HCC-2 enhances metastasis of hepatocellular carcinoma cells

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Background: To analyse differential gene expression in HCC cells induced by FATE/BJ-HCC-2 , and observe tumor genesis and metastasis caused by FATE/BJ-HCC-2 in animals.
Methods: The stable cell clone expressing FATE/BJ-HCC-2 was established by transfection of this gene. The gene expression of above cells was analyzed with Affymetrix Human Genome U133 plus 2.0 GeneChip array. The FATE/BJ-HCC-2 gene-transfected Bel-7402 and mock control cells were inoculated intraperitoneally in nude mice.
Results: GeneChip analysis showed that there were 821 genes up-regulation and 873 genes down-regulation induced by FATE/BJ-HCC-2. The animal experiments showed that tumors were formed in abdominal cavity in 70% mice of the FATE/BJ-HCC-2 gene-transfected group, but only in 10% mice of mock control group. Furthermore we also find tumor formation in livers of the FATE/BJ-HCC-2 gene-transfected group only. It was found that the expression of proteins promoting tumor metastasis, such as ILK, OPN, β-Catenin, MMP-2 and MMP-9 in tumor cells and tumor tissues was higher in experimental group than that in mock control group. However the expression of protein inhibiting tumor

DOI: 10.1002/eji.201970400

metastasis, such as E-Cadherin, was down-regulated in cells and tumor tissues of experimental group. **Conclusion:** FATE/BJ-HCC-2 could induce differential expression of a group of genes in tumor cells and may be an important factor to promote tumor cells growth and metastasis.

This work was supported by grant from Henan natural science foundation (No.182300410343); grant from NSFC(No.81071724)

P0458

Fbxw11-mediated ubiquitination is involved in the proliferation of lymphocytic leukemia cells

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F-box and WD-40 domain protein 11 (Fbxw11) as a constituent of the SCF (Skp1-Cul1-F box) E3 ubiquitin ligase complex, determines the specificity of substrate degradation by identifing and binding to different spectrums of target proteins. The SCF^{Fbxw11} complex regulated many important biological processes including cell cycle, differentiation, development and metabolism by targeting a broad range of substrates. However the role of Fbxw11 in the development of leukemia and the underlying mechanisms remain largely unknown. In this study, we reported that the expression of Fbxw11 was aberrantly up-regulated in lymphocytic leukemia patients. Its expression was dramatically decreased after chemotherapy achieving CR. High-level expression of Fbxw11 in L1210 lymphocytic leukemia cells stimulated cell proliferation *in vitro* and tumor formation *in vivo*. The effects were due to stimulation of cell cycle but not due to induction of apoptosis. Furthermore, bioimformatics analysis suggested concomitant activation of NF-κB and β-catenin/TCF signaling pathways, which were confirmed by reporter gene assays. Moreover, blocking experiments demonstrated the involvement of both pathways in the growth promotion effects of Fbxw11. Our study highlights the mechanism of Fbxw11 on the development of lymphocytic leukemia and implies possible approaches for leukemia therapy.

P0459

Marek's disease virus type 1 MicroRNA miR-M2-5p increases p63 expression by targeting RBM24 and promotes the proliferation of chicken embryo fibroblast cells

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Marek's disease (MD) is a lymphoproliferative disease of chickens caused by Marek's disease virus (MDV). Similar to the other herpesviruses, MDV encodes its own small non-coding regulatory RNAs termed microRNAs (miRNAs). The Meq-clustered miRNAs encoded by MDV-1 have been suggested to play critical roles in the induction of T-cell lymphomas. We have previously found that the deletion of miR-M2 from MDV genome significantly reduced the mortality of virus-infected birds, coupled with the obvious reduction of gross tumour incidence. However, the underlying regulatory mechanism mediated by miR-M2 mature miRNAs requires further investigation. Using the technique of hybrid-PCR and DNA sequencing, we have presently identified the RNA-binding protein 24 (RBM24) as a

DOI: 10.1002/eji.201970400

critical target for miR-M2-5p. We found that during MDV infection, down-regulation of RBM24 expression by miR-M2-5p promotes the proliferation of primary chicken embryo fibroblasts (CEFs), with a significant increase of p63 expression. The biological function of p63 is complex due to the presence of multiple isoforms with opposing functions, which playing a critical role in skin development, aging, metabolism, and tumorigenesis. Our data suggests that the modulation of p63 by miR-M2-5p targeting RBM24 may be one of the important strategies for MDV-1 to trigger the development of MD lymphomas.

P0460

Bioinformatic Analysis and Mechanism Study of Expression Relationship between HSP90AA1 and DAB2IP in Colorectal Cancer

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Loss of DAB2IP, a novel tumor suppressor gene, is associated with the high risk of aggressive CRC. Therefore, the identification of DAB2IP-related genes and signaling pathways will elucidate the exact mechanisms of development, metastasis of CRC. The purpose of this study is to explore the key genes associated with DAB2IP and study the regulatory mechanisms in CRC. We conducted bioinformatics analysis of 355 colon cancer samples provided by the public database of the Gene Expression Omnibus, screening out a key gene, HSP90AA1, negatively related with DAB2IP.Complementary functional HSP90AA1 knockdown experiments were performed. The regulation of molecular mechanism between HSP90AA1 and DAB2IP in CRC was determined. HSP90AA1 was upregulated in colorectal cancer and knockdown of HSP90AA1 expression in colon cancer cells reduced cell apoptosis and inhibited cell growth and migration. In addition, downregulation of HSP90AA1 increased the sensitivity of cells to 5-FU. Expression of DAB2IP is decreased in colorectal cancer and inversely correlates with HSP90AA1 expression. Mechanistically, DAB2IP antagonizes the activation of Protein kinase B (AKT) thereby decreasing the expression of HSP90AA1. Moreover, we demonstrated that DAB2IP and HSP90AA1 are involved in ASK1/JNK apoptosis signaling pathway. DAB2IP promoted cell apoptosis by inducing ASK1 activation, while HSP90AA1 acted the opposite effect. Together, our results indicated that high HSP90AA1 expression is significantly correlated with colorectal cancer progression. DAB2IP regulates the expression of HSP90AA1 through AKT. DAB2IP and HSP90AA1 play opposite role on ASK1/JNK apoptosis pathway in CRC. In addition, DAB2IP activation combined with HSP90AA1 inhibition may be a new therapeutic strategy to combat CRC.

P0461

Evaluation of expression of tumor-associated antigens and cytogenetic characteristics of tumor cells in various forms of urothelial cancer invasion

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Malignant transformed cells of some tumors express immunogenic cancer-testicular antigens (CTAs), which can be a target for immunotherapy. CTAs expression and its relationship with chromosomal mutations (CM) in urothelial cancer (UC) are poorly understood.
Materials and methods: 24 tumor samples with different degrees of invasion were studied: 18(75%) muscle-invasive (MIF) and 6 (25%) muscle non-invasive (MNIF) forms of UC. Immunological and statistical methods were used.

DOI: 10.1002/eji.201970400

Results: It was revealed that with the MIF UC expression of NY-ESO-1 was in 7 (38.9%); MAGE in 15 (83.3%); GAGE in 8 (44.4%) and BAGE in 9 (50%) cases. 2 samples had 4 CTAs (11.1%), 5-three of 4 CTAs (27.8%), 16 - expression of the one CTA was detected (88.9%). But with MNIF UC CTAs expressions were recorded in rare case. GAGE or 2 CTAs - in 33.3%, in 66.7% - one of the 4 studied CTAs was presented. CM research showed the deletion of the 9th chromosome (Ch) 66.7 %, the lack of Y-Ch (50 %), the monosomy of 13th and 17th Ch (33.3%). The increasing of CM was associated with increase of invasion's degrees and malignancy of UC that significantly correlated ($p \leq 0.05$) with the decrease of the CTAs expression.
Conclusions: CTAs expression on UC cells depends on its degree of malignancy, invasion and metastatic propensity. Differences in CM in various clinical forms of UC were revealed, which is promising for the timely selection of the most effective treatment.
The publication has been prepared with the support of the «RUDN University Program 5-100».

P0462

Changes in subsets of T lymphocytes, macrophages, dendritic cells and Th1/ Th2/Th17 cytokines in PBMC and tissue of Peruvian patients with mammary carcinoma

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Breast cancer (BC) is second neoplasm in Peru (19.5%). We evaluated immunocompetent populations percentages and Th1/Th2/Th17 profile both in peripheral blood (PB) and tumor microenvironment (TM) of Peruvian patients in different stages of BC recently diagnosed and without treatment. PB and biopsies samples were used, obtained at National Institute of Neoplastic Diseases (INEN, 2018), diagnosed with BC in stages II (n=6), III (n=8) and IV (n=4). We obtained PB mononuclear cells (PBMC) and TM mononuclear cells (TMNC). In both cases cells were cultured 24h and then flow cytometry was performed for macrophages M1 (CD68+), M2 (CD163+), T cells (CD4+/CD8+) and myeloid dendritic cells (DCm, CD11c+) and plasmacytoid (DCp, CD11c-). In addition, culture supernatants were obtained to measure IFN-g/TNF-α/IL-2; IL-4/IL-6/IL-10 and IL-17 by CBA (Cytometric Bead Array) Becton Dickinson. We demonstrated a clear M2 polarization in TM (> 60% stages II, II and IV) that starts in PB from stage III and IV (< 20%). Although there are no differences in cellular frequencies between healthy controls and BC stages; however, there are differences between PB and TM, there is more CD4+ (III) than in TM and more CD4+ than CD8+ in PB. In both PB and TM, DCp (CD11c-) frecuency are or tend to be higher than DCm (CD11c+) (II, III and IV). There is a tendency towards a Th2 profile. We concluded that M2 polarization, CD8+ lower frecuency and DCp higher frecuency, as well as Th2 tendency are mechanisms to avoid the attack of the immune system.

P0463

Single-cell analysis illuminates dysfunctional CD8+ T cells as a proliferative, dynamically regulated compartment within human melanoma

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DOI: 10.1002/eji.201970400

Tumor immune cell compositions play a major role in response to immunotherapy but the heterogeneity and dynamics of immune infiltrates in human cancer lesions remain poorly characterized. Here we identify conserved intratumoral CD4 and CD8 T cell behaviors in scRNA-seq data from 25 melanoma patients. We discover a large population of CD8 T cells showing continuous progression from an early effector "transitional" into a dysfunctional T cell state. CD8 T cells that express a complete cytotoxic gene set are rare, and TCR sharing data suggest their independence from the transitional and dysfunctional cell states. Notably, we demonstrate that dysfunctional T cells are the major intratumoral proliferating immune cell compartment and that the intensity of the dysfunctional signature is associated with tumor-reactivity. Our data demonstrate that CD8 T cells previously defined as exhausted, are in fact a highly proliferating, clonal and dynamically differentiating cell population within the human tumor microenvironment.

P0464

Programming of macrophages by apoptotic cancer cells inhibits cancer progression and metastasis via PPARγ/PTEN signaling

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Apoptotic cell clearance by phagocytes is essential in tissue homeostasis. We demonstrated that conditioned medium (CM) from macrophages exposed to apoptotic cancer cells inhibits TGFb1-induced epithelial-mesenchymal transition (EMT), migration, and invasion of cancer cells. Apoptotic 344SQ (ApoSQ) cell-induced PPARg activity in macrophages caused increased PTEN levels. PTEN was recovered in the insoluble fraction with the exosomal markers, such as CD63, CD81, and CD9. Transmission electron microscopy revealed the isolates contained nano-sized vesicles and the number of vesicles in a unit area appeared to be decreased by treatment with GW4869. While the size distribution of exosomes from macrophages with or without GW4869, an inhibitor of exosome biogenesis/release, did not change, the concentration of exosomes present was decreased upon GW4869 treatment, secreted in exosomes. Exosomal PTEN was uptaken by recipient lung cancer cells. ApoSQ-exposed CM from PTEN knockdown cells failed to enhance PTEN in 344SQ cells, restore cellular polarity, and exert anti-EMT and anti-invasive effects. Moreover, single injection of ApoSQ cells inhibited lung metastasis in syngeneic immunocompetent mice with enhanced PPARg/PTEN signaling both in tumor-associated macrophages and tumor cells. PPARg antagonist GW9662 reversed PPARg/PTEN signaling, reduction of EMT-activating transcription factors, such as Snai1 and Zeb1, and anti-metastatic effect of ApoSQ injection. Thus, the injection of apoptotic lung cancer cells may offer a new strategy for the prevention of lung metastasis.

P0465

Natural killer cells control the lymph nodes melanoma metastatic spread: the blood CCR7+ NK cells abundance marks melanoma evolution and reveals a CCL19-dependent metastatic pathway

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DOI: 10.1002/eji.201970400

Immune checkpoint blockade therapy has changed prognoses for many melanoma patients. However, immune responses that correlate with clinical progression of the disease are still poorly understood. To identify immune responses correlating with melanoma clinical evolution, we analyzed first the NK cells subsets immune response in melanoma patient´s metastatic lymph nodes. Moreover the serum cytokines as well as circulating NK and T cell subpopulations from melanoma patients were analyses in melanoma patients at stage IV treated with anti immune checkpoints therapy and melanoma patients at stage III. The patients' immune profiles suggested that melanoma progression leads to changes in peripheral blood NK and T cell subsets. Stage IV melanoma was characterized by an increased frequency of $CCR7^{+}CD56^{bright}$NK cells as well as high serum concentrations of the CCR7-ligand CCL19. CCR7 expression and CCL19 secretion were also observed in melanoma cell lines. The $CCR7^{+}$melanoma cell subpopulation co-expressed PD-L1 and Galectin-9 and had stemness properties.nalysis of melanoma-derived cancer stem cells (CSCs) showed high CCR7 expression; these CSCs were efficiently recognized and killed by NK cells. An accumulation of $CCR7^{+}$, PD-$L1^{+}$and Galeactin-9^{+}melanoma cells in melanoma metastases was demonstrated *ex vivo*. Altogether, our data identify biomarkers that may mark a CCR7-driven metastatic melanoma pathway and suggest a new NK cells role in controlling the melanoma progression in the patient´s lymph nodes.

P1038

Deubiquitinase MYSM1 inhibits human colorectal cancer tumorigenesis by facilitating the expression of miR-200 and blocking PI3K/AKT signaling

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Backgrounds: Human colorectal cancer (CRC), characterized by a high morbidity and lethality, seriously threatens human health and lives. MYSM1 is frequently reported to regulate the homeostasis of lymphatic and hematopoietic system, but its function and mechanism in tumor, especially in CRC, are still unclear.
Methods: Expression of MYSM1 was detected in TCGA and GEO databases, patient specimens and CRC cells. Overall survival of the CRC patients was also determined according to the MYSM1 expression. Gain- and loss-of-function assays were used to identify the intrinsic role of MYSM1 in CRC *in vitro* and *in vivo*. qRT-PCR, Western blot, ChIP and restoration assays were occupied to confirm the downstream biotargets of MYSM1.
Results: MYSM1 was significantly lower in CRC specimens and cells than that in each normal group, and constrained multiple malignant phenotypes of CRC cells. Kaplan-Meier analysis indicated a positive correlation between MYSM1 and prognosis of CRC patients. MYSM1 directly bound to the promoter regions of miR-200 family members and CDH1, simultaneously influenced the epigenetic modifications, thus suppressing the initiation of epithelial-mesenchymal transition. In addition, MYSM1 could inhibit the PI3K/AKT signaling and play a role in regulating the progress of CRC.
Conclusions: MYSM1 is a CRC inhibitor that suppresses the epithelial-mesenchymal transition through directly binding to the promoter region of miR-200 family members and CDH1, and blocks the transduction of PI3K/AKT signaling, thus disrupting CRC development and progression. Thus, strategies to increase mysm1 expression may help reduce CRC progression and distant metastasis.

DOI: 10.1002/eji.201970400

P1039

TRIM28 knockdown increases sensitivity to etoposide by upregulating E2F1 in non-small cell lung cancer

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Tripartite motif containing 28 (TRIM28) is a universal corepressor for Kruppel-associated box zinc finger proteins. In our previous study, it was shown that expression of TRIM28 is upregulated in non-small cell lung cancer (NSCLC) cell lines and tissues. Here, we demonstrated that the stable silencing of TRIM28 expression by a specific siRNA lentivirus vector increased the sensitivity of NSCLC cells to chemotherapeutic agent etoposide. Combination of TRIM28siRNA and etoposide significantly inhibited the growth and proliferation of lung adenocarcinoma PAa cells and exerted obvious antitumor effects in nude mice. Using FCM and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay, we found that TRIM28siRNA in combination with etoposide increased apoptosis in vitro and in vivo which was induced by E2F1 activity, since the expression of E2F1 and its target genes was significantly increased in the cotreatment group. Cell proliferation and apoptosis were almost completely abolished in the PAa cells cotreated with TRIM28 siRNA and etoposide following knockdown of E2F1. The results of our study demonstrated that the combination of TRIM28 siRNA and etoposide may be effective against NSCLC and has the potential of being a new therapeutic tool for future treatment.

P1040

Nuclear-localized costimulatory molecule 4-1BBL mediates colon cancer cell proliferation and migration by regulating nuclear Gsk3β, and is linked to the poor outcomes of colon cancer

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4-1BBL is a type II transmembrane costimulatory molecule that can mediate the interaction between antigen-presenting cells and T cells by triggering 4-1BB/4-1BBL bidirectional signaling. Here, we found 4-1BBL molecules localized in the nuclei of colon cancer cells. 4-1BBL knockout (KO) increased the accumulation of Gsk3β in the nuclei of colon cancer cells and consequently decreased the expression of Wnt pathway target genes. 4-1BBL KO arrested colon cancer cell proliferation, migration, and invasion in vitro, and growth in vivo. 4-1BBL is predominantly localized in the nuclei of cancer cells in clinical colon cancer specimens. High levels of nuclear-localized 4-1BBL are positively correlated with tumor size, lymph node metastasis, clinical stage, and a lower survival ratio. Our data suggest that 4-1BBL disappeared from the surface of colon cancer cells, which might avoid triggering the 4-1BB signaling that mediates antitumor immunity in the tumor microenvironment. The nuclear localization of 4-1BBL could decrease the expression level of nuclear Gsk3β molecules and, consequently, enhance the biological activity of the Wnt pathway, mediating the growth, metastasis, and invasiveness of colon cancer cells. Furthermore, nuclear-localized 4-1BBL might be an indicator of colon cancer malignancy and serve as a promising target of immunotherapy.

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DOI: 10.1002/eji.201970400

P1041

Chance to control the cancer in rein - spontaneous regression of lung carcinoma (1988-2018) a 30 year perspective

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Spontaneous tumor regression is an extremely rare phenomenon in the oncology field and even rarer for lung cancer. However, the underlying mechanism is poorly understood. Summarizing available clinical information and supposed potential mechanism as possible may shed new light on lung cancer therapy strategies in the era of immunotherapy blooming. We conducted a PubMed search using the retrieval tactics ("Lung Neoplasms"[Mesh]) AND "Neoplasm Regression, Spontaneous"[Mesh] for reports from 1988 to January 2018, and all references in the relevant literature were subsequently investigated for relevance. Using the criterion of Everson and Cole, 14 cases were finally defined as spontaneous regression as were reviewed in the research. The information regarding patient characteristics, treatments and follow-up has been summarized. The mechanism of spontaneous regression has been discussed to date and presumed to be due to many factors. In this review, we found the spontaneous lung cancer regression cases fall into two categories concerning: (1) neurologic disorders in 6 cases, half of them suffered with paraneoplastic neurological syndromes (PNS) and (2) immunological reactions in 7 cases. For 1 case, we failed to identify possible influence factors with limited data available. Spontaneous regression of lung carcinoma is realistic but rare. The data deposition for more spontaneous regression cases and more detailed information will definitely help understand the mechanism for body's surveillance system - cancer balance, creating a big chance to boom cancer immunotherapy.

P1042

Demethylation-mediated upregulation of melanoma-associated antigen-A11 correlates with malignant progression of esophageal squamous cell carcinoma

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Background: The expression and methylation status of oncogenes are closely related to the onset and progression of cancer.
Aims: To explore the role and methylation status of melanoma-associated antigen-A11 in the pathogenesis of esophageal squamous cell carcinoma.
Methods: 116 esophageal squamous cell carcinoma patients with tumor tissues and corresponding adjacent normal tissues were obtained. The expression level and methylation status of melanoma-associated antigen-A11 in esophageal cancer cell lines and esophageal squamous cell carcinoma tissues were determined respectively.
Results: Significant up-regulation of melanoma-associated antigen-A11 was detected in esophageal cancer cell lines and esophageal squamous cell carcinoma tissues. Up-regulation of melanoma-associated antigen-A11 contributed to proliferation and invasion in cancer cells. Hypomethylation of the CpG site was associated with pathological differentiation, clinical stage, tumor size, lymph node metastasis and distant metastasis. Esophageal squamous cell carcinoma patients in stage III and IV, with high expression of melanoma-associated antigen-A11 or hypomethylation of the CpG site within the promoter demonstrated poor survival.
Conclusion: Melanoma-associated antigen-A11 is up-regulated in esophageal squamous cell

DOI: 10.1002/eji.201970400

carcinoma at least partly by hypomethylation of the CpG site within the promoter and this hypomethylation may affect the prognosis of esophageal squamous cell carcinoma patients.

P1043

Effect of the Hsp90 inhibitor KW-2478 on HepG2 cells

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The study were to investigate the effects of proliferation, apoptosis, cycle, invasion, and senescence of KW-2478 on HepG2 cells, to explore the related mechanism of apoptosis and the cell cycle.HepG2 cells and HL-7702 were cultured with KW-2478, at different doses and for different times, in vitro. The MTT assay was used to detect the effect of KW-2478 on proliferation of HepG2 and HL-7702 cells. Flow cytometry was used to determine the effects of KW-2478 on the cell cycle and apoptosis of HepG2 cells. The Transwell assay was used to determine the effect of KW-2478 on cell invasion. The β-galactosidase assay tested the effect of low-dose KW-2478 on the senescence of HepG2 cells. Western blotting and the quantitative polymerase chain reaction were used respectively to assess changes in protein and mRNA levels of related factors in HepG2 cells after the KW-2478 treatment.·KW-2478 significantly inhibited proliferation of HepG2 cells but did not inhibit proliferation of HL-7702 cells. KW-2478 induced apoptosis and cell cycle arrest of HepG2 cells, and inhibited the invasion of HepG2 cells; low dose KW-2478 promoted HepG2 senescence. KW-2478 inhibited the proliferation of HepG2 cells, induced apoptosis and cell cycle arrest, inhibited invasion, and promoted senescence. However, it had no inhibiting effect on HL-7702 cells. KW-2478 affected the expression of related factors in the mitochondrial apoptotic signaling and cell cycle-related regulatory pathways. KW-2478 downregulated the expression of STAT3, which is a key factor in the JAK-STAT pathway, indicating that KW-2478 may affect the function of HepG2 cells by downregulating STAT3.

P1044

Effect of LncRNA AK093407 on colorectal cancer cell line HCT-116

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Colorectal cancer (CRC) is a common malignant tumor of the digestive system. Long-chain non-coding RNA (lncRNAs) has been proved to play an important role in CRC.In preliminary experiment we found that there was high expression of LncRNA AK093407 in CRC tissue. The present study evaluated the effects and mechanism of lncRNA AK093407 in CRC. For silencing AK093407, cells were transfected using siRNA .Cell cycle and apoptosis were measured using Annexin V/PI staining assay. Then the cell cycle and the expression of apoptosis-related RNA and protein (caspase3,caspase9,caspase10,p21,p27,cyclinA, cyclinB) were detected by qrt-PCR and western blot respectively. The results showed that LncRNA AK093407 was highly expressed in CRC cells and tissues. The levels of cyclin-associated proteins p21, p27, cyclin A and cyclinB after silencing LncRNA AK093407 were lower than those before transfection, suggesting that LncRNA AK093407 silencing induced cell cycle arrest. PI staining also confirmed that silencing LncRNA AK093407 induced cell cycle arrest and that the arrest might occur in G1 S phase. At the same time, the level of apoptosis-related protein caspase3,caspase9,caspase10 increased significantly after silencing LncRNA AK093407. The results of AnnexinV/PI staining showed that the percentage of apoptotic cells was significantly increased after AK093407 was silenced, which indicated that silencing LncRNA AK093407 could induce apoptosis. It can be concluded that there is a high expression of LncRNA

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DOI: 10.1002/eji.201970400

AK093407 in hct-116. When LncRNA AK093407 is silenced, apoptosis and cell cycle arrest can be induced.

P1045

The aryl hydrocarbon receptor mediates tobaccoinduced PD-L1 expression and is associated with response to immunotherapy

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Whether tobacco carcinogens enable exposed cells immune escape resulting in carcinogenesis, and why patients who smoke respond better to immunotherapies than non-smokers, remains poorly understood. Here we report that cigarette smoke and the carcinogen benzo(a)pyrene (BaP) induce PD-L1 expression on lung epithelial cells in vitro and in vivo, which is mediated by aryl hydrocarbon receptor (AhR). Anti-PD-L1 antibody or deficiency in AhR significantly suppresses BaP-induced lung cancer. In 37 patients treated with anti-PD-1 antibody pembrolizumab, 13/16 (81.3%) patients who achieve partial response or stable disease express high levels of AhR, whereas 12/16 (75%) patients with progression disease exhibit low levels of AhR in tumor tissues. AhR inhibitors exert significant antitumor activity and synergize with anti-PD-L1 antibody in lung cancer mouse models. These results demonstrate that tobacco smoke enables lung epithelial cells to escape from adaptive immunity to promote tumorigenesis, and AhR predicts the response to immunotherapy and represents an attractive therapeutic target.

P1046

Research of cancer-derived IgG expression, bio-funtion and preliminary mechanism in prostate cancer

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Objective: The aim of this study was to investigate the expression of cancer-derived immunoglobulin G (CIgG) in prostate cancer and its role in the biological behavior of prostate cancer.
Methods: The expression of CIgG in prostate cancer tissue of 106 cases were detected by immunohistochemistry. The CIgG protein and mRNA in prostate cancer cell lines were detected by RT-PCR and Western blot, respectively. We investigated the migratory and invasive of abilities of prostate cancer cells in vitro and vivo after knocking down and over-expression of CIgG in prostate cancer cells. In addition, we invested the mechanisms of promoting migration and invasion.
Result: It was found that CIgG was widely expressed in prostate cancer, moreover, which correlated with pathological grade, clinical stage and metastasis. The results of experiments in vitro showed that CIgG prompted the proliferation ability, migration and invasive ability of prostate cancer. *Importantly, the results of experiments in vivo showed that a small number of CIgG high cancer cells displayed stronger tumorigenesis ability in NOD / SCID cells than that of CIgG low cancer cells. In addition, we found that CIgG expression could be upregulated by androgen.*
Conclusions: Our data suggested that CIgG has application potential for therapy of prostate cancer as a novel target.

DOI: 10.1002/eji.201970400

P1047

Expression of WT1 gene and clinical significance in childhood acute B-cell lymphocytic leukemia of different immunophenotypes

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Objective: To investigate the expression level and clinical significance of WT1 gene in pediatric patients with B-ALL of different immunophenotypes.
Methods: Expression level of WT1 was detected by real-time quantitative PCR in a total of 77 children patients with newly diagnosed B-ALL in order to compare the differences of the age, gender, immunophenotype, karyotype and clinical risk group among the patients. And the correlation between gene expression and prognosis were observed during a follow-up.
Results: Among 77 cases, WT1 expression positive rate was 96.10%. Younger patients play a higher WT1 level than elder one(p=0.02), but there was no differences between the boys and girls(p=0.229). WT1 showed a highest expression level in Pro-B-ALL (CD34+, CD10-, cIg-), and a lowest expression level in Com-B-ALL (CD34+, CD10+, cIg-). Patients whose WBC more than $50*10^9$/g（p=0.009）at diagnosis, or had translocation of chromosome 4 and 11(p=0.034), or in high risk group in clinic(p=0.041), had a higher expression level of WT1 gene. There were no significant differences in morphology between the remission group and non-remission group after the fisrt chemotherapy(p=0.84). But after a long term follow-up, it showed that patients had a lower WT1 expression level at diagnosis showed a better prognosis, while the higher ones preferred to relapse.
Conclusions: CD10- B cell indicated a high WT1 expression level, the expression level of WT1 maybe corelated with leukemia cell differeation state in childhood acute B-ALL and also showed an close relationship with prognosis.
Keywords: Wilms tumor gene 1 (WT1 Gene)；Childhood acute B-cell lymphocytic leukemia；Immunophenotype of B-ALL

P1048

Exosomes containing miR-142-5p remodel lymphatic vessels and induce IDO to promote immune privilege in the tumor microenvironment

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Purpose: Tumor-associated lymphatic remodeling plays important roles in tumor progression and anti-tumor immunity. We investigate the critical role of miR-142-5p derived from cancer-secreted exosome in lymphatic endothelium cells(LECs) in antitumor immunity through upregulation indoleamine 2,3-dioxygenase(IDO) and facilitating cancer progression and metastasis.
Methods: Exosomes isolated from cervical squamous cell carcinoma (CSCC) patients´ serum or medium from cultured CSCC celllines were purified and characterized by ultracentrifugation and electron microscopy. Gene expression levels and activity of signaling pathways were detected by QT-

DOI: 10.1002/eji.201970400

PCR, immunoblot, ELISA, luciferase assy, *in-situ* hybridization and immunochemistry. IDO activity was assessed for cultured cells and clinical serum samples using HPLC. $CD8^+$ T cells dysfunction was evaluated using flowcytometry.
Results: High levels of miR-142-5p positively correlated with IDO expression in tumor-associated lymphatic vessels in advanced CSCC. miR-142-5p transferring from CSCC cells into LECs induced IDO expression in LECs. IDO^{high} LECs exhausted $CD8^+$ T cells when co-cultured, but IDO inhibitor revered it. AT-rich interactive domain-containing protein 2 (ARID2), upstream mediator of IFN-γ, was the direct target of miR-142-5p. Re-expression or knockdown of ARID2 in CSCC exosome treated LECs respectively reverse or enhance $CD8^+$T cell exhaustion. Increased serum exosomal miR-142-5p, as well as corresponding IDO activity, positively correlated with CSCC progression.
Conclusion: Cancer cells instruct LECs to express immune checkpoint protein IDO, by delivering miR-142-5p via exosomes. miR-142-5p activates ARID2-IFN-γ signaling thus induces IDO and promotes $CD8^+$ T cells exhaustion. Our study suggests that LEC is an integrated component of immunosuppressive tumor microenvironment and provides potential targets for diagnosis and treatment of CSCC.

P1049

Deleting MyD88 signaling in myeloid cells promotes development of adenocarcinomas of the colon

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Intestinal myeloid cells are not only essential for keeping local homeostasis, but also play an important role in regulating the occurrence of colitis and colitis-associated cancer (CAC). In these diseases, the manner in which the myeloid cells work and which molecular pathways influence them are still not fully understood. In our study, we discovered that MyD88 signaling in colonic myeloid cells participates in the development of CAC. Myeloid MyD88-deficient mice showed greater susceptibility to azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced CAC, as evidenced by the increase in the number and sizes of tumors. Myeloid MyD88 deletion markedly increased production of pro-inflammatory and pro-tumor cytokines; recruitment of more IL-1β producing-neutrophils in colon from bone marrow; increased in epithelial cell apoptosis and decreased in epithelial cell proliferation; enhancement of colon mucosal expression of COX-2, p-STAT3, β-catenin, and cyclinD1; induction of further DNA damage and β-catenin mutation. To sum up, these results suggest that myeloid MyD88 signaling protects the intestine from tumorigenesis during the development of CAC.
Supported by 2015CB943203.

DOI: 10.1002/eji.201970400

P1050

Cancer-Derived IgG Expression is associated with poor prognosis in patients with pancreatic cancer

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Background: CIgG has been detected in various cancers and can promote carcinogenesis; however, its clinical significance in pancreatic cancer (PC) remains unclear. This study aimed to clarify the clinical significance of cancer-derived immunoglobulin G (CIgG) in patients with PC.
Methods: Using tissue microarrays (TMAs) and immunohistochemistry, CIgG expression and the infiltration of tumor-infiltrating B cells (B-TILs) were assessed in 326 patients from two independent cohorts who underwent surgical resection for PC. The associations of these parameters with clinicopathological features and clinical outcomes were evaluated.
Results: High expression of CIgG was related to poor tumor differentiation in both the training cohort ($P< 0.001$) and the validation cohort ($P=0.033$). Multivariate Cox regression analysis identified CIgG expression and the N stage as two independent prognostic factors for overall survival (OS) in both the training cohort (hazard ratio (HR)=2.88, $P< 0.001$; HR=1.66, $P=0.017$, respectively) and the validation cohort (HR=1.96, $P=0.004$; HR=1.46, $P=0.077$, respectively). B-TILs were not associated with CIgG expression or OS but potentially contributed to the unfavorable prognostic value of CIgG expression ($P_{interaction}< 0.05$).
Conclusion: CIgG expression in PC was validated to be a powerful poor prognostic factor of survival outcomes.

P1051

CD59 strengthens Cbp/ PAG inhibitory effect on jurkat through palmitoylation sites

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Cbp(C-terminal Src kinase-bingding protein),also known as PAG (phosphoprotein associated with glycosphingolipid-enriched microdomains), has no catalytic activity by itself and can negatively regulates the activity of Src family kinase by binding to other proteins. The proximal membrane region of the intracellular domain of Cbp/PAG contains two potential palmitoylation sites(-CxxC-), and a palmitoylation group is also present in the GPI portion of CD59. Previous studies found that CD59 can be clustered on the cell membrane in the lipid raft region after cross-linking with CD59 monoclonal antibody. In this study, two viral particles of Cbp/PAG overexpressing and mutated Cbp/PAG palmitoylation sites were constructed and transfected into Jurkat to study the relationship between Cbp/PAG and CD59 in signaling pathway, metabolism and apoptosis. We found a significant correlation between the location of Cbp/PAG and CD59 which were co-expressed in the same region of cell membrane before mutation, implicating a potential overlap of the elicited signaling pathways. In addition, Cbp/PAG overexpression reduced the proliferation of Jurkat cells, and CD59 further enhanced the inhibitory effect of Cbp/PAG on Jurkat cells, rather than after mutation. Thus, Cbp/PAG acts as a negative regulator of cellular signaling, and CD59 enhances its negative feedback regulation through palmitoylation sites.

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DOI: 10.1002/eji.201970400

P1052

Downregulated STAG2 expression in de novo acute myeloid leukemia

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Background: STAG2 is a gene that encodes a component of cohesion complex participated in regulating sister chromatid separation during mitosis. It was likely to act as a “caretaker” tumor suppressor gene when inactivated.
Method: As it has been unknown whether STAG2 gene might be responsible for the occurrence and prognosis of acute myeloid leukemia (AML), we analyzed the relative expression levels of STAG2 in 138 AML patients by quantitative reverse-transcription polymerase chain reaction. 17 healthy volunteers were acted as normal controls. In order to understand whether STAG2 gene had something to do with the prognosis, we divided the AML patients into three groups according to risk status based on cytogenetics and molecular abnormalities. And the relative expression levels of STAG2 were also compared between them.
Results: The relative expression levels of STAG2 in AML patients were not normal distribution, while the levels of STAG2 in healthy volunteers were normally distributed. STAG2 expression levels in AML patients were significantly lower than those in healthy volunteers. No significant differences in sex distributions of AML, and no significant differences between risk status based on cytogenetics and molecular abnormalities.
Conclusion: Our data demonstrated reduced expression of STAG2 gene in AML, and indicated that STAG2 gene might play an important role in the development of AML, but had nothing to do with the prognosis.
Keywords: STAG2; acute myeloid leukemia; prognosis; mutation; deletion

P1053

T and B cell deficiencies advance lingual carcinogenic progression by malignant cells spindle transformation and diffuse invasion

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Immunodeficiency turns down the level of immunological surveillance and contributes to carcinogenic progression. However, mechanisms underlying pro-tumor programming of neoplastic tissues due to immunodeficiency remain unknown. Using the 4NQO BALB/c mice (immunocompetent mice), BALB/c nude mice (T cells immunodeficient mice), and CB-17SCID mice (T and B cells immunodeficient mice) model of tongue squamous carcinogenesis, we reported that the development of cancer and local lymph metastases in immunodeficient mice were earlier than that of immunocompetent mice. We also found that maligant tissue invade into adjacent muscle through the pattern formed in a broad, pushing manner with a smooth border in BALB/c mice. By contrast, diffuse growth with tiny sheets of several maliganat cells were widely existed in BALB/c nude mice and CB-17SCID mice. Meanwhile, the shape of these metastatic cells was also lack of polygonal trait in BALB/c nude mice and CB-17SCID mice. In

DOI: 10.1002/eji.201970400

conclusion, immunodeficiency in T or both T and B cells could remarkably advance carcinogenic progression and lymph node metastasis by regulating the spindle transformation of tumor cell and diffuse growth invasion, the mechanism of which will be warranted.

P1054

Predictive value of soluble CD25 during chemotherapy in Breast cancer patients

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Introduction: Breast cancer (BC) is the most common cancer among women. Immunotherapy will be associated to current therapies such as chemotherapy. A better understanding of chemotherapy's effects in immune system is important step and potential biomarker. Treg cells expressing CD25 (IL2-Rα), were widely associated to cancer development and may be influenced by the chemotherapy.
Objectives: To highlight immunological biomarkers in BC, we assessed the serological profile of soluble IL2-Rα and the clinical evolution during chemotherapy.
Methods: Fourty-four (45) Senegalese women with BC treated with Doxorubicin/cyclophosphamide (3 cycles /patient) and 45 healthy women controls (HC) were selected. Peripheral blood samples were taken before each cycle and levels of IL2-Rα were evaluated by ELISA.
Results: Before treatment; levels of IL2-Rα were similar between patients and HC and we did not evidenced significant relationships between IL2-Rα concentrations and tumors' characteristics. However, IL2-Rα levels increases significantly after the first cycle of chemotherapy in the group of patients. This variation concerns patients that having a recurrent BC after the third cycle of chemotherapy. Compared to partially or completely recovered women, patients with recurrent BC, have shown the highest levels of IL2-Rα after the third cycle of chemotherapy ($p = 0.034$). In the group of recovered patients, no significant variation was found during the treatment.
Conclusion: Ours findings show the prognostic value of IL2-Rα levels during BC treatment. and moving towards further exploration of Treg cells such as $CD3^+CD4^+CD25^+Foxp3^+$ and cytokines release.

P1055

Relationship between levels of plasmatic galectin-3 and T cells apoptosis and activation in senegaleses women with breast cancer during chemotherapy

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We investigated relationship between galectin-3 (Gal-3) levels and T lymphocytes apoptosis and activation rates in breast cancer during chemotherapy.
We used plasma samples from 112 women classified in to two groups: 70 women with breast cancer (BC) and submitted to neoadjuvant chemotherapy (3 cycles) and 42 healthy women used as controls (HC). In the group of BC, blood samples were taken before each cycle of chemotherapy and Gal-3 levels was evaluated by ELISA sandwich. Flow cytometry was used to study T cells apoptosis and activation.

DOI: 10.1002/eji.201970400

Before treatment, median values of Gal-3 was 6.31 ng/ml (range 1.07 - 50.74) in BC *and* 0.84 ng/ml (range 0.00 - 4.82) in HC. Gal-3 levels was highest in plasmas from women patients ($p< 0.001$). During the same period, proportions of apoptotic T lymphocytes were highest in BC compared to HC ($p< 0.05$). Similar results were observed about T cells activation. According to clinical symptoms, we observed, in the groups of patients with a low or null response to chemotherapy, a positive correlation between gal-3 levels and tumour size ($rho= 0.48; p= 0.010$). In addition we found a dynamic relationship between gal-3 levels, tumor size and T lymphocytes apoptosis rates during treatment depending to the cure efficiency.
We suggest gal-3 plasma concentration could be used as predictive biomarker for chemotherapy efficiency in breast cancer patients.

P1057

Complete workflows allow comprehensive tumor microenvironment analysis and culture of cell subsets of limited tumor patient samples

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Immunotherapy has proven clinical efficacy and tremendous potential. But clinical benefit is experienced by only a subset of patients, such that additional research is necessary to improve outcomes. In particular, analyze steady-state anti-tumor immunity and monitor the effects of therapy on the tumor microenvironement. However, TIL numbers can be very low. Flow cytometry phenotyping different cell populations requires dividing limited tumor material into multiple samples, reducing the number of cells available for analysis. Therefore, innovative tools and workflows are needed to maximize the quality of data obtained from limited tumor patient samples.
We established workflows combining tissue storage, dissociation, cell isolation and analysis. We developed new reagents for magnetic cell sorting, incorporating novel technology enabling the removal of magnetic beads and antibody fragments (REAlease®). Tumor-infiltrating (TI) T-, B-, Myeloid cells, cancer-associated fibroblasts and tumor cells were sequentially isolated and characterized by flow cytometry, multi-parameter imaging and transcriptomics. Importantly, purification of TI T cells with high viability was essential for high-resolution immune profiling by single cell transcriptomics.
In conclusion, we optimized workflows for the comprehensive analysis of the tumor microenvironement. Sequential isolation of the different cell types maximized the number of cells available for analysis. These workflows reduce experimental time and allow the performance of more complex experimental setups. We believe the use of these innovative tools and workflows can significantly increase the quality of the data obtained in immunooncology and immunotherapy research.

P1058

Rab37 in lung cancer skews macrophages towards tumor-suppressing phenotype via exocytosis of soluble ST2

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DOI: 10.1002/eji.201970400

Interplay between cancer epithelial cells and the surrounding immune cells shape the tumor microenvironment to promote cancer progression. Tumor-associated macrophages (TAM) are well-accepted immune populations that play crucial roles in promoting cancer invasion and metastasis. Accumulating evidence also indicates implication of Rab small GTPase-mediated exocytosis in tumorigenesis. However, it is not clear that the mechanisms for Rab-mediated exocytosis in regulation of macrophage polarization. Here, we report a novel mechanism for regulation of macrophage polarization in tumor microenvironment by Rab37-mediated exocytosis. We identified a new Rab37 trafficking cargo soluble ST2 (sST2), which skewed macrophage polarization toward anti-tumoral M1 phenotype *in vitro*. We also showed that dynamic movement of sST2 via Rab37-containing vesicles was in a nucleotide-dependent manner. We further demonstrated that Rab37-mediated sST2 secretion significantly increased the ratio of M1 v.s. M2 in xenografts and thus reduced tumor growth. Moreover, lung cancer patients with low Rab37, low sST2, and low ratio of M1 v.s. M2 macrophages expression profile correlated with worse overall survival examined by Kaplan-Meier survival analysis. Multivariate Cox regression analysis showed that this Rab37-sST2-M1/M2 expression profile predicted the poor prognosis. Our findings reveal a novel regulation of cancerous Rab37 in microenvironmental macrophages polarization, which preferentially shifts to anti-tumoral phenotype and thereby suppresses lung tumor growth.

P1638

Syncytin induces apoptosis in mouse lymphoma cell line EL4 by promoting mitochondrial depolarization

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Syncytin is the capsule protein of the human endogenous retroviral W family， which is involved in human placental morphogenesis. It has been reported that the expression level of syncytin is associated with the progression of leukemia. But the exact function and mechanism of syncytin on leukemia cell remains unclear. Here， we transfected the mouse lymphoma cells EL4 stablely with syncytin and assessed its effects on cell apoptosis. We demonstrated that overexpression of syncytin enhanced cycloheximide-induced cell apoptosis in EL4 cells. Further， we confirmed that syncytin initiated caspase-3 pathway apoptosis by the depolarization of the mitochondrial membrane potential and releasing the pro-apoptotic protein, but not by the PKC kinase pathway. Collectively, this study provides insights into the function of snycytin involved in leukemia cell apoptosis. It is of great significance in explaining the involvement of human endogenous retrovirus genes in the carcinogenesis of leukemia and in finding new targets for the diagnosis and drug treatment of leukemia.

P1639

Activation of hepatic stellate cells enhanced metastatic potential of liver cancer cell after radiotherapy

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The recurrence and metastasis after radiotherapy in liver cancer was originally thought to be caused by the invasiveness and metastasis ability of the residual cancer cells. But recently, new researches revealed that this phenomenon was also closely related to the enhanced metastatic potential of

DOI: 10.1002/eji.201970400

residual cancer cells triggered by radiotherapy. Changes in the liver microenvironment supplied the "soil" for promoting the metastatic potential of liver cancer. Our previous research found that the activation of hepatic stellate cells by radiotherapy was an important change in the microenvironment of liver cancer. Therefore, we speculate that the activation of hepatic stellate cells may be involved in the process of radiotherapy promoting the metastatic potential of liver cancer. In this study, we found that co-culture human hepatoma cell lines (HCC-LM3, Hep-3B, SK-Hep-1) with irradiation-activated human hepatic stellate cell line (LX2)for 48 hours using transwell chambers could significantly increased the migration and invasion ability of liver cancer cells. Adding the culture supernatant of activated hepatic stellate cells alone was able to enhance the migration and invasion ability of liver cancer cells. Conversely, co-culture with irradiated liver cancer cells or adding their culture supernatants can enhance the migration ability of LX2. Flow cytometry showed that radiotherapy upregulated the expression of α-SMA and desmin in LX2 cells, as well as ICAM1 and TRAILR. These results indicated that the enhanced metastatic potential of liver cancer cell after radiotherapy was related to the activation of hepatic stellate cells.

P1640

Human leukocyte antigen F is a potential prognostic biomarker for glioma patients

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HLA-F (Human leukocyte antigen F) belongs to the non-classical MHC-I molecules, which are responsible for regulating the immune system. It has been reported that HLA-F is involved in regulating microenvironment of tumors. However, there is rarely knowledge about the correlation between HLA-F and gliomas, which are the most common primary tumors of the central nervous system in adults.
In our present study, a total number of 180 patients with gliomas were used to analyze the expression of HLA-F by multiple staining using the Tyramide Signal Amplification Systems. We also investigated its correlation with clinical features and prognosis. Our results showed that glioma patients with low expression of HLA-F had higher tumor recurrence and overall survival. In conclusion, in the present study, we evaluated HLA-F expression in a large number of glioma patient biopsies and demonstrated that their expression correlates with tumor recurrence and overall survival of the patients. Our study suggested that HLA-F could predict the immune state of glioma patients and might be prognostic biomarkers for clinical diagnosis.

P1641

Effect of immunomodulatory factors on prognosis of renal clear cell carcinoma and its mechanism

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Objective: we applied pathological tissues of patients with ccRCC to investigate the controversial relationship between PD-L1 expression and clinical prognosis in ccRCC and to explore the regulatory mechanism of PD-L1 in ccRCC.Then we analyzed the regulatory molecules and the survival prognosis of PD-L1.Finally, We selected differential display genes in ccRCC by Bioinformatics methods and analyzed their relationship with tumor immune infiltration cells and survival prognosis.
Methods: ccRCC cell lines were used to explore the regulatory mechanism of PD-L1 in the hypoxic environment, and tissue samples were used to detect the expression of PD-L1 and its regulatory molecules in ccRCC and its influence on the prognosis of patients. and GEO database was used for

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DOI: 10.1002/eji.201970400

comprehensive bioinformatics analysis to screen out the differential display hub genes in ccRCC tissues and paracancer tissues, and their relationship with immune infiltrating cells and prognosis were analyzed.
Results: By inhibiting HDAC activity, hypoxia enhanced the acetylation level of histone H3K9 in ccRCC cell lines and up-regulated the expression of PD-L1.The expression of PD-L1in ccRCC is positively correlated with HIF-2α and Histone H3K9ac. PD-L1and Histone H3K9ac are independent prognostic factors of ccRCC.ADCY7 and LPAR5 were up-regulated in ccRCC, while CXCL12, BDKRB2, CASR and KNG1 were down-regulated in ccRCC.ADCY7 and LPAR5 were positively correlated with tumor infiltrating lymphocytes and correlated with poor prognosis.

P1642

Preferential expression of B7-H6 in glioma stem-like cells enhances tumor cell proliferation via the c-myc/RNMT axis

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B7 homologue 6 (B7-H6), a newly identified member of the B7 costimulatory molecule family, is not only a crucial regulator of NK cell-mediated immune responses through binding to NKp30 but also has clinical implications due to its abnormal expression in human cancers. Here we show that B7-H6 expression is abnormally upregulated in glioma tissue and that B7-H6 is coexpressed with stem cell marker Sox2. Intriguingly, B7-H6 was rarely detected on the surface of glioma cell lines but was abundantly expressed in glioma stem-like cells (GSLCs) that were derived from the glioma cell line U87 in *vitro*. Surprisingly, B7-H6 was the only one that was preferentially expressed in the GSLCs among the B7 family members. Functionally, knockdown ofB7-H6 in GSLCs by siRNAs led to the inhibition of cell proliferation, with decrease in the expression of the oncogene Myc via the PI3K/Akt and ERK/MAPK signaling pathways. Moreover, we determined that three genes CBL(Casitas B-Lineage Lymphoma Proto-Oncogene), CCNT1(Cyclin T1) and RNMT(RNA guanine-7 methyltransferase) were coexpressed with B7-H6 and c-myc in glioma tissues samples from the TCGA database and found however that only RNMT expression was inhibited by the knockdown of B7-H6 expression in the GSLCs, suggesting the involvement of RNMT in the B7-H6/c-myc axis. Extending this to 293T cells, we observed that knocking out of B7-H6 with CRISPR-Cas9 system also suppressed cell proliferation. Thus, our findings suggest B7-H6 as a potential molecule for glioma stem cell targeted therapy.

P1643

The stemness-related transcription factors SOX2 and OCT4 are essential for the regulation of IgG gene expression in non-small cell lung cancer cells

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Immunoglobulins are known to be synthesized and secreted only by B lymphocytes. However, recent study revealed that cancer cells with stemness features can overexpress IgG, which contributes to the initiation and metastasis of cancer cells, and involves the maintenance of cell stemness. However, the mechanisms underlying the regulation of IgG gene expression in cancer stem cells remain elusive.

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DOI: 10.1002/eji.201970400

Previous studies have shown that stemness-related transcription factors SOX2 and OCT4 regulatory elements were located in the Ig VH gene promoter, suggested that they may regulate IgG gene expression.
In this study, we confirmed that the co-location of SOX2 and IgG in non-small cell lung cancers by Immunohistochemistry, both of them were observed in the stem/progenitor cells and bronchial basal cells in lung adenocarcinoma, or cancer cells in lung squamous sell sarcinoma. Additionally, using a series of Ig VH 5'-truncated luciferase reporter constructs, we found that Ig VH promoter in cancer cell lines was bound and driven by the SOX2 and OCT4, and the activity of the Ig VH promoter and Ig VH gene transcription were attenuated after knockdown of SOX2 or OCT4 by specific small interfering RNAs. Importantly, ChIP assays confirmed that the OCT4 and SOX2 bound directly to the octamer and SOX2 motif located within the promoter region of the Ig VH gene. These results indicate that SOX2 and OCT4 were crucial transcriptional factor for Ig gene transcription and suggested a distinct regulatory mechanism for Ig gene expression in cancer stem cells.

P1644

TFDP3 promotes the immune escape by up-regulating the expression of PD-L1 on breast cancer cells

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PD-L1 is an immune inhibitory molecular that leads to T cell dysfunction and apoptosis by binding to PD-1 expressed on activated T cells. TFDP3, a cancer-testis antigen, plays an important role in cell growth and differentiation by binding to E2F molecules. At present, it´s not quite clear in details how TFDP3 takes part in the process of tumor development and progression. In our present work, the relationship between the expression of TFDP3 and PD-L1 in breast cancer, was studied through the analysis of TCGA and GDC database, and the immunohistochemical analysis of 198 tissue samples from human breast cancer. As the results, we found that the expression of TFDP3 and PD-L1 in breast cancer are positively correlated, and the expression of TFDP3 is also correlated with the TNM stage of breast cancer. The samples from late period showed higher expression level of both TFDP3 and PD-L1, 228 and 3.17 times higher, respectively. Besides, the expression of the two molecules also affects the survival period of breast cancer patients according to our data. In conclusion, it suggests that TFDP3 is a potential key transcription factor in the immune escape due to its roles in the expression regulation of the immune checkpoint molecules, such as PD-L1. Moreover, our previous results showed that TFDP3 may inhibit the function of pRb in breast cancer cells by binding to E2F molecules. Therefore, it is promising that TFDP3 may be a potential target molecules in immunotherapy for breast cancer patients who are received PD-1/PD-L1 inhibitor treatment.

P1645

ISG20 enhances tumor microenvironment and contributes to poor survival in pancreatic cancer

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Pancreatic cancer (PC) remains one of the most challenging solid tumors to treat with a high unmet medical need as patients poorly respond to standard-of-care-therapies. Metastasis is the fatal hallmark

DOI: 10.1002/eji.201970400

of pancreatic cancer and the tumor escape mechanism, the most complex step in the metastatic cascade, remain poorly defined. The cross-talk of tumor cells and tumor-associated macrophages (TAMs) in the tumor microenvironment (TME) play a significant role in tumor immune escape and progression. To identify the key cellular mechanisms that induce an immunosuppressive tumor microenvironment, we adopted co-culture model with pancreatic cancer cells and tumor-associated macrophages. The relationship between tumor cells and TAMs were analyzed by performing tissue microarrays and immunohistochemistry assays using pancreatic cancer tissues from human surgical specimen and mice, along with co-culture, immunofluorescence, invasion assays using pancreatic cancer cell lines. Interferon-stimulated gene of 20 kDa protein (ISG20) turned out to be the key orchestrators in pancreatic tumor microenvironment that regulate the relationship between pancreatic cancer cells and tumor-associated macrophages. Poorer survivals have been seen in patients with higher levels of ISG20. The inhibited invasiveness and metastases have been observed in ISG20 inhibition group both in vivo and in vitro. We show for the first time in vitro and ex vivo that ISG20 seriously affects pancreatic tumor microenvironment by interactions with TAMs. The knockout of ISG20 causes a significant decrease of their interactions. These data strongly support the view that targeting ISG20 may represent a promising new option for the treatment of pancreatic cancer.

P1646

SAE1 protein is overexpressed in lung squamous cell carcinoma and is associated with immunosuppressive status of lung squamous cell carcinoma

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The small ubiquitin-modifying enzyme(SUMO) modification is an important post-translational modification process for proteins, SUMO activating enzyme subunit 1 (SAE1) is the first enzyme in the process of SUMOylation of proteins and an important target for tumor therapy. In this study, the expression levels of SAE1、CD8 and Foxp3 in non-small cell lung cancer (NSCLC) tissues were examined to investigate the effect of SAE1 expression on the immune status of tumor patients.
We used tissue microarray and immunohistochemical staining to detect the expression levels of SAE1, CD8 and Foxp3 in 136 cases of NSCLC and analyzed the relationship between SAE1 and CD8、Foxp3 expression and clinical parameters such as pathological types.
The expression levels of SAE1, CD8 and Foxp3 in NSCLC tissues were significantly higher than those in normal lung tissues. The expression of CD8 was negatively correlated with SAE1, and the expression level of Foxp3 was positively correlated with SAE1. The positive expression rate of SAE1 and Foxp3 in lung squamous cell carcinoma (SCC) tissues was significantly higher than that in lung adenocarcinoma (ADC) tissues, suggesting that high expression of SAE1 may lead to immunosuppression in patients with lung squamous cell carcinoma. That is, SAE1-mediated protein SUMO modification may play a role in tumor immune escape in patients with lung squamous cell carcinoma.

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DOI: 10.1002/eji.201970400

P1647

CD24 is a potential target of non-small cell lung cancer

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Antibody therapy targeting tumor cell surface molecules has achieved remarkable clinical progress. Lung cancer is the leading cause of cancer mortality worldwide, with non-small cell lung cancer (NSCLC) comprising the vast majority (about 80%~85%). However, only gefitinib has been developed to target *EGFR*-mutant NSCLC, there remains no other specific membrane surface target for NSCLC. Our data reveals that CD24, a small GPI-anchored membrane protein, is frequently overexpressed in patients with NSCLC. We found CD24 is overexpressed in human lung cancer cell lines, including NCI-H292, A549. The tumor tissue of NSCLC patient-derived-xenograft model also overexpressed CD24. Simultaneously, we detected the mouse lung cancer cell line LLC and tumor tissue of $Kras^{G12D}$ driven lung adenocarcinoma expressed high levels of CD24, while the lung and liver of normal Balb/c mouse don`t express CD24. Subsequently, we purified three anti-CD24 monoclonal antibodies which could inhibit the proliferation of A549 cells *in vitro*. In summary, we demonstrated CD24 is overexpressed in NSCLC, and anti-CD24 monoclonal antibodies may be as a potential drug to against NSCLC.

P1648

Lipolysis stimulated lipoprotein receptor down-regulates CD8+ T cells and promotes invasion and metastasis of ovarian cancer

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Aim: To study the effect of lipolysis stimulated lipoprotein receptor (LSR) on the development of ovarian cancer and the mechanism of immunity.
Methods: The correlation between lipolysis-activated lipoprotein receptor and ovarian cancer was analyzed by TCGA database, and the expression of LSR in ovarian cancer tissue was studied by immunohistochemistry. The effects of LSR on invasion and metastasis of ovarian cancer cells were observed by transwell assay and subcutaneous tumorigenesis in nude mice using LSR stable interference/overexpression cell lines. We also explore the mechanism of LSR and its correlation with immune cells.
Results: The TCGA database showed that the high expression of LSR was associated with poor prognosis of ovarian cancer ($P < 0.001$). The expression of LSR in ovarian cancer tissues was significantly higher than that in normal ovarian tissues ($P < 0.01$). In vitro and in vivo invasion experiments showed that LSR promoted invasion and metastasis of ovarian cancer. **The mechanism study found that LSR was negatively correlated with CD8 + T cell infiltration** ($P < 0.05$).
Conclusion: LSR down-regulates CD8 + T cells and promotes invasion and metastasis of ovarian cancer.

DOI: 10.1002/eji.201970400

P1649

TAK1 drives immunoresistance in KRAS mutant colorectal cancer

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Mutations in the KRAS oncogene represent one of the most prevalent genetic alterations in colorectal cancer (CRC), the third leading cause of cancer-related death in the world. Besides their well-characterized function in tumor progression, KRAS mutations can induce tumor immunoresistance by interrupting immune checkpoint and cytokines directly or via downstream signal pathways. The transforming growth factor-beta (TGF-beta)-activated kinase 1 (TAK1) is not only required for survival of KRAS-dependent CRC, but also a key regulator in inflammatory and immune signaling pathways. Targeting TAK1 may be a potential therapeutic strategy to overcome tumor immunoresistant and aberrant cell proliferation exhibiting KRAS mutation activation in CRC.
In this study, the subpopulations of CD3+ T cells from mesenteric lymph node were investigated in an azoxymethane/dextran sodium sulfate-induced chemically colitis-associated carcinoma mouse model. The percentage of CD69 was significantly increased as well as PD-1 was upregulated in Villin-Cre/LSL-K-Ras^{G12D} mice. But the changes of CD69 and PD-1 did not occur in wild type mice. After inhibiting TAK1 by a small molecular inhibitor NG25, the percentage of apoptotic KRAS mutant CRC cells was positively correlated with host immunity. Blocking TAK1 by NG25 can modulate immune related-cytokines to recruit CD3+T cells and induce their differentiation. Downregulation of PD-1 expression by NG25 restored the function of T cells.
The above data suggested that KRAS mutation generated immunoresistant during tumor progression. TAK1 may be a key regulator of tumor immune responses in KRAS mutant colorectal cancer.

P1650

Tumor cells release secretory autophagosomes to promote immunosuppression through induction of suppressive immune cells

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The presence of several subsets of suppressive immune cells, including regulatory T cells, TAMs and MDSCs, contribute to a strongly immunosuppressive tumor microenvironment (TME), which inhibits antitumor immune responses. However, the conditions and mechanisms that promote the development of these cells are not completely understood.
The extracellular secretory autophagosomes were isolated and identified from the supernatant of tumor cells or malignant effusions and ascites of cancer patients (termed as TRAPs). We found that TRAPs, with sizes ranging from 300 to 900nm, carry the autophagosomal marker LC3-II, but not exosomal markers CD63 or TSG101, which was completely distinct from exosomes (40-100nm). Our studies have verified that TRAPs can be taken up by B cells, subsequently induce IL-10 production, which potentially suppresses T-cell proliferation and antitumor responses. We subsequently demonstrated that TRAPs were a novel mechanism exploited by tumor cells for immune suppression, including inducing M2 polarization as reflected by increased expression of PD-L1 and IL-10 through TLR4-mediated MyD88-p38-STAT3 signaling pathway. Moreover, treatment of human neutrophils with TRAP promoted the generation of reactive oxygen species through macropinocytosis, contributing to the inhibition of T-cell activation and proliferation in a ROS- dependent manner. Recently, we found that TRAP could directly induce the differentiation of M-MDSC through TRAP-TLR2-PD-L1 axis, and indirectly induce the differentiation of PMN-MDSC by TRAP-induced cytokines. Together, our results have demonstrated that tumor-released autophagosomes could be a major

DOI: 10.1002/eji.201970400

driver of immunosuppression in the TME and provided justification for therapeutic targeting of TRAP in patients.

P1651

YAP is essential for Treg mediated suppression of anti-tumor immunity

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Regulatory T cells (Tregs) are critical for maintaining self-tolerance and immune homeostasis, but their suppressive function can impede effective anti-tumor immune responses. Foxp3 is a transcription factor expressed in Tregs that is required for their function. However, the pathways and microenvironmental cues governing Foxp3 expression and Treg function are not completely understood. Herein, we report that Yes-associated protein (YAP), a co-activator of the Hippo pathway, is highly expressed in Tregs and bolsters Foxp3 expression and Treg function in vitro and in vivo. This potentiation stemmed from YAP-dependent upregulation of Activin signaling which amplifies TGFβ/SMAD activation in Tregs. YAP-deficiency resulted in dysfunctional Tregs unable to suppress anti-tumor immunity or promote tumor growth in mice. Chemical YAP antagonism and knockout or blockade of the YAP-regulated Activin Receptor similarly improved anti-tumor immunity. Thus we identify YAP as an unexpected amplifier of a Treg-reinforcing pathway with significant potential as an anticancer immunotherapeutic target.

P1652

Nonsense-mediated mRNA decay is exploited for immune evasion during colorectal cancer metastasis

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Immunosuppression microenvironment allows primary tumor growth, while tumor-inherent factors that bring about immune evasion during metastasis remain elusive. Here, we observed a stronger nonsense-mediated mRNA decay (NMD) activity with a higher expression of up-frameshift protein 1 (UPF1) in colorectal cancer (CRC) metastasis than in matched primary cancer cells. In metastatic CRC SW620 cells, the higher UPF1 expression was found to arise from the increased stability of UPF1 by ubiquitin specific peptidase 10 (USP10)-mediated deubiquitination. In contrast, in primary CRC SW480 cells, autophagy promotes proteolysis of ubiquitinated UPF1 and inhibits NMD activity. Interestingly, in SW620 cells NMD selectively controlled a cluster of immune-related genes including ICAM1. Furthermore, NMD inhibition exposed SW620 cells but not SW480 cells to natural killer (NK) cell-mediated cytolysis that depended on ICAM1.
Conclusion: NMD activity can be recommissioned in CRC metastasis cells to escape from NK-dependent immune surveillance, and inhibiting NMD to boost anti-tumor immunity could be a novel strategy to treat CRC metastasis.

DOI: 10.1002/eji.201970400

P1653

Runx2 deficiency in osteoblasts induces an immunosuppressive bone microenvironment and promotes myeloma metastasis to bone

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Multiple myeloma (MM) is a plasma cell malignancy that thrives in the bone marrow (BM), with frequent progression to new local and distant bone sites. Our previous studies demonstrated that MM cells at primary sites secrete soluble factors and suppress osteoblastogenesis via the inhibition of Runt-related transcription factor 2 (Runx2) in pre- and immature osteoblasts (OBs) in new bone sites, prior to the arrival of metastatic tumor cells. However, it is unknown whether OB-Runx2 suppression in new bone sites feeds back to promote MM dissemination to and progression in these areas. Hence, we developed a syngeneic mouse model of MM in which Runx2 is specifically deleted in the immature OBs of C57BL6/KaLwRij mice (OB-Runx2$^{-/-}$ mice) to study the effect of OB-Runx2 deficiency on MM progression in new bone sites. In vivo studies with this model demonstrated that OB-Runx2 deficiency attracts MM cells and promotes MM tumor growth in bone. Mechanistic studies further revealed that OB-Runx2 deficiency induces an immunosuppressive microenvironment in BM by increasing the concentration and activation of myeloid-derived suppressor cells (MDSCs). This effect associates with reduced expression of activation markers and enhanced expression of exhaustion markers in cytotoxic CD8^{+} T cells. In contract, gemcitabine-induced MDSC depletion in OB-Runx2$^{-/-}$ mice prevented these effects and inhibited MM tumor growth in BM. These novel discoveries demonstrate that OB-Runx2 deficiency in new bone sites promotes MM dissemination and recruits MDSCs that suppress the antitumor immune response in BM. Depletion of MDSCs by gemcitabine may improve the outcome of MM treatment.

P1655

Interaction between 17β-estradiol, prolactin, and human papillomavirus to induce E6/E7 transcript, modulate the expression and localization of hormonal receptors,and cytokine production

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Human papillomavirus (HPV) type 16 and 18 are the most frequent in cervical cancer (CC) their oncogenes E6 and E7 contribute to the malign transformation.HPV is insufficient to develop CC, other cofactors, such as hormonal ones, are necessary. The estrogen and prolactin receptors´ expression augments as the disease progresses, however the mechanism of cooperation between these hormones and HPV has not been unexplored. This study aimed to evaluate if the oncogenes modulate the expression of ERα, ERβ, GPER, and PRLR, also if these hormones regulate the oncogene expression, as well as to analyze the cytokines profile in a model of keratinocytes transduced with E6 or E7 oncogenes of HPV-16 or -18. The expression of E6/E7 oncogenes was evaluated by qPCR in HeLa andSiHa cell lines treated with 17β-estradiol and prolactin. Hormonal receptors´expression and location were evaluated by Western blot and Immunofluorescencein HaCaT cells transduced with E6

DOI: 10.1002/eji.201970400

and E7 HPV 16/18. Cytokines were quantified by ELISA Multiplex. 17β-estradiol and prolactin induce E6/E7 oncogenes expression in HeLa and SiHa cells. E6 and E7 of HPV16/18 increased the expression of ERα, GPER, and PRLR, only ERβ was positively regulated by E6 oncogenes of HPV16/18. Besides, some of these oncogenes modify the location of PRLR toward cytoplasm, and estrogen receptors mainly to nucleus. Both hormones increase G-CSF, IL-7, and IL-8 production in HaCaT cells transduced with E6/E7 of HPV-16. These results suggest that the mutual regulation between E2, PRL, and HPV oncogenes could cooperate with the carcinogenesis process in CC.

P1656

Upregulation of DAP10 adaptor protein by MICA in NKG2D positive cervical cancer cells

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The induction of MICA and MICB by virus infected cells or tumour cells has been previously reported, and the ability of these ligands to induce cytotoxic activity in NK cells through the interaction with their cognate receptor NKG2D has been well established. Previously we have showed that epithelial cervical cancer cells express de activating NKG2D receptor. In addition, in normal human lymphoid cells the association of DAP10 with NKG2D is essential for signalling and important for its cell surface expression. However, the mechanism of the NKG2D/DAP10 complex upregulation is not completely understood in cancer. This work was undertaken to address the role of MICA in the regulation of DAP10 in cervical cancer cells. Here, we demonstrate the presence of the NKG2D/DAP10 complex in different tumour cell lines. Also, we demonstrate that MICA upregulates the expression of DAP10 in cervical cancer cells in a time dependent manner. We found that the AKT kinase is constitutively phosphorylated and MICA induced an increase in tyrosine phosphorylation. Furthermore, this activation is independent of the PI3K in cervical cancer cell lines. Finally, the possibility that the NKG2D-DAP10 complex is widely expressed in different types of cancer may confer an advantage to transformed cells to survive in the tumour microenvironment and escape from the immune surveillance is discussed.

P2230

Combined expression of B7-H4 and PD-L1 in ovarian cancer detected by immunohistochemistry: association with clinicopathological features

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Background: B7-family ligands have been confirmed to play an important role in negatively regulating the T-cell mediated anti-tumor immunity. These inhibitory molecules are also aberrantly expressed on various human cancers, and significantly associated with cancer progression and poor prognoses.
Methods: Here, the expression of members in B7-family, including PD-L1 and B7-H4 in 46 ovarian cancer (OC) samples was determined by immunohistochemistry. Then, we tried to analyze the value of their combined expression on clinicopathological features for OC patients.
Results: The data showed that PD-L1 and B7-H4 were expressed in the tumor cells, and their expression was restricted to cell membrane and cytoplasm. Membranous and cytoplasmic staining for PD-L1 (22/46, 37/46) and B7-H4 (7/46, 41/46) of tumor cells was detected in OC cases. Interestingly, membranous B7-H4 is co-expression with membranous PD-L1 in carcinoma cells. The status of these patients with high membranous expression of B7-H4 was positively associated with high membranous expression of PD-L1 (P=0.01). Moreover, the high membranous expression of B7-H4 and PD-L1

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DOI: 10.1002/eji.201970400

showed limited co-expression in clear cell OC cases. We also found that the combined expression of both PD-L1 and B7-H4 could be used as a valuable risk factor for predicting the prognosis of OC patients.
Conclusion: Taken together, our study indicated that variable expression and clinical role of B7-family ligands in OC with predominant expression of the candidate target PD-L1 and B7-H4. It was suggested that membranous expression of B7-family ligands can be more effectively used as a clinic marker in OC patients compared to cytoplasmic expression.

P2231

Soluble and membrane anchored calreticulin fragment 39-272 promote B16 melanoma malignancy in vivo in distinct mechanisms

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Calreticulin (CRT), a multifunctional Ca^{2+}-binding glycoprotein mainly located in the endoplasmic reticulum, is a tumor-associated antigen that has been shown to play protective roles in angiogenesis suppression and anti-tumor immunity. We previously reported that soluble CRT (sCRT) was functionally similar to heat shock proteins or damage-associated molecular patterns in terms of ability to activate myeloid cells and elicit strong infammatory cytokine production. In the present study, B16 melanoma cell lines expressing recombinant CRT fragment 39-272 (sCRT/39-272) in secreted form (B16-CRT), recombinant CRT fragment 39-272 (sCRT/39-272) anchoring on membrane (B16-tmCRT) or recombinant enhanced green uorescence protein (rEGFP) (B16-EGFP), were constructed for investigation on the roles of sCRT and in tumor development. When s.c. inoculated into C57BL/6 mice, the B16-CRT cells and B16-tmCRT were signicantly more aggressive (in terms of solid tumor growth rate) than B16-EGFP controls in a TLR4 dependent manner. The B16-CRT-bearing mice showed increased $Gr1^{+}$ MDSC inltration in tumor tissues, accelerated proliferation of $CD11b^{+}Ly6G^{+}Ly6C^{lo}$ (G-MDSC) precursors in bone marrow, and higher percentages of G-MDSCs in spleen and blood, but B16-tmCRT-bearing mice did not affect MDSCs. In *in vitro* studies, recombinant sCRT/39-272 was able to promote migration and survival of tumor-derived MDSCs *via* interaction with TLR4, inhibit MDSC differentiation into DC, and also elicit expression of inflammatory proteins S100A8 and S100A9 which are essential for functional maturation and chemotactic migration of MDSCs. B16-tmCRT could induce polarization of macrophage towards M2. Our data provide solid evidence for CRT as a double-edged sword in tumor development.

P2232

Omega-3 fatty acids inhibit natural killer cell cytotoxicity suppression of multiple myeloma exosomes via altering NKG2D-ligand interactions

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Multiple myeloma (MM) is a common hematological malignancy of plasma cells. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are among the most studied polyunsaturated fatty acids (PUFA) for which various anti-cancer properties have been explained. Acknowledging previous findings that exosomes released from tumor cells including MM play a critical role in tumor survival and progression, in the present study we checked as to whether exosomes released from a myeloma

DOI: 10.1002/eji.201970400

cell line (L363) can affect the function of natural killer cells and that the treatment of L363 cells with sub-lethal doses EPA and DHA can alter the release and function of MM-derived exosomes. Based on our findings, pre-treatment with MM-derived exosomes could significantly reduce the cytotoxic function of NK cells as evidenced by a co-culture system with K562 cells, indicating an immunosuppressive role for these nano-sized vesicles. Interestingly, exosomes from EPA/DHA-treated MM cells had a reduced ability to exert such an immunosuppressive effect. This effect was vanished in the presence of an NKG2D-blocking antibody, indicating a possible role for the involvement of NKG2D ligands in this immunosuppression. Furthermore, we found that EPA/DHA-treated L363 cells could release higher amounts of exosomes compared to the untreated cells. It can be concluded that exosomes released from MM cells play a role in the immunosuppressive microenvironment of MM and that treatment with EPA/DHA can reverse the immunosuppressive effects of MM exosomes. These findings provide evidence on the beneficial effects of PUFAs on the MM microenvironment. Further studies are warranted to explore the precise mechanisms involved.

P2233

HPV and HLA-G mediated immune modulation in head and neck cancer: a study from Northeast India

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HLA-G is an immunomodulator and tumor-mediated ectopic expression of HLA-G is documented to promote carcinogenesis. Considering the role of oncogenic HPV strains and HLA-G in tumorigenesis, the study was designed to understand the role of HPV and HLA-G in immunoregulation in Head and Neck Cancer. Our study cohort comprised of histopathologically confirmed HNSCC patients and samples were collected after obtaining written informed consent. Expression of HLA-G isoforms, immunoregulatory cytokines, chemokines and HPV association were determined by transcript expression and protein quantification. Expression of HLA-G (p=0.002) and sHLA-G isoforms (87.5%) were higher with HPV positivity. Increased expressions of IL-10, TGF-β, and PD1 (fold change - 3.62, 26.66 and 5.70 respectively) were observed in HPV negative tumors while in HPV positive tumors these cytokines were downregulated except proinflammatoryIFN-γ (2.12 fold). HPV positive tumors showed distinct chemokine expression with higher expression of CCL3 and CCL7 (fold change - 26.02 and 38.71) and low levels of CCL5, CCL2 and CX3CL1 (fold change - 0.41, 18.32 and 2.39 fold respectively). Stratifying samples on basis of HPV positivity and sHLA-G status, it was noted that when HPV was present with sHLA-G, studied immunoregulatory cytokines were downregulated whereas, IFN-γ was overexpressed. HPV positive but sHLA-G negative samples showed downregulation of IFN-γ suggesting IFN-γ expression was sHLA-G driven. Difference in chemokine profile with HPV and sHLA-G positivity suggested recruitment of different macrophage populations to tumor tissue. Our data provides evidence for harnessing of sHLA-G mediated pathways by HPV to modulate inflammation for its growth and immune escape.

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DOI: 10.1002/eji.201970400

P2234

Cancer stem cells with high aldehyde dehydrogenase activity in human renal cell carcinoma induce immunosuppression

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Background: Cancer stem cells (CSCs) of renal cell carcinoma (RCC) were considered to be responsible for driving tumor growth. However, the mechanistic contribution of CSCs to tumor-induced immune suppression remains elusive.
Methods: A subpopulation of cells with a high aldehyde dehydrogenase (ALDH) enzymatic activity from RCC xenografts was isolated by using ALDEFLUOR Assay and fluorescence-activated cell sorting (FACS). Flow cytometry was used to characterize the immune phenotype of these cells. Cytokines in culture supernatants of $ALDH^+$ cells and $ALDH^-$ cells were determined by enzyme-linked immunosorbent assay (ELISA). Functional immunosuppressive characteristics were characterized by prompting of T-cell apoptosis, induction of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Treg cells).
Results: In this study, we identified a highly tumorigenic subpopulation of RCC cells with increased ALDH activity using a xenograft model. The $ALDH^+$ cells expressed low levels of HLA-I, HLA-DR. In addition, the $ALDH^+$ cells produced significantly higher levels of GM-CSF and TGF-beta than the $ALDH^-$ cells. Furthermore, $ALDH^+$ cells have been shown to not only more strongly trigger T-cell apoptosis, but also to induce MDSCs and Treg cells more efficiently as compared with $ALDH^-$ cells.
Conclusions: Overall, our findings revealed that CSCs of RCC may contribute to immune suppression by multiple mechanisms.
Keywords: Renal cell carcinoma, aldehyde dehydrogenase, cancer stem cells, immunological phenotype, tumor immune evasion

P2235

Hepatectomy contributes to recurrence of hepatocellular carcinoma via IL-11-STAT3 signaling pathway

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Background: Patients undergoing surgical resection of hepatocellular carcinoma (HCC) are at risk of recurrence, but the mechanism remains poorly understood.
Methods: Through the analysis of gene expression profiles in tumor tissues and matched normal tissues from patients with hepatocellular carcinoma (HCC), we identified difference in interlukine-11 (IL-11) expression. Besides, we utilized a genetic mouse model, orthotopic tumor model, chemically induced model, and orthotopic allograft model to study the correlation between IL-11 and postsurgical recurrence. Furthermore, a series of experiments including histology and immunohistochemistry analysis, three-dimensional culture, immunofluorescence, western blotting, enzyme-linked immunosorbent assay (ELISA) and flow cytometry were conducted to investigate the role of IL-11-STAT3 signaling in recurrence.
Results: We demonstrate that IL-11 levels increase after surgery and trigger HCC outgrowth.

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DOI: 10.1002/eji.201970400

Accordingly, pharmacologically blocking IL-11-STAT3 signaling in these models significantly alleviates tumor cell proliferation and suppresses postsurgical recurrence.
Conclusions: These results establish inhibition of IL-11-STAT3 signaling as a potential therapeutic strategy to prevent recurrence after surgical HCC resection.

P2236

SOX9/miR-203a axis drives PI3K/AKT/mTOR signaling to promote esophageal cancer progression

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Deregulation of SOX9 in esophageal cancer has been reported. However, the regulatory mechanisms underlying SOX9 during esophageal squamous cell carcinoma (ESCC) progression remain poorly understood. Here, we independently confirmed the increased SOX9 expression in two ESCC cohorts and its correlation with poor prognosis. We demonstrated that SOX9 was required for maintaining self-renewal, motility, and chemoresistance in vitro and that ectopic expression of SOX9 promoted tumorigenicity in vivo. Transcriptome-based screening revealed that the mTOR inhibitor rapamycin profile matched the SOX9-associated gene expression signature in ESCC cells and public data sets. Rapamycin treatment reversed SOX9-mediated malignant phenotypes. Mechanistically, SOX9 activation caused repression of miR-203a transcription by binding to miR-203a promoter, thus preventing the miR-203a-mediated inhibition of multiple PI3K/AKT/mTOR components, including *PIK3CA, AKT2*, and *RPS6KB1*. The association between SOX9 expression and PI3K/AKT/mTOR signaling was further validated in clinical samples. SOX9-mediated WNT pathway activation also partly contributed to promoting cell proliferation. Moreover, rapamycin treatment potentiated cisplatin-mediated inhibition of tumor growth in xenografts. Together, these findings uncover a novel activation of the PI3K/AKT/mTOR pathway by the SOX9/miR-203a axis and define a subgroup of patients who may benefit from mTOR-targeted therapy.

P2237

lncRNA-PDPK2P promotes hepatocellular carcinoma progression through the PDK1/AKT/caspase 3 pathway

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Hepatocellular carcinoma (HCC) is one of the malignancies with the worst prognosis. Long noncoding RNAs (lncRNAs) are emerging as an important regulator that regulates gene expression and function, leading to the development of cancer. The aim of this study was to determine the relation between lncRNAs and HCC and to further guide clinical therapy. lncRNAs in HCC and adjacent tissues was screened and the correlation between lncRNA-PDPK2P expression in liver tissues and the pathological characteristics and severity of HCC was assessed by real time PCR. The effects of

DOI: 10.1002/eji.201970400

PDPK2P on HCC proliferation, apoptosis, metastasis and invasion was also systematically investigated *via* CCK-8 assay, flow cytometer, scratch wound healing and transwell assay, separately. The relationship between PDPK2P and PDK1 was verified by RNA pull down, rescue experiment and western blot. lncRNA-PDPK2P was highly expressed in HCC tissues with a distinct positive correlation between PDPK2P and PDK1, and that upregulation was clinically associated with a larger tumor embolus, low differentiation and poor survival of HCC. Mechanistically, lncRNA-PDPK2P interacted with PDK1, and promoted HCC progression through the PDK1/AKT/caspase 3 signaling pathway. lncRNA-PDPK2P can promote HCC progression, suggesting it may be a clinically valuable biomarker and serve as a molecular target for the diagnosis, prognosis and therapy of hepatocellular carcinoma.

P2238

BAP31, a newly defined cancer/testis antigen, regulates proliferation, migration, and invasion to promote cervical cancer progression

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Malignant tumors typically undergo an atavistic regression characterized by the overexpression of embryonic genes and proto-oncogenes, including a variety of cancer/testis antigens (CTAs) that are testis-derived and are not expressed or expressed in trace amounts in somatic tissues. Based on this theory, we established a new method to identify unknown CTAs, the spermatogenic cells-specific monoclonal antibody-defined cancer/testis antigen (SADA) method. Using the SADA method, we identified BAP31 as a novel CTA and confirmed that BAP31 expression is associated with progression and metastasis of several cancers, particularly in cervical cancer. We found that BAP31 was significantly upregulated in stage I, II, and III cervical cancer patients and highly correlated with poor clinic outcomes. We further demonstrated that BAP31 regulates cervical cancer cell proliferation by arresting the cell cycle at the G0/G1 stage and that depletion of BAP31 inhibits hyper-proliferation. Moreover, depletion of BAP31 inhibits cervical cancer cell invasion and migration by regulating the expression and subcellular localization of Drebrin, M-RIP, SPECC1L, and Nexilin, and then affect the cytoskeleton assemblage. Finally, the depletion of BAP31 prevents cervical cancer progression and metastasis in vivo. These findings provide a new method for identifying novel CTAs as well as mechanistic insights into how BAP31 regulates cervical cancer hyper-proliferation and metastasis.

P2239

Cancer associated fibroblasts modify and utilize monocytes and tumor associated macrophages to suppress anti-tumor immune responses

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Cancer-associated-fibroblasts(CAFs) are one of the most abundant cells in tumor-stroma. Monocytes differentiate into M1/M2 macrophages, depending on the environmental-stimuli. We aimed to determine the role of CAFs on monocyte-recruitment and macrophage-polarization in human-breast-cancer. Isolated-fibroblasts were characterized by immunocytochemistry-stainings. Conditioned-mediums(CMs) were obtained from CAFs and normal-fibroblasts(NFs). $CD14^+$ monocytes and $CD4^+$ T-cells were isolated from healthy-volunteers' PBMCs by magnetic-bead-based-selection-protocols. Cells were stained with antibodies against CD206,CD163,CD14,HLA-DR,PD-1 and analyzed with flow-cytometry. Functional effects of CAF-,NF-educated-monocytes on T-lymphocytes were analyzed with flow-cytometry utilizing CFSE-labelled $CD4^+$-T-cells. Effects of CAFs/NFs on monocyte-

DOI: 10.1002/eji.201970400

recruitment were investigated by migration-assays. E-cadherin,vimentin protein-expressions; Snail,Slug,Twist gene-expressions were examined by Western-blot and real-time-PCR, respectively. MDA-MB-231 breast-cancer cell-invasion was evaluated by invasion-assays. Cytokine-levels were assessed by ELISA. MDA-MB-231 breast-cancer cell-proliferation was evaluated via MTT assay and xCELLigence real-time-label-free-system. Our results demonstrated that CAFs, which express α-SMA in contrast to NFs, effectively recruited monocytes. This recruitment may be mediated by MCP-1 or SDF-1 cytokines, since inhibition of MCP-1 or SDF-1 through MCP-1 or CXCR4 blocking-antibodies, significantly reduced monocyte-migration. CAFs differentiated the recruited monocytes into M2-like-macrophages which can demonstrate immunosuppressive effects via PD-1. CAF-educated-monocytes were shown to suppress T-cell-mediated immune-responses; increase expressions of EMT-related-genes and vimentin-protein in cancer-cells; and induce breast-cancer cell-invasion. CAF-educated-M1-macrophages displayed increased expression of M2-markers and production of anti-inflammatory IL-10 in contrast to decreased production of pro-inflammatory IL-12. CAF-educated-monocytes also increased breast-cancer cell-proliferation. We demonstrated that CAFs potently modified the tumor-microenvironment. Identification of such mechanisms that drive macrophage-plasticity and immunosuppression in cancer-microenvironment could provide a basis for macrophage focused diagnostic and therapeutic strategies.

P2240

Cell softness prevents cytolytic T cell killing of tumor-repopulating cells

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Biomechanics is a fundamental feature of a cell. The manner by which a mechanical feature could affect immune evasion of tumor cells remains an enigma. Here we show that **although cytotoxic T lymphocytes (CTL) can effectively destroy stiff differentiated tumor cells, they fail to kill tumor-repopulating cells (TRC), whose softness effectively impedes the pore formation process through perforin released from CTLs.** Mechanistically, **perforin interacting with nonmuscle myosin heavy chain 9 transmits forces to fewer F-actins in soft TRCs, thus inducing a weak contractile force which is inadquate for pore formation by perforin**. Stiffening soft TRCs confers perforin the drilling ability, leading to CTLs killing the TRCs, thus achieving a better tumor immunotherapeutic outcome. More importantly, overcoming the mechanical softness also enhances the killing of human TRCs by human CTLs. These findings provide mechanics-based immunotherapeutic strategies with potential clinical applications.

P2241

PASD1 expression in colorectal carcinoma and polyps patients at UKM medical centre (UKMMC)

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Background: Per ARNT Sim Domain containing Repressor 1 (PASD1) is one of the cancer testis antigens (CTAs) which have restricted expression in normal tissues but widespread expression in cancers. This makes PASD1 as an attractive immunotherapy target. Two *PASD1* transcripts have been identified namely *v1*, which encodes 639 aa N-terminus while *v2*, encodes a longer protein (773

DOI: 10.1002/eji.201970400

aa) with a unique C-terminus. This study aims to investigate the expression of PASD1 in the colorectal patients from UKM Medical Centre.
Method: Formalin-fixed paraffin-embedded (FFPE) from 40 colorectal carcinoma (CRC) and polyps patients' were tested for their mRNA and protein level using Quantitative real-time PCR (qRT-PCR) and Immunohistochemistry (IHC) respectively. For IHC, PASD1 antigen was detected using two antibodies which were 2ALCC136 (recognize both PASD1_v1 and PASD1_v2 proteins) & 2ALCC128 (detects only PASD1_v2 protein). Sample that were positive for PASD1 expression, were tested for the immunogenicity towards the PASD1 peptides using Elispot.
Results: Our results show more than 60% of CRC and polyps samples showed moderate positive expression for PASD1 using both 2ALCC136 & 2ALCC128 antibodies. The positive staining was both in the cytoplasmic and nuclear region. There was a clear association between mRNA, protein expression and immunogenicity. However, no clear association between the location of PASD1 staining and the sample type and stages.
Discussion: This could suggests the important role of PASD1 mRNA and protein in the colon carcinogenesis and polyps. This is the first study reported on the expression of PASD1 in patients' colon tissue.

P2242

Protein expression profiles associated with impaired cell cycle and apoptosis in pyruvate carboxylase deficient MDA-MB-231 breast cancer cells

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The anaplerotic enzyme pyruvate carboxylase (PC) has been demonstrated recently to support breast cancer cell metabolism, and its precise roles in cancer progression remain unclear. In this study, PC siRNA knockdown MDA-MB-231 cells were characterized for viability, cell cycle alterations and apoptosis, and protein expression profiles associated with the PC suppression were explored. Our data demonstrated that PC suppression caused a reduction of cell viability detected by trypan blue exclusion assay, consistent with an increase of apoptotic cell death assessed by flow cytometry using annexin V and Western blot analysis of procaspase 3. DNA analysis by flow cytometry demonstrated G2/M cell cycle arrest, and Western blot analysis indicated a depletion of cyclin B1/CDK1, regulators of G2/M checkpoint, in the PC deficient cells. Protein lysates of the PC knockdown and negative control cells were analyzed by GeLC-MS/MS, and Venn diagram analysis identified 452 proteins upregulated and 399 proteins downregulated in response to PC suppression. Multi-expression Viewer for data analysis further identified 32 proteins whose expressions were correlated with the induction of apoptosis and G2/M arrest. These proteins are implicated in various molecular processes such as cytoskeleton, DNA maintenance, transcription, RNA processing and signaling. Analysis based on STITCH database also predicted interactions of 9 candidate proteins with the signaling networks mediating cell cycle progression and apoptosis. Our finding suggested that PC supports aberrant cell cycle and anti-apoptosis, which are fundamental for the establishment of malignant phenotypes and immune evasion of cancer cells, potentially by influencing the identified protein expression profiles.

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DOI: 10.1002/eji.201970400

P2243

The Glycocode of the tumormicroenvironment in pancreatic cancer, a novel immune check-point

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Pancreatic cancer (PCa) remains as one of the most aggressive malignancies with a 5-year survival rate of only 7%. Despite the increasing understanding of the molecular mechanisms behind cancer development, there is still a clear need for new diagnostic and therapeutic approaches in PDAC. Changes in the glycosylation patters during tumor progression have been extensively reported, but its tolerogenic properties and potential as diagnostic tool as had been widely overlooked in pancreatic cancer. Using big data transcriptomic analysis, cell lines and functional assays we studied the impact of the glyco-code in pancreatic cancer progression and its interaction with the immune system. The analysis of transcriptomic data from patients and cell lines allowed us to identify different clinically relevant glyco-subtypes of PDAC, characterized by specific glycosylation signatures and associated with Epithelial to Mesenchymal transition (EMT). This differential glycosylation during EMT alters the differentiation programmming of monocytes into tumor associated macrophages (TAMs) upon interaction with tumor cells. Our results show a clear impact of the glyco-code in pancreatic cancer progression and highlight its potential as diagnostic tools and targets for therapeutic strategies.

P2244

The mechanism of CSRP2BP in cervial cancer

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CSRP2BP is also known as Lysine Acetyltransferase 14 (KAT14) and ATAC complex 2 Homolog (ATAC2). On the basis of regulating histone acetylation, this complex is involved in cell proliferation and differentiation, cell apoptosis, embryonic development and other biological processes. Cervical Cancer is the second most common Cancer of women in the world and more than 370,000 new Cervical Cancer cases occur each year, accounting for more than 10% of all cancers of women. Our previous study found that CSRP2BP was significantly highly expressed in cervical cancer tissues and cervical cancer cell lines, suggesting that CSRP2BP may be involved in the occurrence and development of cervical cancer by affecting the function of cervical cancer cells. In order to further study, our research group constructed Hela stable cell line with silencing, knockout and overexpression of CSRP2BP to study the effect of CSRP2BP on cervical cancer cell function, so as to explore the mechanism of CSRP2BP on the occurrence and development of cervical cancer. The results showed that CSRP2BP affects the migration, invasion and apoptosis of cervical cancer Hela cells, suggesting that CSRP2BP is involved in the regulation of cervical cancer cell function, which may be related to the pathogenesis of cervical cancer.
This work was financially supported by Major Science and Technology Program of Hainan Province (No.ZDKJ2017007).

DOI: 10.1002/eji.201970400

P2245

A study on the association between nucleostemin and programmed death-ligand-1 in multiple myeloma U266 cells

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Objective: To investigate the correlation between nucleostemin (NS) gene and programmed death ligand-1 (PD-L1) in myeloma cells and cell apoptosis after knockdown *NS* gene. **Estimate the associations between NS and PD-L1 and biological behavior of MM cells and the feasibility of the two as a marker reflecting the status of MM cells**.
Methods: The *NS* gene expression of U266 cells was down-regulated by NS-RNAi-GV248 recombinant lentivirus. Real-time PCR were used to detect the expression of NS, PD-L1 and PI3K/AKT/mTOR mRNA. Western blot and flow cytometry were used to detect the expression of NS and PD-L1. Annexin V-APC/7-AAD staining method was used to detect the apoptosis of U266 cells.
Results: Real-time PCR showed that compared with the negative control group (1.002±0.026), the mRNA expression of *NS, PD-L1* and *PI3K/AKT/mTOR* gene in the transfection group (0.415±0.089) was significantly reduced ($P < 0.05$). The results of flow cytometry and western blot showed that the expression of PD-L1 protein was significantly down-regulated after transfection. After down-regulation of *NS* gene, the apoptosis of U266 cells was increased ($P < 0.05$).
Conclusion: It demonstrated that abnormally high expression of NS and PD-L1 in U266 cells. **Down-regulation of the *NS* gene causes PD-L1 and related pathway PI3K/AKT/mTOR expression decreased. The results showed that the two might play a synergistic role in the changes of U266 cell biology**.

P2246

The upregulation of hsa-mir-181b-1 and downregulation of its target CYLD in the late-stage of tumor progression of breast cancer

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Some microRNAs are usually dysregulated in the cancers by influencing on tumor behavior and progression. Hsa-miR-181b-1 and its target *CYLD* are involved in regulating the inflammatory pathways. This study aimed to investigate the expression levels of hsa-mir-181b-1 and *CYLD* in a cohort of breast tumor tissues and normal adjacent tissues to assess their association with breast cancer stages. A total number of 60 breast samples including cancerous and normal adjacent tissue specimens were collected. After pathological study, the expression of hsa-mir-181b-1 and *CYLD* were measured by qRT-PCR method. The hsa-mir-181b-1 expression level was significantly increased in breast tumor tissues compared to the controls. This increase was associated with the disease progression. Conversely, *CYLD* expression level was decreased in tumor samples compared to normal samples, significantly. ROC curve data added other prestigious information of hsa-mir-181b-1 and *CYLD* by defining cancer and healthy tissues with high specificity and sensitivity at a proposed cutoff point. Also, bioinformatic enrichment for the possible targets of mature sequence of “hsa-mir-181b-5p” was performed. Computational analysis showed the five most significant pathways including metabolic, cancer, calcium signaling, PI3K-Akt signaling and focal adhesion pathways which may be influenced by hsa-mir-181b-1. Thus, we suggested hsa-mir-181b-1 and *CYLD* might be involved in the

DOI: 10.1002/eji.201970400

pathogenesis of breast cancer and could be considered as two biomarkers for prediction, prognosis and diagnosis of the stages of the breast cancer.

P2247

Identification of dynamic regulation of 'brakes' and 'accelerators' of immune system in murine prostate tumor model

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Cancer immunotherapy using monoclonal antibodies (mAb) targeting specific surface receptors expressed by tumor cells yielded encouraging results in clinical trials. However, tumors intelligently evolved escape-mechanism to dampen the effects of immune-checkpoint blockade therapies and continue to chastise cancer patients. Therefore, in this context, urgent development of alternative therapeutic management options is imperative. Negative co-stimulators of tumor-specific T cells such as LAG-3 (Lymphocyte-activation gene), TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), CTLA-4 (cytotoxic T-lymphocyte associated protein 4), PD-1 (Programmed cell death protein 1), TIGIT (T-Cell Immunoreceptor with Immunoglobulin and ITIM Domain), HVEM (Herpes virus entry mediator), VISTA (V-domain Ig suppressor of T cell activation) including others are now identified as the intensive targets of mAb therapies and many are also approved by FDA. For instance, the monoclonal antibody - Ipilimumab (Yervoy^{TM})- is widely used to activate the immune system by targeting CTLA-4 expressed on T cells. Here, we examine that targeting the co-inhibitory receptors in a profile-specific manner regresses the tumor. Anti-tumor response is further enhanced by combing the co-stimulatory receptor CD28 and blockade of a co-inhibitory molecule. Such treatments might work in synergy to optimally suppress the tumor progression by either boosting anti-tumor potential of TILs or suppressing tumor promoting cells with tumor microenvironment.
Keywords: Monoclonal Antibodies (mAbs), T cells, Epigenetics, Co-stimulatory/Co-inhibitory molecules.

P2248

Extracellular vesicles from cancer cell subpopulation affect metastasis and stemness of distinct cancer cell population

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Extracellular vesicles (EVs) have been attracting increasing attention as EVs carry complex molecules that mediate intercellular communication in cancer. Here, we examined whether EVs from subpopulation of cancer cells could affect the pathology of the other cancer subpopulation. For this purpose, we isolated six single cell clones from 4T1 breast cancer cells. Among them, we employed two morphologically distinct cell clones in this study. Upon implantation into mammary part of BALB/c mice, round-shape clone was highly metastasized into the lung (designated HM.4T1 cells) whereas few metastases were found in case of angular-shape clone (LM.4T1 cells). The number of metastatic foci of HM.4T1 cells was less than that of the original 4T1 cells. We then mixed HM.4T1 and LM.4T1 (1:1) and implanted the mixtures into the mice. Although the number of the total implanted cells was equal, the metastasis foci of the mixtures were increased as compared with that of HM.4T1. We assumed that EVs released from each cell population play a role. Therefore, we isolated EVs from HM.4T1 and LM.4T1 and performed cell scratch migration assay in the presence of EVs from distinct cell clone. Interestingly, LM.4T1-EVs enhanced HM.4T1 cell migration and in contrast, HM.4T1-EVs

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DOI: 10.1002/eji.201970400

reduced the mobility of LM.4T1 cells. Three-dimensional spheroid invasion assay showed that LM.4T1-EVs augmented the spheroid invasion ability of HM.4T1 cells. Thus, EVs from cancer cell subpopulation influence distinct cancer cell population, affecting cancer metastasis and stemness in the microenvironment.

P2249

AXL targeting enhances lymphocyte-mediated cytotoxicity of lung cancer cells

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As immunotherapies are now used to treat a large proportion of NSCLC patients, defining mechanisms of immune resistance is critical. Immune resistance may arise from both genetic instability and tumor heterogeneity driven by microenvironmental stresses such as hypoxia that promotes carcinoma cell plasticity as well as extrinsic or intrinsic mechanisms of immune resistance. AXL, a member of the TAM receptor tyrosine kinase family is widely expressed human cancers and increasingly recognized for its role in cell plasticity and drug resistance. In this study, we used a model of hypoxia-induced tumor plasticity to generate multiple lung cancer clones with mesenchymal and epithelial features to address mechanisms of immune resistance. We demonstrate that AXL expression is dramatically increased in mesenchymal lung cancer clones. Moreover, expression of AXL in the cells was correlated with an increased cancer cell intrinsic resistance to both NK and CTL-mediated killing, Notably, small molecule AXL targeting potently sensitized mesenchymal lung cancer cells to cytotoxic lymphocyte-mediated killing. Mechanistically, we showed that attenuation of AXL-dependent immune resistance to immune cells involved a novel molecular network comprising NF-KB activation, increased ICAM1 expression, and upregulation of ULBP1 expression coupled with MAPK inhibition. Congruently, higher ICAM1 and ULBP1 tumor expression, correlated with improved patient survival in two NSCLC cohorts. These results reveal a novel AXL-mediated immune escape regulatory pathway, suggest AXL as a novel candidate biomarker for tumor resistance to NK and CTL immunity, and support AXL targeting to optimize immune response in NSCLC.

LB115

Acute myeloid leukemia immune escape by epigenetic CD48 silencing

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Acute myeloid leukemia is a malignant disorder of haemopoietic stem cells. AML can escape immunosurveillance of NK, et al. by gene mutation, fusions and epigenetic modification. The mechanism of AML immune evasion is not clear understand. Here we showed that the CD48 is a favorable prognosis factor and downregulated in AML patients, which can help AML evade from NK cell recognition and killing. Furthermore, we demonstrated that CD48 expression is regulated by methylation and hypomethylating agent could increase the CD48 expression, which increasing the NK cells killing in vitro. Finally, we showed that CD48 can reversed the AML immune evasion and

DOI: 10.1002/eji.201970400

activated NK cells function in vivo. This study may indicate a new light on combination the hypomethylating agent and NK cell infusion to cure AML.

P3031

ERBB3, IGF1R, and TGFBR2 expression correlate with PDGFR expression in glioblastoma and participate in PDGFR inhibitor resistance of glioblastoma cells

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Aberrant activation of receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor (PDGFR), are frequently observed in glioma. Accumulating evidence suggests that PDGFR plays critical roles during glioma development and progression and is a promising drug target for GBM therapy. However, PDGFR inhibitor (PDGFRi) has failed in clinical trials, at least partially, due to the activation of other RTKs, which compensates for PDGFR inhibition and renders tumor cells resistance to PDGFRi. Therefore, identifying the RTKs responsible for PDGFRi resistance might provide new therapeutic targets to synergetically enhance the efficacy of PDGFRi. In this study, we analyzed the TCGA glioma database and found that the mRNA expressions of three RTKs, i.e. ERBB3, IGF1R, and TGFBR2, were positively correlated with that of PDGFR. Concurrent expression of PDGFR with ERBB3, IGF1R, or TGFBR2 in GBM cells attenuated the toxicity of PDGFRi. Thus, ERBB3, IGF1R, and TGFBR2 might participate in PDGFRi resistance of GBM cells. Consistent with this notion, combination of PDGFRi with inhibitor targeting either ERBB3 or IGF1R more potently suppressed the growth of GBM cells than each inhibitor alone. The positive correlations of PDGFR with ERBB3, IGF1R, and TGFBR2 were further confirmed in 66 GBM patient samples. Intriguingly, survival analysis showed that ERBB3 predicted poor prognosis in GBM patients with high PDGFRA expression. Altogether, our work herein suggested that ERBB3, IGF1R, and TGFBR2 were responsible for PDGFRi resistance and revealed that ERBB3 acted as potential prognostic marker and therapeutic target for GBM with high PDGFRA expression.

P3032

Effects of the combination of As2O3 and AZT on proliferation inhibition and apoptosis induction of hepatoma HepG2 cells following silencing of Egr-1

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Previous studies have demonstrated that 3′-azido-3′-deoxythymidine (AZT) and arsenic trioxide (As2O3), traditional chemotherapy agents, can synergically inhibit the growth of hepatocellular carcinoma cells. However, the molecular mechanisms underlying As2O3 and AZT anti-hepatoma activity are unknown.Objective This study aimed to investigate the role of early growth response protein 1 (Egr-1) in the process of As2O3 combined with AZT inhibiting proliferation and inducing apoptosis of human hepatocellular carcinoma HepG2 cells, and explore mechanism.Materials and methods The expression of Egr-1 was silenced using siRNA, and HepG2 cells were treated with As2O3 (2 μM) and AZT (20 μM). The rates of cell inhibition and apoptosis were determined by the 3-(4,5-Dimethylthiazol-2-yl)- 2,5-diphenyltetrazoliumbromide (MTT) method and flow cytometry, respectively. The mRNA and protein expression of p53, caspase-3, and Egr-1 were detected by real-time quantitative polymerase chain reaction and Western blotting, respectively.Results The inhibitory rate of As2O3 (2 μM) combined with AZT (20 μM) on proliferation of HepG2 cells was significantly higher than that of As2O3 alone. The combination index (CI) values were 0.2< CI< 0.4, showing strong

DOI: 10.1002/eji.201970400

synergic effect. After silencing Egr-1, the proliferation inhibition and proapoptotic ability of As2O3 combined with AZT on HepG2 cells were decreased, and the CI value was greater than 1, showing antagonistic effect. In addition, the expression of p53 and caspase-3 mRNA/protein was also significantly decreased. Conclusion It shows that AZT increase the sensitization of As2O3 for inhibiting proliferation and promoting apoptosis of HepG2 cells through regulating the expression of Egr-1, which control the expression of p53 and caspase-3.

P3033

SAHA promote lung cancer cell immune response by activating STAT1 to up-regulate HLA-I expression

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Human leukocyte antigen class I antigens (HLA-I) is crucial in immune response by presenting antigenic peptides to cytotoxic T lymphocytes. Among the tumor immune escape mechanisms, the alterations in the expression of HLA-I molecules play an important step in lung cancer development. Currently, SAHA is considered to be a promising anticancer agent ,which approved for the treatment of cutaneous T-cell lymphoma by the U.S. Food and Drug. However, the mechanism of anti-tumor effect of SAHA remains unclear. In this study, our results demonstrated that SAHA was able to upregulated the expression of HLA-I in A549 and H520 cells. At the same time, we found that the membrane expression of HLA-I were also increased after SAHA treatment by flow cytometry, thereby attenuated the cytotoxic T cell mediated lysis of lung cancer cells. Then, we revealed that SAHA upregulated HLA-I by promoting STAT1 and smad2/3 phosphorylation, then demonstrated that both STAT1 and smad2/3 occurred nuclear translocation. At last, we indicated that SAHA affect the expression of HLA-I by regulating STAT1 and smad2/3 by silencing STAT1 and smad2/3. These results provide a new teeatment by improving tumor immunotherapy of lung cancer cells.

P3034

Exosomal miR-590-3p promotes HCC metastasis through regulating vascular endothelial cell permeability

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Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death worldwide. Postsurgical recurrence or metastasis is a main cause of death.
However, the mechanism of HCC to break through blood vessel barrier and metastasis has not been clarified. We have found that more exosome is released in advanced HCC blood, and miR-590-3p is highly enriched in serum exosome. Data from TCGA database indicates that miR-590-3p level in HCC tissue is negatively correlated with patient survival rate and positively correlated with pathological stage. Further study shows that miR-590-3p could break the stability of vascular endothelial cells in vitro and promote the metastasis of liver cancer in mouse model. Using bioinformatics tools, we identify DCN as the target of miR-590-3p. DCN plays an essential role in maintaining endothelial cell integrity by inhibiting MMP2 and MMP9 expression. Our date indicates that miR-590-3p may involve in vascular metastasis of HCC through diminishing DCN expression. We proposed that metastatic HCC can break the vascular homeostasis and promote the metastasis of liver cancer by secreting exosomal

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DOI: 10.1002/eji.201970400

miR-590-3p. We will establish a clinical cohort for HCC, and analyze the correlation between miR-590-3p and metastasis, disease progression, HCC recurrence after operation. We hope to provide a new molecular mechanism for the metastasis of hepatocellular carcinoma and provide a new diagnostic target for Early HCC metastasis screening and personalized treatment.

P3035

Metabotropic glutamate receptor 5 opens a unique "neutrophil-cancer cell" friendship mode in non-small cell lung cancers

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Neutrophils in lung cancer-bearing hosts can potentiate cancer progression. However, many of the underlying mechanisms are still unknown. In 200 patients with non-small cell lung cancers (NSCLCs), we identified the significant inducible metabotropic glutamate receptor 5 (mGluR5) expressions on both neutrophils and tumor cells, naming the phenotypes as mGluR5+ neutrophils or tumor cells. Furthermore, we found that the inducible level of mGluR5 expression was negatively associated with the clinical stages and prognosis of NSCLC patients. In neutorphils and tumor cell co-culture system, normal neutrophils from healthy persons (mGluR5- phenotype) killed tumor cells by their innate immune activities, while neutrophils from NSCLSs patients (mGluR5+ phenotype) had longer life span and potentiated tumor cell proliferation and metastasis instead of killing them. Further investigation showed that mGluR5+ neutrophils recognized mGluR5+ tumor cells as "self" rather than "nonself", so that they exhibited discrete cancer-promoting properties rather than cleaning them up. Deprivation of mGluR5 on neutrophils or on NSCLC cells by knocking out mGluR5 gene or blocking mGluR5 with specific antagonist MTEP could restore the anti-tumor activity of neutrophils and inhibit tumor cell migration both in vitro and in vivo. These results indicate that inducible mGluR5 expressions on neutrophils and tumor cells just like labeling a recognizable logo, which opens a friendship mode between neutrophils and NSCLCs cells. This is a novel mechanism mediated by neutrophils to facilitate NSCLCs progression and also a new mode of neutrophils being educated by NSCLCs.

(This work is supported by NSFC No. 81572885, No.81572830).

P3036

Identify human long-coding RNAs regulating PD-L1 using a paired-guide RNA CRISPR-cas9 library

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Long non-coding RNA (lncRNA) represent a large subgroup of RNAs that are over 200 nucleotides long and have a limited protein-coding potential. They play an important role in diverse cellular processes through a variety of mechanisms. This ability of lncRNAs to modulate gene expression renders them indispensable for normal development.
Cancer cells express the programmed death-1 (PD-1) ligand 1(PD-L1) to evade T-cell mediated immunosurveillance. Antibodies that block the PD-1 and PD-L1 axis have been approved for seven different tumor types. The success of therapies that overcome PD-L1 mediated tumor tolerance has highlighted the need to understand the molecular regulation of PD-L1 expression. Some groups have

DOI: 10.1002/eji.201970400

identified novel PD-L1 regulators based on CRISPR/Cas9 screens, however these screens are all based on the coding-gene screen.
In view of the limited understanding of PD-L1 expression regulated by the LncRNAs, we perform a genome-scale deletion screening of human LncRNAs using a paired-guide RNA (pgRNA) CRISPR library in multiple cancer cell lines to identify some PD-L1 regulators. Applying this screening method, we designed a pgRNA library targeting around 1200 human lncRNA genes and sort the cells with PD-L1 high expression or low expression by fluorescence-activated cell sorting (FACS), before sorting, the cancer cells pre-treated with IFN-γ to stimulate the expression of PD-L1. We use the MAGeCK algorithm to identify the top hits by comparing samples in PD-L1 high expression with low expression ones. We successfully identified some known regulators which imply the reliability of our screen. We also identify some LncRNAs that regulate PD-L1potentially.

P3037

Monoclonal antibodies against non B-Ig κ$^{(Vκ4-1/Jκ3)}$ inhibit the response of human T cells in vitro.

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Background: Secretion of immunoglobulin (Ig) is highly specialized by plasma cells. However, immunologists gradually agree with the concept of Ig imparted by non B cells. Of all, non B cell-derived Ig κ was assembled by similar rearrangements, in which the bias toward Vκ4-1/Jκ3 recombination. All the biological function of these Ig κ in T cells were not yet completely understood.
Method: Our team had previously made 1A3 mAb and 6G5 mAb against non B-Ig κ$^{(Vκ4-1/Jκ3)}$. Then, the CFSE-labeled CD2^{+} T cells from healthy volunteers were stimulated with anti-CD3/CD28 antibodies in the presence of 1A3 mAb or 6G5 mAb respectively for 4 days. CFSE dilution assay and the phenotypic characteristics were used to assess the effect of non B-Ig κ$^{(Vκ4-1/Jκ3)}$ on T cells.
Results: Compared with isotype antibodies, 1A3 mAb or 6G5 mAb could obviously dampen the proliferation of T cells in a dose-dependent manner. Furthermore, the expression of activation markers CD69, CD25 and CD71 were significantly decreased when cocultured with specific antibodies. The detection of cytokines in culture supernatants showed the addition of 1A3 mAb would result in a decreasing secretion of IFN-γ. Besides, a considerable necrotic rate for T cells was also found in the presence of the antibodies.
Conclusion: Generally, these results revealed the 1A3 and 6G5 mAb against non B-Ig κ$^{(Vκ4-1/Jκ3)}$ could quell the T cells response, hinting the novel strategy targeting B-Ig κ$^{(Vκ4-1/Jκ3)}$ for tumor escape.

P3038

Curcumol potentiates celecoxib-induced growth inhibition and apoptosis in human non-small cell lung cancer

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Combinatorial therapies that target multiple signaling pathways may provide improved therapeutic responses over monotherapies. Celecoxib and curcumol are two highly hydrophobic drugs which show bioavailability problems due to their poor aqueous solubility. In the present study, we evaluated the effects of celecoxib and curcumol alone and in combination on cell proliferation, invasion, migration, cell cycle and apoptosis induction in non-small cell lung cancer (NSCLC) cells using *in vitro* and *in vivo* experiments. Our data showed that the sensitivity of a combined therapy using low concentration of

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DOI: 10.1002/eji.201970400

celecoxib and curcumol was higher than that of celecoxib or curcumol alone. Suppression of NF-κB transcriptional activity, activation of caspase-9/ caspase-3, cell cycle G1 arrest, and inhibition of survival MAPK and PI3K/AKT signaling pathway contributed to the synergistic effects of this combination therapy for induction of apoptosis. Additionally, either celecoxib alone or in combination with curcumol inhibited NSCLC cell migration and invasion by suppressing FAK and matrix metalloproteinase-9 activities. Furthermore, the combined treatment reduced tumor volume and weight in xenograft mouse model, and significantly decreased tumor metastasis nodules in lung tissues by tail vein injection. Our results confirm and provide mechanistic insights into the prominent anti-proliferative activities of celecoxib and/or curcumol on NSCLC cells, which provide a rationale for further detailed preclinical and potentially clinical studies of this combination for the therapy of lung cancer.

P3039

The association of five preoperative serum tumor markers and pathological features in patients with breast cancer

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The utility of frequently used serum tumor markers in breast cancer remains controversial. The study aimed to investigate the role of preoperative carcinoembryonic antigen(CEA), cancer antigen125(CA125),cancer antigen153(CA153),cancerantigen724(CA724),and ferritin (FER) in the management of breast cancer and their relationships with pathological features. A total of 804 patients with breast mass who underwent breast surgery and 305 healthy volunteers were enrolled. Preoperative serum levels of CEA, CA125, CA153, CA724, and FER were measured. And the pathological features of all the patients were recorded. The association of preoperative serum tumor markers and pathological features was analyzed. Among the 804 patients, 355 were identified as malignant cases and 449 as benign cases. CEA, CA153, and FER of patients with breast cancer were higher than those of healthy volunteer group and patients with benign breast diseases. The area under curve (AUC) of CEA, CA153,and FER for distinguishing patients with breast cancer and subjects with non-breast cancer was 0.688 (95%CI:0.656-0.721),0.609(95%CI:0.574-0.645), and 0.623(95%CI:0.586-0.660), respectively.CA153 correlated with tumor size, node status, and TNM stage, whereas CA125 with node status. No statistic differences of the five markers were observed among the four molecular subtypes. Preoperative levels of CEA, CA153,and FER exhibit low diagnostic accuracy for breast cancer (stage I-III). CA153 correlates with tumor burden, suggestingits prognostic value. The five serum markers do not correlate with molecular subtypes

P3040

The function and mechanism of EIF4B activation mediated by PDGF-AA/PDGFRA signaling pathway in glioblastoma

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Glioblastoma(GBM) is a malignant brain tumor, which possesses poor prognosis and with limited therapeutic modalities. Previous study indicates that translational dysregulation plays vital roles in tumorigenesis, therefore, targeted translation is a new orientation for cancer therapy. EIF4B is a key regulator in process of translation, its role in GBM as well as its clinical significance remain unclear. Based on our preliminary study, in GBM, activating the PDGFRA pathway can regulate the phosphorylation of EIF4B. Mass spectrometry analysis indicates there are new phosphorylation site of

DOI: 10.1002/eji.201970400

EIF4B during PDGFRA pathway activation. Meanwhile, in GBM subtype overexpressing PDGFRA, patients overexpressing EIF4B possess favorable prognosis. Nevertheless, there is no relevance between the expression of EIF4B and the prognosis of patients in other GBM subtypes. We speculate that PDGFRA phosphorylates EIF4B, which change the translational efficiency of prognostic genes in patients with GBM and then infect the prognosis of patients. This study is to investigate the effect of PDGFRA pathway on translational activity of EIF4B through methods such as cell experiments, xenograft in mice and mass spectrometry analyze. Moreover, we will verify the viewpoint above by clinical samples analysis. This study may provide new targets and new strategies for diagnosis and therapy in GBM.

P3041

The effect of SAMHD1 on the chemosensitivity of triple negative breast cancer

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Objective: TNBC is a special subtype of breast cancer, which is lack of effective therapeutic targets because of a lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Compared with other subtypes, TNBC is more prone to metastasis and recurrence. Surgery and chemoradiotherapy are main treatments for TNBC. Studies have shown that SAMHD1 inhibits the killing effect of paclitaxel on tumor cells. SAMHD1 is a natural antiviral factor which can inhibit the replication of retroviruses such as HIV and DNA viruses in myeloid cells by degrading intracellular dNTPs. However, the function of SAMHD1 in TNBC cells is still unclear. Therefore, elucidate the regulatory mechanism of SAMHD1 in TNBC cells is helpful to explore the role of SAMHD1 in affecting the sensitivity of TNBC to chemotherapy and to better apply chemotherapeutic drugs to clinical treatment.
Method: We intend to clarify whether SAMHD1 phosphorylation at different sites could reduces chemosensitivity through Co-IP, mass spectrometry and kinase assay. Elucidate the effect of SAMHD1 phosphorylation on TNBC chemosensitivity through expression, knockout and site mutation cell models. And clarify the regulating molecular mechanism of chemosensitivity by SAMHD1 through RNAseq and Western blot experiments.
Results: Phosphorylation of SAMHD1 promote the DNA damage repair and apoptosis of tumor cells, enhance the proliferation, migration and invasion ability of TNBC cells and phosphorylation of SAMHD1 at different sites reduce the killing effect of taxol and other therapeutic drugs on TNBC.
Conclusion: The overexpression of SAMHD1 can inhibit the chemosensitivity of TNBC.

P3042

Capillary morphogenesis Protein 2 is a novel prognostic biomarker and plays oncogenic roles in glioma

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Capillary Morphogenesis Protein 2 (CMG2) is originally noticed for its participation in capillary morphogenesis, and then it is identified as the second receptor for anthrax toxin. In this study, we revealed a remarkable association of CMG2 overexpression to increased tumor grade and poor patient survival in glioma. The *in vitro* and *in vivo* assays further demonstrated that CMG2 promoted G2/M-phase transition during cell cycle of GBM cells and contributed to tumor growth. We also observed that CMG2 was implicated in the activation of extracellular signal-regulated kinases (ERK),

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DOI: 10.1002/eji.201970400

epithelial-mesenchymal transition (EMT), migration and invasion in GBM cells. Then, we compared the whole-transcriptomes of GBM cells with or without CMG2 overexpression to interpret the molecular mechanism underlying oncogenic roles of CMG2. The transcriptomic analysis indicated that a panel of oncogenic signaling pathways was altered with CMG2 upregulation, implying that CMG2 might orchestrate these signaling pathways to regulate the growth of GBM cells. Moreover, our results showed that yes-associated protein 1 (YAP1) activity was enhanced by CMG2 overexpression but suppressed with CMG2 deficiency, which identified a novel link between CMG2 and YAP1. Since YAP1 is critically implicated in GBM, the oncogenic roles of CMG2 might be mediated, at least partially, by YAP1 in GBM cells. Altogether, our work herein demonstrated that CMG2 functioned as an oncogene in glioma cells and implied that CMG2 was a potential prognostic biomarker or therapeutic target for clinical treatment of glioma.

P3043

Effects of NDUFA4 overexpression on growth of human colon cancer cells in vitro

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Objective: To investigate the effects of NDUFA4 overexpression on the growth of human colon cancer cells in vitro and to explore its potential mechanism.
Methods: The recombinant plasmid p-NDUFA4 (p- NDUFA4) and the control plasmid (p-Cont) were transiently transfected into human colon cancer HCT 116 cells, respectively. Then, the relative expression level of NDUFA4 was determined by Real-time PCR and Western blot. The proliferation and colony formation ability of HCT116 cells were detected by CCK-8 assay and clone formation assay. The mRNA expression levels of cyclin-dependent kinase (CDK) 2, CDK3, CDK4 and CDK6 were determined by Real-time PCR. The expression levels of AKT (p-AKT) and ERK1/2(pERK1/2) were analyzed by Western blot.
Results: Compared with control groups, the expression levels of NDUFA4 were increased remarkably in p-NDUFA4 group . The proliferation and clone formation
capacity of HCT116 cells in p-NDUFA4 group were obviously increased. The expression of CDK2 and CDK3 mRNA were increased signifcantly. The expression of p-AKT and p-ERK1/2 were increased obviously .
Conclusion: The overexpression of NDUFA4 could significantly promote the growth of human colon cancer HCT116 cells in vitro, which might be closely related to the change of AKT and ERK signaling transduction.
Key words: NDUFA4; Eukaryotic expression; Colon cancer; HCT116; Proliferation

DOI: 10.1002/eji.201970400

P3044

Inhibitory effects of resveratrol in combination with DAPT on precursor T cell lymphoblastic leukemia molt-4 cells

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Background: A Notch1 activating mutation is the most common mutation in T-cell acute lymphoblastic leukemia (T-ALL). The clinical development of γ-secretase inhibitors has been hindered due to their prominent gastrointestinal toxicity in clinical trials. Resveratrol (Res) is believed to have anticancer effects via multiple signaling pathways.
Methods: The inhibition rate of Molt-4 cells was assessed by MTT assays. Apoptosis was analyzed using Annexin-V/propidium iodide (PI) double staining and flow cytometry. Notch1 and *Hes-1* mRNA expression was detected by RT-PCR. Molt-4 cells in the log phase of growth were tested with Res, DAPT, and Res plus DAPT.
Results: Res and DAPT significantly inhibit the proliferation of Molt-4 cells and promote apoptosis in a dose- and time-dependent manner. Notch1 and *Hes-1* gene expression in Molt-4 cells was significantly downregulated by Res in a dose-dependent manner. Notch1 and *Hes-1* mRNA expressions were significantly decreased in Res, DAPT and Res plus DAPT groups compared with control group. Res combined with DAPT exhibited synergistic effects on the inhibition of proliferation and the induction of apoptosis in Molt-4 cells.
Conclusions: Res may function through the Notch1 signaling pathway. A combination of Res and DAPT may decrease γ-secretase inhibitor toxicity due to synergistic effects.

P3045

Mechanism of resveratrol-mediated reversal of drug resistance of HL-60/ADR cells via PI3K/Akt/Nrf2 signaling pathway

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Resistance to chemotherapy drugs, such as adriamycin (ADR), is a common problem in acute myeloid leukemia (AML) patients. We hypothesized that the natural compound resveratrol (Res) may reverse AML drug resistance through the PI3K/Akt/Nrf2 pathway. We investigated the *in vitro* effect of Res using human promyelocytic leukemia cells (HL-60) and the adriamycin-resistant cell line (HL-60/ADR) and treated with either Res or ADR+Res. Cellular proliferation inhibition rate, auto-fluorescence intensity of ADR in HL-60/ADR cells, mRNA and protein expression of PI3K, Akt and Nrf2 and the drug-resistance gene MRP1 were measured. Our results showed the half inhibitory concentration of HL-60/ADR cells treated with Res alone was significantly decreased in a dose-response manner. In addition, ADR+Res had a more significant inhibitory effect than Res alone on HL-60/ADR cells. Auto-fluorescence intensity of ADR in HL-60/ADR cells treated with ADR+Res was significantly increased, suggesting lower efflux of ADR from the cells. mRNA and protein expression of PI3K, Akt, Nrf2 and MRP1 were significantlydecreased in HL-60/ADR cells treated with both Res and ADR+Res. In conclusion, Res enhances ADR's inhibitory effect on AML cells and reverses the drug resistance of AML HL-60/ADR cells through inhibition of the PI3K/Akt/Nrf2 signaling pathway and down-regulating MRP1 expression.

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DOI: 10.1002/eji.201970400

P3046

Expression of TIM-3 on T cell from peripheral blood of cervical cancer

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Objective: To investigate the expression of TIM-3 on T cell from peripheral blood of cervical cancer patients, and explore the correlation of immunization between TIM-3 and cervicalcancer.
Methods: This study analyzed the expression of TIM-3 on T cell from peripheral blood with the method of flowcytometer. The samples included 21cases of control groups,15 cases of CIN patients and 24cases of cervical cancer patients. The expressions of TIM-3 on T cell from peripheral blood of different stages of cervical cancer patients were analyzed further.
Results: Comparison between CIN groups and control group: the expression of TIM-3 on CD4+T and CD8+T cells from peripheral blood increased significantly ($P< 0.05$). Comparison between cervical cancer patients and control group, the expression of TIM-3 on CD4+T and CD8+T cells from peripheral blood was significantly elevates ($P< 0.05$). Comparing with different clinical factors, there was no statistically significant association between the expression of TIM-3 on peripheral blood T cells and age, lymph node positive, tumor size and SCC level($P>0.05$).
Conclusion: The increased expression of TIM-3 on T cells from peripheral blood promoted the occurrence of cervical carcinoma in high-risk HPV infection, and it was positively correlated with the staging.

P3047

Knockdown of SNHG12 suppresses tumor metastasis and epithelial-mesenchymal transition via the Slug/ZEB2 signaling pathway by targeting miR-218 in NSCLC

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Non-small cell lung cancer (NSCLC) is a type of lung cancer which has a high mortality and low survival rate. Previous studies have revealed that long non-coding RNAs participate in tumorigenesis and metastasis in NSCLC. In the present study, the function of small nucleolar RNA host gene 12 (SNHG12) was investigated in NSCLC. Using reverse transcription-quantitative polymerase chain reaction analysis, it was identified that SNHG12 was significantly overexpressed in NSCLC specimens. Furthermore, overexpression of SNHG12 was identified to be associated with tumor progression and poor overall survival rates. Knockdown of SNHG12 in NSCLC cells could effectively induce cell apoptosis and suppress cell viability, proliferation, migration and invasion via inhibition of the epithelial-mesenchymal transition process. Furthermore, a direct interaction between microRNA (miR)-218 and the binding site of SNHG12 was identified. SNHG12 acted as an endogenous sponge for miR-218. Knockdown of SNHG12 upregulated the expression level of miR-218 as well as downregulating the Slug/zinc finger E-box-binding homeobox 2 EMT signaling pathway, and thus inhibited cell migration and invasion. Therefore, SNHG12 may serve as a key biomarker and a potential therapeutic target for the treatment of NSCLC.

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DOI: 10.1002/eji.201970400

P3048

Autophagy impacts on oxaliplatin induced hepatocarcinoma apoptosis via the IL-17/IL-17R-JAK2/STAT3 signaling pathway

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The interleukin (IL)-17/IL-17 receptor (IL-17R) complex has been shown to be important for the regulation of inflammation; however, its role in the regulation of tumor processes has recently emerged as a research focus. The present study demonstrated that oxaliplatin was able to increase the levels of IL-17/IL-17R in hepatocellular carcinoma (HCC) patients and cells lines, and that it had important roles in reducing the susceptibility of the cells to oxaliplatin-induced apoptosis. Furthermore, the expression of autophagy-related proteins was induced by IL-17/IL-17R and autophagy was shown to induce resistance to oxaliplatin in HCC. In addition, the janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway was shown to be an impor-tant pathway in the induction of autophagy in response to oxaliplatin. Autopjhagy was inhibited by 3-methyladenine and JAK2/STAT3 signaling was blocked by AG490, which induced apoptosis in SMMC7721 cells treated with oxaliplatin. The results of the present study may help to elucidate the mecha-nism underlying the role of IL-17/IL-17R-induced autophagy in the chemoresistance of HCC, as well as help to establish and develop measures to overcome chemoresistance in HCC.

P3049

Tumour necrosis factor-α-induced protein 8-like 2 is a novel regulator of proliferation, migration, and invasion in human rectal adenocarcinoma cells

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Tumour necrosis factor-α-induced protein 8-like 2 (TIPE2) is a tumour suppressor in many types of cancer. However, the mechanism of action of TIPE2 on the growth of rectal adenocarcinoma is unknown. Our results showed that the expression levels of TIPE2 in human rectal adenocarcinoma tissues were higher than those in adjacent non-tumour tissues. Overexpression of TIPE2 reduced the proliferation, migration, and invasion of human rectal adenocarcinoma cells and down-regulation of TIPE2 showed reverse effects. TIPE2 overexpression increased apoptosis through down-regulating the expression levels of Wnt3a, phospho (p)-β-Catenin, and p-glycogen synthase kinase-3β in rectal adenocarcinoma cells, however, TIPE2 knockdown exhibited reverse trends. TIPE2 overexpression decreased autophagy by reducing the expression levels of p-Smad2, p-Smad3, and transforming growth factor-beta (TGF-β) in rectal adenocarcinoma cells, however, TIPE2 knockdown showed opposite effects. Furthermore, TIPE2 overexpression reduced the growth of xenografted human rectal adenocarcinoma, whereas TIPE2 knockdown promoted the growth of rectal adenocarcinoma tumours by modulating angiogenesis. In conclusion, TIPE2 could regulate the proliferation, migration, and invasion of human rectal adenocarcinoma cells through Wnt/β-Catenin and TGF-β/Smad2/3 signalling pathways. TIPE2 is a potential therapeutic target for the treatment of rectal adenocarcinoma.

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DOI: 10.1002/eji.201970400

P3050

Lactoferrin plays an important role in AKT signaling pathway induced anoikis resistance and metastasis in nasopharyngeal carcinoma

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Lactoferrin (LF) gene is located in the high frequency deletion region on the chromosome of tumor patients, and its expression is down-regulated in many kinds of tumors. The lactoferrin the gene encodes is a member of the transferrin family and has the function of inhibiting tumor. In this study, HNE1 and HONE1 cells were cultured in suspension culture to construct the anoikis-resistant nasopharyngeal carcinoma cells (NPC), and the biological characteristics were detected to find that their survival, proliferation, invasion and metastasis, and anti-apoptotic ability were enhanced. The anoikis-resistant NPC (HNE1 cell) which Up-regulated LTF decreased cell survival and invasion ability, increased cell proportion in G1 phase, decreased cell proportion in S phase, increased apoptosis rate, down-regulated expression of vimentin, matrix metalloproteinase-9 and vascular endothelial growth factor A, and up-regulated expression of e-cadherin. The results were reversed in the anoikis-resistant NPC (HNE1 cell) which down-regulated LTF(HONE1 cell). Western blot indicated that the expression of AKT and TrkB in anoikis-resistant NPC cells were significantly increased, meanwhile, the expression of AKT and TrkB in untreated cells were higher than LTF overexpression cells and lower than LTF knockdown cells. Our results showed that the anoikis-resistant NPC cell had strong ability of proliferation, invasion and anti-apoptosis. LTF could inhibit anoikis resistance and metastasis of NPC, which molecular mechanism may be related to AKT signaling pathway.
Keywords: lactoferrin, nasopharyngeal carcinoma, anoikis, TrkB/ AKT

P3051

M2 macrophages-derived CXCL6 up-regulates PD-L1 expression through STAT3 pathway in esophageal squamous cell cancer

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Objective: To investigate the underlying mechanism by which CXCL6, mainly derived from M2 macrophages in esophageal squamous cell cancer (ESCC) in our previous study, works in the malignant behaviors of ESCC cells.
Methods: Immunohistochemistry was performed to detect the expression of CXCL6 with the ESCC tissue microarray. *In vitro*, wound-healing and Transwell assays were used to detect the effect of CXCL6 on the migration and invasion ability of ESCC cell lines Eca109 and KYSE150 in the absence of serum. Western blot was used to detect the expression variation of PD-L1 and phosphorylated STAT3 in Eca109 and KYSE150 cells treated with CXCL6.
Results: The positive expression rate of CXCL6 was shown to be significantly higher in ESCC than in adjacent normal control tissues (P < 0. 05). The migration and invasion of ESCC cells were markedly enhanced after treatment with CXCL6 recombinant protein compared with the control group (P < 0 05). The expression of PD-L1 was remarkably elevated in Eca109 and KYSE150 cells treated with CXCL6 relative to control group untreated with CXCL6. Meanwhile, as compared with control group, Eca109 and KYSE150 cells whose STAT3 were observed to be heavily phosphorylated after treatment with CXCL6. In addition, abolishment of phosphorylated STAT3 (p-STAT3) using the specific chemical inhibitor targeting p-STAT3 was found to be able to down-regulate the expression of PD-L1.

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DOI: 10.1002/eji.201970400

Conclusions: Collectively, M2 macrophages-derived CXCL6 can up-regulate the expression of PD-L1 through phosphylation of STAT3 in ESCC cells, suggesting that CXCL6 might be druggable in the immunotherapy of ESCC.

P3052

Stromal infiltration of neutrophil, M2 macrophage and cytotoxic CD8 T cells and its clinicopathological significance in esophageal squamous cell carcinoma

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Objective: To explore and quantitatively analyze the infiltration, distribution, prognostic significance of neutrophils, M2 macrophages and cytotoxic CD8 T cells in the tumor microenviroment of esophageal squamous cell carcinoma (ESCC)
Methods: Immunohistochemistry and immunofluorescence were used to identify the neutrophils, M2 macrophages and cytotoxic CD8T cells by means of the well-established surface biomarker CD15, CD163 and CD8α, respectively in ESCC tissue microarray consisting of 90 paired ESCC and its matched normal control dots. Prognostic significance of infiltration of these three immune cells was analyzed using Kaplan-Meier survival curve.
Results: The infiltration of neutrophils, M2 macrophages and cytotoxic CD8T cells were predominantly seen in the stroma rather than in the epithelial of ESCC tissues. Kaplan-Meier survival analysis showed that there was significant difference between patients with high infiltration of neutrophils and M2 macrophages versus those with low infiltration of neutrophils and M2 macrophages (P < 0. 05). On the contrary, it is the other way around for the infiltration of cytotoxic CD8T cells in terms of prognostic meaning. Patients with high infiltration of cytotoxic CD8T cells showed remarkably better overall survival rate than those with low infiltration of cytotoxic CD8T cells (P < 0 05).
Conclusions: Taking together, herein we showed the real-time infiltration and distribution of neutrophils, M2 macrophages and cytotoxic CD8 T cells in the tumor microenviroment of ESCC using multiple immunofluorescent staining platform by Perkin-Elmer finding that infiltrative intensity of the three kinds of tumor-associated immune cells was significantly linked with the overall prognosis of ESCC patients.

P3053

SPAG9/MKK3/p38 axis is a novel therapeutic target for liver cancer

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Background: Sperm-associated antigen 9 (SPAG9) is a biomarker and potential therapeutic target for several cancers; however, its involvement in liver cancer progression is not clear.
Objective: To determine whether SPAG9 regulates proliferation of liver cancer.
Methods: Immunohistochemistry and cell immunofluorescence were used to confirm the expression and the localization of SPAG9 in human liver cancer tissues and the liver cancer-derived HepG2 cells. A small interfering RNA (siRNA) designed to target *SPAG9* was transiently transfected into HepG2 cells using Lipofectamine™ 2000, and proliferation, apoptosis, and cell cycle progression were analyzed using the CCK8 assay and flow cytometry; western blotting was used to detect the expression of SPAG9, JNK, p38, MKK3, and MKK6, and co-immunoprecipitation was used to measure

DOI: 10.1002/eji.201970400

the interaction between SPAG9 and JNK.
Results: SPAG9 was overexpressed in 16 of 20 (80%) patients with hepatocellular carcinoma. The protein was localized in both cytoplasm and nucleus of liver cancer cells obtained from patients and in HepG2 cells. Depletion of SPAG9 inhibited the proliferation of HepG2 cells, promoted apoptosis, and arrested the cell cycle at S phase. Moreover, cells deficient in SPAG9 had decreased expression of JNK, p38, and MKK3 compared to HepG2 cells not treated with an siRNA targeting *SPAG9*.
Conclusion: SPAG9 regulates cell proliferation, apoptosis, and cell cycle progression in liver cancer cells through the SPAG9/MKK3/p38 axis. This axis is a novel therapeutic target for liver cancer.

P3054

IL-1β up-regulates PD-L1 via STAT3-dependent and independent mechanism in esophageal squamous cell carcinoma

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Objective: IL-1β derived from M2 macrophages was observed to be able to up-regulate PD-L1 expression in esophageal squamous cell carcinoma (ESCC) cells; however, the regulatory mechanism remains far from unknown. To investigate the working mechanism by which IL-1β up-regulates PD-L1 in ESCC.
Methods: ESCC cell lines Eca109 and KYSE-150 was treated in the presence or absence of recombinant human IL-1β, followed by phosphorylated STAT3 (p-STAT3), PD-L1 and KDM3A were detected with western-blot. Meanwhile, p-STAT3 as well as PD-L1 was evaluated in Eca109 and KYSE-150 cells treated with p-STAT3 specific inhibitor. Moreover, the methylated status of PD-L1 promoter was assayed with methylated PCR method with commercial kit.
Results: *In vitro* in ESCC cell lines Eca109 and KYSE-150, in which STAT3 can be significantly phosphorylated relative to control upon treatment with IL-1β, with the PD-L1 being markedly up-regulated; abolishment of phosphorylated STAT3 (p-STAT3) using specific inhibitor targeting p-STAT3 was shown to be remarkably capable of decreasing PD-L1, strongly suggesting that PD-L1 expression was STAT3-dependent. On the other hand, KDM3A, a contraction of Lysine (K)-specific demethylase 3A, was significantly elevated with treatment of IL-1β. Besides, methylated status of PD-L1 promoter was found to be mitigated, indicating that PD-L1 expression was STAT3-independent in ESCC as well.
Conclusions: Taken together, our data suggests that M2 macrophages-derived IL-1β up-regulates PD-L1 may be through STAT3-dependent and independent mechanism in ESCC, supporting the drugability of IL-1β in the immunotherapy of ESCC.

P3055

Regulation of PD-L1 in mouse B cell lymphoma

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Genetic alteration, viral infection and other mechanisms can induce high expression of programged death 1-ligad (PD-L1) on tumor cells, when binding to PD-1 on the surface of tumor-infiltrating

DOI: 10.1002/eji.201970400

lymphocytes, can promote tumor immune escape, thus participate in the pathogenesis of lymphoma.To investigated the regulation of PD-L1 in mouse B cell lymphoma, three RNA interference target sequences were designed for the target sequence of PD-L1, after ligated into pGMVL-SC5 interference vector, transiently transfected into A20 lymphoma cells. qRT-PCR was performed to detected the interference efficiency of each target, the shRNA vector with optimal interference efficiency was packaged with lentivirus and a stable and low expression of PD-L1 A20 lymphoma cell line (A20 PD-L1-KD) was obtained. The normal A20 and A20 PD-L1-KD cell lines were used to carry out tumor-bearing BALB/c mice with normal immune function. The tumor formation was observed every 2 days. The changes of body weight were recorded. The tumor formation was evaluated by fluorescence imaging. The survival time of each group was recorded. The results showed in vivo fluorescence imaging and pathological anatomy all indicated that the tumor size of PD-L1 low expression tumor-bearing mice was significantly lower than that of the common tumor-bearing group. The survival time was longer than that of the common tumor-bearing group. PD-L1 plays an important role in the development of lymphoma. Interference its expression has a certain inhibitory effect on the growth of tumor.
Keywords: PD-L1; RNA interference; lymphoma

P3056

Down-regulated miR-339-5p leads to aberrant over-expression of B7-H3 on glioma associated with tumor progression and invasion

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B7-H3, an immune-checkpoint molecule, is aberrantly over-expressed in various cancers, and is a predictive indicator for tumor metastasis and prognosis. However, the mechanisms underlying abnormal B7-H3 expression on tumor cells remains largely unclear. In this study, we investigated the expression and the regulatory mechanism of B7-H3 in glioma, which is one of the most common malignant brain tumors in human. We found that B7-H3 expression was significantly higher in human glioma tissues and tumor board infiltrating tumor cells, which was correlated with advanced tumor stage. We have identified that the B7-H3 gene could be directly targeted by miR-339-5p. Then we presented evidence to elucidate miR-339-5p inhibited glioma cell invasion by inhibition of IL-6 and IL-8 secretion through the JAK2/STAT3 signal pathway. Furthermore, we also explored the clinical value by similarly analyzing expression of miR-339-5p and B7-H3 and as well as the possibility of miR-339-5p-based therapy.

P3057

Enrichment of N-Cadherin-bearing leukemic stem cells by chemotherapy-resistance

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DOI: 10.1002/eji.201970400

Acute myeloid leukemia (AML) arises from genetic changes at the level of stem cell, various mutations have been elucidated, including AML1-ETO fusion gene has been shown as the representative target of cellular transformation for LSCs originating from hematopoietic stem cells (HSCs) compartment. LSCs resemble HSCs with respect to self-renewal capacity and chemotherapy-resistance. And the interaction mediated by adhesion molecules between LSCs and niche played a role in chemoresistance of LSCs. Therefore, study on the LSCs surface makers related to niche is helpful for the potential target therapy in the future. In this study, the proportions of LSCs compartment co-expressing the adhesion molecules, N-Cadherin (Ncad) from AML patients before and after chemotherapy were analyzed. We demonstrated Ncad positive LSCs population could be enriched by chemotherapy. Furthermore, AML1/ETO fusion signals and MDR1 expression were detected on the LSCs populations expressing Ncad. To determine the role of Ncad in maintaining LSCs self-renewal and stationary properties, Leukemic cell lines KG1a were used, and cells were divided into $Ncad^+$ and $Ncad^-$ fraction by FACS. The results showed that $Ncad^+$ cells had remarkable increased numbers of colonies with cytokines stimulation when compared with the negative control. However, IC50 of VP-16 was higher for $Ncad^+$ population than $Ncad^-$ population, and most cells in $Ncad^+$ fraction stayed in the G0-G1 stage. The results of engraftment showed that nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice transplanted with $Ncad^+$ cells had a shorter lifetime than the negative control. Therefore, Ncad is probably the potential marker for identification of LSCs.

P3058

Metastasis-associated protein 2 (MTA2) promotes the metastasis of esophageal squamous cell carcinoma via epithelial-mesenchymal transition

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Metastasis-associated protein 2 (MTA2) is frequently amplified in many types of cancers. Here, we report that MTA2 was highly expressed in esophageal carcinoma tissues and cell lines, and that further analysis revealed that high MTA2 expression was closely related to ESCC progression and prognosis. Moreover, the results from the gain- and loss-of-function experiments indicated that compared to the controls, MTA2 knockdown significantly suppressed ESCC proliferation and migration in vitro and viability and metastasis in nude mice in vivo. The forced expression of MTA2 accelerated the growth and migration of esophageal cancer cells compared to those of the controls. Microarray analysis indicated that MTA2 partially positively regulated the expression of genes that are associated with epithelial-mesenchymal transition (EMT), including EIF4E. We demonstrated that EIF4E was typically upregulated in ESCC and was associated with accelerated ESCC cells migration, invasion and EMT progression. EIF4E overexpression partially weakened the decrease in cell migration and invasion capacity in the MTA2 knockdown cells. Our results revealed that MTA2 promoted the development of ESCC via the regulation of EMT progression. In addition, forced EIF4E expression partially abrogated MTA2- knockdown induced EMT suppression. In contrast, the promotion of EMT progression that was caused by MTA2 overexpression was rescued by EIF4E depletion. Mechanistically, Twist, which was also modulated by EIF4E, recruited MTA2 to the E-cadherin promoter for repression the expression of E-cadherin. In conclusion, our study demonstrated that MTA2 has an oncogenic role in ESCC progression and suggested that MTA2 may be a potential target for metastatic ESCC therapy.

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DOI: 10.1002/eji.201970400

P3059

Coexpression of B7-H3 and α-SMA on cancer-associated fibroblast infiltration in gastric adenocarcinoma was a poor prognostic outcome indicator

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B7-H3 is an essential immune checkpoint abnormally expressed on tumor microenvironment and conducts tumor immune escape. Caner-associated fibroblasts play an important role in tumor evolution and progression; however, the impact of B7-H3 on tumor fibroblasts has not been fully clarified. Here, we examined B7-H3, CD31, and α-SMA protein expression in 268 gastric adenocarcinomas (GACs) by immunohistochemistry. We found B7-H3 was expressed in both tumor and stromal GAC cells. Immunofluorescence staining showed that B7-H3 was expressed in tumor cells and α-SMA-positive fibroblasts. In addition, the prognosis of patients with positive B7-H3 and α-SMA expression was significantly worse than that of other combination types. Our founding suggests that B7-H3 and α-SMA expression status can be used as an indicator of a poor prognosis for GAC patients.

P3060

Expression of TRIM28 correlates with proliferation and Bortezomib-induced apoptosis in B-cell non-Hodgkin lymphoma

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Tripartite motif containing 28 (TRIM28) as a transcriptional co-repressor has been reported playing a role in regulating DNA damage response (DDR), cell differentiation, immune response, and tumorigenesis. The present study was performed to explore the biological function and clinical significance of TRIM28 in B-cell non-Hodgkin lymphoma (B-NHL). Results of the study displayed that high expression of TRIM28 was positively associated with the poorer survival of B-NHL patients as an independent prognostic factor. In addition, TRIM28 could promote the B-NHL cells proliferation through modulating cell cycle progression. The change of cyclinA, P21, and PCNA expression after TRIM28 expression modified further illustrated the mechanism in which TRIM28 participated in cell proliferation progression. Moreover, inhibition TRIM28 expression in B-NHL cells enhanced the sensibility to Bortezomib by regulating p53-mediated apoptosis pathway. Taken together, the present study showed that TRIM28 functions as a tumor promoter in B-NHL and may be a novel target for drug resistance to Bortezomib.

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DOI: 10.1002/eji.201970400

P3061

Application of multiparameter flow cytometry for diagnosis and differential diagnosis of chronic myelomonocytic leukemia

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Objective: To analyze the response phenotypes of mononcytes in chronic myelomonocytic leukemia(CMML), so as to explore the value of flow cytometry in diagnosis and differential diagnosis of CMML.
Methods: The bone marrow immunophenotypic patterns of 60 CMML patients、50 healthy donors、15 patients with reactive monocytosis and 40 patients of AML-M5b were analyzed, and antigens with stable expression of monocytes were selected to be compared between CMML and other groups.
Results: percentage of myeloblasts and monocytes、expression of CD14、CD15、CD16、CD56 、HLA-DR in monocytes were compared between groups . Compared with healthy donors and patients with reactive monocytosis or CMML, AML patients demonstrate a characteristic increase in the percentage of myeloblasts($P<0.01$); Compared with healthy donors and patients with reactive monocytosis, CMML patients demonstrate a characteristic increase in the fraction of CD14+/CD16- cells, patterns with two or more abnormal antigens were more frequently observed in CMML patients than in other diseased group and healthy donors ($P<0.05$). Compared with patients with reactive monocytosis and AML-M5b, bone marrow monocytes with abnormal expressions of CD56、CD2、HLA-DR、CD15、and CD14 in CMML patients were significantly different ($P<0.05$).
Conclusion: immunophenotypic patterns of monocytes between CMML、AML-M5b、healthy donors and reactive were significantly different. Flow cytometry could be used to distinguish them.

P3062

Single cell transcriptomics reveal immune phenotype heterogeneity and cell interaction networks in the pancreatic cancer microenvironment

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Compelling evidence has suggested the pivotal role of immune cells in carcinogenesis and metastasis in pancreatic ductal adenocarcinoma (PDAC). Interrogation on the cell diversity is essential to delineate the tumor microenvironment and the cross-talks between immune cells and tumor. In the current study, we systemically profiled single-cell transcriptomes of PDAC covering 63,265 cells from 16 patients, providing the most comprehensive single-cell map of the PDAC microenvironment to date. Interestingly, intratumoral T cells exhibit a continuous dynamic transformation trajectory from the naive state to the dysfunctional state, providing rich resources for detailed mechanistic studies. Strikingly, fibroblasts displayed previously unidentified heterogeneity containing 3 subpopulations with different gene signatures, potentially leading to varied degrees of tissue fibrosis. Our analyses highlighted the connection between infiltrated Treg cells and monocytes, dendritic cells, epithelial cells by different chemokine and chemokine receptor pairs and provided insight into the cellular cross-talk of PDAC at single-cell resolution. Results from this study will further our understanding of immune landscape in PDAC with implications for therapeutic development.

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DOI: 10.1002/eji.201970400

P3063

Expression of CD81 and CD117 in plasma cell myeloma and the relationship to prognosis

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In this study the immunophenotype was retrospectively analyzed in 131 patients who received initial treatment for plasma cell myeloma (PCM) and the relationships of CD81 and CD117 with the clinicopathologic characteristics and prognosis were further evaluated. The Kaplan and Meier method and Cox regression survival analysis model were used to determine whether CD117 and CD81 were factors affecting the overall survival (OS) and progression-free survival (PFS) of PCM patients. CD117- and CD81-positivity was demonstrated in 35.88% and 40.46% of the 131 patients, respectively. Kaplan-Meier analysis showed that CD117 and CD81 were potential predictors of a patient's prognosis. Specifically, CD117(+) patients had longer PFS (p = 0.033) and OS (p = 0.002), while CD81(+) patients had shorter PFS (p = 0.001) and OS (p = 0.002). CD117(+) and CD81(-) patients had the longest PFS [p = 0.0183 compared to the CD117(-)CD81(-)/CD117(+)CD81(+) group ; p = 0.0007 compared to the CD117(-)CD81(+) group] and the longest OS [p = 0.0331 compared to the CD117(-)CD81(-)/CD117(+)CD81(+) group ; p = 0.0005 compared to the CD117(-)CD81(+) group]. Our results show that CD81 is an independent factor affecting the OS and PFS of PCM patients, and CD117 is an independent factor affecting the OS of PCM patients. CD117-positive and CD81-negative patients with PCM have a better prognosis.

P3064

The expression of Foxp3 and TLR4 in cervical cancer: association with immune escape and clinical pathology

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Objective: Purpose To explore the expression of forkhead/winged helix transcription factor p3(Foxp3) and toll-like receptor 4(TLR4) in cervical cancer and evaluate their clinical significance.
Methods: Foxp3 and TLR4 protein expression was detected in 105 cervical tissue specimens including cervical cancer, cervical intraepithelial neoplasia (CIN), and healthy control samples using immunohistochemistry. Their relationship with clinicopathologic parameters was also determined.
Results: Foxp3 and TLR4 had high levels of expression in cervical cancer cells (91.43 and 82.86%, respectively). Foxp3 levels were significantly associated with FIGO stage (P< 0.001) and tumor size (P = 0.034), while TLR4 levels were associated with clinical FIGO stage (P = 0.033) and lymph node metastasis (P = 0.031). Their expression levels were not correlated with age, histologic type, or differentiation (all P> 0.05). These findings suggest that Foxp3 and TLR4 may be useful prognostic indicators of cervical carcinoma. In addition, there were significant positive relationships between Foxp3 and TLR4 expression (r = 0.703, P< 0.001), which shows a possible link and synergistic role of Foxp3 and TLR4 in promoting the immune escape of cervical cancer.
Conclusions: Foxp3 and TLR4 may be useful biomarkers for patient prognosis and cervical cancer prediction and treatment.

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DOI: 10.1002/eji.201970400

P3065

Interleukin (IL)-33/ST2 axis promotes glioma mesenchymal transition and stemness via JNK activation

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IL-33 is a member of the IL-1 family which has pleiotropic activities in innate and adaptive immune responses. Recently, more researched have focused on the function of cellular immunity in the onset and development of tumor, and the expression and biological role of IL-33 in glioma is poorly understood. In this study, we found that glioma cells and tissues expressed higher levels of IL-33 and its receptor ST2 compared to normal brain. Clinically, tumor IL-33 expression was associated with poor survival in patients with glioma. Treatment with recombinant human IL-33 enhanced cell migration, invasion, epithelial to mesenchymal transition and stemness. Anti-ST2 blocked these effects of IL-33 on tumor. Mechanistically, IL-33 activated JNK signaling pathway via ST2 and improved the expression of key transcription factors that controlling EMT and stemness. Our study sheds lights on the role of IL-33/ST2 axis in brain glioma and offers a potential approach to the treatment of glioma patients.

P3066

Preliminary study on serum exosome microRNA-17-5p as a biomarker of colorectal cancer

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Objective: The purpose of this study is to investigate the expression level of microRNA-17-5p in serum exosomes of patients with colorectal cancer and its clinical significance.
Method: The serum exosome was extracted by Minute™ Hi-Efficiency Exosome Precipitation Reagent kit, and the specific surface marker protein CD9 of serum exosome was detected by Western blot. MicroRNA was extracted from the exosome by using the microRNA Neasy Mini Kit kit. The expression of microRNA-17-5p in the serum exosome of the subjects was detected by RT-q PCR, and the correlation was analyzed with clinical indicators.
Result: RT-q PCR results showed that the expression level of microRNA-17-5p in healthy control group was (1.048±0.561), in non-metastasis group was (3.173±1.943), and in metastasis group was (4.763±1.729). The expression levels of microRNA-17-5p in the three groups were not identical ($F = 10.750$, $P < 0.05$), and increased among healthy control group, non-metastasis group and metastasis group.The expression of serum exosome microRNA-17-5p in patients with colorectal cancer was analyzed with clinicopathological characteristics. It was found that microRNA-17-5p was related to the stage of cancer ($P < 0.05$) and distant metastasis ($P < 0.05$), but not to age, sex, tumor markers, location, pathological type, differentiation degree, depth of invasion and lymph node metastasis.
Conclusion: The expression of microRNA-17-5p in serum exosomes of patients with colorectal cancer was higher than that of healthy controls, and the content was positively correlated with tumor stage and distant metastasis.

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DOI: 10.1002/eji.201970400

P3067

Abnormal expression of RBM39 gene and prediction of related lncRNAs in PBMC of patients with esophageal squamous cell carcinoma (ESCC)

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Efficacy of early diagnosis and treatment of ESCC is unsatisfied, so it is necessary to further explore the molecular mechanism. In this study, the differential gene expression profiles of PBMC of 5 patients with ESCC were screened by high throughput sequencing and iTRAQ, compared to 5 healthy volunteers. Adjust p value < 0.05 and $\log_2$FC >1 were used as the criterion for differentially expressed genes. For mRNA, 529 up-regulated and 442 down-regulated genes were obtained. The RNA-binding protein gene, RBM39, was down-regulated in PBMC both in mRNA and protein levels. Down-regulation of RBM39 mRNA was validated by RT-qPCR in 30 ESCC patients and 30 healthy controls. Gene co-expression analysis of transcriptome data showed that lncRNA RP3-453C12.14 could be a candidate gene targeting RBM39. Further analysis of their expression and function in subtypes of PBMC will help to reveal the mechanism of abnormal immune status of ESCC.

P3068

Prognostic significance of circulating soluble programmed death ligand-1 in patients with solid tumors: a meta-analysis

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The aim of this meta-analysis was to explore the prognostic significance of circulating soluble programmed death ligand-1 (PD-L1) in patients with solid tumors. Several electronic databases were searched from January 1970 to May 2017. The hazard ratios (HRs) and 95%CI were calculated to determine the relationship between the level of soluble PD-L1 in peripheral blood and patient overall survival. A total of 1040 patients with solid tumors from 8 eligible studies were included in the present meta-analysis. The pooled HR suggested that high level of soluble PD-L1 (sPD-L1) in peripheral blood was significantly correlated with a worse overall survival (HR=2.26, 95% 1.83-2.80, Z=7.51, *P*< .001). The present meta-analysis demonstrated that high level of soluble PD-L1 in peripheral blood significantly predicts poor prognosis in patients with solid tumors, suggesting that high level of sPD-L1 may serve as a predictive biomarker for poor prognosis.

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DOI: 10.1002/eji.201970400

P3069

Decoding the information of tumor microenvironment embedded in cell free DNA of patients with esophageal cancer

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Cell free DNA (cfDNA) in human plasma carries abundant information, which has therefore been the key sample for noninvasive liquid biopsy. Especially by using the rapidly developed next-generation sequencing (NGS) techniques, the genetic and epigenetic information embedded in cfDNA has been effectively and extensively decoded. We have developed a new method(SALP-seq) for constructing the NGS library of cfDNA, which is a key step of NGS, and successfully sequenced 20 cfDNA samples from 16 esophageal cancer(EC) patients and 4 healthy people. By analysing the epigenetic difference between patients and healthy people,we identified 23 epigenetic altered esophageal cancer-specific genes, which has significant differences in chromatin accessibility around transcription start site (TSS). It´s worth mentioning that some of these genes played critical roles in programmed cell death(PCD),such as ATG4B, and upregulation of this pathway in tumor microenvironment (TME) would inhibit the anti-tumor immune response of effector T cells. Differences in gene expression has been identified by comparing the RNA-seq data of EC and normal samples collected by TCGA. Although further research is needed, this study sheds new light on the value of cfDNA NGS by providing new clue to the liquid biopsy of EC, deepens our understanding of immune escape mechanism and probably provides new biomarkers and ideas for cancer immunotherapy.

P3070

Direct binding and activation of c-Met by B7-H3 in colorectal cancer and glioblastoma promotes cancer cell stemness

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Immune checkpoint protein B7-H3 has been widely detected in the various types of tumors, while rarely in normal healthy tissues. However, the receptor of B7-H3 in tumor cell has not been identified. Here, we demonstrate that a novel interaction of B7-H3/c-Met labels a group of cancer initiating cells and maintains the cancer cell stemness via its downstream STAT3 signaling in colorectal cancer and glioblastoma. In addition, we observed the anti-tumor effects of Crizotinib (a c-Met inhibitor) and Cryptotanshinone (a STAT3 inhibitor), in B7-H3 positive CRC tumor in vivo. Last, we developed a functional B7-H3 antibody interfering B7-H3/c-Met binding, c-Met activation. Our findings suggest that targeting B7-H3/c-Met can be an effective strategy in tumor therapy.

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DOI: 10.1002/eji.201970400

P3071

Importance of TFEB acetylation in control of its transcriptional activity and lysosomal function in response to histone deacetylase inhibitors

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Background: TFEB is a master regulator of lysosomal biogenesis, function and autophagy. The transcriptional activity of TFEB is mainly controlled by phosphorylation mediated by the mTOR.
Purpose: At present, little is known whether other forms of posttranslational modifications such as acetylation also affects is transcriptional activity.
Methods: Lysosomal function was measured using LysoTracker and Magic Red assay for cathepsin B or L. Mass spectrometry was performed to identify the acetylation sites of TFEB. Immunoprecipitation was conducted to determine the interaction of TFEB with its upstream ACAT1 and HDAC2. siRNA specific for ACAT1 or HDAC2 was transfected into cells to knock down their expression. Luciferase assay was used to examine the transcriptional activity of TFEB.
Results: In this study, we first observed that a well-established histone deacetylase inhibitor SAHA activated lysosomal function in human cancer cells, a process independent of the mTOR pathway. Second, SAHA treatment activated TFEB transcriptional activity, as evidenced by increased TFEB luciferase activity and expression of its target genes. Third and more importantly, we observed the enhanced TFEB acetylation in SAHA-treated cells, with identification of 4 acetylation sites. Mutation of these 4 sites markedly diminished TFEB transcriptional activity and lysosomal function induced by SAHA. Finally, we found that TFEB acetylation was functionally implicated in SAHA-mediated autophagy and cell death in cancer cells.
Conclusion: Taken together, our results demonstrate that TFEB acetylation is a novel form of posttranslational modifications in TFEB that plays an important role in determining its transcriptional activity, lysosomal function and autophagy in cancer cells.

P3072

The role of PRR13 in the pathogenesis of breast cancer and the underlying molecular mechanism

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Breast cancer is one of the most common malignant tumors in women.At present, in addition to the natural factors, cell apoptosis and adhesion molecule expression are also closely related to the occurrence of breast cancer.In this study, a new tumor/testis antigenPRR13 was identified by selfestablished SADA method, and it was first found not to be expressed in human normal tissues, but highly expressed in breast cancer and other tumor tissues.Taking human breast cancer cells McF-7 as the model, it was found that after the expression of PRR13was down-regulated, the proliferation rate of McF-7 cells was slowed down, and the invasion ability was significantly decreased, and the sensitivity to apoptosis induced by chemotherapy drugs was increased.It is suggested that PRR13may be involved in the occurrence and development of breast cancer by affecting the biological behavior of breast tumor cells.This study aims to systematically evaluatethe correlation between PRR13and the occurrence, development and metastasis of breast cancer.The effects of RNAi and gene transfection on the biological function of breast cancer or tissue were comprehensively observed from in vivo and in vitro experiments, and the feasibility of using RNAi and gene transfection as immunotherapy targets for breast cancer was

DOI: 10.1002/eji.201970400

discussed.Immunoprecipitation combined with mass spectrometry and chip screening were used to identify the molecular and signal transduction pathways.It will lay a foundation for elucidation of the pathogenesis of breast cancer and provide new targets and ideas for its early diagnosis and immunotherapy.

P3073

Study on the drug resistance of HepG2 cells induced by adenosine triphosphate (ATP)-binding cassette transporter G2

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Objective: The study investigated the correlation between the expression of adenosine triphosphate-binding cassette transporter G2 (ABCG2) and drug resistance of liver cancer.
Methods: MTT assay was used to detect the IC50 of adriamycin (ADM) in the liver cancer HepG2 cells after treatment of ADM for 24h. An ADM-resistant subline, HepG2/ADM, was generated from the HepG2 live cancer cells by a stepwise selection in ADM from 0.001 to 0.1 µg/ml. The resulting subline was designated HepG2/ADM cell. The HepG2/ABCG2 cell line was established by transfecting ABCG2 gene into HepG2 cells. MTT was used to detect the IC50 of ADM on HepG2/ADM and HepG2/ABCG2 cells after the treatment of ADM for 24h, and the resistance index was calculated. The apoptosis, cell cycle and ABCG2 protein expression level in cells were detected by flow cytometry. Flow cytometry was used to detect the efflux effect of cells to ADM.
Results: IC50 of ADM to HepG2 cells was (0.72±0.03) µg/ml. After 3 months, HepG2/ADM cells grew stably in the cell culture medium containing 0.1 µg/ml ADM. The cell apoptosis rate of HepG2/ADM and HepG2/ABCG2 cells was no significant difference compared with HepG2 cells, $P>0.05$, but G_0/G_1 phase decreased and the proliferation index increased significantly, $P< 0.01$. The expression level of ABCG2 protein and efflux effect of ADM in HepG2/ADM and HepG2/ABCG2 cells was significantly higher than that in HepG2 cells, $P< 0.05$.
Conclusions: ABCG2 is highly increased in drug-resistant liver cancer cells, and high expression of ABCG2 is involved in the drug resistance of hepatocellular carcinama.

P3074

DHA inhibits melanoma involving in cytokine expression and STAT1 signaling pathway

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In recent years, although Dihydroartemisinin (DHA) has exhibited anti-tumor potential in a variety of tumors, its effect on melanoma has been rarely reported. To investigate the effects of DHA on melanoma, we performed a series of experiments. Firstly, we applied different concentrations DHA in B16F10 melanoma cells and found that DHA inhibited the proliferation and invasion of melanoma cells in a time-dependent and dose-dependent manner. Then, we established the melanoma bearing model with BALB/c mice by subcutaneous injection of B16F10 melanoma cells and gave DHA gavage (25 and 50mg/kg/d) for 14 days. Compared with the model group, a smaller tumor size was observed in the DHA group. In addition, apoptosis-related proteins cleaved caspase 9 and cleaved caspase 3 were significantly increased in the DHA group by Western Blot. Furthermore, DHA significantly reduced IL-6 and IL-10 levels-expression and increased IFN-γ levels-expression at mRNA level. Moreover, IFN-γ and signal transducers and activators of transcription 1 phosphorylation (p-STAT1) increased significantly, but PD-L1 and p-STAT3 remained unchanged through Western Blot. Lastly,

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DOI: 10.1002/eji.201970400

we established the mouse melanoma lung metastasis model by intravenous injection B16F10 melanoma cells with BALB/c mice and gave DHA gavage (20 and 40mg/kg/d) for 28 days. We found that the number of metastatic melanoma pulmonary nodules in the DHA group was significantly lower than that in the model group. These results suggest that DHA may regulate anti-tumor immunity by regulating STAT1 signaling pathway and cytokine levels.
Keywords: melanoma; DHA; B16F10; cytokine; STAT1

P3075

IL-23 production of liver inflammatory macrophages to damaged hepatocytes promotes hepatocellular carcinoma development after chronic hepatitis B virus infection

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Liver inflammation after chronic hepatitis B virus (HBV) infection is essential for hepatocellular carcinoma (HCC) development. We did a nested case-control study based on QBC chronic HBV infection cohort to identify HCC-related inflammatory cytokines. Serum levels of distinct Th-cell representative cytokines at varied periods before HCC diagnosis were determined in 50 HCC cases and 150 age- and gender-matched controls who did not develop HCC in 8-10 years. The individuals with HCC outcome had statistically higher serum levels of IL-23 than controls ($P < 0.01$). Further analysis in HCC tissues showed that $CD14^+$ inflammatory macrophages were the major IL-23 producers. Monocytes-derived macrophages generated more amount of IL-23 after being stimulated with cell-associated HBV core antigen from damaged HBV-infected hepatocytes than the cells being stimulated with HBV-S and HBV e antigen, which are secreted from infected hepatocytes. IL-23 upregulated IL-23 receptor expressions on macrophages, enhanced macrophage-mediated angiogenesis. In HBV-transgenic (Alb1HBV) mice, administration of diethylnitrosamine induced more liver tumors than in wild-type mice. The livers of Alb1HBV mice had higher concentrations of IL-23 and vascular endothelial growth factor (VEGF) than the wild-type mice. Neutralizing IL-23 activity, diethylnitrosamine-treated Alb1HBV mice developed significantly less tumors and produced less VEGF, tumor angiogenesis was inhibited with dramatically decreased $CD31^+$ cells within tumor mass (all $P < 0.01$).
Conclusion: Persistent IL-23 generation of liver inflammatory macrophages responding to damaged hepatocytes after chronic HBV infection altered macrophage function for HCC promotion. Blocking IL-23 activity might be helpful for the intervention in chronic hepatitis B patients who had high risk to HCC.

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DOI: 10.1002/eji.201970400

P3076

TIPE3 promotes NSCLC cell growth and metastasis via a AKT/ERK1/2-GSK3β-β-catenin/Snail pathway

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TIPE3 can be detected in a variety of human organs and is highly upregulated in various types of human tumor tissues. However, the role and mechanism of TIPE3 in the growth and metastasis of human lung cancer are not fully understood. Here, we demonstrated that the expression of TIPE3 in human lung cancer tumor tissues was elevated, which was positively associated with the tumor size, the presence of lymph node metastasis and the pathologic stage. We also found that TIPE3 was upregulated in a panel of human non-small cell lung cancer (NSCLC) cell lines. We furthermore established TIPE3-knocked down H1299 (H1299-shTIPE3) and H292 (H292-shTIPE3) and TIPE3-overexpressed A549 (A549-TIPE3) human NSCLC cell lines. We revealed that knockdown of TIPE3 remarkably suppressed in vitro proliferation, migration, invasion and induced G1 phase cell cycle arrest in H1299、H292 NSCLC cells and in vivo in athymic BALB/c nude mouse, whereas overexpression of TIPE3 promoted these processes in A549 NSCLC cells. Mechanistically, knockdown of TIPE3 significantly downregulated level of p-AKT, p-ERK1/2, p-GSK3β, β-catenin, Snail1, Slug, Cyclin D1, N-cadherin, Vimentin while upregulated E-cadherin in H1299、H292 NSCLC cells in vitro and in vivo in athymic BALB/c nude mouse. However, overexpression of TIPE3 exhibited an opposite effect in A549 NSCLC cells, and it has passed the test of interference suppression assays. Our data suggest that TIPE3 promotes growth and metastasis in human lung cancer very probably via a AKT/ERK1/2-GSK3β-β-catenin/Snail pathway. TIPE3 may be a novel therapeutic target for human lung cancer.

P3077

TIPE1 involves in tumor development by regulating hepatocyte death and macrophage activation

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in human beings, and its mortality ranks third among human tumor diseases, thus it is extremely harmful to human health. TIPE1was reported to be involved in regulating both necroptosis and apoptosis. However, the exact role of TIPE1 in the pathological milieu remains largely unknown. In the present work, we found that TIPE1 is involved in HCC pathogenesis by regulating both hepatocyte death and macrophage activation. Evaluated by immunohistochemical staining, hepatocytes in HCC tissues showed significantly downregulated TIPE1 expression compared with those in adjacent non-tumor tissues, which positively correlated with tumor pathologic grades and patient survival. We discovered that TIPE1 significantly inhibited tumor growth in vitro and in vivo by interacting with Rac1, and inhibiting the activation of Rac1 and its downstream p65 and c-Jun N-terminal kinase pathway. In addition, TIPE1 was highly expressed in mouse PM and BMDM cells and M2 macrophages showed much higher TIPE1 expression than M1 macrophage. HCM and TGF-β dose-dependently upregulated TIPE1 expression in macrophage, while the TGF-β receptor inhibitor SB431542 decreased TIPE1 level. TIPE1 knockdown reverses M2-like phenotype of BMDM. In conclusion, Hepatocyte TIPE1 expression is downregulated in HCC tissues, which is involved in promoting cell growth of hepatocytes. In contrary, TIPE1 is highly expressed in macrophage in HCC tissues, which might

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DOI: 10.1002/eji.201970400

induce macrophages to M2-like phenotype. Altogether, TIPE1 might involve in HCC development by two distinct mechanisms.

P3078

SIRT7 promotes NSCLC cell growth and metastasis via facilitation of G1 to S phase transition and EMT and activation of AKT and ERK1/2 signaling

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Increasing evidence indicates the roles of sirtuin 7 (SIRT7) in various human cancers. Nonetheless, the effects and the clinical significance of SIRT7 in human lung cancer remain largely unclear. In the present study, we demonstrated that SIRT7 was elevated in human lung cancer tumor tissues. Upregulation of SIRT7 was associated with lung cancer malignant clinicopathological characteristics. We also found that SIRT7 was upregulated in a panel of human NSCLC cell lines. We furthermore established SIRT7-overexpressed A549 (A549-SIRT7) and SIRT7-knocked down H292 (H292-shSIRT7) human NSCLC cell lines. Using above cell and xenograft mouse models, we revealed that overexpression of SIRT7 remarkably promoted growth, G1 to S cell cycle phase transition, migration, invasion and distant lung metastasis of A549 NSCLC cells *in vitro* and *in vivo*, whereas knockdown of SIRT7 repressed these processes in H292 cells. Mechanistically, SIRT7 overexpression significantly enhanced activation of AKT and ERK1/2 and also dramatically downregulated p21 and p27 CDK inhibitors as well as upregulated Cyclin D1 and Cyclin E1 cyclins and CDK2 and CDK4 CDKs. Interestingly, EMT process in A549 NSCLC cells was facilitated by SIRT7 overexpression, as evidenced by downregulation of E-cadherin epithelial marker and upregulation of N-cadherin, Vimentin, Snail and Slug mesenchymal markers. Meanwhile, knockdown of SIRT7 in H292 NSCLC cells exhibited opposite effects. Our data suggest that SIRT7 accelerates human NSCLC cell growth and metastasis possibly by promotion of G1 to S phase transition and EMT, and activation of AKT and ERK1/2 signaling. SIRT7 may be a novel therapeutic target for human NSCLC.

P3079

IKKα deficiency in dendritic cells triggers spotaneous adenocarcinoma through cGAS-STING pathway

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The pathway of cGAS-STING plays a pivotal role in dendric cells (DCs) and anti-tumor immune responses. The interplay between DCs homeostasis and tumor progression remains less known. Here we demonstrated a key role for the non-canonical nuclear factor κB (NF-κB) subunits, IKKα, in dendritic cell function. We found that spontaneous tumors were induced in mice lacking IKKα in DCs. IKKα deficient impaired the effector T cells homeostasis and the antigen-presentation of DCs. Moreover, inhibition of IKKα delayed cGAS-STING pathway by down-regulated STING, IRF3 and

DOI: 10.1002/eji.201970400

phosphorylation of STAT3. Our studies also found that loss of IKKα in DCs impaired the generation and maintenance of γσT cells, suggesting a therapeutic strategy to improve anti-tumor immune responses.

P3080

Aspirin inhibits colon cancer cell migration through EMT

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The mechanism responsible for the initiation of tumor metastasis and epithelial-mesenchymal transition (EMT) is not well understood. During EMT, epithelial cells lose their polarity and adhesion to surrounding cells and migrate, resulting in transition into mesenchymal cells. Canonical Wnt signaling has been implicated in controlling gene transcription and body axis pattern formation during development. However, canonical Wnt signaling has also been indicated to servea role in carcinogenesis by regulating EMT. In the present study, it was demonstrated that the expression of several positive regulators of EMT and Wnt signaling was repressed by aspirin treatment in SW480 tumor cells, and that this reduction was due to alterations in the localization of zinc finger E-box binding homeobox 1and Snail family transcriptional repressor 2. It was also demonstrated that aspirin may be an effective inhibitor of EMT, reducing the viability and migration ability of SW480 tumor cells, including cells induced by TGF-β1.

P3081

Circulating tumor microparticles promote lung metastasis by reprogramming inflammatory and mechanical niches via a macrophage-dependent pathway

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Despite the frequency of lung metastasis and its associated mortality, the mechanisms behind metastatic tumor cell survival and colonization in the lungs remain elusive. Here, we show that tumor cell-released microparticles (T-MPs) from the primary tumor site play a critical role in the metastatic process. The T-MPs remodeled the lung parenchyma via a macrophage-dependent pathway to create an altered inflammatory and mechanical response to tumor cell invasion. Mechanistically, we show that circulating T-MPs readily enter the lung parenchyma where they are taken up by local macrophages and induce CCL2 production. CCL2 recruits CD11b+Ly6Chigh inflammatory monocytes to the lungs where they mature into F4/80+CD11b+Ly6C- macrophages that not only produce IL6 but also trigger fibrin deposition. IL6 and the deposited fibrin facilitate the survival and growth of tumor-repopulating cells in the lungs by providing chemical and mechanical signals, respectively, thus setting the stage for lung metastasis. These data illustrate that T-MPs reprogram the lung microenvironment promoting metastasis

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DOI: 10.1002/eji.201970400

P3082

The multifunction of YB-1 in cancer

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Y-box binding protein 1(YB-1) is an ubiquitously expressed protein and involes in multiple biological functions.YB-1 is a family member of cold shock protein,the cold shock domain of YB-1 can bind to both DNA and RNA thus regulates the transcription and translation of genes.**YB-1 has a variety of important biological functions in proliferation, migration, invasion and apoptosis of cancer cell.** It has confirmed that YB-1 is increased in various cancer, and closely related to poor prognosis and drug resistance. However,inhibitory effect of YB-1 is also showed on cancer cell proliferation and differentiation.It can be indicated that **YB-1 may play a dual role in promoting or inhibiting cancer in the process of tumorigenesis and development. As a signaling molecule, YB-1 is involved in many intracellular signal transduction.** YB-1 can regulate multiple proteins and may be an effective target for cancer treatment.In addition,YB-1 can regulate systemic or local inflammatory reaction by regulating the expression of proinflammatory and anti-inflammatory factors.**YB-1 can increase the expression of proinflammatory factor in initial inflammatory response stage and activates inflammatory cells.YB-1 can be secreted to extracellular space by granulocytes,lymphocytes and other cells and play a crucial role in inflammation and cancer. This review is attempt to summarize the molecular structure, tissue expression,impact on cancer development of YB-1.**
Keywords: Y-box binding protein 1; signal pathway; cancer

P3083

Prognostic value of TIM-1 expression in human non-small-cell lung cancer

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T-cell immunoglobulin and mucin domain 1 (TIM-1) is an important co-stimulatory molecule which serves as a surface marker for T cell activation, especially for Th2 cells. Recently, many studies have also shown that TIM-1 can be abnormally expressed in human cancers and may have a potential role in promoting cancer progression. We found that the TIM-1 expression was increased in human NSCLC tissues compared with the adjacent normal tissues, and the OS rate of NSCLC patients with higher TIM-1 expression was significantly lower compared with the ones with lower TIM-1 expression. The COX model showed that higher TIM-1 expression in lung cancer tissues could be used as an independent prognostic predictor for the patients. Furthermore, we depleted TIM-1 in NSCLC cell lines A549 and SK-MES-1, and the cellular functional studies also revealed that depletion of TIM-1 could significantly inhibit the cell viability as well as the abilities of migration and invasion. In addition, our microarray data showed that certain signaling pathways were altered and enriched after depletion of TIM-1. We subsequently verified that PI3K/Akt signaling pathway was involved in the TIM-1-mediated regulation of cellular functions in NSCLC cells. Our findings supported the notion that TIM-1 could serve as a potential therapeutic target for NSCLC.

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DOI: 10.1002/eji.201970400

P3084

Overexpression of HHLA2 in human clear cell renal cell carcinoma is significantly associated with poor survival of the patients

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HHLA2 could be uniquely expressed in humans but not in mice, and play an important role in the functional inhibition of CD4 and CD8 T cells. Herein, we found that higher HHLA2 mRNA expression level in human ccRCC tissues compared with that in adjacent normal tissues based on TCGA data, and the HHLA2 expression at mRNA level was positively and significantly correlated with PD-L1, PD-L2, B7-H6, but negatively and significantly correlated with B7-H3. Moreover, our immunohistochemistry study showed that the staining intensity of HHLA2 in human ccRCC tissues was significantly higher than that in the adjacent normal tissues, and the overall survival rate of ccRCC patients with higher HHLA2 expression was significantly poorer than that of the patients with lower HHLA2 expression. Higher expression of HHLA2 in ccRCC tissues was positively and significantly associated with larger tumor size and advanced TNM stage. The COX model revealed that the parameters including patient's age, TNM stage and HHLA2 expression level could be used as the independent risk factor respectively for the prognostic prediction of the patients. Our cellular study showed that upon knockdown of HHLA2 expression in human ccRCC cell lines, the cell viability, the migration and the invasion ability were significantly inhibited, while the cell cycle arrest at G1 phase was induced and the expressions of Cyclin D1, c-Myc and Cyclin E1 were decreased. In addition, according to the microarray data, the expressions of epithelia-to-mesenchymal transition markers, such as E-cadherin, N-cadherin and Vimentin, were significantly decreased after knockdown of HHLA2 expression.

P3085

CTLA-4 positive breast cancer cells suppress dendritic cells maturation and function

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Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a potent immunoregulatory molecule, can down-regulate T-cell activation and inhibit anti-tumor immune response. This study showed that LPS-stimulated human dendritic cells (DCs) decreased the expression of HLA-DR, CD83 and costimulatory molecules (CD40, CD80 and CD86) following coculturing with CTLA-4+ breast cancer cells. Moreover, the suppressed DCs further inhibited proliferation of allogeneic CD4+/CD8+ T-cells, differentiation of Th1 and function of cytotoxic lymphocytes (CTLs). However, CTLA-4 blockade in breast cancer cells could recover DC maturation and cytokine production, elevate antigenpresenting function of DCs, reverse Th1/CTLs response and cytokine secretion. Subsequent study demonstrated that the activation of extracellular-signal regulated kinase and signal transducer and activator of transcription 3 of DCs caused by CTLA-4+ breast cancer cells were the predominant mechanism of DC suppression. In addition, CTLA-4 blockade treatment also directly inhibited proliferation and induced apoptosis of CTLA-4+ breast cancer cells. Collectively, CTLA-4 was expressed and functional on human breast cancer cells through influencing maturation and function of DCs in vitro, and CTLA-4 blockage not only recovered the antigen-presenting function of DCs and T-cells activation but also suppressed the biological activity of breast cancer cells themselves. This study highlights the clinical application of CTLA-4 blockade therapy in breast cancer.

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DOI: 10.1002/eji.201970400

P3086

Low-dose total body irradiation can enhance systemic immune related response induced by hypo-fractionated radiation

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A systemic immune related response (SIME) of radiotherapy has been occasionally observed on metastatic tumors, but the clinical outcomes remain poor. Novel treatment approaches are therefore needed to improve SIME ratio. We used a combination of hypo-fractionated radiation therapy (H-RT) with low-dose total body irradiation (L-TBI) in a syngeneic mouse model of breast and colon carcinoma. The combination therapy of H-RT and L-TBI potentially enhanced SIME by infiltration of CD8+ T cell and altering the immunosuppressive microenvironment in non-irradiated subcutaneous tumor lesions. The frequency of IFN-γ, as a tumor-specific CD8+ T cells producing, significantly inhibited the secondary tumor growth of breast and colon. Our findings suggest that L-TBI could serve as a potential therapeutic agent for metastatic breast and colon cancer and, together with H-RT, their therapeutic potential is enhanced significantly.

P3087

The study of molecular mechanism of ABCE1 in hepatocarcinogenesis

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As an essential and highly conserved protein, ATP-binding cassette E1 (ABCE1) involves all kinds of biological functions. In detail, ABCE1 improves replication of human immnnodeficiency virus (HIV) through specifically binding to RNase L and inhibiting its activity. Via blocks the 2-5A/RNaseL pathway which correlates with cell growth, ABCE1 inhibits cell proliferation as well. Moreover, the expression of ABCE1 is tissue specific, which shows high level in the spleen, liver, lung, intestine and most of immune cells such as T, B cells and macrophages. Researches have shown that ABCE1 might play roles in both innate immunity and tumor promotion. However, the upstream mechanism of ABCE1 in carcinogenesis is still uncovered. It is well-known that c-myb is a proto-oncogene and which is overexpressed in all kinds of malignant tumor cells. Meanwhile, it has been confirmed c-myb as a key regulator of $CD8^+$ T cell stemness. Prediction with the PROMO database has shown that c-myb might be the transcription factor of ABCE1. Preliminary data have shown that both ABCE1 and c-myb were significantly increased in clinical liver cancer tissues and liver cancer cells, and might play an important role in liver carcinogenesis. We suppose that ABCE1 could be an effective molecular target in clinic and highlight its therapeutic potential. First, we try to confirm the relationship between ABCE1 and c-myb. Then we are going to explore the upstream mechanism of ABCE1 in tumor immunology and liver tumorigenesis by using all kinds of experiments of molecular and cell biology.

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DOI: 10.1002/eji.201970400

P3088

Interferon-stimulated gene ISG12a exerts antitumor effects through crosslinking the canonical Wnt/Beta-catenin signaling and cancer immunity

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Regulatory mechanism of interferon (IFN)-stimulated genes (ISGs) in immunity and cancers remains ambiguous. ISG12a, an IFN-stimulated gene, reverses anticancer drug resistance and enhances innate immune response to viral infection, suggesting its potential role in anticancer. Here, low expression level of ISG12a is found in cancer cells and majority tissue samples with hepatic or gastric cancers, and significantly correlated with the poor prognosis of patients. Characteristics of cancer cells in vitro and in vivo is upregulated with ISG12a knockdown. Mechanically, ISG12a promotes the proteasome degradation of β-catenin through inhibiting the ubiquitination of Axin induced by E3 ubiquitin ligase Skp2, thus blocking the abnormal activation of canonical Wnt/β-catenin signaling. Moreover, ISG12a restricts surface PD-L1 expression and sensitizes the cancer cells to NK cell killing, thus enhancing the anticancer immunity. Together, our study reveals the anticancer effect of ISG12a, and partially explains the promising therapeutic efficacy of IFNs in clinic.

Reproductive Immunology

O409

Antibiotic use during pregnancy impacts neonatal microbiota composition and increases offspring asthma severity

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Antibiotic use during pregnancy is associated with increased allergic asthma risk in children. Given that antibiotics are prescribed to approximately 25% of pregnant women, it is important to understand the mechanisms contributing to this phenomenon. Despite this fact, there are currently no studies that directly test this association experimentally. Herein, we develop the first mouse model to test the effect of antibiotic treatment during pregnancy with regard to offspring asthma. We found that daily maternal treatment with the antibiotic vancomycin (from gestation day 8 to 17) resulted in increased offspring asthma severity in a dose dependent manner. Maternal antibiotic use was also significantly associated with decreased weight in allergic offspring. Quantitative real-time PCR analysis of the fecal microbiota at several time points (gestation day 17, weaning and after offspring allergy induction), revealed that both dams and pups displayed microbial dysbiosis associated with maternal antibiotic use. The increased asthma severity and intestinal microbiota changes in pups were also correlated with

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DOI: 10.1002/eji.201970400

significant decreases in caecal short chain fatty acid concentrations. Consistent with the "Developmental Origins Hypothesis", our results confirm that exposure to antibiotics during pregnancy shapes the neonatal intestinal and immune environment and increases offspring allergic asthma severity.

O410

Placental endovascular extravillous trophoblasts (enEVTs) educate maternal regulatory T cell differentiation via producing TGFβ1

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The healthy growth of a semi-allogeneic fetus requires the adaptive regulation of the maternal immune system to establish an immune tolerant environment at the maternal-fetal interface. Along the placental-maternal circulation pathway, the maternal blood perfuse into the placenta through the uterine spiral arteries (SPA), in which the endothelial cells are extensively replaced by endovascular extravillous trophoblasts (enEVTs). The placenta villi immerse into the maternal blood that perfuse from SPA, while are not attacked by the maternal lymphocytes. The mechanism of immune tolerance in this pathway remains unclear. Here we demonstrate that the maternal blood naïve $CD4^+$ T cells can be induced differentiation to Tregs by enEVTs in the remodeled SPA, and therefore building up an immune-tolerant environment along the placental-maternal circulation pathway. Our results of *in situ* hybridization, flow cytometry and ELISA demonstrated that enEVTs, but not interstitial extravillous trophoblast cells (iEVTs) or decidual endothelial cells (dECs), could actively produce TGFβ1. The conditioned media from the highly-purified primary enEVTs significantly promoted $FOXP3^+$ Tregs differentiation, and the effect could be blocked by the TGFβ1 blocking antibody. Neither iEVTs nor dECs affected Tregs differentiation. In recurrent spontaneous abortion (RSA) patients, the proportions of TGFβ1-producing enEVTs and Tregs in SPA and intervillous space significantly reduced compared to normal pregnancy. Our results demonstrate a unique immune-regulatory characteristic of enEVTs, and provide new insight in revealing the mechanism of immune tolerance in the placental-maternal circulation system. We anticipate our study will help to understand the mechanisms that contribute to placental pathology of RSA.

O411

The correlation between vitamin D and Treg/Th17 and the effects of vitamin D supplementation on Treg/Th17 balance in recurrent pregnancy loss

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Problem: This study aimed to determine the correlation between vitamin D and Treg/Th17 and the effects of vitamin D supplementation on Treg/Th17 balance in women with recurrent pregnancy loss (RPL).

DOI: 10.1002/eji.201970400

Methods of study: The level of vitamin D was determined in women with normal pregnancy (NP) and RPL by electrochemiluminescence. The percentages of $CD4^+Foxp3^+$ Treg, $CD4^+IL\text{-}17^+$ Th17 and $CD4^+Foxp3^+IL\text{-}17^+T$ cells were determined by FCM before and after vitamin D supplementation. Changes about Treg/Th17 balanceafter culturing with active vitamin D*in vitro*were determined. Vitamin D metabolic activity of PBMC was also detected by RT-PCR.
Results: Compared with NP, both the level of vitamin D and the Treg/Th17 ratio were significantly decreased in RPL. There was a positive correlation between the level of vitamin D and the Treg/Th17 ratio in RPL. Within the RPL group, those who received 2 months of vitamin D supplementation showed a significantly increased Treg/Th17 ratio compared with those without vitamin D supplementation. *In vitro*analysis showed that adding different concentrations of active vitamin D increased the Treg/Th17 ratio, also the mRNA levels of the vitamin D receptor and the metabolic enzyme CYP24A1 increased significantly.
Conclusion: The occurrence of RPL may be related to vitamin D insufficiency and Treg/Th17 imbalance. The Treg/Th17 imbalance seen in RPL can be restored by vitamin D supplementation both *in vivo*and *in vitro*. The effects of vitamin D on the immune regulation of RPL indicate that vitamin D might be used as an alternative therapyin the future.

O412

Role of Tim-3 in decidual Macrophage functional polarization during abnormal pregnancy with Toxoplasma gondii infection

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Vertical transmission of the intracellular parasite *Toxoplasma gondii* (*T. gondii*) can lead to devastating consequences during gestation. Tim-3, a negative immune regulator, is constitutively expressed on decidual macrophages, but its specific role during *T. gondii* infection has not yet been explored. In the present study, we discovered that Tim-3 plays an important role in the abnormal pregnancy due to *T. gondii* infection using Tim-$3^{-/-}$ pregnant mice and anti-Tim-3 neutralizing antibody treated human decidual macrophages. The results showed that abnormal pregnancy outcomes were more prevalent in Tim-$3^{-/-}$ infected pregnant mice than in wild-type infected pregnant mice. Tim-3 expression in decidual macrophages was significantly down-regulated after *T. gondii* infection both in vitro and in vivo. Tim-3 down-regulation by *T. gondii* infection could strengthen M1 activation and weaken M2 tolerance by changing the M1 and M2 membrane molecule expression, arginine metabolic enzymes synthesis, and cytokine secretion profiles of decidual macrophages. Moreover, Tim-3 down-regulation by *T. gondii* infection led to PI3K-AKT phosphorylation inhibition, downstream transcription factor C/EBPβ expression, and SOCS1 activation, which resulted in enzymes synthesis regulation and cytokines secretion. Our study demonstrates that Tim-3 plays an indispensable role in the adverse pregnancy outcomes caused by *T. gondii* infection.

DOI: 10.1002/eji.201970400

O413

The PD-1 signaling regulates macrophage polarization by reprogramming metabolic pathways to facilitate pregnancy establishment

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Problem: The regulatory effects of PD-1 signaling on macrophage polarization during early pregnancy are unclear.
Methods: The profile of decidual macrophages (DMs) and the expression of PD-1 and PD-L1 on DMs from women with early normal pregnancy (NP) and recurrent miscarriage (RM) were determined by FCM. PD-L1 expression on villous from NP and RM was analyzed by WB and qRT-PCR. Monocytes from NP were cultured in vitro with rhGM-CSF with or without PD-1 activation. The percentages of M1 and M2 subsets and phagocytosis were determined by FCM. Their metabolism was analyzed by qRT-PCR. Normal pregnant mice were treated with PD-1 antagonist. The percentages of M1 and M2 macrophages and PD-1 expression were determined by FCM.
Results: Apart from higher percentages of M1 DMs, and lower frequencies of M2 DMs, decreased PD-1 expression on the two subsets were observed in RM compared with NP. Low PD-L1 expression on villus was determined in RM. Using *in vitro* experiments, we found that PD-1 activation by PD-L1 Fc drove the differentiation of macrophages biased to M2 subset and showed enhanced phagocytic activity. PD-1 blockade enhanced pro-inflammatory cytokines and IRF5 expression, while IRF4 expression was inhibited. In addition, PD-1 blockade promoted glycolysis and inhibited FAO. Both PI3K/AKT/m-TOR and MEK/ERK pathways underlies PD-1 signal during DM metabolism reprogramming. *In vivo* data showed PD-1 blockade or deficiency may be associated with increased M1 percentages at the maternal-fetal interface and embryo loss.
Conclusions: PD-1 signaling is a major regulator of macrophage differentiation and function, and it is critical for the success of a pregnancy.

O414

Mechanism and antiviral function of hypoxia-induced trophoblast autophagy

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Objective: To investigate the molecular mechanism and potential antiviral function of hypoxia-induced autophagy of trophoblast cells at the maternal fetal interface.
Method: Trophoblast cell line HTR-8 was cultured under normoxia and hypoxia condition respectively. Autophagy level was evaluated by electron microscope and by the expression of LC3B and p62. Autophagy-related PCR array was used to screen potential molecules regulated by hypoxia. PTEN inhibitor and siRNA was uesd to determine the regulatory role of PTEN in hypoxia-induced autophagy. RNA-seq was used to investigate potential function of hypoxia-induced autophagy.
Results: We found that hypoxia significantly induced HTR-8 autophagy. Autophagy-related PCR array showed that hypoxia significantly upregulated PTEN expression. Mechanismly, hypoxia inhibits Akt/mTOR pathway through PTEN, and further promotes HTR-8 autophagy. Treatment with autophagy inhibitor 3-MA up-regulated 1459 gene and down-regulated 1496 gene expression. GO and KEGG analysis indicated that trophoblast autophagy was involved in the regulation of antiviral immunity. We further showed that hypoxia regulated Poly I:C stimulated IFN-β1 via autophagy in HTR-8. **Conclusion:** Our study revealed the regulatory role of PTEN in hypoxia-induced trophoblast

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DOI: 10.1002/eji.201970400

autophagy, and firstly investigated potential antiviral role of hypoxia-induced autophagy at maternal-fetal interface.
This work was supported by grants from the National Natural Science Foundation of China (No. 81772879) and National Key Research and Development Program of China (2018YFC1002803).

O415

Targeting iron-induced inflammatory T cells in endometriosis immunotherapy

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Endometriosis is a high incidence of chronic inflammatory disease that is a leading cause of pelvic pain and infertility in women of gestational age. However, the immune microenvironment of ectopic tissue and the activating mechanism of inflammation are still unclear.The ovaries are the organs most susceptible to endometriosis due to ectopic endometrial invasion. Here, we have illustrated the tissue structure and distribution of immune cells of ectopic tissue in ovarian endometriosis, identifying a class of pro-inflammatory T cells induced by iron deposition. First, we found that a large number of patients with endometriosis were associated with anemia, and repeated bleeding at the ectopic endometrium resulted in excessive deposition of iron ions during menstruation. At the same time, we found that a large number of inflammatory cytokines are contained in cyst fluid by cytokine antibody microarray. In addition, we revealed that lots of T cells are accumulated in ectopic endometrium. High dose of ferrous ion can convert endometrium resident T cell to inflammatory states through X signaling. Inflammatory T cells secrete high levels of pro-inflammatory cytokines and chemokines such as Y, IL-6, IL-8, IL-18 and CCL2. Sustained inflammatory factor stimulation leads to severe pain and follicle development disorders in patients with ovarian endometriosis.Our study revealed the pathological immune microenvironment of endometriosis and the pathogenesis of iron-induced T cell inflammatory response, providing new ideas and targets for immunotherapy of diseases.

O416

The role of Tim-3 on decidual NK cells dysfuction during abnormal pregnancy with Toxoplasma gondii infection

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Vertical transmission of *Toxoplasma gondii* infection during gestation can result in severe complications such as abortion, congenital malformation and fetal teratogenesis. Immune inhibitory molecule Tim-3, discovered to be expressed on some decidual immune cells and participate in the maintenance of maternal fetal tolerance. The dysregulation of Tim-3 expression on dNK cells were observed in many pregnancy complications, while the role of Tim-3 on dNK cells during *T. gondii* infection is still unclear. In this study, *T.gondii* infected Tim-3$^{-/-}$ pregnant mice and anti-Tim-3 neutralizing antibody treated human dNK cells were successfully established to explore the role of Tim-3 on dNK dysfuction during abnormal pregnancy with *T. gondii* infection. Our results showed that

DOI: 10.1002/eji.201970400

Tim-3$^{-/-}$ pregnant mice displayed worse pregnancy outcomes after *T.gondii* infection compared to WT infected pregnant mice. Also, it demonstrated that Tim-3 expression on dNK cells was significantly down-regulated after *T. gondii* infection in vivo and in vitro. Data suggested remarkable activation of dNK cells in Tim-3$^{-/-}$ mice and anti-Tim-3 neutralizing antibody treated group, with higher ratio of NKG2D/NKG2A, INF-γ/IL-10 and more granules production (Granzymes, Perforin) compared with WT infected mice and infected dNK cells. Also we proved that *T. gondii*-caused Tim-3 decrease significantly activated phosphorylation of PI3K-AKT and Jak-Stat pathway, which further upregulated the secretion of GranzymeB, Perforin, INF-γ and IL-10. Our research demonstrated that the decrease of Tim-3 on dNK cells caused by *T. gondii* infection further lead to dNK cells dysfunction, which finally contribute to the development of abnormal pregnancy outcomes.

P0466

Phenotypic heterogeneity and cytokine expression by tissue-resident memory T cells in human and murine uterus

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Recently, tissue-resident memory T cells (Trm) that are different from Tem and Tcm were identified in the tissues and organs, which can provide protective immunity against microbial agents that enter through the local tissues. After removing blood cells from tissues we found that most of the T cells in murine uterus were memory T cells and expressed significantly higher levels of chemokine receptors including CCR2, CCR5, CX3CR1, CXCR4 and CXCR5. Most of CD4^{+}T cells and CD8^{+}T cells expressed the surface marker of Trm , CD69 and CD103. Compared with T cells in blood, we found that CD69^{+}T and CD103^{-}T cells in uterus expressed higher percentages of IFN-γ and IL-17 than CD69^{-}T and CD103^{+}T cells. In addition, we found that CD69^{+}CD103^{+} and CD69^{+}CD103^{-}T cells expressed significantly more proportion of IFN-γand IL-17 than CD69^{-}CD103^{+} and CD69^{-}CD103^{-}T cells. Finally, we found that T cells in the human uterus were mainly distributed in the endometrium and there were few T cells in the myometrium. Our data indicated that Trm cells played an important role against uterine reinfections.

P0467

Palmitate-TLR4 pathway induces decidual dysfunction via GLN metabolism reprogramming

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Gravida serum contains an amount of palmitate (PA), the simplest long-chain fatty acid and the substrate of mitochondrial respiration. Its receptor, Toll-like receptor 4 (TLR4), is a critical immunoregulatory factor in decidual stromal cells (DSCs). This study was aimed to explore the regulatory mechanisms of PA-TLR4 signaling pathway on the biological functions and immunometabolism in DSCs. Here, we observed the abnormal formation of lipid droplet in the DSCs of gravidas with hyperlipidemia. Activation of PA-TLR4 signaling induced the apoptosis and pro-inflammatory cytokine production in DSCs, which was accompanied by the abnormal accumulation of intracellular lipid droplets. Specifically, PA-TLR4-induced apoptosis and inflammatory cytokine production, particularly IL-6, in DSCs were dependent on JNK pathway and NK-κB pathway. In addition, the measurement of the oxygen consumption rate (OCR) demonstrated that PA-TLR4 signaling facilitated the basal mitochondrial respiration in DSCs, and that the alteration of the proportion of three mitochondrial oxidation pathways occurred, among which glutamine (GLN)

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DOI: 10.1002/eji.201970400

oxidation was significantly activated, fatty acid oxidation was suppressed, and aerobic glycolysis remained unchanged. Meanwhile, PA-TLR4 signaling up-regulated the expression of glutaminase (GLS) in DSCs. Inhibition of GLN oxidation worsen the PA-TLR4-triggered apoptosis and inflammation in DSCs. These findings identify PA-TLR4-mediated GLN metabolism reprogramming as a key alternative energy metabolism pathway is involved in regulating the apoptosis and inflammation in DSCs, suggesting a potential therapeutic target for gestational hyperlipemia-induced decidual dysfunction.

P0468

Endonuclease APE1 treatment of miR-92b formation, thereby regulating the expression of tumor suppressor LDLR in cervical cancer cells

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Apurinic/apyrimidinic endodeoxyribonucuclease 1 (APE1) is a multifunctional AP endonuclease that regulates the transcription of many cancer-associated genes through the regulation of the microRNA group. Here, we investigated the role of miR-92b-3p (miR-92b) in the progression of cervical cancer, and its treatment may be regulated by APE1.
A ratio based on qRT-PCR was measured for APE1 treatment of miR-92b from its pri-miR form. APE1 endonuclease activity was measured by AP site incision assay. Ape1-drosha interactions were studied by immunofluorescence confocal and adjacent junction analysis. MiR-92b targeting low density lipoprotein receptor was studied by luciferase reporter assay. Silencing of mir-92b and shRNA-based mir-92b, as well as LDLR overexpression and siRNA-based LDLR silencing were used in CaSki and SiHa cervical cancer cells. Cell proliferation and chemical sensitivity to paclitaxel and cisplatin were determined. Cell cycle progression and apoptosis were assessed by flow cytometry. Tumor growth was studied in mouse xenograft model.
The endonuclease activity of APE1, through association with the DROSHA-processing complex, is necessary for the miR-92b maturation thereby regulating the expression of LDLR. miR-92b promotes cell proliferation in vitro and in vivo, promotes cell cycle progression, and reduces apoptosis and chemosensitivity. Silencing LDLR summarizes the transformation of miR-92b, while overexpression of LDLR rescues these effects.
APE1 enhances the treatment of miR-92b, thereby inhibiting the expression of LDLR and promoting the progression of cervical cancer. Our identification of the novel APE1-miR-92b-LDLR axis enhances our understanding of the complex pathogenesis of cervical cancer and reveals a new therapeutic strategy to combat this disease.

P0469

The appropriate frequency and function of decidual Tim-3$^+$CTLA-4$^+$CD8$^+$T cells are important in maintaining normal pregnancy

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Maternal decidual CD8$^+$T (dCD8$^+$T) cells must integrate the antithetical demands of maternal-fetal tolerance and anti-viral immunity to establish a successful pregnancy. T-cell immunoglobulin mucin-3 (Tim-3) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are two important co-inhibitory molecules that regulating CD8$^+$T cells responses during infection and tumor. In the present study, we

DOI: 10.1002/eji.201970400

examined the co-expression of Tim-3 and CTLA-4 on $CD8^+$T cells during pregnancy and found the higher frequency of Tim-3^+CTLA-4^+d$CD8^+$T cells in response to trophoblasts. This Tim-3^+CTLA-4^+d$CD8^+$T cells subset showed an active status and produced more anti-inflammatory cytokines. Furthermore, the decreased number and altered function of Tim-3^+CTLA-4^+d$CD8^+$T cells correlated to miscarriage. Combined targeting Tim-3 and CTLA-4 pathways were highly effective in inhibiting the production of anti-inflammatory cytokines and were detrimental to the maintenance of pregnancy. Together, these findings supported that Tim-3 and CTLA-4 pathways might play positive roles in the establishment and/or maintenance of maternal-fetal tolerance so to promote the maintenance of normal pregnancy. So the reproductive safety must be considered, especially when anti- Tim-3/CTLA-4 antibody (and other immune checkpoint inhibitors) are used in pregnancy.

P0470

Altered frequency and function of spleen CTLA-4^+Tim-3^+ T cells are associated with miscarriage

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Normal pregnancy is associated with several immune adaptations in both systemic and local maternal-fetal interface to allow the growth of semi-allogeneic conceptus. A failure in maternal immune tolerance to the fetus may result in abnormal pregnancies, such as recurrent spontaneous abortion (RSA). The regulation of T cell homeostasis during pregnancy has important implications for maternal tolerance and immunity. Cytotoxic T-lymphocyte antigen-4(CTLA-4) and T-cell immunoglobulin mucin-3 (Tim-3) are important negative immune regulatory molecules involved in viral persistence and tumor metastasis. Here we described the lower frequency of splenic T cells co-expressing CTLA-4 and Tim-3 accompanied by higher levels of pro-inflammatory but lower anti-inflammatory cytokines production in abortion-prone mouse model. Blockade CTLA-4 and Tim-3 pathways leaded to the dysfunction of splenic T cells. By the higher expression during normal pregnancy, CTLA-4 and Tim-3 co-expression on splenic T cells linked to immunosuppressive phenotype. As the spleen is an important site for peripheral immune activation, our data suggest potential non-invasive biomarkers and therapeutic targets for miscarriage.

P0471

Predictive value of peripheral blood lymphocyte subsets for early pregnancy outcomes of recurrent miscarriage

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Objective: Recurrent miscarriage is associated with abnormal lymphocyte subsets. This study discussed the relationship between lymphocyte subsets abnormalities and early-pregnancy outcomes.
Methods: 263 women' data were collected in the Second Affiliated Hospital of Zhengzhou University from January 2017 to August 2018. There were 153 RM patients, including 66 RM non-pregnant patients and 87 RM early-pregnant patients. 110 healthy women were selected as controls, including 42 non-pregnant women and 68 early-pregnant women. The percentage of peripheral blood lymphocyte subset was measured by flow cytometry and the pregnancy outcomes were followed up to 10 weeks.
Results: Compared to healthy women (9.56±4.68%), the percentage of NK cell of RM patients (14.26±5.42%)was significantly increased, P< 0.001. ROC analysis (AUC = 0.7378) showed that there was high specificity (94.55%) for RM women when NK percentage > 17.5%, and RM patients with

DOI: 10.1002/eji.201970400

early pregnancy were more likely to have miscarriage when NK percentage >13.5%. T and helper T lymphocytes were significantly increased in RM patients with early pregnancy than in RM non-pregnant patients (P< 0.001). For all women, NK percentage of pregnant women was significantly lower than those without pregnancy (P< 0.001). The percentage of NK in RM abortion patients was significantly higher than that in RM pregnant continuation patients(P< 0.001). In control pregnant women, there is no difference in the percentage of NK between abortion women and pregnant continuation women (P>0.05).
Conclusion: An increase in the percentage of NK is a risk factor which would cause early pregnancy with RM history to loss .

P0472

Spontaneous delivery is associated with increased levels of cord blood endothelial cell-derived microvesicles compared to elective caesarean section

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Introduction: Endothelial activation and dysfunction is associated with inflammatory reaction. Inflammatory processes are suggested to accompany the initiation of spontaneous labor. Aim of our study was to explore biomarkers of inflammation in different modes of delivery.
Methods: Biomarkers were measured in cord blood and in newborns (48-72 hours) using multiplex immunoassays Luminex®xMAP multi-analyte profiling platform and flow cytometry (BD FACS CantoII). Term healthy newborns after uncomplicated pregnancy and spontaneous delivery (n=24), elective (n=12) and emergency (n=13) caesarean section (C-section) were included.
Results: The concentration of MV in cord blood showed significant increase in spontaneous delivery compared to elective C-section (2020±1187 vs. 1336±513 MV/µL; p=0.03). Endothelial-specific MV were significantly increased in spontaneous delivery cord blood compared to elective C-section determined by expression of: VCAM/CD106 (10 ± 6 vs. 6±3 MV/µL; p=0.01), EPCR/CD201 (67±38 vs. 37±25 MV/µL; p=0.02), endoglin/CD105 (225±230 vs. 128±61 MV/µL; p=0.05), ICAM1/CD54 (34±27 vs. 18±9 MV/µL; p=0.03) and MadCAM (156±88 vs. 104±36 MV/µL; p=0.01). No significant differences in endothelial MV were found between spontaneous and emergency C-section groups or between both C-section groups. We measured soluble VCAM, ICAM1 MadCAM, and endoglin in cord blood and neonatal samples. VCAM was the only marker which showed significantly higher levels in cord blood in spontaneous delivery compared to elective C-section.
Conclusion: Spontaneous delivery seems to be accompanied by increased total and endothelial microvesicles in cord blood compared to C-section suggesting activation of endothelium and increased inflammatory activity during spontaneous delivery compared to elective C-section.

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DOI: 10.1002/eji.201970400

P0473

The participation of microvesicles of leukocyte origin in reproductive processes

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Microparticles are present in the peripheral blood during normal pregnancy and at preeclampsia, the functional characteristics of them differ. It is thought that MV can influence surface receptor expression on circulating immune cells migrating to the decidual tissue.
The aim of this work was to study the content and morphometric properties of microparticles originating from various peripheral blood leukocyte populations in normal pregnancy and preeclampsia, and to evaluate the impact of them on phenotypic properties of intact and activated monocytes.
Microparticles were isolated by a series of differential centrifugations of peripheral blood samples of nonpregnant women, healthy pregnant women and women with preeclampsia. Morphometric analysis of samples of blood plasma microparticles was performed by atomic force microscopy (NT-MDT, Russia). THP-1 cell line was used as a model of monocytes. THP-1 cells were disseminated in a 24-well plate, some cells were activated with TNFα. Samples of microparticles were transferred to the THP-1 cells and cultivated for 24 hours. The resultant microparticles and THP-1 cells were stained with cocktail of antibodies. The expression of receptors was assessed using flow cytometer (BD, USA). The statistical analysis was performed using the Mann-Whitney nonparametric test.
We showed that normal pregnancy is associated with high levels of microparticles formed by the main leukocyte populations. Preeclampsia is associated with low levels of microparticles formed by NK cells. These types of microvesicles have different influences on the expression of surface molecules on THP-1 monocytes.
The work was supported by the RFBR grant 17-04-00679 and the RSF grant 17-15-01230.

P0474

Investigation of mother to child transmission of HLA antibodies

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Human leukocyte antigens (HLA) are proteins responsible for presentation of peptide fragments to T lymphocytes. HLA-encoding genes are extremely polymorphic, which may lead to alloimmunity mediated by HLA antibodies. Late in pregnancy, women produce and transfer high amounts of antibodies, including HLA antibodies, to the fetus. However, very little is known about their specificity, transfer rate, and role in pregnancy and newborns.
The purpose of this study was to describe the prevalence of HLA antibodies in serum of thirty mother-infant pairs six weeks post-partum. We used Luminex technology that allows for detection of low-level antibodies. We found that 72% of babies and 80% of mothers expressed HLA antibodies. In babies, 77% of HLA-I antibodies and 50% of HLA-II antibodies matched those of their mothers, suggesting that the remaining antibodies were self-made. In contrast, the proportion of maternal antibodies that matched those of their babies was only 16% for HLA-I and 24% for HLA-II, suggesting that not all HLA antibodies produced during pregnancy cross to the fetus.
Production of self-made antibodies by six weeks of age indicates early capacity of the neonatal

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DOI: 10.1002/eji.201970400

immune system to generate antibodies; whether they are natural antibodies or preceded by exposure to specific HLA antigens, remains to be clarified. Our findings further indicate that there is selective antibody transfer of particular antibody specificities. Because HLA antibodies may act against paternal antigens present in the neonate, the mechanism of selective antibody transfer or depletion may give insights into mechanisms of immune tolerance.

P1059

Decreased expression of TIGIT on NK cells in patients with recurrent implantation failure (RIF)

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Recurrent implantation failure (RIF) refers to not achieving clinical pregnancy after repeated transfers of morphologically good quality embryos into a normal uterus. Patients with idiopathic RIF were considered to be thrombophilia, hysteroscopy and endometrial culture normal, and no chromosomal factor was suspected. The increased number of peripheral blood NK (pNK) cells has been supposed as a factor for RIF. However the dysregulation of NK cells activity was poorly understood, especially for the inhibitory receptors expression level on NK cells in the patients. T cell immunoglobulin and ITIM domain (TIGIT) is an inhibitory immune checkpoint receptor, which is involved in mediating T cells and NK cells exhaustion in tumors. The aim of this study was to investigate whether TIGIT was expressed differentially on NK cells in RIF patients. Here we found that the expression level of TIGIT on pNK cells was significantly lower than that of control women, and what's more, the TIGIT+ cell desity in endometrium was also lower than that of control women. Low TIGIT expression may lead to overactivity of NK cells both in the peripheral blood and endometrium, which caused the inability of the embryo to implant. Then we detected the secretory cytokines of pNK cells, such as IFN-γ, perforin and granzyme B, all of which were pretty higher than those of control women. This study suggested that testing for the expression of TIGIT receptor on NK cells might be useful in women with idiopathic RIF before the embryo transfer.

P1060

Human monocytes undergo functional re-programming during differentiation to dendritic cell mediated by human extravillous trophoblasts

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Maternal immune adaptation is required for a successful pregnancy to avoid rejection of the fetal-placental unit. Dendritic cells within the decidual microenvironment lock in a tolerogenic profile. However, how these tolerogenic DCs are induced and the underlying mechanisms are largely unknown. In this study, we show that human extravillous trophoblasts redirect the monocyte-to-DC transition and induce regulatory dendritic cells. DCs differentiated from blood monocytes in the presence of human extravillous trophoblast cell line HTR-8/SVneo displayed a DC-SIGN(+)CD14(+)CD1a(-) phenotype, similar with decidual DCs. HTR8-conditioned DCs were unable to develop a fully mature phenotype in response to LPS, and altered the cytokine secretory profile significantly. Functionally, conditioned DCs poorly induced the proliferation and activation of allogeneic T cells, whereas promoted CD4(+)CD25(+)Foxp3(+) Treg cells generation. Furthermore, the supernatant from DC and HTR-8/SVneo coculture system contained significant high amount of M-CSF

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DOI: 10.1002/eji.201970400

and MCP-1. Using neutralizing antibodies, we discussed the role of M-CSF and MCP-1 during monocyte-to-DCs differentiation mediated by extravillous trophoblasts. Our data indicate that human extravillous trophoblasts play an important role in modulating the monocyte-to-DC differentiation through M-CSF and MCP-1, which facilitate the establishment of a tolerogenic microenvironment at the maternal-fetal interface. This work was supported by grants from the National Natural Science Foundation of China (No. 81772879) and National Key Research and Development Program of China (2018YFC1002803).

P1061

The expression of LEP/ sLEPR and Th17/Treg in preeclampsia

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Objective: The study aimed to confirm the interaction of leptin(LEP)/ soluble leptin receptor(sLEPR) with T-helper type 17 (Th17)/ regulatory T (Treg), cytokines IL-35 and IL-17 in cases of preeclampsia (PE).
Methods: Peripheral blood was obtained from 50 PE patients and 50 normal control (NC) pregnant women. The mRNA expressions of LEP/ sLEPR, Treg transcription factor FoxP3 and Th17 transcription factor RORγt, IL17A,IL17F and TGFβ were determined by Quantitative real-time PCR (Q-PCR). The serum levels of IL-17 and IL-35 were measured by ELISA.
Results: It was observed that the mRNA expressions of Foxp3 in PE patients were lower compared to the normal control pregnancy, whereas the RORγt expression was significantly higher. The results also demonstrated that PE patients exhibited decreased levels of Treg-related cytokine IL-35, whereas Th17-related cytokine IL17A,IL17F and TGFβ were significantly increased. PE patients expressed higher mRNA levels of LEP, whereas sLEPR was much lower. Furthermore, the mRNA levels of FoxP3 T cells correlated negatively with LEP. In addition, there were positive correlations between the mRNA level of IL-17 and LEP.
Conclusion: Our results suggest that imbalance of Th17/Treg cells, affected possibly by LEP / sLEPR, may be involved in the pathogenesis of PE, emphasizing on the importance of these changes in maternal immunological cross-talk.

P1062

Homing and restorative effects of bone marrow-derived mesenchymal stem cells on cisplatin injured ovaries in rats

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Objective: Premature ovarian failure (POF) is a long-term adverse effect of chemotherapy treatment. We aim to evaluate the homing and distribution of BMSCs in ovaries injured by cisplatin after intravenous injection, and to evaluate its restorative effects on ovarian structure and function.
Methods: The Sprague-Dawley rats were divided into three groups (10 rats/group): normal control group, cisplatin-induced POF group and BMSCs treatment group. At days 15 and 30, Serum estradiol levels were monitored in each group, and ovarian morphologic, and tracked BMSCs by immunohistochemical staining.
Result: The BMSCs treatment group's antral follicle count and estradiol levels increased after 30 days, compared with the POF group. The granulosa cell (GC) apoptosis induced by cisplatin was

DOI: 10.1002/eji.201970400

reduced when BMSCs were migrated to granulosa cells (GCs) in vitro. Chemotherapy-induced POF was induced by intraperitoneal injection of cisplatin in rats. BMSCs labeled with enhanced green fluorescent protein (EGFP) were injected into the rats via the tail vein to investigate the homing and distribution of BMSCs in vivo. The number of BMSCs in the ovarian hilum and medulla was greater than in the cortex, but no BMSCs were found in the follicles and corpus lutea.
Conclusion: In conclusion, ovarian function and structure was restored, after intravenously injecting BMSCs and intravenously delivered BMSCs can home to the ovaries,and restore its structure and function in POF model rats.

P1063

Alteration of peripheral blood cytokines of infertile patients with recurrent spontaneous abortion after vitamin D supplementation

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Objective: To investigate the effect of vitamin D supplementation on the levels of cytokines in peripheral blood of infertile patients with recurrent spontaneous abortion (RSA).
Methods: A prospective study was conducted on 155 infertile patients with RSA who were admitted to the department of obstetrics and gynecology of Shengjing Hospital affiliated to China Medical University from January 2017 to January 2018.All the patients were excluded from the cause except for the immune factor. They were given the same kind of vitamin D preparation and calcium agent combined treatment, and the levels of serum vitamin D and peripheral blood cytokines were determined before and after the treatment.
Results: The level of serum vitamin D of the patients with RSA was higher than that before treatment, and the levels of IL-2 and TNF-α were decreased. All the difference was statistically significant($P<0.05$).
Conclusion: The occurrence of RSA may be related to vitamin D insufficiency and Th1/Th2 imbalance. Vitamin D supplementation can decrease the level of Th1 cytokines in peripheral blood. The Th1/Th2 imbalance seen in women with RSA can be adjusted by vitamin D supplementation in *vivo*. Vitamin D might be used as an immunotherapy because of its immunomodulatory effects on RSA in the future.

Key words: recurrent spontaneous abortion; vitamin D;supplementation

P1064

Characteristics of lymphocyte subsets in patients with non-criteria obesteric antiphospholipid syndrome

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Object: The clinical characteristics of non-criteria antiphospholipid syndrome (oAPS) proposed in 2000 remains unclear compared with criteria oAPS. We investigate the difference of lymphocyte subsets between criteria and non-criteria oAPS to reveal clinical characteristics and provide treatment evidence of non-criteria oAPS.
Methods: 38 patients with criteria oAPS (Sydney criteria, Criteria-Group) and 49 age-matched non-criteria oAPS,(Non-criteria Group) were enrolled in our study between June 2015 and June 2018. Flow cytometry was used to examined the percentage of the lymphocyte subsets in peripheral blood.

DOI: 10.1002/eji.201970400

Results: The relative proportion of $CD3^+CD8^+$T cells(Ts cell) and $CD3^+CD8^+$ $CD28^+$(activated Ts cell) were significantly higher in criteria-group than non-criteria group(p< 0.05).The percentage of $CD3^-CD56^+$(natural killer cell, NK cell) shows lower percentages in criteria-group compared with non-criteria group(p< 0.05),whereas there was no significantly difference of $CD3^-CD56^{dim}CD16^+$($CD16^+$NK cell) and $CD3^-CD56^{bright}CD16^-$($CD16^-$NK cell) between criteria-group and non-criteria group,(p>0.05).
Conclusion：Non-criteria oAPS exhibits lower activation degree of Ts cell and percentage of NK cell compared with criteria oAPS which reflects a lightly autoimmune activation status in Non-criteria oAPS patients. Activation degree of Ts cell and percentage of NK cell in peripheral blood may help us fomulate treatment plan for non-criteria oAPS in contrast to criteria oAPS.
Keywords: oAPS; lymphocyte subsets；Ts cell

P1065

Proteases regulating the immunological system in recurrent pregnancy loss

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Object: Proteases hydrolyze proteins that play an important role in the reproductive and immune system. Immune factors are one of the causes of recurrent pregnancy loss (RPL), and the function of the immune system is largely dependent on interleukins. In this study, proteases (ITI-H4, KLKB1, and RPL-serine protease A)-interleukins loop axis was confirmed, indicating a novel pathogenesis of RPL.
Materials and methods: Human blood samples were collected from patients with RPL in the Fertility Center of the CHA General Hospital in Korea. Flow-cytometric assay: Cytokine concentrations in the supernatants of the cells and sera were measured with the BD cytometric Bead Array Human Th1/Th2/Th17 Inflammatory Cytokines Kit. Quantitative RT-PCR: It was performed using the SYBR Green Master Mix, in the ABI 7500 Real-time PCR System.
Results: Expression of a cysteine protease ITI-H4 (ΔN688), an aspartic protease KLKB1 and pro-inflammatory cytokines was higher in the RPL human serum than that of the control group. ITI-H4 increased the level of anti-inflammatory cytokines and ITI-H4 (ΔN688) increased the level of pro-inflammatory cytokines, respectively. KLKB1 also increased the level of pro-inflammatory cytokines, but decreased the level of anti-inflammatory cytokines. Interestingly, IL-6 promotes expression of ITI-H4 (ΔN688) via ERK, STAT3 and JNK signaling pathway. IL-6 also promotes KLKB1 expression and cleaves long ITI-H4 to ITI-H4 (ΔN688).
Conclusion: KLKB1 produces ITI-H4 (ΔN688) from long ITI-H4 and these isoforms have opposing roles in immune system through regulating cytokines. RPL-serine protease A previously identified by subtractive hybridization was demonstrated to be involved in immune signaling.

P1066

Postpartum metabolites, and their implications on postpartum ovarian cyclicity of Friesian Dairy Cows

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Viability and profitability of livestock enterprise is evaluated by the percentage of pregnant females over a period of time after exposure to bulls. Transition Cows undergo a lot of metabolic changes.

DOI: 10.1002/eji.201970400

Adaptation is more pronounced in early lactation. During this time feed intake is insufficient resulting in negative energy balance. There is interaction between metabolic hormones and follicular development. In Kenya**,** information on effects of postpartum metabolites and hormones on re-initiation of ovarian cyclicity is scarce. The objective of this research was to determine adaptive processes and how they affect postpartum ovarian cyclicity. The study used 20 Cows at 7 months in-calf supplemented with total mixed ration and 10 Cows at 7 months in-calf as negative Control. Weekly milk samples were collected into sodium azide containers for progesterone analysis using radioimmunoassay. Biweekly blood samples were collected and analyzed for metabolites. Data was analyzed using SAS 9.1.3 portable. Metabolites highly ($P< 0.05$) varied across treatments. There was significant ($P< 0.05$) difference in glucose levels between prepartum and postpartum periods in both groups. Non-esterified fatty acids (NEFA) peaked 2 weeks postpartum. Beta-hydroxybutyrate (BHBA) peaked 4 weeks postpartum. There was high (R^2=0.9, $P< 0.05$) correlation between postpartum cyclicity and glucose. Reinitiation of ovarian cyclicity started immediately NEFA and BHBA declined to thresholds.
Keywords: Metabolites, effects, ovarian rebound
*

P1067

Features of certain parameters of the immune system in men with disturbed fertility

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One of the factors of male infertility is the absence of the spermatic duct, the cause of which may be cystic fibrosis. Changes in the reproductive system affect the work of the immune system. The purpose of the study is a clinical and immunological examination of men with primary infertility of unknown origin. 95 men of reproductive age were examined. Anamnesis was collected, seminal fluid examination, spermograms, determination of the level of sex hormones, as well as ultrasound of the scrotum, kidney, and Doppler sonography of the spermatic cord. The control group consisted of 20 healthy men, married and having children. Of the 95 surveyed men aged 28-40, 56 experienced primary infertility of unknown origin, who were married for more than 3 years. Of these, azoospermia was detected in 32.6%, in 28.6% -varocell, chronic bacterial viral infection was diagnosed in 72.5%. Testicular hypoplasia was found in 18.4% of men and 36.8% had changes in hormonal status. Homozygote for allele 1 in the CFTR_Del 508F gene was detected in 8 patients. It was revealed that this contingent had an imbalance in the content of some parameters of the immune system. Thus, the level of CD3 + - and CD4 + was reduced, CD8 +, CD16 + and CD95 + were elevated. The number of CD20 + and the level of IgG and IgA were elevated. Thus, in men with infertility, along with other clinical and laboratory changes in the body, there are deviations in the state of the immune system.

P1658

The expression and function of Myosin-11 in mouse testis development

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Smooth muscle myosin heavy chain (Myosin-11/SMMHC/SM-MHC/MYH11) is a well-known biomarker of vascular physiology, which is involved in vascular or blood disease and different kinds of cancer. However, there are no reports about the function of MYH11 in male reproductive system. Here, we aimed at revealing the expression of MYH11 in mouse testis and its functions in male sterility. As a

DOI: 10.1002/eji.201970400

result, we found that MYH11 was expressed widely in the mouse testis by immunohistochemistry, which was consistent with the results from RT-PCR and western-blot analyses. In addition Myh11 was down-regulated both in mRNA and protein levels in the testes of male sterile pcd (Purkinje cell degeneration) mouse caused by the mutation of cytosolic carboxypeptidase 1 (CCP1). Dramatically, immunoprecipitation combined mass spectrometry revealed that MYH11 might be interacted with CCP1 and USP9Y. The structural model of MYH11 was predicted by Phyre2 software and the functional protein sites were analyzed with Antheprot Editor software, respectively. Moreover, Bioinformatic analysis results suggested that MYH11 might play crucial role in tight junction pathway besides its well-known muscle construction pathway. Our results may shed light on the novel function of MYH11 related to male reproductive system.

P1659

Comparison of experimental autoimmune orchitis as a model for immune male infertility

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Objective: About 60-75% of male infertility is caused by idiopathic spermatogenesis in clinical practice. Lymphocyte infection and immune deposition were found in multiple testicular biopsy specimens in previous studies, suggesting that inflammatory reaction or immune factors were the cause of the lesion. However, there is little intuitive evidence to prove the effect of different modeling methods on male immune infertility at present. Therefore, we compared the effects of different treatments on experimental autoimmune orchitis (EAO) in BALB/c mice.
Methods: 24 balb/c mice were randomly divided into 4 groups, 6 mice in each group. Mice in the control group were injected subcutaneously with PBS or adjuvant. Testicular homogenates completely emulsified with Fresno adjuvant from Balb/c ,C57 mice ,SD rats were injected subcutaneously into balb/c mice. The homogenates were injected 3 times, 0.2ml each time, and the interval was 2 weeks. Fifty days after the first immunization, the cauda epididymis of mice was taken to test the quality of semen. Mice testes were taken and sectioned for pathological comparison after HE staining.
Results: The results showed that compared with the control group, the sperm density of the EAO(SD) group was significantly reduced, and the sperm density of the control group, the EAO(balb/c) group and the EAO(C57) group was slightly reduced. Testicular injury was severe in the EAO (SD) group, but less than in the EAO (C57) and balb/c groups.
Conclusion: The results showed that the mice of balb/c strain were immunized with SD rats´ testicular homogenate.

P1660

Generation of hypertrophic cardiomyopathy specific stem cell lines from a patient who carries myosin binding protein C (*MYBPC3*) p.Cys1124Leufs*25 mutation

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Objective: We idendified an autosomal dominant mutation of the myosin binding protein C (MYBPC3) gene p.cys1124leufs *25 in a Chinese family with familial cardiomyopathy (HCM). In this study, we aim to generate iPSCs from a 62-year-old male patient with p.cys1124leufs *25 mutation.

DOI: 10.1002/eji.201970400

Methods and results: We generated a human induced pluripotent stem cell (hiPSC) line from a patient's peripheral blood mononuclear cells with p.cys1124leufs *25 mutation by using episomal vectors for transgene delivery. Induced pluripotent stem cells expresses pluripotent stem cell markers such as SSEA4 and TRA-1-81 ； in vitro formation of teratoma demonstrated trilineage differentiation potential；We also confirmed that the iPSCs has a normal karyotype；All iPSCs cells express genes markers characteristic of hES cells, including POU5F1 and NANOG.
Conclusions：This hcm-specific iPS cell line with *MYBPC3* p.Cys1124Leufs*25 mutation was successfully established to provide a useful resource for studying and modeling hypertrophic cardiomyopathy.
Keywords: HCM,；induced pluripotent stem cells；MYBPC3

P1661

The study of the relationship between anti-thyroid antibodies and peripheral blood lymphocyte subsets in nonpregnant women with recurrent spontaneous abortion

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Background: It has been reported that there is a correlation between anti-thyroid antibodies (ATA) and recurrent spontaneous abortion (RSA), but the mechanism is not clear.
Methods: In a prospective controlled trial, nonpregnant RSA patients (n=200, group A) and age-matched fertile women (n=40, group B) were enrolled and further grouped based on the presence of ATA and levels of thyroid-stimulating hormone (TSH). Group A was divided into ATA positive with mild thyroid dysfunction group (MTD, defined as TSH≥2.50uIU/mL) (n=36, group A+MTD), ATA positive with euthyroid group (ET, defined as TSH< 2.50uIU/mL) (n=50, group A+ET), ATA negative with MTD group (n=50, group A-MTD) and ATA negative with ET group (n=64, group A-ET). Women in all groups were tested for the ATA, levels of TSH and peripheral blood lymphocyte subsets.
Results: Our data indicated a statistically significantly higher prevalence of women with increasing of CD16+CD56+natural killer cells (cutoff value: >15%) and decreasing of CD3+CD4+helper T cells (cutoff value: < 31%) were observed in 3 conditions: (1) RSA patients (group A) compared to fertile women (group B); (2) group A+MTD compared to group A+ET; and (3) group A+MTD compared to group A-MTD. The prevalence of increasing/decreasing of other peripheral blood lymphocyte subsets among groups were not statistically significant.
Conclusions: Our study revealed a certain correlation between ATA and peripheral blood lymphocyte subsets in RSA patients; suggesting that immune factors may contribute to the pathogenesis of RSA with ATA positive and the possible association between peripheral blood lymphocyte subsets and thyroid function.

DOI: 10.1002/eji.201970400

P1662

Aberrant Tim-3/Gal-9 pathway involved in the pathogenesis of pre-eclampsia in rat model by regulating the polarization of decidual macrophages

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Dysfunction of decidual macrophages (DM) is considered a critical event in the pathogenesis of pre-eclampsia (PE). Tim-3 induces immune tolerance by interacting with its ligand Gal-9 and thus modulating function of various immune cells, including macrophages. However, the regulatory effects of Tim-3/Gal-9 signaling on DM polarization and its role in PE remain unclear. In this study, we established a PE-like rat model by administering 1.0 µg/kg LPS to normal pregnant Sprague-Dawley rats via the tail vein on E5. Apart from the pre-eclamptic manifestations, increased M1 and decreased M2 subtypes were observed at the maternal-fetal interface, with decreased Tim-3 expression on DM. After administration of rGal-9, we found that liver and renal injuries and placental functional deficiency, including shallow trophoblast cells invasion, impaired spiral artery remodeling and fetal capillary development, were reversed. In addition, the polarization of DM was inclined to M2 subtype, which was similar to the polarization of DM in the control rats but contrary to the PE-like rats. Interestingly, at E9, Tim-3 expression in DM significantly increased to near the level in normal pregnancy in the rGal-9 protein intervention group. Taken together, our findings showed that administration of rGal-9 protein can alleviate PE-like rat manifestations induced by LPS. This finding may be related to the activation of the Tim-3/Galectin-9 signaling pathway, which promotes the polarization of DM dominantly shifting to M2. Moreover, Tim-3 upregulation on DM starting from E9 suggests that Tim-3 may play some important roles in early pregnancy and even embryo development.

P1663

Cytokine synthesis during development of uterine myoma in women of reproductive age

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Uterine fibroids - the most common tumor that is found in 15-20% of women of reproductive age. In the structure of gynecological diseases, it is 27%. The role of immune disorders in the pathogenesis of uterine fibroids is currently being discussed. The purpose of the study is to study serum levels of proinflammatory cytokines (IL-6, IL-8, IL10, IL-18, IFNγ and TNFα) in women with uterine myoma. The study included 29 women of fertile age with uterine myoma. The control group consisted of 25 practically healthy women of the same age. It was revealed that in women with uterine myoma the level of IL-6, IL-8, IL10, IL-18, IFNγ and TNFα was higher than in women in the control group. IL-10 levels were elevated in patients with adenomyosis. An increase in serum IL-6 levels was observed in patients with single myoma nodules compared with a group of women with multiple nodes and a control group. hus, in women with uterine myoma there is an imbalance in the level of pro- and anti-inflammatory cytokines, which indicates the active participation of the cytokine network in the development of the tumor process.

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DOI: 10.1002/eji.201970400

P1664

The nature of interactions in the immune system in cervical intraepithelial neoplasia

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Women with a reduced immune response are more at risk of developing cervical intraepithelial neoplasia (CIN) and cervical cancer (CC). The aim of the study was to study the content of some subpopulations of lymphocytes in women with CIN living in the Aral Sea region. 32 women aged from 21 to 40 years with CIN of varying degrees living in the Aral Sea region were examined. The survey included the study of clinical and laboratory data. We studied the number of lymphocytes with the phenotype CD3, CD4, CD8, CD16, CD20. It was revealed that the content of the total pool of T-lymphocytes in patients with CIN was significantly reduced ($P < 0.01$). The level of CD4 + - lymphocytes was 1.17 lower than the control values, and the number of CD8 + cells was 1.3 times higher than in women of the control group ($P < 0.01$). The level of lymphocytes with a CD20 marker was significantly increased compared with the control group ($P < 0.05$). In women with CIN, activation of CD25 + cells was observed ($P < 0.01$). A significant decrease in CD95 + - lymphocyte content in women with CIN was shown, in contrast to the indicators of women in the control group ($P < 0.01$). Since lymphocytes of subpopulation composition play an important role in the formation of antitumor immunity, the change in their level as the clinical manifestations worsen indicates the involvement of the immune system in the pathogenesis of CIN.

P1665

Uterine dendritic cells modulation by mesenchymal stem cells improved the outcome of pregnancy in abortion prone mice

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Uterine Dendritic cells (uDCs) play a pivotal role in the establishment and maintenance of the pregnancy. Immunological disorders are known to be a major cause of unexplained recurrent spontaneous abortion (RSA). Since MSCs have been shown to exert immunomodulatory effects on immune cells especially DCs, this study was done to investigate the capability of MSCs to reduce the abortion rate in mouse RSA model and simultaneously modulate the frequency, maturation state and phenotype of uDCs as one probable mechanism for improvement of pregnancy outcome.
MSCs were derived from mouse adipose tissue and following characterization were administered to abortion-prone pregnant mice at the fourth day of gestation. On the day 13.5 of pregnancy, the abrtion rates and the frequency, phenotype and maturation state of uDCs were determined in the test and control groups.
Our results showed that administration of MSCs, at the implantation window, significantly decreased the abortion rate while the frequency of uDCs increased. MSCs therapy also really decreased the expression of co-stimulatory molecules and maturation markers on uDCs.
Regarding the indisputable role of uDCs in establishment of appropriate immunological conditions for implantation and proper maternal immune responses for promotion of successful pregnancy, it seems that uDCs modulation by MSCs could be one of the main mechanisms responsible for the abortion rates reduction following MSCs therapy of RSA.

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DOI: 10.1002/eji.201970400

P2250

Obesity intestinal microbes and testicular immunity

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Obesity is a complex metabolic disease and the current population of obesity is big data on a global scale. A high-fat diet may cause overeating and obesity by altering the composition and function of colonizing microorganisms (microbiota) in the gastrointestinal tract. Increased intestinal permeability and systemic inflammation, while reducing the ability to produce short-chain fatty acids that inhibit obesity. Numerous data suggest that changes in gut microbes are closely related to innate immune responses, and colonization and development of gut microbiota in all vertebrates is critical to producing a fully functional immune system. Many literatures have shown that the sperm motility of obese people is significantly reduced, morphological analysis of the testes showed that the spermatogenic epithelium was severely atrophied, and the cell adhesion between the spermatogenic cells and the supporting cells was loosely arranged in the HFD mice. In addition, severe disruption of the integrity of the blood-testis barrier is consistent with a decrease in tight junction-associated proteins, occludin, ZO-1 and androgen receptors. which is consistent with our findings. Excessive activation of autophagy was also observed in sperm samples from obese infertile male patients. and diet-induced obesity in male C57BL/6 mice decreases fertility as a consequence of disrupted blood-testis barrier, So the increase in intestinal permeability and interference from colonized flora may affect the testicular immune system.

P2251

The expression and localization of programmed cell death 1 and its ligand in the testicular tissue of mice

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Problem: To determine the expression and localization of PD-1 and PD-L1 in the testes of mice at different developmental stages.
Methods: By means of RT-qPCR, western blot and immunofluorescence, the expression and localization of PD-1 and PD-L1were detected in the testicular tissues of mice at different postnatal times: P7, P14, P21, P28, P35 and adulthood. The level of soluble PD-L1 (sPD-L1) was evaluated by ELISA in the testicular interstitial fluid (IF) of the adult mice, culture supernatants of TM4 cell lines and primary Sertoli cells at P14
Results: *Pd-1*mRNA levels were unexpectedly low. From P7 to P21, there was limited PD-1 protein detected while PD-1 was evident at P28 and afterwards at significantly higher levels than at P14 and P21. Despite being found in the interstitial areaat P7, P14 and P21, PD-1 was also detected in the germ cells of the seminiferous tubules after P28. *Pd-l1*mRNA exhibited age-related changes, peaking at P21, while PD-L1 protein was constitutively expressed at any stage, specifically localized in the nucleus of Sertoli cells. Moreover, the level of sPD-L1 in IF was significantly higher than that in the culture supernatants of both TM4 and primary Sertoli cells at P14.
Conclusions: The expression and localization of PD-1 fluctuated with age, and PD-1 was mainly localized to advanced germ cells, suggesting that it may play a role in spermiogenesis. PD-L1 was

DOI: 10.1002/eji.201970400

constitutively expressed in the nucleus of Sertoli cells, which could secrete sPD-L1 into the testicular interstitial space and thus may be involved in testicular immune privilege.

P2252

Forkhead box P3 is selectively expressed in human trophoblasts and decreased in recurrent pregnancy loss

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Problem: Forkhead box P3 (Foxp3) is necessary for induction of the immunosuppressive functions in regulatory T cells. Recent data indicate that some non-lymphoid cells, such as certain tumor cells, also express Foxp3, which participates in tumorigenesis and is related to the invasive and proliferative behavior of the tumor. However, the expression of Foxp3 in trophoblasts has not been studied previously.
Methods: Localization of Foxp3 in the villi and decidua in the first trimester was determined using immunohistochemistry. Foxp3 expression was detected by flow cytometry, and the expression levels in the first trimester placenta were compared with those in the term placenta. Additionally, the Foxp3 expression in trophoblasts in recurrent pregnancy loss (RPL) was analyzed by real-time polymerase chain reaction (RT-PCR), immunohistochemistry and western blot.
Results: Foxp3 is expressed by trophoblasts in early pregnancy in the villi and decidua. Foxp3 is expressed in the nuclei of both trophoblast cell columns and cytotrophoblasts. However, low Foxp3 expression was observed in syncytiotrophoblasts. Foxp3 was also expressed by the extravillous trophoblasts in early pregnancy in decidua. The Foxp3 expression in trophoblasts in the term placenta was significantly decreased compared with that in the normal early pregnancy. Moreover, decreased levels of Foxp3 mRNA and protein were observed in women with RPL.
Conclusion: The selective expression of Foxp3 in human trophoblasts suggest that Foxp3 expression may be associated with the proliferation and invasion behavior of trophoblasts. The decreased Foxp3 expression in the trophoblasts in RPL maycontribute to better understanding of the pathological characteristics of RPL.

P2253

CD49a$^+$ Natural Killer cells promote fetal development at the maternal-fetal interface

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Background: Decidual NK cells have many differences compared to periphery NK cells, which have been regarded as innate surveillance defender in circulation. Decidual NK cells, with low cytotoxic function, take large population at maternal fetal interface during early pregnancy and their roles in fetal growth are not clear.
Methods: Both mouse models and human decidual tissues have been used in this study, including *Nfil3*$^{-/-}$mice, *T-bet*$^{-/-}$mice, aged mice, early decidual tissues from normal human and RSA patients. Flow cytometry, Quantitative PCR analysis, histological analysis, Coculture cells system and adoptive transfer experiment have been used in this study.
Results: Here we identify a NK subset, occupying the uteruses of both human and mouse, that has

DOI: 10.1002/eji.201970400

the capability to secret growth promoting-factors (GPF) and have the CD49a+Eomes+ phenotype. The decreased GPFs-secreting NK subset impairs the development of fetuses, and leads to fetal growth restriction. Nfil3 expression and appropriate maternal age play key roles in the existence of the GPFs-secreting NK subset. Adoptive transfer of these NK cells can reverse the impaired fetal growth and rebuild an appropriate local microenvironment.
Conclusions: These findings reveal new properties of NK cells for promoting fetal growth and novel approaches for therapeutically exploiting NK cells.

P2254

Recent advances in immune deficiency-related neonatal IBD caused by single gene mutation

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Neonatal inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract. The abnormality of the intestinal immune system caused by single gene mutation is one of the main causes of neonatal IBD. Abnormal activation and function of B lymphocytes, destruction of epithelial barrier function, decreased bacteriostatic ability of neutrophils and phagocytic cells, and immune disorders, leading to excessive reaction of the human immune system to common bacteria in the digestive tract. Simultaneously, because of its genetic susceptibility, clinical course is heavier, traditional hormones and surgical treatment are ineffective, and tolerance to immunosuppressive therapy is strong, the incidence rate is increasing year by year, and neonatal IBD is receiving more and more attention. Seeing that the different treatments and prognosis of different types of primary immunodeficiency disease combined with IBD, early identification of neonatal gene mutation phenotype by gene sequencing can achieve early diagnosis and selection of appropriate treatment methods.

P2255

Novel application of natural killer cell activity test for recurrent miscarriages and repeated implantation failure: a case report

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Recurrent miscarriages (RM) and repeated implantation failure (RIF) remain obstacles of pregnancy that affect thousands of women worldwide. Statistically, about 50% of RM cases are of unknown causes, making it hard to carry out assisted reproductive treatment. In the present study, we report a case of an infertile woman whose peripheral blood natural killer (NK) cell activity (NKA) tested by NK Vue test kit (ATGene) showed elevated result. The woman underwent prednisolone therapy (15mg p.o, q.d) for 4 weeks; retested NKA showed a normal value, and she got an embryo transferred, followed by a successful pregnancy. She continued prednisolone therapy until 10th week of pregnancy and then tapering of prednisolone before stopping. She gave birth to a healthy child after a 42-week gestation. This is for the first time the NKA test has been used to evaluate a possible cause of female infertility.

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DOI: 10.1002/eji.201970400

P2256

Prorrenin/renin receptor expression in immune cells of the placenta from rats with pregnancy-induced hypertension

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Pregnancy-induced hypertension (PIH), known before as preeclampsia, is a multisystemic disorder characterized by hypertension, proteinuria and low intrauterine development. Its etiology remains unknown. Several systems seem to participate in PIH genesis. The renin-angiotensin system (RAS) has been implicated in PIH development. RAS is essential for blood pressure control and hydro-saline balance. The prorrenin/renin receptor (PRR) is a component of this system. Recently have suggested that in addition to the components of the RAS, the immune system (IS) also participates in the pathogenesis of PIH. The most characteristic immunological finding is the activation of the innate and adaptive immune system which induces an inflammatory response during this syndrome, losing the balance that occurs in healthy pregnancy. These data support a new paradigm in which RAS components other than the angiotensin II receptor modulate inflammation in the target organ. It is possible that the PRR, through the RAS and the immune system, plays a role in the pathophysiology of PIH. In the present work we determined PRR expression in immune cells of the placenta from normal pregnant and pregnancy-induced hypertensive rats. We induced PIH in Wistar rats using sub-renal aortic coarctation (CASR). We measured proteinuria, systolic and diastolic blood pressure, pups body weight, length, and number. The expression of PRR was determined by flow cytometry in leukocytes from placenta. Our results show a significant decrease in PRR in the PIH model compared to normal pregnancy groups. Our findings indicate that PRR is functional in immune cells during healthy and complicated pregnancy.

P2257

Epigenetically regulated immune dysfunction is associated with chronic pelvic pain syndrome

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Chronic pelvic pain syndrome (CPPS) is a complex multi-symptom disorder with unknown etiology and limited effective therapies. Previously published data from our laboratory demonstrated increased IL7 expression in expressed prostatic secretion (EPS) samples from CPPS patients compared to healthy controls. These increases were also positively correlated with increased patient-reported symptom severity. IL7 is known modifier of CD4 T-cell activation and differentiation and has been implicated in a number of autoimmune disorders, including diabetes. This evidence is thus suggestive of an auto-immune-like phenotype in these patients that could drive pathology. To delineate this more succinctly we performed QRTPCR profiling on voided bladder (VB) urine samples from CPPS patients and relevant controls for markers associated with CD4 T-cell function. Here we demonstrate immune skewing towards increased Th17 responses and decreased T-regulatory cell responses, including FoxP3. A methylation array was then performed on PBMCs isolated from CPPS and control blood samples to determine whether these alterations in immune response were epigenetically regulated. The data shown here demonstrate hypermethylation of the IL10 promoter and hypomethylation of the ITGAL promoter in CPPS patients compared to healthy controls. Furthermore, we also demonstrate that PBMCs from patients show altered capacity to respond to LPS than their control counterparts. Taken together this novel data demonstrates that the etiology of CPPS may be the result of an epigenetically regulated immune dysfunction that is exacerbated upon damage to the prostate. We hypothesize that lack of a fully functioning regulatory network facilitates development of autoimmunity and pain in these patients.

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DOI: 10.1002/eji.201970400

P2258

Maternal immunological adaptation to pregnancy

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Pregnant women undergo a series of metabolic and immunologic changes to ensure provision of nutrients to, and prevent rejection of, the fetus. To ensure continuous supply of glucose to the fetus, the mother increases glucose production, glucose intolerance and insulin resistance. To meet her own energy demands, the mother transitions from lipid storage to lipolysis. To prevent rejection of the fetal semi-allograft, the mother's immune system must be regulated, whilst maintaining protection against pathogens. The hypothesis is that well recognised metabolic changes in pregnancy could impact maternal immune function. The aims of this project are to determine whether monocytes undergo metabolic adaptation and when during pregnancy this might occur.
Monocytes are isolated from whole blood of term (> 37 weeks of gestation) pregnant and not pregnant women using magnetic microbeads. Extensive flow cytometry analysis has shown that not only do pregnant women have significantly more non-classical CD16+ monocytes but that this subset from pregnant women expresses more CD36 (a fatty acid transporter; p = 0.0025 and less CD98 (an amino acid transporter; p = 0.0043). Bioenergetic analysis of resting monocytes has shown that oxidative phosphorylation is significantly reduced in pregnant women compared to non-pregnant women; glycolysis is unchanged.
Ongoing work will include analysis of mitochondrial health and measuring transcriptomics, biosynthetic lipids, cytokines, and enzyme responses. Results will be vital to understanding immunological adaptation during pregnancy. This could provide insight into mechanisms and treatment of adverse pregnancy outcomes and women's long-term health in relation to immune-mediated diseases.

P2259

Anti tumoral response of multiparous women compared with nulliparous women in co-culture breast tumor cell lines

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According to the Microchimerism hypothesis, the association between breast cancer and multiparity is controversial. We aimed to compare the antitumoral response between multiparous and nulliparous women, using the co-culture modeling of peripheral blood mononuclear cells in exposure to breast tumor cell lines.
A total number of 48 multiparous and nulliparous women were recruited; PBMNC was purified by Ficoll density gradient centrifugation. Cell proliferation, concentrations of TNF-α cytokine and percentage of CD3 + CD8 + lymphocytes at two-assessments after co-culture with breast tumor cell lines (MDA-231 and MCF-7) with PBMC's of these women were compared .
The proliferative response of effector cells in MCF-7 co-culture is higher than MDA-231 with a statistical significant difference in both cell lines between multiparous and nulliparous women, 48h after co-culture (P< 0.001). Mean of lymphocyte proliferation in multiparous women was significantly higher than nulliparous group after 48 and 72 hours (P < 0.001). Mean of TNFα production in multiparous women after 72 hours was significantly higher than nulliparous ones (P< 0.001). Despite no significant difference in MCF-7 culture between multiparous and nulliparous women, there was a significant difference in co-culture of MDA-231 (P=0.012). There was no significant difference in proportion of cytotoxic lymphocytes (CD3 + CD8 +) between nulliparous vs. multiparous.

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DOI: 10.1002/eji.201970400

P3089

AP-1 transcription factor complexes affect HLA-G expression and immune regulation ability of JEG-3

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Background: AP-1 complexes play vital role in the migration and invasion of EVTs (extravillous trophoblast cells), however its function in the development of immune tolerance in early pregnancy still remains unclear.
Object: This study will demonstrate the immune regulation function of AP-1 complexes in JEG-3 and detect their possible mechanism in the first trimester of pregnancy.
Method: RNA-seq and bioinformatic analysis were firstly used to detect the expression of AP-1 complexes and potential reputational interaction with HLA-G, a main immune regulation factor that is uniquely expressed in EVTs. Then, c-JUN, c-FOS and JUND siRNA were transfected in the JEG-3 to downregulate the expression of AP-1 complexes. The expression of AP-1 complexes and HLA-G mRNA was detected by qRT-PCR. Dual luciferase reporter assay was used to directly demonstrate the binding of AP-1 complexes and HLA-G Enhancer L. Two co-culture systems of JEG-3-PBMCs and JEG-3-dNK were built to evaluate the effect of AP-1 on the decidua immune cells.
Result: c-JUN, c-FOS and JUND (main subunits of AP-1 complexes) were highly expressed in first-trimester trophoblasts and JEG-3 cells. Knocking down AP-1 complexes in JEG-3 cells can inhibit the HLA-G expression. $Foxp3^+$ Treg transformation was deceased in the co-cultured system of PBMCs - JEG3 with knockdown of AP-1 complexes, and the cytotoxicity of dNK treated by JEG3 with knockdown of AP-1 complexes on HTR8-SVneo were significantly increased. All changes mentioned above can be eliminated after HLA-G blockage.
Conclusion: AP-1 may involve immune tolerance on fetal-maternal interface through regulating the expression of HLA-G.

P3090

Peripheral neutrophils combined with IL-10 to evaluate the efficacy of G-CSF for recurrent pregnancy loss

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Granulocyte colony-stimulating factor（G-CSF） is one of promising therapic strategies for unexplained recurrent early pregnant loss. But still have argument in different researches.
We got the clinical data of 232 pregnant patients diagnosed with unexplained recurrent early pregnant loss in the second affiliated hospital of Zhengzhou University from January 2015 to December 2018. All of Patients were divided into two groups -- the G-CSF group contains 185 patients who received conventional anti-abortion treatment added G-CSF and the control group that 47 patients who only got conventional anti-abortion treatment. The records contained peripheral neutrophil count (NEUTC), Th1/Th2 cytokine group .The outcome was followed up to 10 weeks. We found that the abortion rate 21.08% (39/185) in the G-CSF group is lower than that 36.17% (17/47) in the control group (P=0.0309). In the G-CSF group, interleukin-10 (IL-10), as well as NEUTC, were significantly higher than those in the control group (P< 0.05).We used receiver operating characteristic (ROC) curves to calculate optimal cut-off values (0.78) for IL-10 and NEUTC to predict G-CSF to treat recurrent early pregnant loss. The AUC, sensitivity, specificity of the combined NEUTC with IL-6 were 0.865, 80.8%

DOI: 10.1002/eji.201970400

and 84.6%.
These results suggest that G-CSF could increase IL-10 and decrease the abortion rate of RM patients with early pregnancy and **the peripheral neutrophils combined with interleukin-10 (IL-10) can predict the clinical efficacy of G-CSF for RM patients**. G-CSF could be used as an immunomodulatory to treat recurrent pregnant loss in the future.

P3091

Testicular immune privilege and spermatogenesis

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Immune privilege Is an important physiological self-protection mechanism that can be weakly immune to foreign antigens and autoantigens to prevent tissue damage and dysfunction caused by immune response. The testis is an immune-privileged organ that has an important protective effect on spermatogenesis. Testicular immune privilege is regulated by its tissue structure, cellular, and molecules. The balance of the testicular immune environment is necessary to maintain its normal function. When the testicular immune balance is destroyed, it can cause orchitis and damage male fertility. When we could deeply understand the effects of testicular immune privilege on spermatogenesis, it might provide new ideas for the diagnosis and treatment of immune-related male infertility. This article will review the research progress in this field and propose the direction for further research.

P3092

Effects of prokineticin 2 on testicular inflammation in rats

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Problem: Prokineticin 2 (PK2), a pro-inflammatory peptide, is highly expressed in primary spermatocytes. However, systematic research on PK2 and testicular inflammation is lacking to date.
Method of study: An experimental autoimmune orchitis (EAO) model was established to detect the expression of PK2 and its receptor (prokineticin receptor 1, PKR1) 50 and 80 days after immunization. PK2 siRNA sequence was injected into the rat rete testis to downregulate the expression of PK2. PK2 was over-expressed in the testis by injecting PK2 protein through the rat rete testis at different concentrations. Testicular morphology and expression of inducible nitric oxide synthase (iNOS) were detected after the intervention.
Result: Results showed that PK2 and PKR1 were upregulated in EAO at 50 days and downregulated at 80 days. PK2 over-expression contributed to the apoptosis of spermatogenic epithelial cells and increased infiltration of the inflammatory cells, whereas PK2 under-expression showed no change. Furthermore, iNOS expression was increased significantly when PK2 was over-expressed.
Conclusion: This finding demonstrated that the PK2/PKR1 signals may have an essential role in the regulation of testicular inflammation through iNOS. PK2 interference may represent a novel and promising therapeutic strategy for the clinical management of orchitis.

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DOI: 10.1002/eji.201970400

P3093

Primordial follicle activation is affected by the absence of Sohlh1 in mice

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In this study, we used Sohlh1−/−transgenic mice to explore the role of Sohlh1 in folliculogenesis. The results showed that only primordial follicles and empty follicles can be observed in Sohlh1−/− ovaries from 0.5 to 23.5 dpp. The expression of Foxo3 and FOXO3 was downregulated; nucleocytoplasmic shuttling of FOXO3 was normal in 7.5-dpp Sohlh1+/+ but not Sohlh1−/− ovaries; and primordial follicle activation (PFA) was not observed in 7.5-dpp Sohlh1−/− mice. The expression levels of KIT, AKT, and P308-AKT were downregulated ($p < 0.05$), whereas that of P473-AKT was not significantly changed ($p > 0.05$). The KIT/PI3K/AKT pathway was inhibited.The absence of Sohlh1 may affect PFA in mouse ovaries via downregulation of Kit and inhibition of the KIT/PI3K/AKT pathway.

P3094

Inflammasome activation in trophoblast cells contributes to Listeria monocytogenes induced animal pregnancy failure

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Listeria monocytogenes (LM), a foodborne pathogen, can cause pregnancy failure in human and animals. Recent studies have shown that LM can activates inflammasomes, however, whether the inflammasome activation regulates LM-induced pregnancy failure remains largely unknown. Here we used mouse model to investigate the molecular mechanism by which LM-induced inflammsome activation contributes to LM-associated pregnancy failure. Results showed that wild-type, but not Listeriolysin O-deficient (Δ*hly*) LM, significantly reduced mouse embryo survival, accompanied by the increase of IL-1β release. IL-1β neutralization significantly reduced the LM-induced embryo losses, suggesting that LM-induced pregnancy failure was associated with LLO-induced inflammasome activation. To explore the mechanism of LM-induced pregnancy failure, we used LM and recombinant LLO to treat mouse trophoblast cells, the main cells in palanceta, and test the IL-1β production and the invasion ability of trophoblast cells in vitro. We found that the inflammasomes in trophoblast cells can be activated by LM and LLO, suggesting that the inflammasome activation in trophoblast cells contributes to LLO-induced inflammasome activation in palanceta. And the invasion ability of trophoblast cells decreased after treated by LM and IL-1β, which was adverse to the animal pregnancy maintenance. Our data demonstrated that the LLO-induced inflammasome activation in trophoblast cells plays a key role in LM-induced pregnancy failure, and inflammasome-mediated the decrease of trophoblast cell invasion ability might be involved in the pregnancy failure.
Keywords: *Listeria monocytogenes*, Listeriolysin O, Pregnancy failure, Inflammasome, Trophoblast cells
Acknowledgments: This study was supported by the Natural Science Foundation of Hebei Province (C2013204130; C2017201213).

DOI: 10.1002/eji.201970400

P3095

Role of the TGF-β/SMAD pathway in the oogenesis in autoimmune ovarian disease

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Objective: To investigate the characteristic of oogenesis in autoimmune ovarian disease.
Methods: Autoimmune ovarian disease (AOD) was inducted by Day3 thymectomy in neonatal mice. The TGF-β, SMAD2 and SMAD3 in ovarian tissues were analyzed by Q-PCR in mRNA level and by Western blot in protein level. The TGF-β/SMAD pathway was compared in the adult female mice through the inhibition of TGF-β via RNA interference in follicle culture system in vitro.
Results: The AOD mice had lower E2 and AMH, higher FSH and LH, fewer of follicles and more stromal lymphocytes in ovary, which were similar to human premature ovarian failure (POF). It was observed that the TGF-β in the ovarian tissue was decreased significantly. However, both smad2 and smad3 did not shown obvious difference in the AOD. The number of mature follicles was decreased after medicated by siRNA expression vectors in vitro, while the granulosa apoptosis increased significantly.
Conclusion: The TGF-β/SMAD signal pathway has an essential role in AOD in D3tx mice. TGF-β might involves the oogenesis and could be a potential method in the treatment of POF.

P3096

Clinical features of microextraction of testicle in patients with non-obstructive azoospermia complicated with mumps

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Objective: to investigate the clinical characteristics of microscopic testicular sperm extraction in patients with non-obstructive azoospermia complicated with mumps.
Methods: retrospective analysis was performed on the clinical data of microextraction of testicle from 33 patients with non-obstructive azoospermia complicated with mumps from May 2017 to February 2019, including previous medical history, physical examination, sex hormone levels, etc. All patients were confirmed by surgery.
Results: of the 32 patients with non-obstructive azoospermia complicated with mumps, 17 (50.0%) obtained sperm.Sperm was obtained in 10 (90.9%) of 11 cases of postpubertal mumps (14 years old), while only 7 (33.3%) of 21 cases of prepubertal non-obstructive azoospermia with mumps (< 14 years old).
Conclusion: the age of onset of non-obstructive azoospermia with mumps may be an important factor in determining the success rate of sperm extraction.

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DOI: 10.1002/eji.201970400

P3097

Global characterization of decidual immune microenvironment in patients with recurrent spontaneous abortion by single-cell sequencing

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Maintaining homeostasis of the decidual immune microenvironment is indispensable at the maternal-fetal interface. Although several distinct immune cell subpopulations have been identified, a comprehensive understanding of the heterogeneity and alteration of the decidual immune microenvironment from human with recurrent miscarriage still remains unclear. Here, we profiled the respective transcriptomes of 21684 primary human decidual immune cells isolated from patients with recurrent spontaneous abortion (RSA) and healthy controls at single-cell resolution. By comparing with decidual immune cell atlas from healthy controls, we revealed dramatically differential distributions of decidual NK (dNK) cell subsets in RSA patients. We further illustrated distinct chromatin accessibility landscape of three dNK subsets as well as under RSA disease state using ATAC-seq. We discovered that KIRs-prone dNK1 subsets diminished in proportion due to chemotaxis and enhanced cytotoxicity of other dNK subsets in RSA patients. In addition, decreased macrophages, increased T cells, altered receptor-ligand communications and differential signaling pathway were also identified in RSA patients. Our study provides deeper insights into the decidual immune microenvironment in RSA patients, and reveal the regulatory mechanisms of the immune cells driving this disease.

P3098

Single-cell transcriptomic landscape of nucleated cells in umbilical cord blood

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Umbilical cord blood (UCB) transplant is a therapeutic option for both pediatric and adult patients with a variety of hematologic diseases such as blood cancers, myeloproliferative disorders, genetic diseases, and metabolic disorders. However, the level of cellular heterogeneity and diversity of nucleated cells in the UCB has not yet been assessed in an unbiased and systemic fashion. In the current study, nucleated cells from UCB were subjected to single-cell RNA sequencing, a technology that enables simultaneous profiling of the gene expression signatures of thousands of cells, generating rich resources for further functional studies. Here, we report the transcriptome of 17,637 UCB cells, covering 12 major cell types. Many of these cell types can be further divided into distinct subpopulations. Pseudotemporal ordering of nucleated red blood cells (NRBC) identifies wave-like activation and suppression of transcription regulators, leading to a polarized cellular state, which may reflect NRBC maturation. Progenitor cells in UCB also consist of two subpopulations with divergent transcription programs activated, leading to specific cell-fate commitment. Detailed profiling of cytotoxic cell populations unveiled granzymes B and K signatures in NK and NKT cell types in UCB. Collectively, we provide this comprehensive single-cell transcriptomic landscape and show that it uncovers previously unrecognized cell types, pathways and gene expression regulations that may contribute to the efficacy and outcome of UCB transplant, broadening the scope of research and clinical innovations.

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DOI: 10.1002/eji.201970400

P3099

Detection of T lymphocyte subsets and related functional molecules in follicular fluid of patients with polycystic ovary syndrome

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Immune responses play an important role in the pathogenesis of polycystic ovary syndrome (PCOS). However, the characteristics of T lymphocyte subsets in PCOS remain insufficiently understood. In this study, lymphocytes of follicular fluid (FF) were obtained from oocyte retrieval before in-vitro fertilization (IVF) in infertile women with or without PCOS. The levels of cluster of differentiation 25 (CD25), CD69, programmed death 1 (PD-1), interferon-γ (IFN-γ), interleukin 17A (IL-17A) and IL-10 in T lymphocytes were determined by flow cytometry. Our results showed that the percentage of FF $CD8^+$ T cells was significantly decreased in infertile patients with PCOS ($P< 0.05$). Furthermore, the levels of CD69 and IFN-γ were significantly decreased and the level of PD-1 was increased in both $CD4^+$ and $CD8^+$ T cells from infertile patients with PCOS ($P< 0.05$). Moreover, the expression of PD-1 on $CD4^+$ or $CD8^+$ T cells was positively correlated with the estradiol (E2) levels in the serum and reversely correlated with the expression of IFN-γ in $CD4^+$ or $CD8^+$ T cells in infertile patients with PCOS. These results suggested that T cell dysfunction may be involved in the pathogenesis of PCOS.
Keywords: PCOS, follicular fluid, T cell dysfunction, cytokines, PD-1.

P3100

Effect and mechanism of MDSCs in pathogenesis of endometriosis

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To our knowledge, little is known about the role of MDSCs in the pathogenesis of endometriosis, though MDSCs were found to be important in maintaining materno-fetal tolerance. Therefore, the aim of our present study was to investigate the level and function of MDSCs in the peripheral blood and peritoneal fluid of patients with endometriosis. We collected the peripheral blood and peritoneal fluid from endometriosis patients and controls and analyzed M-MDSCs level using specific monoclonal antibodies recognizing HLA-DR, CD33, CD11b, CD14 markers by flow cytometry. We found that there existed abnormal expansion of monocytic MDSCs (M-MDSCs) (HLA-$DR^{-/low}$CD33+CD11b+ CD14+) in peripheral blood and peritoneal fluid of patients with endometriosis. Functional studies revealed that M-MDSCs from endometriosis patients significantly suppressed T-cell responses and produced high level of reactive oxygen species (ROS). The elevation of M-MDSCs from endometriosis patients may contribute to the disease progression.
Keyword: MDSCs ; Pathogenesis ; Endometriosis

DOI: 10.1002/eji.201970400

P3101

Premature ovarian insufficiency may be associated with the mutations in mitochondrial tRNA genes

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Premature ovarian insufficiency (POI) is a critical fertility defect characterized by an anticipated and silent impairment of the follicular reserve, but its pathogenesis is largely unexplained. Although some studies have reported increased levels of reactive oxygen species (ROS) in idiopathic POI, the role of mitochondrial DNA (mtDNA) mutations in the pathogenesis of POI has not been studied extensively. In this study, we used the PCR-Sanger sequencing to detect the mt-tRNA mutations from 50 POI patients and 30 healthy subjects. Moreover, we assessed the mitochondrial functions used the trans-mitochondrial cybrid cells containing the candidate pathogenic mt-tRNA mutations. We identified five mutations: $tRNA^{Leu(UUR)}$ C3303T, $tRNA^{Met}$ A4435G, $tRNA^{Gln}$ T4363C, $tRNA^{Cys}$ G5821A and $tRNA^{Thr}$ A15951G. We noticed that these mutations localized at the highly conserved nucleotides of the corresponding mt-tRNAs and may result the failure in mt-tRNA metabolism and subsequently cause the impairment in mitochondrial protein synthesis. In addition, biochemical analysis of the cybrid cells containing these mutations showed a significantly lower level of ATP when compared with the controls, whereas the ROS levels were higher in POI patients carrying these mt-tRNA mutations, suggesting that mt-tRNA mutations may cause the mitochondrial dysfunction, and contributed to the pathogensis of POI. Taken together, our study provided novel insight into the molecular pathogenesis of POI that was manifestated by mt-tRNA mutations.

Veterinary and Comparative Immunology

O349

CD14 and *TLR2* gene polymorphisms in Chinese Holstein cattle and their associations with bovine tuberculosis

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Cluster differentiation antigen 14 (*CD14*) and Toll-like receptor 2 gene (*TLR2*) are important pattern recognition receptor proteins in innate immunity. The aim of this study was to identify and assess the association of single-nucleotide polymorphisms (SNPs) in the *CD14* and *TLR2* gene with susceptibility to bovine tuberculosis (BTB) in Chinese Holstein cows. DNA samples were collected from 517 and 447 Chinese Holstein cows, respectively. SNPs in the entire genes, including exonic regions, intronic regions and close to the 5´- and 3´-terminal untranslated regions, were detected by PCR, followed by direct sequencing. Significantly increased BTB susceptibility was evident in T allele carriers of *CD14* -5C/T ($P < 0.001$; odds ratio, OR 2.02; 95% confidence interval, CI 1.57-2.77), G allele carriers of *CD14* 613G/A ($P < 0.001$; OR 2.17, 95% CI 1.50-3.08) and TG haplotype carriers of both SNPs ($P < 0.001$; OR 3.14, 95% CI 1.24-4.50). Additionally, the allele and genotype distributions of *TLR2* A631G and *TLR2* T1707C were not different between case and control groups, indicated that these SNPs were not associated with susceptibility to BTB. These results suggested that *CD14* -5C/T and *CD14* 613G/A were risk factors for BTB in Chinese Holstein cattle and might be used as candidate genetic markers in breeding cows with natural resistance to BTB, while SNPs in the *TLR2* gene might not play a significant role in the BTB risk in Chinese Holstein cattle.

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DOI: 10.1002/eji.201970400

O350

Prolactin and 17β-estradiol induce pro-inflammatory cytokines in bovine mammary epithelial cells inhibiting Staphylococcus aureus internalization and modulating epigenetic marks

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Cattle are more susceptible to mastitis during the peripartum, which is characterized by changes in the 17β-estradiol (E2) and prolactin (PRL) levels, compromising the animal innate immune response (IIR). Bovine mammary epithelial cells (bMECs) are target tissue of these hormones and previously we showed that they regulate IIR of bMECs affecting the internalization of *Staphylococcus aureus*: PRL induces it (5 ng/ml) and E2 decreases it (50 pg/ml). In spite of both hormones play a relevant role for bovine mammary tissue *in vivo*, their combined effects on the IIR of bMECs during infection are unknown, as well as the modulation of epigenetic marks. The results showed that the hormonal combination [PRL (5 ng/ml) and E2 (50 pg/ml)] decreases *S. aureus* internalization into bMECs (~50%); which was associated with a reduction of integrin α5β1 (~80%) in membrane. The hormonal mixture inhibited p38 and ERK1/2 kinases activation. Also, both hormones reduced the antimicrobial peptide DEFB1 secretion (~2 fold) respect to infected bMECs, whereas the gene expression of IL-6 and TNF-α was up-regulated (~4 and 12-fold, respectively). Respect to the epigenetic marks, H3K9Ac was induced by E2 (~40%), and the hormonal combination (~20%). In addition, both hormones reduced HDACs activity (~25%) at 6 h. PRL reduced the expression of the enzymes KDM4C (~60%) and DnMT1-b (~80%), whereas E2 abolishes the expression of KDM4C in infected bMECs. In conclusion, the hormonal combination of PRL and E2 induces elements of the pro-inflammatory response in bMECs, while reducing *S. aureus* internalization and modulates epigenetic marks.

LB066

Double deletion Marek's disease virus as a potential vaccine candidate

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Marek's disease virus (MDV) is a highly contiguous alphaherpesvirus that causes rapid onset lymphomas in chickens. Marek's disease is effectively controlled by vaccination; however, field strains continue to breakthrough vaccinal immunity due to the emergence of highly virulent field strains. Earlier studies have shown that deletion of meq gene from MDV resulted in attenuated vaccines capable of protecting against highly virulent field strains. However, meq deleted viruses maintain the ability to induce lymphoid organ atrophy raising safety concerns. Also, we have earlier shown that deletion of vIL8 results in loss of lymphoid organ atrophy. Here we describe the generation of a potential vaccine candidate that lacked expression of both vIl8 and meq genes. In vitro studies showed that double deletion mutant viruses replicated with similar kinetics as parental virus. Animal studies confirmed that the double mutant (vIl8 and meq) virus had the ability to confer superior protection compared to currently available vaccines and overcame the induction of lymphoid organ atrophy induced by the deletion of meq.

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DOI: 10.1002/eji.201970400

P0475

The structure and function of the channel-nerve-endocrine-immune-network

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Immunology as a new subject of physiological function, network regulation and nerve development, endocrine system, gas regulation, that is, the structure and function of meridian-neuroendocrine immune system play a special role in regulating the immune function of the body. Each system has its own structure, path and function. It also crosses vertically and horizontally, transfers information, integrates information, exchanges material energy, and plays the role of feedback regulation system. Cytokines in the immune system play an active or inhibitory role through synergistic or antagonistic effects. Cytokines, peptide hormones and neurotransmitters are used in conjunction with neuroendocrine gases to produce extensive and close network connections. Studies had found that more than 20 hormones and neurotransmitters have immunomodulatory effects, and immune cells can produce endocrine hormones. Traditional Chinese medicine holds that the body maintains the balance of yin and yang, the neuroendocrine immune network maintains the functional balance, and the body is in a steady state of health. Bullet theory of traditional Chinese medicine is actually a multi-target action system. It can act on unbalanced neuroendocrine network by multi-target, multi-link, multi-level and multi-channel after entering the body, which can reconcile Yin and yang, and make the body return to steady state and restore health.

P0476

Newcastle disease virus-like particles induce DC maturation through TLR4/NF-κB pathway and facilitate DC migration by CCR7-CCL19/CCL21 axis

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Newcastle disease virus-like particles (NDV VLPs) are a potential candidate vaccine, as shown by eliciting specific immune response against NDV in mice and chickens. Activation of dendritic cells (DCs) is critical to initiate immune response. However, the mechanism of how NDV VLPs induce DC maturation and migration remains elusive. In this study, we found that NDV VLPs are efficient in DC activation by up-regulating surface MHC II and costimulatory molecules, and proinflammatory cytokines through the TLR4/NF-κB pathway. Furthermore, NDV VLPs elevated CCR7 expression on DCs, resulting in DC migration towards CCL19/CCL21 both *in vitro* and *ex vivo*. As a consequence of DC maturation and migration, CD4+ T cells were also activated *in vivo*, demonstrating increased intracellular IFN-γ and IL-4 levels. Together, these results present new insights for NDV VLPs induced DC maturation and migration, providing a better understanding of VLP-triggered innate immune responses.

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DOI: 10.1002/eji.201970400

P0477

Molecular and functional characterization of an NLRP1 inflammasome from zebrafish reveals a unique activation mechanism of inflammatory caspases

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NLRP1 inflammasome is one of the well known inflammasomes in humans and other mammals. However, the occurrence of this inflammasome in non-mammalian species remains limited. In the present study, we identified an NLRP1 homolog (*Dr*NLRP1) from a zebrafish (*Danio rerio*) model. This *Dr*NLRP1 has similar structural architecture to mammalian NLRP1s. It is able to trigger the formation of a classical inflammasome for the activation of zebrafish inflammatory caspases (*Dr*Caspase-A and *Dr*Caspase-B) and maturation of *Dr*IL-1b in a *Dr*ASC-dependent manner. In this process, *Dr*NLRP1 promotes the aggregation of *Dr*ASC into a filament with *Dr*ASC^{CARD} core and *Dr*ASC^{PYD} cluster. The assembly of *Dr*NLRP1 inflammasome depends on the CARD-CARD homotypic interaction between *Dr*NLRP1 and *Dr*ASC^{CARD} core, and PYD-PYD interaction between *Dr*Caspase-A/-B and *Dr*ASC^{PYD} cluster. The FIIND domain in *Dr*NLRP1 is necessary for inflammasome assembly. To understand the mechanism of how the two *Dr*Caspases are coordinated in *Dr*NLRP1 inflammasome, **we propose a two-step sequential activation model. In this model, the recruitment and activation of *Dr*Caspase-A/-B in the inflammasome is shown in an alternate manner, with a preference for *Dr*Caspase-A followed by a subsequent selection for *Dr*Caspase-B.** By using MO-based knockdown assays, the *Dr*NLRP1 inflammasome was verified to play important functional roles in anti-bacterial innate immunity *in vivo*. **These observations demonstrate that the NLRP1 inflammasome originated as early as in teleost fish.** This finding not only gives insights into the evolutionary history of inflammasomes but also provides a favorable animal model for the study of NLRP1 inflammasome-mediated immunology and diseases.

P0478

Cd58 and Cd2 interaction plays a costimulatory role in adaptive humoral immunity in a zebrafish model

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CD58 and CD2 have been known as a pair of reciprocal adhesion molecules involved in the immune modulations of CD8$^+$ T and NK-mediated cellular immunity in humans and several other mammals. However, the functional roles of CD58 and CD2 in CD4$^+$ T-mediated adaptive humoral immunity remain poorly defined. Moreover, the current functional observations of CD58 and CD2 were mainly acquired from *in vitro* assays, and *in vivo* investigation is greatly limited due to the absence of a *Cd58* homology in murine models. In the present study, we identified *cd58* and *cd2* homologs from a zebrafish (*Danio rerio*) model. These two molecules share conserved structural features to their mammalian counterparts. Functionally, *cd58* and *cd2* were significantly upregulated on antigen-presenting cells and Cd4$^+$ T cells upon antigen stimulation. Blockade or knockdown of Cd58 and Cd2 dramatically impaired the activation of antigen-specific Cd4$^+$ T and mIgM$^+$ B cells, followed by the

DOI: 10.1002/eji.201970400

inhibition of antibody production and host defense against bacterial infections. **These results indicate that CD58/CD2 interaction was required for the full activation of CD4$^+$ T-mediated adaptive humoral immunity.** The interaction of Cd58 with Cd2 was confirmed by co-immunoprecipitation and functional competitive assays by introducing a soluble Cd2 protein. **This study highlights a new costimulatory mechanism underlying the regulatory network of adaptive immunity and makes zebrafish an attractive model organism for the investigation of CD58/CD2-mediated immunology and disorders.** It also provides a cross-species understanding of the evolutionary history of costimulatory signals from fish to mammals as a whole.

P0479

Apoptosis of granulocytes and lymphocytes of peripheral blood in rabbits experimentally infected with antigenic variants RHDVa (lagovirus europeus GI.1a)

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Documented role of phagocytosis of granulocytes and lymphocytes B and T and their subpopulations in peripheral blood in rabbits infected with RHDV (GI.1), including RHDVa (GI.1a) in pathogenesis of this disease, became a reason to undertake the analysis of apoptosis of granulocytes and lymphocytes in rabbits infected with RHDVa (Lagovirus europeus GI.1a). The aim of the study was the assesment of apoptotic granulocytes and lymphocytes in rabbits infected with 6 European RHDVa (GI.1a). Apoptosis was measured on the basis of ApoFluor®Green Caspase (MP Biomedicals, USA) with the use of FACScan Canto II (Becton Dickinson) cytometer on the Cell Quest (USA) programme. Total amount of caspases 1,3,4,5,6,7,8 and 9 was measured in granulocytes and lymphocytes in peripheral blood in rabbits infected with RHDVa (GI.1a). The study was performed in 0, 4, 8, 12, 24, 36 h p.i. The results showed that apoptosis of examined cells in peripheral blood of rabbits infected with 6 RHDVa (GI.1a) is more intensive in granulocytes comparing to lymphocytes. Moreover, the apoptosis in granulocytes begins 8 h p.i. and lasts till 36 h p.i, to death of rabbits. In lymphocytes this process begins a bit earlier in 4 and 8 h p.i. and also last until 36 h p.i. Summing up it may be stated infection of rabbits with RHDV activates apoptosis in granulocytes and lymphocytes and it is, as it seems, a new important element of pathogenic mechanism of RHDV (GI.1a).

P1068

Construction and identification of A cDNA library for screening the host tumor cells mRNA targets for the miR-M4-5p encoded by marek´s disease virus

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Marek's disease virus (MDV), an important member of the subfamily *Alphaherpesvirinae*, causes immunosuppression and rapid-onset lymphoma in its natural host chickens namely Marek's disease (MD). A total of 26 viral microRNAs (miRNAs) have been reported in MDV serotype 1 genomes and possibly play critical roles in virus replication, latency, and/or oncogenesis. The tumor in chickens induced by MDV-1 infection has been historically regarded as an ideal natural model for virally-

DOI: 10.1002/eji.201970400

induced cancer research. As a viral analog of cellular miR-155, the MDV-1 encoded miR-M4-5p has been shown to be crucial for the MD tumorigenesis. Previously we have demonstrated that the deletion of miR-M4-5p from the viral genome significantly reduces virus pathogenicity and oncogenicity, suggesting a putative role as tumour promotor in MDV-1 tumorigenesis. To further reveal the regulatory mechanism mediated by miR-M4-5p, we used the reverse transcribed splenic RNAs from MDV-1-infected chickens as the template to produce a cDNA library for screening the host mRNA target genes for miR-M4-5p utilizing the hybrid-PCR. A total of 52 candidate target genes were obtained, of which 31 binding sites were located in the 3´-UTRs of the mRNA genes. After constructing the reporter gene vectors, two chicken mRNA genes, DPT and TMEM230 targeted by miR-M4-5p, were primarily confirmed by the dual luciferase reporter assay (DLRA). Our work indicates that the approach of using hybrid-PCR to construct a cDNA library for screening miRNA targets is efficiency and provides an important basis for further elucidating the regulatory mechanism of miR-M4-5p in MDV oncogenesis.

P1069

Marek´s disease virus serotype 1 encoded miR-M11-5p/3p targets the host gene HSPA4 and suppresses the ZO-1/ZONAB signaling pathway

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Marek´s disease virus (MDV) is a representative alpha herpesvirus that induces Marek´s disease (MD) in natural host chickens, which is characterized by rapid-onset T-cell lymphoma. MDV infection is an important biological model for studying human virus-induced diseases and tumors, such as the human herpesvirus type 6 (HHV-6) and Epstein-Barr virus (EBV) caused lymphoid tissue proliferative cancers. The MDV-1 genome encodes a total of 26 mature microRNAs (miRNAs) derived from 14 miRNA precursors and distribute in three gene clusters, namely the Meq-cluster, the Mid-cluster and the LAT-cluster, respectively. Our previous works focused on the pathogenic phenotype of individual miRNA precursor genes from the Mid-cluster have demonstrated their potential role as tumor suppressors in the pathogenesis of MDV-1. The miR-M11 is one of them. However, the molecular regulatory mechanism mediated by the miR-M11 derived mature miRNAs in MD pathogenesis remains unknown. Herein, we have identified the chicken gene HSPA4 as a host target for miR-M11-5p/3p. In the MDV-infected or miRNA overexpressed CEF cells, miR-M11-5p/3p specifically down-regulates the HSPA4 expression, resulting in the decreased expression of PCNA and CCND1 in ZO-1/ZONAB signaling pathway and inhibiting cell proliferation.

P1070

Structural and immunological characterization of haemagglutinin-neuraminidse glycoprotein of Newcastle disease virus expressed in transgenic rice seeds

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DOI: 10.1002/eji.201970400

Newcastle disease (ND) is one of the most important avian diseases throughout the world with frequent epidemics occurring, and has caused considerable economic losses to the poultry industry. Current NDV modified live-virus vaccines and inactivated vaccines are protective, but do not allow differentiation of infected from vaccinated animals (DIVA), this is a critical aspect of disease surveillance programs. Here, we report the production of Oryza sativa recombinant tandem HN (Osr2HN) protein from transgenic rice seeds. The design of flexible linker makes HN to form the stable dimer and tetramer to improve immunogenicity. Crystal structural characterization of plant-made HN revealed it to be equivalent to the HN obtained from insect cell lines and HEK293T cells. Immunological characterization in chicken revealed that Osr2HN was efficient to induce a faster antibody response, and 1.5 g of Osr2HN conferred complete protection against high virulence NDV challenge. Compared to the commercial inactivated vaccine from LaSota strain, immunization with Osr2HN vaccine elicited haemagglutination inhibition antibody titres more than sixfold on the twelfth day after immunization. Furthermore, Plant-derived vaccines have significantly less effect on animal weight gain. These results suggest that Ors2HN from transgenic rice seeds is effective, safe and inexpensive, and can be used as a candidate vaccine against NDV.

P1071

The structure, potential antigenic epitope regions, and variability of novel porcine circovirus type 3

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Porcine circovirus type 3 (PCV3), with a circular genome of 2000 nucleotides distantly related to known circoviruses, is a novel porcine circovirus which may play an etiologic role in reproductive failure and porcine dermatitis and nephropathy syndrome (PDNS). Current evidence suggests that PCV3 is widespread. Its high incidence may pose a threat to the world pig industry. However, the pathogenesis and immunity of PCV3 remain poorly understood. Therefore, the structure, antigenic B-cell epitope regions, and MHC II binding T cell epitopes of Cap protein based on PCV3 strain (PCV3-US/MO2015, KX778720.1) were predicted. The structure of PCV3 Cap protein was predicted by I-TASSER and SWISS-MODEL, respectively. The antigenic epitope regions were acquired by comprehensive analysis of the results by BepiPred and IEDB. Eight dominant B-cell epitope regions at amino acids NO.5-25, 42-61,71-81,95-105, 112-134, 138-160, 169-175, 191-204 were obtained. These epitopes are located in the flexible region and distribute almost the entire Cap protein surface. Helper T (Th) cell epitopes were identified by IEDB. Three peptides (4-18aa, 139-153aa, 161-175aa) that may activate Th cells were obtained. The homologies of PCV3 Cap protein and conservative of the predicted epitopes were analyzed using online BLAST. Amino acid sequence analysis indicated that PCV3 Cap had low homology with other swine viruses. B-cell epitope regions are highly conserved, except for the epitope region 5-25aa. Overall, this study has important implications for understanding humoral immune mechanism and further vaccine design.
The research was supported by grants from National Key Research and Development Program of China (2016YFD0500704).

DOI: 10.1002/eji.201970400

P1072

Anthrax-like disease outbreak in cattle in West Africa from atypical *Bacillus* species: preliminary report

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Globally, outbreaks of anthrax disease, caused by *Bacillus anthracis* are making headlines everyday and across the sub regions and continents of the world. In the recent times however, anthrax-like outbreaks caused by other species of *Bacillus* (other than *B. anthracis*) are creating new and extended challenges to health workers and farmers in the field. Recently, in April 2016, an outbreak of anthrax-like disease was reported in three herds of cattle in North Central Nigeria of West Africa. Clinically, the signs and symptoms observed in the diseased cattle were anthrax-like and include: anorexia, lethargy, blood at the nose and anal area, extended neck, darkening of certain areas of the animal skin resulting in four deaths. In humans who came in contact with the diseased cattle or carcasses there were reports of skin lesions akin to cutaneous anthrax. Some of the villagers also reported abdominal pain after the consumption of the contaminated meat although no human fatalities were reported. Microbiologically, the cultural and biochemical characteristics of the pathogens presumptively incriminated a co-infection of *Brevibacillus laterosporus* and *Virgibacillus pantothenticus* isolates from the spleen, blood, nasal swabs and faecal samples of diseased cattle. *Bacillus anthracis*, the established causative agent of anthrax was not isolated either microscopically, culturally or with use of USA Navy field Anthrax ELISA test kits for antigen detection. The genomic and virulence factors determination of these 'pathogens' is underway. The recent discoveries of anthrax-like *Bacillus* species could undermine the current vaccine in use to mitigate anthrax.

P1073

Characterization of rainbow trout CD3ζ signaling: common mechanism between fish and mammals?

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In mammals, CD3ζ subunit is an essential component of the T cell receptor (TCR) complex.
It has been described CD3ζ structural and functional homologues in *Gallus gallus* and *Xenopus laevis*, being the last one considered the immune system link between mammals and ancestral vertebrates as rainbow trout; however, it is still not clear its role on the fish T cell activation.
Here we report a cDNA and gene encoding CD3ζ in rainbow trout, representing an ORFs of 468 bp for 156 amino acids. A multiple alignment of trout and CD3ζ sequences from mammals revealed well-conserved ζζ and ITAM domains. Gene expression of CD3ζ was analyzed and detected in sorted $CD3\varepsilon^{+}$cells from PBL and also detected from different tissues.
Structural model of the trout homodimer CD3ζζ was obtained using an NMR structure from human CD3ζ as template, which was aligned with a 55% identity with trout CD3ζζ sequence. The predicted interface residues of the trout model showed a conserved polar nature, with the S-S bond C_2-$C_{32}$and the aromatic stacking Y_{12}-Y_{42}, conserved as in the human structure.
We constructed a chimera that fused the extracellular and transmembrane domains of trout CD8α to the cytoplasmic domain of CD3ζ. Cells expressing the chimera interacts with proteins having tyrosine kinase activity. Our results show that the kinase activity associated to the CD3 complex is quite similar to the previously characterized kinase activity in mammals. This finding suggests that trout and mammals TCR components may initiate signaling events using a common molecular mechanism.

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DOI: 10.1002/eji.201970400

P1666

Comparisons of immunological parameters in the different strains of mice

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Based on the gene loci of major histocompatibility complex (MHC), strains of mice can be divided into H-2^S (SJL, A.SW, B10.S), H-2^q (FVB/N), H-2^b (C57BL/6, 129/ SvJ), H-2^d (BALB/c and DBA/2) and other different strains of mice. So far, there are few reports describing systematic comparisons of immunological parameters of different strains of mice. Here, we compared the proportion of immune cellsof C57BL/6, DBA/2, BALB/c, SJL, and FVB/N mice. The results of cell number showed that FVB/N had the largest number of spleen cells, C57BL/6 had the largest number of thymocytes,SJL had the largest number of mediastinal lymph node cells. The percentage of T cells in DBA/2 spleen was 20% that of other strains was about 34%; the percentage of DBA/2 B cells was 67%, and other strains were about 56%. The percentage of regulatory T cells ($CD25^+FoxP3^+$) among $CD4^+$cells showed a maximum in DBA/2. The percentage of myeloid dendritic cells (mDCs, $MHCII^+CD11c^{High}$) among spleen cells is approximately the same.The anti-mouse MHCII (I-A/I-E, clone M5/114.15.2) antibody does not bind to SJL immune cells. The percentage of plasmacytoid DCs (pDC, $CD317^+CD11c^{Med}$) of C57BL/6 was the least, while there was no differencebetween the other four lines. Taken together, different strains of mice have different immunological properties, which provide a new basis for the design and analysis of mouse experiments.
Fundings: National Natural Science Foundation of China (81460503), Jilin Provincial Science & Technology Department (20150101207ZC), Education Department of Jilin Province (JIJIAOKEHEZI2015No.3), Health and Family Planning Commission of Jilin Province (2014Z089).

P1667

Generation, identification and functional analysis of monoclonal antibodies against atypical porcine pestivirus NS3 protein

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The atypical porcine pestivirus (APPV), a genetically distinct pestivirus, was reemerged in many countries in recent years and threatening the world's pig industry. It is hard to differentially diagnose the infection caused by APPV and other viruses, such as classical swine fever virus and pseudorabies virus, causing similar neurological symptoms. To facilitate the development of effective methods for detection APPV infection, we prepared a series of monoclonal antibodies (mAbs) targeting NS3 protein of APPV. Isotyping analysis results demonstrated that all these seven mAbs were IgG1 isotype with a kappa light chain. Epitope mapping results indicated that five out of these seven mAbs recognized the same linear epitope (GRIKSAYSDE). However, the 6H3 recognized another linear epitope while the 7E10 recognized a conformational epitope. The applications of these mAbs were evaluated by enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), western blotting assay, and flow cytometry analysis. The results demonstrated that all these seven mAbs were applicable to ELISA, western blotting, IFA, and flow cytometry assay. Taken together, we developed seven functional mAbs against APPV-NS3 protein, contributing to detection of the early infection of APPV by ELISA, western blotting, IFA, and flow cytometry.
Keywords: atypical porcine pestivirus; NS3 protein; monoclonal antibodies;

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DOI: 10.1002/eji.201970400

P1668

Porcine deltacoronavirus engages the transmissible gastroenteritis virus functional receptor porcine aminopeptidase N for infectious cellular entry

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Identification of cellular receptors used by coronavirus (CoV) entry into the host cells is critical to understand pathogenesis and to develop intervention strategies. The fourth CoV genus, *Deltacoronavirus*, evolutionally related to the *Gammacoronavirus*, has just been defined recently. In the current study, we demonstrate that porcine aminopeptidase N (pAPN) acts as a cross-genus CoV functional receptor for both enteropathogenic porcine DeltaCoV (PDCoV) and AlphaCoV (transmissible gastroenteritis virus, TGEV) based upon three lines of evidences. First, the soluble S1 protein of PDCoV efficiently bound to surface of target porcine cell lines known to express pAPN as TGEV-S1 did, which could be blocked by soluble pAPN pre-treatment. Second, either PDCoV-S1 or TGEV-S1 physically recognized and interacted with pAPN by co-immunoprecipitation in pAPN-cDNA-transfected cells and by dot blot hybridization assay. Finally, exogenous expression of pAPN in refractory cells conferred susceptibility to PDCoV-S1 binding and for PDCoV entry and productive infection. PDCoV-S1 appeared to have a lower pAPN-binding affinity and likely consequent lower infection efficiency in pAPN-expressing refractory cells as compared to TGEV-S1, suggesting that there may be difference in virus-binding regions in pAPN between these two viruses. This study paves the way for dissecting the molecular mechanisms of PDCoV-host interactions and pathogenesis as well as facilitates future vaccine development and intervention strategies against PDCoV infection.

P1669

Genomic organization and transcriptional analysis of the immunoglobulin locus in the Mexican axolot, Ambystoma mexicanum

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Most of our knowledge of amphibian adaptive immune system derives from *Xenopus* metamorphosing frogs. The axolotl, *Ambystoma mexicanum* is a unique biological model for complete tissue regeneration, neoteny and one of the largest genome described so far. It is an endangered species highly susceptible to environmental stress, including infectious disease. Seminal studies described that the *axolotl* immune system is deficient, with features resembling that of newborn mammals. Using the published genome sequence, we have mapped, characterized and compared the immunoglobulin locus of *A. mexicanum*. We validated gene models and quantitated relative Ig gene expression using spleen transcriptomic data from NCBI/SRA.
The *A. mexicanum* Ig locus shares the same general genome architecture as most studied vertebrates. The complete locus, including the preB cell receptor is distributed in 71 scaffolds spanning 25.2 Mb. The J_H-C_H cluster was mapped to chr13, adjacent to the TRAV-TRDC (TCR) cluster, and it follows the same architecture as in *Xenopus*, including the *IGHD* composed of eight Ig domains. Also, the syntenic *X. tropicalis IGHF* ortholog is a pseudogene. Of 96 *IGHV* and 72 *IGVL* genes, 68% and 50% are pseudogenes. Clan I *IGHV* segments were not detected. The kappa locus is encoded in chr1, but consists of only three functional genes. Consistent with its large genome,

DOI: 10.1002/eji.201970400

intergenic regions are larger, suggesting no restriction of intergenic region length for VDJ recombination. Our work is a first step to determine if the observed extensive pseudogenization compromises the *axolotl* potential repertoire diversity and contributes to its impaired antibody response.

P1670

Characterising the early protection of C strain vaccine against subsequent classical swine fever virus infection

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Classical Swine Fever Virus (CSFV) is a single stranded RNA virus belonging to the *Flaviviridae* family. It is an ongoing threat to the livestock industry due to its high transmission and mortality rates or high mortality associated with infection. The primary sites of replication are the tonsils and oropharangyeal lymph nodes. Interferon signalling is a key component of how the innate immune system responds to challenge with CSFV, where high levels of interferon-α (IFN-α) are a characteristic feature of acute disease that are not protective, but lead to the development of disease-associated immunopathology.
Live attenuated vaccines such as the CSFV C strain vaccine are capable of protecting against infection within 5 days of vaccination, where IFNg$^+$ CD8$^+$ cells precede the detection of a humoral, virus neutralising response; but the molecular mechanisms through which this early protection is mediated have yet to be established. In this study, we compared the response of pigs vaccinated with the C strain to non-vaccinated pigs both challenged with a pathogenic strain of CSFV. Analysis of transcriptomic data from the tonsils of these animals during the early stages after vaccination and challenge reveals a set of regulated genes that appear throughout the analysis. Many of these are linked to the ISG15 antiviral pathway that is upregulated suggesting it plays a key role in the rapid and early response protection conferred by C strain vaccination.

P2260

A broad species tropism in vitro and a mouse model for swine acute severe diarrhea syndrome coronavirus infection

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Outbreaks of severe diarrhea in neonatal piglets in Guangdong, China in 2017 resulted in isolation and discovery of a novel swine enteric alphacoronavirus (SeACoV), derived from the bat CoV HKU2. SeACoV was later referred to as swine acute diarrhea syndrome (SADS) CoV by another group. The present study investigated whether there may have been transmission via an unknown intermediate host. We first demonstrated that SADS-CoV possesses a broad species tropism and is able to infect cell lines from diverse species including bats, mice, rats, gerbils, hamsters, pigs, chickens, nonhuman primates and humans. To further demonstrate whether SADS-CoV has the potential to infect rodents, wild-type C57BL/6J mice were inoculated with the virus via oral or intraperitoneal routes. Interestingly, although the mice exhibited only subclinical infection, they supported viral replication and persistent infection in the spleen. SADS-CoV nonstructural proteins and double-stranded RNA were detected in

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DOI: 10.1002/eji.201970400

splenocytes of the marginal zone on the edge of lymphatic follicles, indicating active replication of SADS-CoV in the mouse model. Furthermore, we identified that splenic dendritic cells (DCs) are the major targets of virus infection by immunofluorescence and flow cytometry approaches. The ability of SADS-CoV to replicate in various cells lines from a broad range of species and the unexpected tropism for murine DCs provide important insights into the biology of this bat-origin CoV, highlighting its possible ability to cross interspecies barriers and the potential role of rodents as intermediate hosts in the field.

P2261

Pseudorabies virus variant strain induced necropotosis of PK-15 cells to promote viral release

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Pigs infected by the PRV variant strain are characterized by the pathology of the necrotizing inflammatory, while the mechanism of it is not clarified. Necroptosis is a recently reported cell death manner which is regulated by the cascade reaction of RIPK1, RIPK3 and MLKL. In our study, we offer the first evidence that the PRV variant strain induced necroptosis in PK-15 cells. The PRV variant strain induced cell death was proved by using propidium iodide (PI) staining. And the cell death progress can't be prevented by caspase inhibitor Z-VAD-FMK. Meanwhile, cleaved caspase-3 or caspsed-8 were not detected in PK-15 cells infected by the PRV variant strain. These results indicated that the PRV variant strain induced cell death was not related to apoptosis. However, phosphorylation of RIPK3 and MLKL protein was up-regulated in PK-15 cells infected by the PRV variant strain. And the ratio of cell death was decreased by the silencing of RIPK3 or MLKL in the PRV variant strain infected PK-15 cells. Notably, we found the silencing of RIPK3 or MLKL reduced viral release out of PK-15 cells during the PRV variant strain infection. Our data indicated that necroptosis is likely to be involved in the cell death and viral replication progress during the PRV variant strain infection. This may reveal a unique view of the pathogenesis of the PRV variant strain infection and provide new avenues for the development of antiviral strategies.

P2262

Regulation of *Lactobacillus rhamnosus* LGG on the development and differentiation of intestinal associated immune cells in mice and piglets

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Lactic acid bacteria (LAB) is a kind of food-grade microorganism (GRAS), which has been mainly used in food preservation and fermentation for a long time. Lactobacillus has many advantages, such as easy culture, high safety and colonization in the intestinal tract. At present, Lactobacillus has become the best choice for the expression of heterogenous proteins and the preparation of live vector vaccine to prevent intestinal related diseases. However, the mechanism on its regulation of mucosal immune system is not clear enough. In this study, *Lactobacillus rhamnosus* (LGG ATCC53103) was selected as the experimental strain. After LGG intervention, it could promote the development of Pro-B cells in lamina propia (LP), promote the differentiation of B lymphocytes into Mature B cells in bone marrow (BM), and promote the mature differentiation of B lymphocytes in peripheral immune organs of mice. LGG could affect the differentiation of Th1, Th2, Th17 and Tregs in peripheral lymphoid organs (Spleen) and intestinal secondary lymphoid tissues (mesenteric lymph node, MLN; peyer's patch, PPs). We also confirmed that the lamina propria of pig intestine, like bone marrow, is a place for the

DOI: 10.1002/eji.201970400

origin and development of B cells as primary lymphoid tissue. LGG can promote the early differentiation and development of B cells, and affect the production of antibody diversity in the intestinal lamina propria of piglets. LGG intervention could also promote the development and differentiation of intestinal associated T lymphocytes in piglets.

P2263

Cellular immune response induced by avian influenza virus H7N9 in a natural host of chicken

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Emerged from live poultry, avian influenza A H7N9 virus (AIV) poses a great threat to human health. Both avian-H7N9 and human-H7N9 viruses have been shown delayed clearance in chicken and human after infection, suggesting distinct kinetics of immune responses elicited by these AIV subtypes. However, due to that H7N9 replication in mice was not well recapitulated, the host cellular defense response against H7N9 was incompletely defined. In this study, using a newly-established multi-parameter flow cytometry suitable for chicken, we examined innate and adaptive cellular immune responses in chicken after intranasal infection with low pathogenicity AIV H7N9 (A/Chicken/Zhejing/JX148/2014). We found that KUL01$^+$heterophils significantly increased especially in lung after H7N9 JX148 infection and maintained about 30% of leukocytes during this study. Interestingly, KUL01$^+$ heterophils from infected chicken significantly up-regulated MHC II expression, suggestive of the potential of antigen-presenting. CD8a$^+$CD3$^-$ NK cells increased significantly in the lung at early stage of infection but decreased to baseline level later. The percentage of Bu-1$^+$ B cells also increased in lung and spleen but decreased in PBMC after infection. The percentage of TCR1$^+$ γδ T cells increased drastically in the lung but decreased in spleen and PBMC. While CD3$^+$CD4$^+$ T cells increased markedly in all three organs and sustained after infection, CD3$^+$CD8$^+$ T cells increased in spleen and lung but decreased in PBMC. Furthermore, CD3$^+$CD8$^+$ T cells exhibited an activated phenotype with significant down-regulation of CD8α expression. These results suggest that H7N9 infection induced distinct systemic and local cellular immune responses in chicken.

P2264

Protective efficacy of oral immunization with a novel inactivated enterotoxigenic *Escherichia coli* vaccine candidate against post weaning piglet colibacillosis

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Enterotoxigenic *Escherichia coli* (ETEC) strains producing each F4, and F18 fimbriae were lysed by the extract of silkworm inoculated with some antigens. The lysed cells were used as ETEC vaccine candidate in post weaning piglets. This study was carried out to examine whether oral immunization of piglets with the novel vaccine candidate could effectively protect the piglets against ETEC colibacillosis. All piglets were inoculated with commercial feed from 4 weeks of age till 6 weeks of age. Group A piglets were fed with PBS. Group B piglets were bred with 2×10^6 the mixture of the F4+ ETEC lysed cells and the F18+ ETEC lysed cells/piglet in commercial feed. Seral IgG titers from all piglets were evaluated using ELISA kit. All piglets were challenged with the mixture of wild-type F4+ ETEC and the F18+ ETEC at 6 weeks of age. All piglets were monitored daily for diarrhea and

DOI: 10.1002/eji.201970400

mortality for 14 days after challenge. Seral IgG titers from group B piglets were significantly higher than those of group A piglets. After challenge with wild-type ETEC, diarrhea and mortality in group B piglets was not observed. However, diarrhea was observed in 60% of group A piglets, but mortality was not observed. These findings indicate that oral immunization with the mixture of the novel inactivated vaccine cells can effectively protect piglets from ETEC infection.

P3102

Nuclear export signal of PRRSV NSP1α is necessary for type I IFN inhibition

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The nonstructural protein 1α (NSP1α) of porcine reproductive and respiratory syndrome virus (PRRSV) is a nucleo-cytoplasmic protein that suppresses the production of type I interferon (IFN). In this study, we investigated the relationship between the subcellular distribution of NSP1α and its inhibition of type I IFN. NSP1α was found to contain the classical nuclear export signal (NES) and NSP1α nuclear export was CRM-1-mediated. NSP1α was shuttling between the nucleus and cytoplasm. We also showed that the nuclear export of NSP1α was necessary for its ability for type I IFN inhibition. NSP1α was also found to interact with CBP, which implies a possible mechanism of CBP degradation by NSP1α. Taken together, our results describe a novel mechanism of PRRSV NSP1α for type I IFN inhibition and suppression of the host innate antiviral response.

P3103

Dynamic analysis of antibody growth and decline after immunization with porcine viral diarrhea vaccine

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To investigate the growth and decline of maternal and immune antibody after immunization with porcine viral diarrhea vaccine. 100 Double-Negative pregnant sows were screened for PEDV, TGEV and PoRV antibodies and antigens.included the test of A, B, C, D and control group. The "PEDV -TGEV -PoRV live vaccine" (PTR) and "PEDV -TGEV inactivation vaccine" (PT) were used to immunized 100 Pregnant sows and piglets were immunized differently. PEDV IgA was detected in the milk collected from sows after delivery at 0 to 14 days , IgG was detected in piglet serum at 7 to 80 days . The results showed that IgA antibody titers of sows immunized with PTR and PT alternately on 40 d and 20 d before parturition were significantly better than those of single immunization PTR or PT. Piglets were intranasally vaccinated with PTR at the age of 3 days, twice immunized with PTR at the age of 28 days and thrice immunized with PT at the age of 65 days, IgG antibody titers were on the rise after 15 days. This experiment analyzed the growth and decline of maternal antibody and immune antibody levels after porcine viral diarrhea vaccine immunization, and provided a theoretical reference for the porcine viral diarrhea vaccine immunization program.

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DOI: 10.1002/eji.201970400

P3104

Dynamic effects on cytokine by different immune procedures for porcine reproductive and respiratory syndrome (PRRS) vaccine

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To analyze the effect of porcine reproductive and respiratory syndrome virus (PRRSV) attenuated vaccine on serum cytokine secretion levels in piglets by different immune procedures. 40 piglets of 7 days of age were randomly divided into 4 groups, which were negative for both PRRSV antigen and antibody. The test group (groups A, B, and C) were immunized with attenuated vaccine for the first time at 14 days of age, Group A and B were inactivated vaccine and attenuated vaccine at 35 days of age respectively, normal saline was injected into group C at 35 days of age, and the control group was injected with normal saline. Serum was collected at 1, 2, 3 weeks after the first immunization and 1, 2, 3, 4, 5, 6 weeks after the second immunization. The levels of cytokines IFN-γ, TNF-a, IL-2, IL-10 and IL-12 were detected by ELISA. The results showed that the secretion of IFN-γ and TNF-a in the immunized group were significantly higher than that in the control group ($P< 0.01$), and the secretion of IL-2 was significantly higher than that in the control group ($P< 0.05$) less than 3 weeks after the first immunization of piglets. The secretion of cytokines in the immunized group was not significant after the second immunization ($P>0.05$). The results indicated that the first PRRSV attenuated vaccine at 14 days of age can effectively induce the cellular immune response.

P3105

Molecular cloning and functional characterization of porcine DEAH (Asp-Glu-Ala-His)-box polypeptide 15 (poDHX15)

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DHX15, a member of the DEXDc helicase family, was recently identified as an intracellular RNA sensor in mouse and human. However, it is unclear whether porcine DHX15 (poDHX15) is also an important RNA sensor. In this study, poDHX15 was cloned and its tissue distribution, subcellular localization, and functions in the induction of type I interferon (IFN) signaling pathway were investigated. Full-length poDHX15 cDNA is 2388 bp long, encodes 795 amino acid residues and contains a DEADc domain, a HELICc domain, a HA2 domain, and a DUF domain. poDDX41 mRNA is widely expressed in different tissues, especially the lung, lymph node and small intestine. The subcellular distribution of poDHX15 is not only in the cytosol and nucleus, but also on mitochondria, Golgi apparatus and the endoplasmic reticulum membrane. Overexpression of poDDX41 in HEK293T cells induced IFN-β by the activation of transcription factors IRF3 and NF-κB, which was dependent on critical adaptor molecule VISA and STING. Deletion mutant analysis revealed that the DEADc domain, a HELICc domain and a HA2 domain are required for the activation of IFN-β, but the DUF domain causes some kind of obstruction of the IFN signaling pathway. Knockdown of poDHX15 in porcine kidney epithelial cells with siRNA significantly reduced IFN-β expression induced by poly(I:C), a double-stranded RNA (dsRNA) analogue. Therefore, poDHX15 is an important RNA sensor, involved in the dsRNA-mediated type I IFN signaling pathway in the porcine innate immune system.

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DOI: 10.1002/eji.201970400

Acknowledgments: The work was supported by the China National Natural Science Funds (31302072, 31372423 and 30871884).

P3106

Molecular mechanism of the RLRs-mediated type I IFN signaling pathway inhibited by the non-structural protein C of peste des petits ruminants virus

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In order to study the innate immune mechanism mediated by PPRV non-structural protein C, the effects of C gene on IFN and downstream factors expression in RLRs pathway were detected by qPCR using co-transfection plasmids into HEK293T cells. The dual-specific luciferase reporter assay was performed to investigate the interaction between C with the key molecules in the RIG-I signaling pathway. Furthermore, the antiviral effects of endogenous and exogenous INF that caused by C were tested. The phosphorylation of signal transducers and activators of transcription I (STAT1) were also studied.
1. C protein inhibits the expression of INF-β and its downstream factors of ISG15, ISG56, CXCL10 at transcriptional level mediated by RLRs signaling pathway.
2. C protein inhibits the activity of reporter gene NF-kappa-B and ISRE. PPRV C protein significantly inhibited the activity of IFN-beta promoter that activated by signal molecule RIG-IN and MAVS ($p<0.001$), but did not inhibit the promoter activity of other signal molecules of IKKε, TBK1, TRAF3, IRF3 and IRF7 ($p>0.05$), indicating that the target molecule of C protein in RLRs pathway is MAVS.
3. C protein can significantly inhibit the anti-PPRV, EMCV and VSV produced by either endogenous or exogenous IFN.
4. C protein can inhibit the phosphorylation of STAT1, but the antiviral mechanism of PPRV C protein blocking JAK-STAT signal transduction to inhibit IFN production still needs further study.
Overall, we concluded that the inhibition of STAT1 phosphorylation in JAK-STAT pathway by C protein was the reason for its inhibition of IFN production.

P3107

Establishment and preliminary application of an indirect elisa for detecting antibody based on N protein of porcine epidemic diarrhea variant virulent strain

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In order to establish an indirect ELISA method for detecting porcine epidemic diarrhea virus variant virulent strain, the N gene of PEDV mutant strain was amplified by RT-PCR and cloned into pQE30 vector for prokaryotic expression. Then the expressed protein was purified and identified. Purified recombinant N protein was used as coating antigen, and ELISA reaction conditions were optimized by matrix method. PEDV indirect ELISA detection method was established, and specificity, sensitivity and repeatability tests were carried out.500 clinical serum samples from different pig farms were tested and the results were compared with imported commercial kits. The results showed that the expressed recombinant protein was approximately 50.4 ku, and the results of western blot showed that the recombinant protein had good immunogenicity. The optimal coating concentration of indirect ELISA was 2 µg/mL, the dilution of serum was 1:100 and the duration of action was 1 h, the goat anti-pig

DOI: 10.1002/eji.201970400

HRP-IgG was 1:5 000 . This method could specifically detect PEDV antibodies with no cross reaction with PRV, PRRSV, CSFV, PCV, TGEV and other pathogens. The highest dilution factor of the same serum using this indirect ELISA was consistent with the result of neutralization test. The variation coefficient of repeatability test was less than 10 % in batch and between batches, which showed this method had good repeatability and stability. Using this method to detect 500 clinical porcine serum, the coincidence rate compared with the imported commercial kit was 99.6 %.

P3108

The influences on chickens' T-lymphocyte subsets and the proliferation activities of T/B lymphocytes by different concentrations of recombinant chicken interleukin-6/2 fusion protein in vivo

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To study the biological activity of recombinant chicken interleukin-6/2 fusion protein (rChIL-6-Linker-ChIL-2, recombinant fusion protein) with different concentrations, rChIL-6-Linker-ChIL-2 at 1:8 dilution, rChIL-6 at 1:8 dilution, rChIL-2 at 1:8 dilution, and mixture of equal volume rChIL-6 and rChIL-2 at 1:4 dilution (rChIL-6+rChIL-2) were inoculated in 28-day SPF chickens, and the specific immune reactivity of rChIL-6-Linker-ChIL-2 and anti-ChIL-6/ChIL-2 monoclonal antibody was detected by ELISA. The index of promoting proliferation activities of T/B-lymphocytes, the CD3+CD4+, CD3+CD8+ T-lymphocytes and the content of Th1/Th2-type cytokines in chicken were detected by MTS, FCM(flow cytometry).The results showed that, the proliferation activity of T/B-lymphocytes effected by rChIL-6-Linker-ChIL-2 in dilution of ≤1:8 was significantly higher than that in ConA, but there was no significant difference with its stoste. When the dilution higher than 1:8, the activity in vivo was decreased with the decrease in concentration. The activity of rChIL-6-Linker-ChIL-2 dilute to 1:8 was higher than that to 1:32 or 1:64, (the index of promoting proliferation activities of T/B-lymphocytes, the CD3+CD4+, CD3+CD8+ T-lymphocytes and the content of Th1/Th2-type cytokines in vivo), which had no obvious difference with its stoste. The index was higher than rChIL-6 and rChIL-2, and was not different with proteins mixed, which included the promoting T/B-lymphocytes proliferation activity, the percentage of CD3+CD4+, CD3+CD8+ T-lymphocytes, the content of Th1/Th2-type cytokines and the level of specific immune antibody in vivo.

P3109

Expression of African swine fever virus protein P30 in pichia pastoris

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African swine fever (ASF) is an acute and often fatal disease affecting domestic pigs and wild boar, with severe economic consequences for affected countries. The causative agent, the African swine fever virus (ASFV), is a large, enveloped, double-stranded DNA virus. As eukaryotic expression system, Pichia pastoris has many advantages, such as protein processing, folding, post-translational modifications, and its characteristics of faster, simpler, cheaper, and higher levels of expression than other eukaryotic expression systems (Baculovirus or mammal). To prevent and control ASF, we successfully generated the recombinant plasmid ppiczαA-p30 to express ASFV P30 protein. Then recombinant ASFV P30 protein (rP30) was successfully expressed by the recombinant plasmid ppiczαA-p30 in *Pichia pastoris* X-33, and identified by SDS-PAGE and western blot with a band at

DOI: 10.1002/eji.201970400

37KD higher than the target band (30KD), which may be the result of glycosylation. In conclusion, the recombinant plasmid ppiczαA-p30 has a strategic significance for the prevention and control of ASF, and the expression of the ASFV rP30 would be useful for developing diagnosis techniques of ASF infection.
This work was supported by grants from the national key research and development plan(2018YFCD840405).
Keywords: ASF, ASFV, Pichia pastoris, rP30

P3110

H9N2 avian influenza A virus infection is associated with an elevated innate immune response and suppression of antigen presentation in chicken dendritic cells

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H9N2 avian influenza, as a mild and highly contagious disease, is associated with the decrease of the efficacy of vaccine interventions, and the increase of susceptibility to secondary infections in poultry. However, the immune evasion mechanism of H9N2 avian influenza virus (AIV) in chicken remains poorly understood. Dendritic cells (DCs) are immune cells of major importance, involved in innate immune responses against viruses, but also in the setting of adaptative immune response due to their high ability to present viral antigen. Therefore, in this study we analyze the differentially expressed genes (DEGs) at the transcriptome level in chicken DCs infected with H9N2 AIV compared to mock infection by high-throughput RNA-sequencing technology. In total, 4151 up-regulated and 2138 down-regulated DEGs were identified. Further enrichment analysis showed that the up-regulated DEGs were enriched in immune-related biological processes mainly involved in defense response to virus, and inflammatory response, while the down-regulated DEGs were associated with the immune-related biological processes mainly including MHC class I and II antigen processing and presentation, and protein catabolic process in proteasome and lysosome. Additionally, 49 of these immune-related DEGs were validated by quantitative real-time PCR, and the decrease in the ability to stimulate T cells of H9N2 AIV infected DCs was also confirmed by mixed lymphocyte reaction assay. **Collectively, these data proposed that H9N2 AIV infection induced a strong innate immune response in chicken DCs, but impaired the antigen-presenting response of this cell, which may provide helpful insights into the pathogenesis of H9N2 AIV in chickens**.

P3111

Identification of novel B-cell epitope in gp85 of subgroup J avian leukosis virus and its application in diagnosis of disease

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Background: The gp85 protein is the main envelope protein of avian leukosis subgroup J (ALV-J) and involved in virus neutralization. Here, we mapped the epitope in ALV-J gp85 protein by ELISA using synthetic peptides and developed epitope based diagnostic methods for ALV-J infection.
Results: The results revealed that monoclonal antibody (mAb) JE9 recognized ^{83}WDPQEL^{88} motifs, which was highly conserved in gp85 among different ALV-J strains by homology analysis. Moreover, after evaluation with two hundred and forty sera samples obtained from different chicken farms, the

DOI: 10.1002/eji.201970400

epitope-based peptide ELISA had much higher sensitivity than commercial ELISA kit for antibody detection of ALV-J.
Conclusions: A novel B-cell epitope recognized by the mAb JE9 was identified. The developed peptide-ELISA based on this novel B-cell epitope could be useful in laboratory viral diagnosis, routine surveillance in chicken farms, and also in understanding the pathogenesis of ALV-J.

P3112

Riemerella anatipestifer extracellular protease S blocks complement activation via the classical and lectin pathways

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Riemerella anatipestifer (RA) is the causative agent of infectious serositis in ducklings and other avian species. It is difficult to control the disease due to its 21 serotypes, poor cross-protection, and bacterial resistance to antimicrobial agents. The complement system is an important component of the innate immune system. However, bacterial pathogens exploit several strategies to evade detection by the complement system. Here, we purified and identified a 59-kDa RA extracellular protease S (EcpS) consisting of a gelatinase. In this study, we aimed to determine how EcpS interferes with complement activation and whether it could block complement-dependent neutrophil function. We found that EcpS potently blocked RA phagocytosis and killing by duck neutrophils. Furthermore, EcpS inhibited the opsonization of bacteria by complement 3b. EcpS specifically blocked complement 3b and complement 4b deposition via the classical and lectin pathways, whereas the alternative pathway was not affected. In summary, we show that RA can survive the bactericidal activity of the duck complement system. These results indicate that RA has evolved mechanisms to evade the duck complement system that may increase the efficiency by which this pathogen can gain access and colonize the inner tissues where it may cause severe infections.

Vaccines for Infection and Cancer

O121

Listeria based anti-hepatocellular carcinoma vaccine facilitates immune checkpoint blockade therapy by regulating tumor microenvironmental macrophage polarization

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Hepatocellular carcinoma (HCC)is a common liver malignancy and in advanced HCC patients, limited therapeutic options lead to high mortality and recurrence rate. Recently, immune checkpoint blockade immunotherapy has benefited the patients with advanced cancers greatly. However, in HCC patients, immune checkpoint blockade is still suboptimal and needs more immune modification to achieve an efficient therapeutic goal. Here, we investigated the combined administration of a *Listeria* based HCC

DOI: 10.1002/eji.201970400

vaccine, Lmdd-MPFG, and an anti-PD1 immune checkpoint blockade antibody. We found Lmdd-MPFG could promote the expression of PD-L1 in HCC cells and resensitize the tumor local T cell to response to the anti-PD1 immunotherapy. Mechanistically, through TLR2 and MyD88 pathway, Lmdd-MPFG vaccine activatesNF-κB pathwayin the tumor associated macrophages (TAMs). It results in the recruitment p62 and the activation of the autophagy pathway. The overall effects skew the TAMs from M2-polarized TAMs into the M1-polarized TAMs, and most importantly, skews the cytokine profiles in the tumor microenvironment (TEM). This change restores the T cell reactivity to the anti-PD1 blockade.Our study suggested that Lmdd-MPFG combined with PD-1 blockade exerted synergistic anti-tumor effects through modifying TAMs in tumor microenvironment (TME) and removing T cell inhibitive signals thereby providing a new potential strategy in treatment with HCC.

O122

Leishmania tarentolae secreting the sand fly salivary antigen PpSP15 and CpG motifs confers protection against Leishmania major infection in a susceptible BALB/c mice model

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Cutaneous leishmaniasis is a zoonotic, vector-borne disease causing a major health problem in several countries. No vaccine is available and there are limitations associated with the current therapeutic regimens. Immune responses to sand fly saliva have been shown to protect against Leishmania infection. A cellular immune response to PpSP15, a protein from the sand fly Phlebotomus papatasi, was sufficient to control *Leishmania major* infection in mice. This work presents data supporting the vaccine potency of recombinant live non-pathogenic *Leishmania (L.) tarentolae* secreting PpSP15 in mice and its potential as a new vaccine strategy against *L. major*. We generated a recombinant *L. tarentolae*-PpSP15 strain delivered in the presence of CpG ODN and evaluated its immunogenicity and protective immunity against *L. major* infection in BALB/c mice. In parallel, different vaccination modalities using PpSP15 as the target antigen were compared. Humoral and cellular immune responses were evaluated before and at three and eight weeks after challenge. Footpad swelling and parasite load were assessed at eight and eleven weeks post-challenge. Our results show that vaccination with *L. tarentolae*-PpSP15 in combination with CpG as a prime-boost modality confers strong protection against *L. major* infection that was superior to other vaccination modalities used in this study. This approach represents a novel and promising vaccination strategy against Old World cutaneous leishmaniasis.

O123

Ubiquitinated proteins enriched from drug-resistant breast cancer cells by Vx3-functionalized alumina nanoparticles as a potent cancer vaccine

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Nowadays the major treatments for breast cancer include surgery, chemotherapy, radiotherapy and immune therapy. However, resistance to chemotherapy drugs is the main cause for therapeutic failure, and immunotherapy is a promising treatment. In previous studies, we have demonstrated that ubiquitinated proteins (UPs), enriched by Vx3 (ubiquitin binding protein)-conjugated α-Al_2O_3 (α-Al_2O_3-

Vx3) from tumor cells lysate, could be used for cancer vaccine for immunotherapy. In this study, UPs were enrich from lysate of Epirubicin-resistant stem cell-like 4T1 cells (4T1/EPB) to investigate their anti-tumor efficacy against drug-resistance breast cancer.
Both prevention trial and therapeutic trial showed that α-Al_2O_3-UPs (4T1/EPB) have an advantage over α-Al_2O_3-UPs(4T1/WT) of preventing and inhibiting tumorigenesis and metastasis in the 4T1/EPB tumor model. Moreover, the combination of UPs-4T1/EPB vaccination and chemotherapy significantly improved the anti-tumor effect. More importantly, the combination of STING agonists can further enhance the anti-tumor efficacy of UPs-4T1/EPB vaccine to cause complete regression of transplanted 4T1/EPB tumors through facilitating $CD8a^+$ DC migration in TDLN and enhancing 4T1/EPB-special T cell responses. Our results demonstrated that combination of UPs-4T1/EPB and STING agonists may have the potential immunotherapy for drug-resistance breast cancer.
Keywords: Ubiquitinated proteins; Alumina Nanoparticles; Drug-resistance breast cancer; Cancer Vaccines; STING

O124

Leishmania major MAPK10 offers cross-protection against L. donovani infection

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Leishmaniases are complex of neglected tropical poverty associated vector borne diseases caused by different eukaryotic species of dimorphic, obligate, protozoan flagellated parasites of the genus *leishmania* belongs to the order kinetoplastida and the family trypanosomatidae. In the host body, these *Leishmania* parasites reside and multiply within the host macrophages, which serve the dual function of being the principal host cells for the parasites as well as the cells primarily involved in parasite elimination. Thus, host-parasite interaction dictates the fate of *Leishmania* infection outcome. Treatment options are related with high cost, toxicity and emerging drugs resistance. Despite these problems an effective anti-leishmanial vaccine remains elusive. MAPK10 (Mitogen associated protein kinase10) a cytosolic signalling intermediate protein, shows homology with mammalian host p38 MAPK. As *L. major* expressed MAPK10 showed significant host-protective effect in susceptible BALB/c mouse model of experimental cutaneous leishmaniasis, we tested whether priming with the same gene would offer cross-protection against *L. donovani* infection. **We found that MAPK10 DNA vaccination using a mammalian expression vector significantly reduces the parasite burden predominantly, visceral organ spleen and liver accompanied by NO production.** The protection is accompanied by host-protective T cell functions, TH_1-type cytokines and elevated leishmanial antigen-specific IgG2a isotype response. T cell response to the challenge *L. donovani* infection is associated with increase in IL-12 and IFN-γ, but reduced IL-10 and IL-4, production. These findings clearly suggest the cross-protective vaccine potential of LmjMAPK10 against *L. donovani* infection.

O125

Mechanical force of soft fibrin gel promotes selection of colon tumor-repopulating cells through DAB2IP/PI3K/FOXA1/Nanog regulation

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Cancer stem cells have been identified to be the initiator for the recurrence and metastasis of colon cancer. Recently, fibrin deposition in stroma of several cancers, including colon cancer, was

DOI: 10.1002/eji.201970400

demonstrated correlated with tumor development. As a physical factor, mechanical force generated by extracellular fibrin may play a key role in cancer cell differentiation and function. In this study, we investigated the effects of fibrin as supportive framework on colon tumor-repopulating cell (TRC) selection and regulation and elucidated the underlying mechanisms. Results showed that 90 Pascal (Pa) soft fibrin matrix was most efficient in isolation and enrichment of colon TRCs compared with stiff 420 Pa and 1 050 Pa gels. The size and number of colony formation negatively correlated with gel stiffness. These colon TRCs exhibited efficient tumorigenicity, enhanced stem cell markers as well as anti-chemotherapy. Among the up-regulated stem cell markers, the self-renewal molecule Nanog was sharply increased and behaved indispensable in TRC formation. Importantly, such process was extensively controlled by the stiffness of fibrin matrix. Mechanism study illustrated that Nanog expression was negatively regulated by DAB2IP through PI3K/FOXA1 signaling pathway. Overall, our study elucidated that stiffness of extracellular fibrin could direct dynamic shaping of colon TRCs. The soft fibrin matrix at 90 Pa stiffness facilitated colon TRCs growth through mechanical force mediated DAB2IP/Nanog regulation.

O126

Obesity and gender affect the immune-response to Tick-borne encephalitis (TBE) booster vaccination

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Obesity is a global epidemic which, apart from related co-morbidities, directly affects the immune system leading to immune dysfunction and increased susceptibility to infectious diseases. Thus prophylaxis against vaccine-preventable diseases is very important, yet responsiveness to some vaccines is impaired.
In order to assess efficacy of tick-borne encephalitis (TBE) vaccine, we booster-vaccinated 37 obese individuals and 36 normal-weight controls in a phase IV clinical trial. General immunologic and metabolic profiles along with vaccine-specific humoral and cellular immune responses were evaluated in sera and PBMC.
Obese adults had significantly increased metabolic (leptin, insulin, triglycerides, cholesterol) and pro-inflammatory (CRP) markers. Also total IgM and IgA concentrations were elevated, yet natural IgM specific for pneumococcal Ags were significantly reduced. Obese individuals showed a stronger fold-increase of TBE-specific Ab titers 4 weeks after booster and a significantly faster decline after 6 months, both were positively correlated with BMI, leptin and insulin. While in controls titer-increases are generally higher in females (due to estrogen), in the obese group males featured highest fold-increases in association with reduced testosterone levels. On cellular level, TBE-specific IL-2 and IFNg/L10 ratios were higher in obese and also B/T/Tfh lymphocyte subsets showed effects of the metabolic state. Vaccine reactogenicity (local and systemic) was increased in obese subjects.
Our results indicate that TBE booster-vaccination was effective in obese subjects yet differed depending on gender. However, higher decline rates might lead to shorter long-term protection, thus a follow-up study with administration of TBE booster after 3 years is currently conducted.
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DOI: 10.1002/eji.201970400

O127

The cooperative complex of Argonaute-2/MicroRNA-146a regulates hepatitis B virus replication through FEN1

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Objective: To investigate the effect of miR-146a on the replication of chronic hepatitis B virus and its molecular mechanism.
Methods: The miR-146a mimic and inhibitor were transfected into the HepG2.2.15 cell line that can produce HBV, and the HBV-DNA copies were detected by qRT-PCR. The downstream target gene IRAK1/TRAF6 of miR-146a was screened and analyzed, and the mRNA and protein levels of IRAK1/TRAF6 were detected by qRT-PCR and western blot. HepG2.2.15 cells were further transfected with FEN1 plasmid and its siRNA and miRNA-146a mimic and inhibitor to detect HBV-DNA levels. The Ago2 protein in HepG2.2.15 cell line was precipitated by Ago2 RIP and qRT-PCR detected miR-146a levels.
Results: After transfection of miR-146a mimic and inhibitor for 48h, HBV-DNA copies were (3.22±0.001), (2.62±0.08), respectively and significantly increased and decreased compared with the control group (2.81±0.02) ($P< 0.05$); Corresponding qRT-PCR and Western blot showed that the expression levels of IRAK1/TRAF6 were (0.114±0.01), (1.18±0.04), respectively were significantly lower and higher than the control group (1.000±0.03) ($P< 0.05$). . After co-transfection of miR-146a mimic with FEN1 plasmid and miR-146a inhibitor with FEN1 siRNA, HBV Copies were (4.02±0.02), (2.37±0.01) significantly higher and lower than the control group (3.45±0.03) ($P< 0.05$). Ago2 RIP showed a significant decrease in miR-146a levels in the Ago2 siRNA group ($P< 0.05$).
Conclusion: Ago2 cooperates with miR-146a to regulate the transcription of FEN1 protein through the downstream target gene IRAK1/TRAF6, thus promoting HBV replication.

O128

CIMAvax-EGF: Toward long-term survival of advanced NSCLC

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Lung cancer remains one of the leading causes of cancer related deaths. Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer. The human Epidermal Growth Factor Receptor (EGFR) family plays a key role in tumor development. This cluster of genes is associated with augmented proliferation, angiogenesis, cell survival and migration of the tumor cells. CIMAvax-EGF vaccine consists of a chemical conjugate of the EGF with the P64 protein derived from the Meningitis B bacteria and Montanide ISA 51, as adjuvant. The vaccine induces antibodies against EGF that results in EGF withdrawal. CIMAvax-EGF demonstrated to be safe and immunogenic in advanced NSCLC patients in 5 exploratory studies. Then, two randomized studies were completed in advanced NSCLC, where CIMAvax-EGF was administered after chemotherapy, as 'switch maintenance'. The vaccine was very well tolerated and the most frequent adverse events consisted of grade 1/2 injection site reactions, fever, headache, vomiting and chills. Subjects receiving a minimum of 4 vaccine doses had a significant survival advantage. NSCLC patients with high EGF concentration at baseline had the largest benefit, comparable with best maintenance therapies. Recently, cimavax-EGF is being combined with an anti-PD1 antibody, as second line therapy for patients with advanced NSCLC. The combination of nivolumab with CIMAvax-EGF was safe and demonstrated promising

DOI: 10.1002/eji.201970400

efficacy even in PD-L1 low patients. Combination of cimavax-EGF and nivolumab increased the proportion of good responders. Patients with objective response had lower serum HB-EGF, TGF alpha, IFN alpha, IL7, 1L15, CXCL11 and CRP concentration.

O273

A multipotent artificial antigen-presenting cell delivering eleven kinds of immune molecules powerfully expands tumor antigen-specific CTLs ex vivo and in vivo

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Antigen-presenting cells are powerful tools to expand antigen-specific T cells *ex vivo* and *in vivo* for tumor immunotherapy, but suffer from time-consuming generation and biosafety concerns raised by live cells. Alternatively, the cell-free artificial antigen-presenting cells (aAPCs) have been rapidly developed, but only present two or three kinds of immune molecules. Here, a multipotent artificial antigen-presenting cell (MaAPC) dilevering eleven kinds of immune moleclues was developed to comprehensively simulate natural APCs by cocoupling tartget antigens (H-2K^b/TRP2$_{180-188}$-Ig dimers, H-2D^b/gp100$_{25-33}$-Ig dimers), costimulatory molecules (anti-CD28, anti-4-1BB, anti-CD2) and "self-marker" CD47-Fc onto surface-modified polylactic-co-glycolic acid microparticles (PLGA-MPs) and co-encapsulating cytokine (IL-2, IL-15), chemokine (CCL21) and checkpoint inhibitors (anti-CTLA-4, anti-PD-1) inside. One-week co-culture of the MaAPCs with naïve T cells elevated the frequencies of TRP2$_{180-188}$-specific and gp100$_{25-33}$-specific CTLs to 51.0% and 43.3%, respectively, with enhanced cytotoxicity. Three infusions of the MaAPCs powerfully inhibited the subcutaneous melanoma growth, and expanded the TRP2$_{180-188}$ and gp100$_{25-33}$-specific CTLs for 59-86 folds in peripheral blood, 76-77 folds in spleen and 205-212 folds in tumor tissue, in an antigen-specific manner. As compared with the conventional aAPCs carrying 2 or 3 kinds of immune molecules, the eleven-signal MaAPCs exerted much more powerful impacts on T cells, including activation, proliferation, cytotoxicity, differentiation to memory CTLs or regulatory T cells, and cytokines profiles, without visible side effects. This approach provides a new-generation aAPCs, termed MaAPCs, by presenting as many as eleven kinds of immune signals to T cells for the patient-individual and antigen-specific immunotherapy of tumors.

O275

miR-5119-engineered DC vaccine for cancer immunotherapy via regulating ligands of iRs

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Dendritic cell (DC)-based immunotherapy is a promising approach to clinical cancer treatment. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that bind to RNAs mediating multiple events including encoding proteins. miRNA mimics may be potent agents to enhance DC-based immunotherapy against cancers. We firstly used the miRNA array analysis to identify a representative microRNA (miR-5119) potentially regulating PD-L1 in DC-mediated immune tolerance. We evaluated expression of ligands of immune cell inhibitory receptors (iRs) and miR-5119 in DCs from 4T1 mouse breast cancer tumor-bearing mice, and examined the molecular targets of miR-5119. miR-5119-transfected DC cells were generated and their capacity to induce recovery of exhausted CD8$^+$ T cells and enhance anti-tumor immunity. Our results showed that miRNA-5119 was

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DOI: 10.1002/eji.201970400

downregulated in spleen DCs from breast cancer-bearing mice. Our data also showed that miRNA-5119 targeted mRNAs encoding multiple negative immune regulatory molecules, such as PD-L1 and IDO2. DCs engineered to express an miR-5119 mimic downregulated PD-L1 and prevented T cell exhaustion in mice breast cancer. Moreover, miR-5119-engineered DCs effectively restored function to exhausted $CD8^+$ T cells, resulting in anti-tumor cell immune response and upregulated cytokine production. Treatment with miR-5119-engineered DC vaccine reduced T cell exhaustion and suppressed mouse breast tumor homograft growth. This study shows, for the first time, a novel therapeutic approach using miRNA-5119-engineered DC vaccines to regulate inhibitory receptors and enhance anti-tumor immune response in a mouse model of breast cancer. miRNA/DC-based immunotherapy has potential for advancement to the clinic as a new strategy for DC-based anti-cancer immunotherapy.

O277

Mucosal heterologous prime/boost vaccination strategy induces polyfunctional systemic immunity improving protection against Trypanosoma cruzi

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Chagas disease is a potentially life-threatening disease caused by the protozoan parasite *Trypanosoma cruzi*, that is recognized by WHO as a NTD in Latin America. Despite several efforts, there is no approved vaccine against Chagas disease. We have previously introduced Traspain, a chimeric antigen rationally designed to display B/T cell epitopes of key parasitic proteins: Cruzipain, ASP-2 and Transialidase. Traspain proved to be immunogenic and protective against *T. cruzi* murine infection in a protein-subunit vaccine model. Here we analyzed immunogenicity and efficacy of a mucosal prime/boost protocol using attenuated *Salmonella* as Traspain-DNA-delivery system, followed by boosts of rTraspain adjuvanted with cyclic-di-AMP (CDA) or CpG-ODN. In agreement with previous results, mice that received Traspain/CDA displayed a Th1/Th17 bias. However, the single Cruzipain and ASP2 combined, failed to achieve similar levels of vaccine potency despite receiving CDA. Traspain/ODN-CpG boost showed a Th1 profile. Traspain/CDA immunized mice showed the more equilibrated immune response during the acute phase that correlated with a reduction of circulating parasites and increase in survival rates upon a lethal *T. cruzi* challenge. We found a similar scenario in a chronic infection models, when Traspain/CDA boost produced the strongest reduction of blood parasites and body weight losses compared with non-vaccinated control. We concluded: CDA outperformed another adjuvant in mucosal prime/boost strategy inducing a Th17 immune response; the chimeric Traspain improve the magnitude of the immune response compared with the single antigens combined; and the increase of polyfunctionality of cell immunity is associated with better protection as anti-*T. cruzi* vaccine.

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DOI: 10.1002/eji.201970400

O278

Efficient induction of cytotoxic T cells by viral vector vaccination requires STING

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Modified Vaccinia virus Ankara (MVA) is currently investigated as a promising vaccine vector against infectious diseases and cancer. MVA acquired mutations in host range and immunomodulatory genes, rendering the virus deficient for replication in most mammalian cells. MVA has a high safety profile and induces robust immune responses. However, the role of innate immune triggers for the induction of cytotoxic T cell responses after vaccination is incompletely understood. Stimulator of interferon genes (STING) is an adaptor protein which integrates signaling downstream of several DNA sensors and therefore mediates the induction of type I interferon in response to many dsDNA viruses. Since the type I interferon response is entirely STING-dependent during MVA infection, we studied the effect of STING on primary and secondary cytotoxic T cell responses and memory T cell formation after MVA vaccination in STING KO mice. Moreover *in vitro*, we analyzed the impact of STING on the maturation of dendritic cells (DC) and their functionality as antigen presenting cells for cytotoxic T cells during MVA infection. Our results show that STING played a crucial role for DC maturation and for the induction of efficient cytotoxic T cell responses *in vivo* since we observed significantly decreased short-lived effector and effector memory T cell responses after priming in STING KO mic**e**. These findings indicate that STING probably integrates innate immune signaling downstream of different DNA sensors in DC and shapes the cytotoxic T cell response via the DC phenotype which strictly depends on type I interferon in this infection model.

O279

Recent advances in therapeutic lung cancer vaccines: a unique role of TAP-independent CD8 T-cell epitopes

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An effective antitumor immune response is often associated with the presence of T cells directed toward cancer neoepitopes, a class of major histocompatibility complex (MHC)-bound peptides that arise from tumor-specific mutations. However, tumors frequently use multiple mechanisms to evade cytotoxic T lymphocyte (CTL) recognition and destruction. Among these mechanisms, defects in genes relevant for antigen presentation by MHC class I molecules to $CD8^+$ T lymphocytes, such as loss of b2m and alterations in transporter associated with antigen processing (TAP), play a major role by inducing a sharp decrease in surface expression of MHC-class I/β2m-peptide complexes. Therefore, developing therapeutic cancer vaccines based on tumor neoantigens that are selectively presented by cancer cells carrying defects in antigen processing and presentation was a major challenge over the last two decades. In this regard, we have previously identified a non-mutant tumor neoepitope, $ppCT_{16-25}$, derived from preprocalcitonin (ppCT) leader sequence and processed independently of proteasomes/TAP by a novel mechanism involving signal peptidase (SP) and signal peptide peptidase (SPP). We have recently shown that most human lung tumors display altered expression of TAP and frequently express ppCT self-antigen. ppCT includes several HLA-A2-restricted T-cell epitopes that are processed by proteasome/TAP-independent and -dependent pathways. We have also provided *in vitro* and *in vivo* proof of concept of an active immunotherapy based on ppCT-derived peptides capable of controlling growth of immune-escaped tumors expressing low levels of MHC-class I molecules. Non-mutant and mutant neoepitopes are promising targets for therapeutic cancer vaccines in combination with immune checkpoint inhibitors, such as anti-PD-1.

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DOI: 10.1002/eji.201970400

O280

TLR2-activated dendritic cells prevent tumor growth

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Immune system eliminates neoplastic transformations induced by various carcinogens, but loss of tumor antigens and APC dysfunction eventually promotes tumor succession. Addressing APC dysfunction, dendritic cell (DC) mediated anti tumor immunization was extensively studied in both human and mouse. Vaccination with tumor antigen-pulsed dendritic cells (DCs) or administration of the ligands for Toll-Like Receptor 3 (TLR), TLR7 and TLR9 led to significant declining in tumor burden in challenge experiments. Herein, using RM-1 murine tumor relapse model, we show that the elicitation of the anti-tumor immune response by priming with tumor antigen-pulsed DCs accentuated by further stimulation of the DCs with TLR2 ligands. However, the efficiency of the TLR1/2 or TLR2 or TLR2/6 ligands in eliciting host-protective anti-tumor response differed. The TLR2 ligand peptidoglycan (PGN), when used to stimulate tumor antigen-pulsed DCs, induced complete resistance to repeated tumor challenge. The protection was associated with reduced T-reg cell and Th2 cell numbers and heightened CTL functions. High IL-27, T-bet and IFNγ expression in tumor tissue suggests tumor regression by the infiltrating IFNγ-secreting Th1 cells. Adoptive transfer of T cells from these protected mice confers anti-tumor immunity to naïve mice upon RM-1 challenge. **Thus, this study show that PGN-activated tumor antigens-pulsed DCs enhance host-survival by establishing host-protective, adoptively transferable, robust anti-tumor T cells, as the mice remain resistant to tumor rechallenge, instilling a protocol that removes a major bottleneck in anti-tumor immunotherapy impeding the relapse and tumorigenesis.**

LB029

B19 parvovirus-like particles as a delivery system of tumor antigens to prime cellular and humoral immunity against triple-negative breast cancer

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Triple negative breast cancer (TNBC) continues to be a major health problem which lacks molecular targets for therapy. Tumor-associated antigens (TAAs) and neoepitopes may represent a viable option for the induction of anti-tumor immune responses in TNBC. However, its use as synthetic peptides presents low immunogenicity and induction of tolerance. Virus-like particles (VLPs) of parvovirus B19 may be used as a delivery system of TAAs or neoepitopes to prime cellular and humoral immunity. In this work, we evaluated the prophylactic and therapeutic effect of chimeric B19 VLPs with multiple neoepitopes (m-neoVLPs) or multiple TAAs (m-taaVLPs) in the 4T1 model. Balb/c mice of 6-8 weeks with or without breast tumors received intraperitoneally and peritumorally simultaneous 4 immunizations with m-neoVLPs, mtaaVLPs, native VLPs, vehicle, or VLPs plus adjuvant; and tumor growth, lung macro-metastasis and specific humoral and cellular immune responses were evaluated. Our results showed that m-neoVLPs were able to significantly delay the tumor growth. While administration of mtaaVLPs protects mice of tumor establishment, both chimeric VLPs decreased the number of lung macro-metastasis in mice immunized. induces specific proliferative responses of CD8 and CD4 T lymphocytes towards the neoepitopes in lymphatic nodes, these CD8 T lymphocytes were positive to the production of Granzyme B. Moreover, mtaaVLPs induces high titers of specific epitope

DOI: 10.1002/eji.201970400

antibodies in serum. Our results show that B19-VLPs as a delivery system of tumor antigens can induce specific cellular and humoral responses able to impact tumor establishment, tumor growth, and lung macro-metastasis in vivo in an aggressive TNBC model.

LB030

The Nano-plasmid vector as an alternative for the conventional pcDNA platform for vaccine design

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Background: DNA vaccine is a promising technology that has proven to be effective in inducing both humoral and cellular immune responses. Particularly, inducing the potent cellular responses is crucial for providing protection against intracellular pathogens. To date, several approaches have been studied for improving the efficacy of the plasmid DNA vaccines (pcDNAs), including modifications in the sequence, size and vector design. Nano-plasmids are novel vectors containing minimal prokaryotic backbone, antibiotic-free sucrose based selection and modified CMV promoter.
In this study, the potential advantages of the Nano-plasmid DNA vaccine over the conventional pcDNA have been evaluated.
Methods: The EGFP gene has been cloned into pcDNA3.1+ and NTC9 385-R MCS. Following purification, COS-7 and CHO-K1lines were subjected to transfection with the same concentration (0.5 µg) of cloned vectors using Effectence® transfection reagent. Subsequently, the EGFP expression was investigated by florescent microscope and confirmed by flow cytometry.
Results: Our results showed that, in both cell lines the transfection rate with Nano- plasmid was significantly (~2 times) higher than that of pcDNA. This leads to higher expression of the gene of interest under *in vitro* and *in vivo* conditions which is under investigation.
Conclusion: Because of the small size of the NTC Nano-plasmid (~1700bp), the efficacy of its transfection is higher than pcDNA3.1 (~5400bp). Additionally, in the absence of antibiotic resistance genes, the risk of resistance gene transfer to endogenous microbial flora and antibiotic propagation in the environment could be minimized. Altogether, the Nano-plasmid can be an effective alternative for pcDNA in immunization studies.

LB067

The Cry1Ac protoxin confers antitumor adjuvant effect in the 4T1 breast cancer model by avoid the increase of MDSC and increasing dendritic cells in omentum

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Due to the lack of specific treatments in triple negative breast cancer (TNBC), the immunotherapy could be an attractive strategy for the treatment of patients. The adjuvants have been used in immunotherapy to enhance the cellular and/or humoral response of tumor antigens. The Cry1Ac protoxin is a delta-endotoxin produced by *Bacillus thuringiensis*. It´s a systemic and mucosal adjuvant, able to activate antigen presenting cells by upregulate costimulatory molecules expression and increase the production of pro-inflammatory cytokines. The Cry1Ac toxin is obtained by the proteolysis of Cry1Ac protoxin, despite not being an adjuvant, also has the ability to activate antigen presenting cells. We investigated the antitumor adjuvant effect and the underlying immune mechanisms conferred by the Cry1Ac protoxin and Cry1Ac toxin in a triple negative mouse model of breast cancer. We

DOI: 10.1002/eji.201970400

observed that prophylactic intraperitoneal immunizations with lysates cellular 4T1 + Cry1Ac protoxin as adjuvant protected mice from developing tumors, while Cry1Ac toxin showed a partial antitumor adjuvant effect. The antitumor effect conferred by the Cry1Ac protoxin prevents the decrease of TCD3+, TCD4+ and TCD8+ lymphocytes as well as the increase in MDSC in spleen. Some possible immune mechanisms that explain the antitumor adjuvant effect of Cry1Ac protoxin are the increase in the number of dendritic cells in the omentum after immunization intraperitoneal, the increased proliferation of TCD4+ and TCD8+ lymphocytes in spleen and mesenteric nodule after *in vitro* re-stimulation. These results support the potential use of Cry1Ac protoxin as antitumor adjuvant in TNBC with the capacity to improve cellular responses.

LB068

Targeting IL-10/STAT-3 mediated anti-inflammatory signaling to augment the protective efficacy of rBCG30 vaccine against tuberculosis

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Several recent studies have contributed to our understanding of the need for host directed immunotherapeutic strategies against *Mycobacterium tuberculosis* (*Mtb*), either concomitantly with classical chemotherapy or also as standalone approach. Nevertheless, most of these studies were focused on enhancing chemotherapeutic potential of standard anti-tuberculosis chemotherapy and only few were aimed towards improving prophylactic immunity offered either by BCG or its potential replacements. In the present study we tried improving anti-TB immunity offered by 2nd generation rBCG30 vaccine. We attempted to bolster rBCG30 invoked anti-TB immunity in mice by modulating IL-10/STAT-3 interaction mediated anti-inflammatory program in mononuclear phagocytes. A potent immunomodulator that blocks IL-10/STAT-3 signaling axis was administered in immunized mice to reinforce the anti-pathogen capabilities of mononuclear phagocyte system and thereby, to improve T cell mediated secondary immune responses. We envisage that modulation of this crucial axis may result in reduced expansion of pathogen permissive AAMs/AADCs (Alternatively Activated Monocytes/Macrophages/DCs) and augmented abundance of pro-inflammatory CAMs/CADCs (Classically Activated Monocytes/Macrophages/DCs), the cells that resist establishment of successful *Mtb* infection. The study was also aimed towards generating optimum activated APCs early either after vaccination or infection, which can process and present mycobacterial antigens more efficiently to T cells.

LB103

Prophylactic potential of a recombinant bivalent fusion protein LcrV-HSP70 against Yersiniosis

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Yersiniosis is mainly caused by *Y. pseudotuberculosis* and *Y. enterocolitica*. It is typically a self-limiting disease in humans. The infection is transmitted through soil, water and uncooked animal food. The disease symptoms range from mild diarrhea to mesenteric lymphadenitis, acute gastroenteritis to reactive arthritis in immuno-compromised individuals. In this context, we cloned *lcrV* gene encoding LcrV protein of 37 kDa, a *hsp70* DNA fragment of *M. tuberculosis* encoding heat shock protein domain II of 23 kDa. We also designed a chimeric *lcrV-hsp70* construct and cloned in pET28a vector. All three recombinant constructs i.e, *lcrV, hsp70 and lcrV-hsp70* were expressed in *E. coli* individually. The

DOI: 10.1002/eji.201970400

recombinant proteins *i.e.,* LcrV, HSP70 and a bivalent fusion protein LcrV-HSP70 were purified using Ni-NTA column. The prophylactic potential of bivalent fusion protein LcrV-HSP70 was evaluated against *Y. pseudotuberculosis* and *Y. enterocolitica* in Balb/c mice. The purified antigens were formulated with alum, a human compatible adjuvant and animals were immunized. The humoral and cellular immune response was evaluated in the sera of vaccinated animals. There was significantly high IgG titer in the sera collected from LcrV-HSP70 vaccinated animals. Significant differences were noticed in the titers of IgG isotypes, *i.e.,* IgG1, IgG2a, IgG2b, and IgG3 in anti LcrV-HSP70 sera. A significant difference was noticed in the expression of IFN-γ and TNF-α in the splenocytes of LcrV-HSP70 immunized mice. There was a significant reduction in the CFU count (CFU/ml) in blood and spleen of LcrV-HSP70 immunized mice in comparison to control animals.

LB104

Effect of synthetic short intron sequences on transient gene expression of EGFP in HEK 293T and CHO cell line

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DNA vaccine offers many potential advantages over traditional approaches, including the stimulation of both B- and T-cell responses, improved vaccine stability, the absence of any infectious agent and the relative ease of large-scale manufacture. Recent researches have shown significant improvements in vaccine efficacy against several infectious diseases and immunotherapy of certain carcinomas such as Leukemia. DNA vaccines are usually expression vectors derived from bacteria that containing eukaryotic promoter/virus-derived promoter, transgene, poly A tail, a bacterial origin of replication, and an antibiotic selection marker. The transgene expression can be improved by incorporating expression enhancer sequences such as introns and Kozak sequence and by reducing the overall size of the vector by removing the bacterial DNA from the expression vector like a Micro-Linear vector and MIDGE (minimalistic immunogenically defined gene expression) vector. Our study involves in designing synthetic short intron sequences derived from the 2nd intron of Human β-globin gene and examine the transient expression of the reporter gene with and without Kozak sequence in HEK 293T and CHO cell line. The vector used for this study is pVAX-1. It is a 3.0 kb non-viral mammalian expression vector. It consists of CAG Enhancer sequence with CMV immediate early promoter for high-level expression in a wide range of mammalian cells, BGH Poly A tail for efficient transcription termination and polyadenylation mRNA, Kanamycin resistance gene for selection in *E. coli*, and pBR322 origin of replication. The expression studies using the HEK 293T and CHO cell lines will be discussed in the conference.

DOI: 10.1002/eji.201970400

LB105

Characterization of *Tp53* derived neoantigens as potential vaccine candidates for lung cancer immunotherapy

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Advances in next generation sequencing and bioinformatics have allowed the prediction and the identification of neoantigens that are endowed with exquisite tumor specificity and ability to induce T cell reactivity, which render them promising targets for cancer immunotherapies. Herein, taking into consideration the prevalence of the HLA-A0201 allele, we aimed at identifying a set of HLA-A0201 restricted neoantigens in NSCLC patients and evaluating their immunogenicity and specificity.Results showed the presence of immunogenic neoantigens; derived form *tp53 gene* that are experimentally good binders with high affinity and ability of inducing a T cell response in vitro as compared to their WT counterparts suggesting a higher immunogenicity and specificity. These findings warrant more investigation of the therapeutic applications of these neoantigens aiming at enhancing anti-tumor immunity.

P0480

Immunization with the XCL1/glypican-3 fusion gene generates potent antitumor cellular immunity and enhances the anti-PD1 effect to suspend hepatocellular carcinoma development

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Cancer vaccines could amplify existed anti-tumor responses or prime naïve T cells to elicit effector T cell functions in patients through active immunization. The participation of antigen-specific $CD8^+$ T cells is crucial to reject established tumors. We linked the chemokine XCL1 to tumor antigen glypican-3 (GPC3), which is over-expressed in hepatocellular carcinoma (HCC), and constructed the XCL1-GPC3 fusion molecules. The XCL1-GPC3 expressed products chemoattracted murine $XCR1^+CD8\alpha^+$DCs and human $XCR1^+CD141^+$DCs *in vitro* via XCL1/XCR1 interaction. After XCL1-GPC3 plasmid injection into mice subcutaneously, expressed XCL1-GPC3 protein was mainly detected in $CD8\alpha^+$DCs of the draining lymph nodes. XCL1-GPC3-targeted DCs enhanced antigen-specific $CD8^+$T cell proliferation and induced the *de novo* generation of GPC3-spcific $CD8^+$T cells, which abolished GPC3-expressing tumor cells both in murine system and in human system. In an autochthonous liver cancer murine model induced in AlBlHBV mice, that express hepatitis B envelop proteins persistently in hepatocytes, by administrating 25 mg/kg diethylnitrosamine intraperitoneally,

DOI: 10.1002/eji.201970400

we immunized the mice with *mXCL1-GPC3* plasmid starting at week 6, or at week 14 when the mice had developed liver tumor nodules. **The 3-dose (totally 30 µg) immunization suspended tumor development with significantly reduced tumor incidence and decreased tumor numbers compared with the *GPC3*-immunized mice.** After mXCL1-GPC3 immunization, the infiltration of activated NK and NKT cells also increased in the mouse liver. The anti-tumor effect of *mXCL1-GPC3* plasmid immunization was further enhanced by administration of anti-PD1 antibody. XCL1-GPC3 might be a promising cancer vaccine to compensate the deficiency of the checkpoint blockades to intervene in HCC development.

P0481

Evaluation of immunotherapeutic efficacy of Vaccae vaccine and identification of differentially expressed genes in BALB/c mice infected with *Mycobacterium tuberculosis*

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Tuberculosis (TB) is the leading cause from a single infectious agent and one of the top 10 causes of death. Mycobacterium vaccae (M. vaccae) vaccine is the first preventive TB vaccine in phase III trials besides BCG. However, the differentially expressed (DE) genes of host infected with M. tuberculosis before and after M. vaccae vaccine treatment is still poorly understood. In the present study, the immunotherapeutic efficacy of M. vaccae vaccine were assessed in BALB/c mice, and the DE genes were identified by microarray analysis. **Our results suggested that M. vaccae vaccine had a significant immunotherapeutic effect in mouse model**, and 2,326 upregulated and 2,221 downregulated genes were identified to be DE genes in M. vaccae group compared with control group. Additionally, a total of 123 signaling pathways with significant differences (68 upregulated and 55 downregulated) were identified, and our speculation suggested that **the upregulated and downregulated pathways most related to M. vaccae might be MyD88-dependent TLR signaling pathway and PI3K-Akt signaling pathway**. These findings enhanced our understanding of the mechanism of M. vaccae vaccine immunotherapy, and provided new insights into the development of novel improved TB vaccines.

P0482

Construction of recombinant oncolytic vaccinia virus rVV-CCL5-SSTR2-Luc+ carrying both CCL5 and SSTR2 genes *in vitro* and preliminary verification of its antitumor activity

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Oncolytic virus refers to a genetically engineered virus that can lead to tumor cell lysis. Both *in vivo* and *in vitro* experiments have confirmed the synergistic tumor cells killing effect of oncolytic virus which constructed multiple tumor therapy genes into viral vectors. CCL5 and SSTR2 have powerful anti-tumor effects. This study aims to construct a recombinant oncolytic vaccinia virus with CCL5 and SSTR2 genes *in vitro* and to detect its antitumor activity. Result shows that the recombinant poxvirus eukaryotic expression plasmid pSC65-CCL5-SSTR2-Luc**+** was constructed successfully after homologous recombined with WR poxvirus and 23 rounds of purification; Purified recombinant oncolytic vaccinia virus rVV-CCL5-SSTR2-Luc+ with titer as 9.4×10^8 PFU/ml was obtained after homologous recombination, screening, purification and concentration; ELISA and WB results showed

DOI: 10.1002/eji.201970400

that, after Hela cells were infected with rVV-CCL5-SSTR2-Luc+, the expression levels of CCL5 and SSTR2 protein in Hela cells were significantly higher than those in the control group rVV-Luc**+** (*P*< 0.05 and *P*< 0.01, respectively); The results of CCK8 indicated that the killing activity of rVV-CCL5-SSTR2-Luc+ on two tumor cell lines increased gradually with the infection time. In addition, the killing activity of oncolytic vaccinia virus rVV-CCL5-SSTR2-Luc+ on tumor cells was significantly increased compared with that of the control group rVV-Luc+ (HCT116: 48h after infection **P*< 0.05; Hela: at 48h, **P*< 0.05; at 72h, ***P*< 0.01). A purified recombinant oncolytic vaccinia virus rVV-CCL5-SSTR2-Luc+ was constructed and purified successfully, its tumoricidal activity was verified preliminarily, this work laid the experimental foundation for subsequent *in vivo* and *in vitro* experiments.

P0483

Local mucosal immunization with self-assembled nanofibers elicit robust cellular immune responses in a murine orthotopic model of cervical tumour

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Introduction: Human papillomavirus (HPV) is the causative agent of cervical cancer, the second most common malignant tumour threatening women´s health. The current therapeutic HPV candidate vaccines generally lack significant clinical efficacy. One of the limitations of preclinical assessment for those vaccines was the use of a subcutaneous transplant model of tumour cells. This study employed an orthotopic model of cervical tumour, and thus allowed to put emphasis on evaluating the effects of eliciting effective local antitumor immunity through a mucosal immunization route.
Methods: HPV16 $E7_{44-62}$ peptide was fused to N terminus of a self-assembling peptide Q11, and self-assembled into nanofibers in a salt solution. The orthotopic model was established firstly with TC-1 cells, and then immunization of the nanofibers was performed via a subcutaneous, intranasal or intravaginal route.
Results: Comparing with subcutaneous immunization, mucosal especially local intravaginal immunization produced more significant suppression on tumor growth. The underlying mechanisms might include promoting not only the production but also tumor infiltration of tumor-specific Th1/CTL cells and the high expression of effector molecules IFN-γ and granzyme-B, suppressing Th2-like responses, and reducing the production and tumor infiltration of immunosuppressive Treg cells and MDSCs; in addition, chemokines CXCL9 and CXCL10 was elevated significantly in tumor tissue in locally immunized mice, which may account for the improved migration of effector T cells into tumour.
Conclusion: Local mucosal immunization with self-assembled nanofibers is superior to subcutaneous immunization for eliciting the systemic generation and cervical migration of effector T cells, presenting a promising therapeutic HPV vaccine strategy.

P0484

A universal influenza nanovaccine for "mixing vessel" hosts confers potential ability to block cross-species transmission

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Influenza A viruses (IAV), the Sword of Damocles that hangs over humans and animals, pose a tremendous threat and burden to global health systems. Pigs act as “mixing vessel” hosts to support

DOI: 10.1002/eji.201970400

and generate new pandemic viruses, making IAV difficult to control. Preventing the spread of IAV in pigs effectively can delay or even block cross-species transmission. Porcine circovirus type 2 (PCV2), the most susceptible pathogen of pigs, could promote IAV infection. Virus-like particles (VLPs) are self-assembling protein nanoparticles with excellent immunogenicity, providing platforms for the development of nanovaccines. In this study, the highly conserved ectodomain of influenza matrix protein 2 (M2e) was inserted into the C-terminal of capsid protein (Cap) of PCV2 without burying neutralizing epitopes and self-assembled to form VLPs. Inoculation with Cap-M2e VLPs nanovaccine induced robust M2e- and PCV2-specific humoral and cellular immune responses. The nanovaccine conferred protection against lethal challenges of IAV from different species in mice, and significantly reduced swine influenza virus (SIV) titers in pigs respiratory tract and blocked SIV transmission. These results suggest that the nanovaccine is an inexpensive and promising PCV2 and universal IAV bivalent vaccine, which is more acceptable to farmers. The nanovaccine confers dual protection against IAV and PCV2, which will synergistically and powerfully offer potential ability to block IAV cross-species reassortment and transmission, reducing the pressure on the global health system.

P0485

Alarmin-painted exosomes elicit persistent antitumor immunity in large established tumors in mice

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DC vaccines have good safety profiles and the potential to induce innate and adoptive antitumor immunity for long-term efficacy, thus one of the emerging trends in DC vaccine research based on registered DC clinical trials is to improve the immunogenicity of next-generation DC vaccines. However,treating large established tumors remains a challenge for dendritic cell (DC)-based immunotherapy, resulting partly from the low immunogenicity of activated DCs. High mobility group nucleosome-binding protein1 (HMGN1)can enhance responses to cancer vaccines as a potent adjuvant.However,HMGN1 has to be intracellular and intracellular delivery is inefficient. Here, we describe efficient delivery of HMGN1 to DCs by painting the functional domain of HMGN1(N1ND)on tumor cell-derived exosomes (TEXs)via an exosomal anchor peptide(CP05) identified by our group. N1ND-painted TEX (TEX-N1ND) potentiated DC immunogenicity (DCTEX-N1ND)and elicited long-lasting antitumor immunity in different syngeneic mouse models with large tumor burdens, most notably in large poorly immunogenic orthotopic hepatocellular carcinoma (HCC), which to our knowledge are unresponsive to published DC treatments. TEX-N1ND promotes DC homing to lymphoid tissues and contributed to accelerate the generation of long-lived protective T cell memory response which accounted for the persistent antitumor immunity observed. Importantly, cancer patients' serum exosomes painted with N1ND also augmented DC activation against different cancer cells *in vitro*.
Our study demonstrates that the efficient delivery of HMGN1 to cells will unlock the full therapeutic potential of HMGN1 as an immunoadjuvant and the potency of TEX-N1ND to strengthen DC immunogenicity and to suppress large established tumors, and thus provides an avenue to improve DC-based immunotherapy.

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DOI: 10.1002/eji.201970400

P0486

Plasmodium infection inhibits tumor angiogenesis through effects on tumor-associated macrophages in a murine implanted hepatoma model

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Our previous studies demonstrated that *Plasmodium* infection provides a potent antitumor effect by inducing innate and adaptive immunity and inhibiting angiogenesis via plasma exosomes that contain endogenous functional microRNAs in a murine Lewis lung carcinoma (LLC) model. Here, we report that *Plasmodium* infection efficiently inhibited tumor progression and prolonged survival in tumor-bearing mice in a murine implanted hepatoma model. The inhibition of tumor progression by *Plasmodium* infection is related to the suppression of tumor angiogenesis within the tumor tissue and the decreased infiltration of tumor-associated macrophages (TAMs). Further study demonstrated that TAM-produced matrix metalloprotease 9 (MMP-9) contributed to tumor angiogenesis in the tumor tissue and that the reduced expression of MMP9 in TAMs mediated by parasite infection resulted in the suppression of tumor angiogenesis. A mechanistic study revealed that the *Plasmodium*-derived hemozoin (HZ) that accumulated in TAMs inhibited IGF-1 signaling through the PI3-K and MAPK signaling pathways, which led to decreased expression of MMP-9 in TAMs. Our study suggests that this novel method of inhibiting tumor angiogenesis by *Plasmodium* infection is of high importance for developing new therapies for cancer patients.

P0487

Development of subunit vaccine and rapid detection techniques based on rabies virus G protein

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Rabies is a global acute lethal zoonotic disease which is caused by rabies virus. The disease is common in rural areas of developing countries. It will inevitably lead to the death of humans or animals after the appearance of clinical symptoms. Currently, effective immunization is the best way to prevent rabies. The rabies virus G protein (glycoprotein) is the only protein which can trigger the production of neutralizing antibodies in the body among the five viral structural proteins. Besides, G protein plays an important role in the induction of humoral and cellular immunity. In the present study, we will describe the development of a candidate subunit vaccine and antibody test strips against rabies virus based on the expression of glycoprotein G. Firstly, the protein G was obtained by using the bac-to-bac baculovirus expression system. And then it was identified and analyzed through SDS-PAGE and western blot. After purification, the protein was supplemented with different adjuvants to make the subunit vaccine. The immunogenicity of the candidate vaccine was evaluated in mice, and it could induce high levels of neutralizing antibodies. The difference between this candidate vaccine and the commercial inactivated rabies vaccine (control group) was significant. Furthermore, we used G protein for screening of monoclonal antibodies to develop the antibody test strips. Therefore, this G protein-based candidate subunit vaccine lays a foundation for the development of rabies subunit vaccines and diagnostic reagents. It may be a promising approach for the prevention and diagnosis of rabies.

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DOI: 10.1002/eji.201970400

P0488

The immunogenicity of a novel human papillomavirus type 11 L1 epitope recombinant protein vaccine

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Objective: The currently marketed HPV vaccines use virus-like particles (VLPs) as antigens. But the production system is complicated and the yield is low. The antigenic epitope recombinant protein is easy for production. However, the epitope is difficult to stimulate the immune response, due to the weak immunogenicity. In our study, a novel carrier protein, IMX315, was used to enhance the immunogenicity of HPV epitopes, and then the neutralizing antibody titers induced by the recombinant protein were detected in mice.
Methods: HPV11 L1 epitopes were selected and inserted into IMX315. The IMX315-HPV11 L1 epitope recombinant protein was mixed with aluminum adjuvant for immunization experiments in mice. The IgG antibody response to HPV11 L1 VLP was determined by ELISA.
Results: The DE, FG and HI loops of HPV11 L1 were inserted into IMX315. Fifty C57 mice were randomly divided into 5 groups, and immunized with PBS, IMX315, IMX315-DE loop, IMX315-FG loop and IMX315-HI loop, respectively. After 3 immunizations, serum was collected and the ELISA result indicated that IMX315-DE loop and IMX315-FG loop can effectively stimulate HPV11 L1 VLP-specific antibody responses in mice.
Conclusion: The current study shows that the IMX315-DE loop and IMX315-FG loop fusion proteins are highly immunogenic and are expected to be new vaccine candidates against HPV11.

P0489

Potential therapeutic application of Orf virus strain NA1/11 in non-small cell lung cancer

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Oncolytic viral therapies against cancers, using variously attenuated or recombinant viruses, have appeared as a promising method in cancer treatment in recent years. Being a zoonotic, epitheliotropic, DNA parapoxvirus, orf virus (ORFV) infects principally sheep and goats, can repeatedly infect a host even in the presence of antibody, owing to its identified virulence genes and evasive mechanisms, which contribute to the development of potent immunemodulators in various animal species. The unique host immune-evasion ability of ORFV has made it an important candidate for vaccine vectors and biological agents (as an oncolytic virus). Here we report ORFV strain NA1/11, isolated from a sheep in Jilin province of China, inhibited the growth of non-small cell lung cancer (NSCLC) cells lines including A549, PC9, H1299, H1975 cells in vitro. Moreover, ORFV strain NA1/11 significantly inhibited the growth and the metastasis of NSCLC cells in vivo by inducing an antitumor immune response, which is mediated largely by the macrophage and the potent activation of tumoricidal NK cells. These in vitro and in vivo results showed the clinical potential of the ORFV strain NA1/11.

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DOI: 10.1002/eji.201970400

P0490

Design and preparation of a four-linked multi-epitope CpG 2395-Amastin-CaNA2-KMP-11 vaccine against Leishmania donovani

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Kala-azar is a serious systemic disease caused by *Leishmania donovani*, which currently has no effective vaccine for prevention. This study aims to use bioinformatics tools to guide the design of anti-*Leishmania* vaccines. Bioinformatics tools were used to predict HLA-A2, HLA-A24 and HLA-DR1 epitopes of Amastin, CaNA2 and KMP-11 proteins of *Leishmania donovani*. Combining the secondary and tertiary structures, surface properties and topological structures of the three proteins, some important epitopes were selected and linked with GGGGS linker to form Amastin-CaNA2-KMP-11 multi-epitope protein. CpG ODN 2395 sequence as an adjuvant of nucleic acid vaccine was serialized upstream of Amastin-CaNA2-KMP-11 gene sequence, and a Kozak sequence was inserted between CpG 2395 and Amastin to enhance gene expression. Finally, the four-linked multi-epitope gene sequence of CpG 2395-Amastin-CaNA2-Kmp-11 was designed. The gene sequence was synthesized and cloned for construction of recombinant eukaryotic plasmid pCMV-CpG-ACK. The recombinant plasmid was transferred into NIH3T3 cells to verify its transient expression and stable expression. The sequencing results showed that the recombinant plasmid pCMV-CpG-ACK was successfully constructed. The results of immunofluorescence, qPCR and Western blot suggested that the plasmid could be successfully expressed in NIH3T3 cells. Our future work is to prepare DNA vaccine by encapsulating pCMV-CpG-ACK plasmid with liposome, and to immunize mice to explore the immune effect of the vaccine.

P0491

Toll-like receptor agonist rMBP-NAP enhances antitumor cytokines production and CTL activity of peripheral blood mononuclear cells from patients with lung cancer

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Toll-like receptor (TLR) agonists are known for their ability to inhibit tumor progression via enhancing antitumor cytokines production and cytotoxic T lympho-cyte (CTL) activity. Recombinant Helicobacter pylori neutrophil-activating protein fused with maltose-binding protein (rMBP-NAP) has been reported as a novel TLR agonist for antitumor treatment in murine models. The present study aimed to determine the potential and efficacy of the rMBP-NAP for antitumor treatment prior to further clinical trials. The rMBP-NAP was expressed and purified for subsequent experiments. Peripheral blood mononuclear cells (PBMCs) from health donors and patients with lung cancer (LC) were incubated with PBS and 0.2 mg/ml rMBP-NAP. Antitumor cytokines production was assayed using ELISA and reverse transcription-quantitative polymerase chain reaction analysis. The cytolytic activity of PBMCs and the number of Interferon-γ (IFN-γ)-secreting cells were assayed using lactate dehydrogenase and Enzyme-linked ImmunoSpot assays, respectively. The results from the present study revealed that the expression of IFN-γ, interleukin (IL)-2, tumor necrosis factor-α and IL-12 of PBMCs from patients with LC and healthy donors were significantly increased following treat-ment with rMBP-NAP ($P< 0.05$). Additionally, rMBP-NAPsignificantly upregulated the number of IFN-γ-secreting cells in PBMCs and prominently increased the cytotoxic activity of PBMCs ($P< 0.05$). Furthermore, the expression of TLR2 was significantly enhanced following rMBP-NAP stimula-tion ($P< 0.05$), which indicated that rMBP-NAP may serve an antitumor role via TLR2 signaling pathways. Overall, these results demonstrated

DOI: 10.1002/eji.201970400

that rMBP-NAP possesses the potential to be a novel immunomodulatory candidate drug and requires further evaluation in clinical trials.

P0492

Heterologous human/rat HER2-specific exosome-targeted T cell vaccine stimulates potent humoral and CTL responses leading to enhanced circumvention of HER2 tolerance in double transgenic HLA-A2/HER2 mice

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DNA vaccines composed of heterologous human HER2 and rat neu sequences induce stronger antibody response and protective antitumor immunity than either HER2 or neu DNA vaccines in transgenic mice. We previously developed HER2-specific exosome-targeted T-cell vaccine HER2-T_{EXO} capable of stimulating HER2-specific $CD8^+$ T-cell responses, but only leading to partial protective immunity in double transgenic HLA-A2/HER2 mice with self-immune tolerance to HER2. Here, we constructed an adenoviral vector AdV_{HuRt} expressing HuRt fusion protein composed of NH_2-HER_{21-407}(Hu) and COOH-$neu_{408-690}$(Rt) fragments, and developed a heterologous human/rat HER2-specific exosome-targeted T-cell vaccine HuRt-T_{EXO} using polyclonal CD4+ T-cells uptaking exosomes released by AdV_{HuRt}-transfected dendritic cells. We found that the HuRt-T_{EXO} vaccine stimulates enhanced $CD4^+$ T-cell responses leading to increased induction of HER2-specific antibody (~70 µg/ml) compared to that (~40 µg/ml) triggered by the homologous HER2-T_{EXO} vaccine. By using PE-H-$2K^d$/$HER2_{23-71}$ tetramer, we determined that $HuRtT_{EXO}$ stimulates stronger HER2-specific CD8+ T-cell responses eradicating 90% of HER2-specific target cells, while HER2-T_{EXO}-induced $CD8^+$ T-cell responses only eliminating 53% targets. Furthermore, $HuRtT_{EXO}$, but not HER2-T_{EXO} vaccination, is capable of suppressing early stage-established HER2-expressing $4T1_{HER2}$ breast cancer in its lung metastasis or subcutaneous form in BALB/c mice, and of completely protecting transgenic HLA-A2/HER2 mice from growth of HLA-A2/HER2-expressing BL6-$10_{A2/HER2}$ melanoma. HuRt-T_{EXO}-stimulated HER2-specific $CD8^+$ T-cells not only are cytolytic to trastuzumab-resistant HLA-A2/HER2-expressing BT474/A2 breast tumor cells in vitro but also eradicates pre-established BT474/A2 tumors in athymic nude mice. Therefore, our novel heterologous human/rat HER2-specific T-cell vaccine HuRt-T_{EXO}, circumventing HER2 tolerance, may provide a new therapeutic alternative for patients with trastuzumab-resistant $HER2^+$ breast tumor.

P0493

The optimized fusion protein HA1-2-FliCΔD2D3 promotes mixed Th1/Th2 immune responses to influenza H7N9 with low induction of systemic proinflammatory cytokines in mice

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H7N9 influenza virus has unusually high fatality rate, approximately 40%, and a safe and effective vaccine is urgently needed. Flagellin, TLR5 agonist, has been deemed as a potent adjuvant candidate. However, its high antigenicity and potential for causing inflammatory injury might restrict its clinical application. Previously, we demonstrated that HA1-2-FliC, comprising the hemagglutinin globular head protein (HA1-2) of H7N9 influenza virus and the full-length *Salmonella typhimurium* flagellin protein (FliC), had high efficiency against H7N9 in mouse and chicken models. Here, we

DOI: 10.1002/eji.201970400

constructed an improved fusion protein, HA1-2-FliC△D2D3, with HA1-2 fused to the FliC△D2D3 (lacking the hypervariable-region domains D2 and D3 of FliC). HA1-2-FliC△D2D3 exhibited efficient immunoreactivity and TLR5 agonist efficacy, and promoted innate immune-response activation in mouse macrophages, peripheral blood mononuclear cells, and splenocytes, based on cytokine- and chemokine-expression profiles. Mice immunized with HA1-2-FliCΔD2D3 showed significantly lower systemic inflammatory responses (compared with HA1-2-FliC) and highly reduced flagellin-specific antibody production, without affecting HA1-2-specific antibody production and cellular immune responses. Enhanced IFN-γ/IL-4 generation suggested that HA1-2-FliCΔD2D3 maintained balanced Th1/Th2 immune responses. Furthermore, virus challenge was performed in a chicken model. The results showed that chickens receiving FliCΔD2D3 adjuvant vaccine induced high levels of serum neutralizing antibodies, and exhibited a significant reduction of viral loads in throat and cloaca compared to chickens receiving only HA1-2. In conclusion, we constructed the H7N9 influenza subunit vaccine candidate HA1-2-FliCΔD2D3, with reduced immunogenicity against FliC and lower adverse events. The improved adjuvant FliCΔD2D3 can potentially help in developing safe and effective universal protein-based influenza vaccines for humans.

P0494

Heat-shock conditioned tumor lysates as immunogenic sources for cancer vaccines

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Clinical strategies using immune-checkpoint blockers, such as anti-CTLA4, (Ipilimumab) or anti-PD1 antibodies (Nivolumab), have recently demonstrated durable survival benefits in patients with melanoma and other tumors. Nevertheless, an important percentage of treated patients remain refractory, suggesting that combination with other kind of active immunization is required for increasing responses rate. In this context, cancer vaccines become again a complementary alternative. The optimal delivery of antigens (Ags) and the use of adequate adjuvants are crucial for vaccine success. Here, a prototype of a generic therapeutic vaccine for the treatment of malignant melanoma named TRIMELVax™ was tested in an experimental model. This vaccine is based on heat-shock conditioned melanoma allogeneic tumor lysates combined with specific adjuvants. The vaccine is intended to activate immune responses against tumor *in vivo*, inhibiting its growth. TRIMELVax was evaluated, in terms of safety and efficacy in C57Bl/6 murine model. In short, immunocompetent mice was vaccinated with three doses of TRIMELVax, and then challenged with B16 murine melanoma cells. Alternatively, immunization was tested therapeutically in tumor bearing mice. Our results showed that only TRIMELVax was capable to reduce the occurrence of tumor. In contrast the use of non-conditioned tumor lysate, or adjuvant alone did not impact tumor growth. Observed response was associated with CD8+ T cells intratumoral accumulation and antibody production in sera. Moreover, anti-PD1 therapy was strongly potentiated by combination with TRIMELVax in immunocompetent mice, which encourage testing of the combined treatment in future clinical trials.
Financed by grants FONDECYT 1171213; FONDEF ID16I10148 and MIII P09/016-F.

DOI: 10.1002/eji.201970400

P0495

Defective BCG performance against tuberculosis challenge in mice of the B10.M (*H2*^{f}) strain and immune response to mycobacterial antigens

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Tuberculosis (TB) pathogenesis is multistage, highly complex sequence of events involving many types of cells. Earlier our laboratory demonstrated that a high-dose vaccination with bacillus Calmette-Guerin (BCG) before *Mycobacterium tuberculosis* H37Rv challenge significantly prolonged survival times of genetically resistant and susceptible inbred mice, except the strain B10.M (*H-2*^{f}). In the present study we applied a more relevant TB model based upon aerosol infection of B10.M and *H2*-congenic B10 (*H-2*b) control mice, which readily respond to BCG vaccination after intravenous challenge. It was shown that spleen and lung T cells of B10.M mice are defective for IFN-γ production in response to prolonged stimulation with mycobacterial antigens during chronic infection. However, immunologic recognition of these antigens was unaltered (proliferative response), as was the capacity of secrete IFN-γ after non-specific signaling via T cell receptor (anti-CD3 response). We demonstrate that lung T cells from infected B10.M mice produce significantly lower amounts of IFN-γ in response to mycobacterial antigens compared to B10 cells, and that this phenotype does not depend upon BCG vaccination. These results suggest that immune response in infected lungs of B10.M mice may be less Th1-biased. However, further research is needed to answer the question whether “fail-of-vaccination” phenotype of B10.M mice is due to abnormal memory T cell development or exhaustion of IFN-γ-producing T cell pool, perhaps, because of activation-induced cell death.
Animal House of the CIT is supported by the Federal Program AAAA-A16-116032560061-9. This study was supported by the RSF grant 18-45-04015.

P0496

Immunomodulatory nanoparticle as a novel platform for HIV-1 vaccine development

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A major challenge for the design of an effective HIV vaccine is the high rate of mutation of human immunodeficiency virus-1 (HIV-1). The discovery of broadly neutralizing antibodies (bNAbs) that bind conserved sites on the viral surface envelope protein trimer (Env) and neutralize diverse clades of viruses provides new insight into immunogen design. VRC01 is one of the most potent bNAbs and neutralizes 90% of circulating viral isolates. The mature form (mVRC01) arises from its germline reverted form (gVRC01) through the process of antibody affinity maturation, and the goal of current vaccine immunogen design is to activate B cells expressing gVRC01. In this study, we aim to test protein nanoparticles as a vaccine delivery vehicle for native-like HIV envelope proteins (Bg505 SOSIP.664 Env) in combination with co-stimulation of the B cell co-receptor, CD19 using the anti-CD19 monoclonal antibody. Cross-linking of CD19 and mVRC01 synergistically enhanced B cell signaling using Bg505 SOSIP.664 Env -anti-CD19- nanoparticles; however, activation of gVRC01 was poor. Neither increasing the size of the nanoparticle nor the density of incorporated molecule in the context of nanoparticles improved activation of gVRC01. Incorporating anti-CD19 along with the newly engineered germline targeting Env, however, synergistically enhanced activation signal in both mVRC01 and gVRC01 expressing B cells. This result is promising as increased B cell repertoire expanded through such germline targeting Env and anti-CD19 crosslinking could potentially engage

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DOI: 10.1002/eji.201970400

gVRC01 B cells of low affinity BCR towards Env. A sequential immunization strategy utilizing such immunogen design may favour bNAbs production.

P0498

Development of Pan-filovirus T cell vaccines against Ebola and Marburg virus challenges

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The 2013-2016 epidemic of Ebola virus disease (EVD) in West Africa and the 2018-2019 EVD outbreak in the Democratic Republic of Congo, have raised awareness of ebolaviruses as pathogens of global relevance. Unfortunately, currently available experimental vaccines and immunotherapeutics, such as rVSV-ZEBOV and ZMapp™, while efficacious against EBOV by design, have no demonstrable activity against the related pathogenic filoviruses, Sudan virus (SUDV), Bundibugyo virus (BDBV) and Marburg virus(MARV). In contrast, T-cell vaccines offer an opportunity to design a single pan-filovirus vaccine protecting against all members of the Filoviridae family. In this study FILOcepX vaccines were constructed targeting the four most conserved regions among the viral proteomes with the aim to induce protective T-cell responses against different filoviruses. BALB/c and C57BL/6 mice were immunized with FILOcep 1 and 2 vaccines vectored by non-replicating engineered simian adenovirus and poxvirus MVA. High frequency T cells specific responses were detected in mice receiving the test vaccines by IFN-γ ELISPOT kits. All the FILOcepX vaccinated mice maintained their normal weight and survived after either mouse adapted EBOV or MARV-angola challenge, while all the mice in the control groups succumbed to death. These FILOcepX vaccines provided 100% protection against the lethal challenges with filoviruses of two different genera. This suggests a possibility that our T-cell vaccine also may protect against other known as well as yet unencountered viruses of the filovirus family.

P0499

Development of a generalizable DNA-launched nanoparticle platform to enhance adaptive immunity of DNA vaccines

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Nanotechnologies are considered to be of growing importance to the vaccine field. Through decoration of immunogens in multivalent fashions, designed nanovaccines are reported to elicit improved humoral immunity in several animal models. However, challenges in large-scale production of nanovaccines have impeded their clinical translation. Here, we describe a new platform to use synthetic DNA and adaptive electroporation for *in vivo* production of a prototypical nanovaccine eOD-GT8-60mer. We demonstrated DNA-launched nanoparticles spontaneously self-assemble *in vivo* in a multimeric form to enhance humoral responses in a dose-sparing fashion. We discovered that the nanoparticle scaffold domain from lumazine synthase provides robust CD4-help in two different mice models; and the CD4 help is necessary but not sufficient for the observed immunological phenotypes as knockout of the epitope partially decreased observed humoral and cellular responses. Comparisons of protein-

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DOI: 10.1002/eji.201970400

based and DNA-launched GT8-60mer nanovaccines reveal that while both routes of vaccination induce similar levels of humoral responses, DNA-launched nanovaccines uniquely induce CD8 T-cell responses, potentially because DNA-launched nanoparticle immunogens can be taken up by antigen presenting cells through phagocytosis of apoptotic cells that carry the immunogens in a manner independent of the complement opsonization and uptake. Finally, the platform is generalizable for *in vivo* production of alternative GT8 and influenza hemagglutinin-based nanovaccines to induce promising immunological phenotypes and may have broader impact for the treatment of cancer and other infectious diseases.

P1074

Colorectal cancer stem cells: an effective vaccine for colorectal cancer

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The morbidity and mortality of colorectal cancer (CRC) have increased in the China. Several approaches have suggested that the immunization with CRC vaccine is useful in eliciting antitumor immunity. However, validation of the clinical trial is limited due to CRC vaccine's weak immunogenicity. In the study, we developed the CRC stem cell (CCSC) vaccine to evaluate its efficacy of inhibiting CRC growth. $CD133^+$CCSCs were isolated from human LOVO, SW620, HCT116, and mouse CT26 cell lines respectively by the magnetic-activated cell sorting system. Mice were subcutaneously immunized with the freezing and thawing whole CCSC, followed by a challenge with LOVO, SW620, HCT116, and CT26 cells respectively at 7 days after final vaccination. The results showed the CRC growth was significantly inhibited in the xenogeneic or syngeneic mice immunized with the CCSC vaccines compared with the non-CCSC vaccines. The CCSC vaccines markedly increased the cytotoxic activities of NK and spleen cells, serum IFN-γ, and anti-MUC1 levels in the vaccinated mice. Furthermore, the efficacy against CRC was decreased or increased while the MUC1 expression was down or up regulated by shRNA or Lenti-MUC1 in CT26 CCSC vaccine, which was reflected in decreasing or increasing the $CD8^+$CTL activity, IFN-γ level, and changing the subset of CCSC and the aldehyde dehydrogenase 1 positive cells in the CRC tissues. The findings suggest the $CD133^+$CCSC vaccines with high MUC1 expression were highly effective in eliciting immunity against CRC in mouse model, and it may act as a valid vaccine for immunotherapy of CRC.

P1075

Synthesis and functional studies of multi-component anti-breast tumor vaccines based on HER2-derived CH401peptide

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Multi-component tumor vaccines containing tumor associated antigens and immune adjuvants can enhance specific immune responses to target antigens and overcome the immune tolerance, and are potential alternative therapies for cancer treatment. Previous studies proved that HER2 derived CH401 peptide (YQDTILWKDIFHKNNQLALT) contains B and T cell epitopes and can induce specific IgG antibody production against HER2-overexpressed tumor-bearing mice, which indicated CH401 peptide was a promising epitope to be used in the design of novel cancer vaccines[1-2]. Toll-like receptor ligands (TLRs), such as Pam_2CSK_4 (TLR2/6 ligands) and Pam_3CSK_4 (TLR2/1 ligands), are strong immunostimulators and have great potential for using as adjuvants [3]. It has reported that TLRs and Nod-like receptor ligands can regulate the release of cytokines in a synergistic manner. However,

DOI: 10.1002/eji.201970400

design and syntheses of tumor vaccines containing mono- or double- adjuvants (nature ligands) and evaluating the differences of *in vivo* antitumor activity have not been investigated. Therefore, the purpose of this study is investigating the *in vivo* antitumor activity of synthesized tumor vaccines to select the most effective vaccine molecule as well as exploring the changes of related gene and protein expression in the immune response pathway and understanding the immune response mechanisms of CH401-based multi-component tumor vaccines, which could provide important guidance for the further optimization of vaccine design.

References

1. T. Ishida, A. Yachi et al. (1994) *Jpn J Cancer Res.*, **85**, 172-178.
2. H. Miyako, Y. Tokuda et al. (2011) *Anticancer research*, **31**, 3361-3368.
3. R. Spohn, K. H. Wiesmuller et al. (2004) *Vaccine*, **22**, 2494-2499.

P1076

Synergized immunotherapy via simultaneous DC-mobilization and DC-targeted gene silencing of IDO using a novel man-GNR-siIDO nanosystem

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Purpose: Dendritic cell (DC)-mediated immunotherapy is an emerging strategy in anti-cancer therapy but, unfortunately, the clinical outcome has been disappointing, largely due to immune regulatory molecules such as indoleamine 2,3-oxygenase (IDO) that attenuates DC-mediated immunotherapy. We previously reported that knockdown of IDO using siRNA can reinstall anti-tumor immunity. However, a combinatorial strategy for mobilizing DCs and targeting them specifically with a siRNA delivery system in vivo remains to be developed.

Experimental Design: We developed a novel DC-targeted siRNA delivery system man-GNR-siIDO using gold nanorods (GNR) as a nanocarrier of siRNA specific for IDO (siIDO) and mannose (man) as a guide molecule for targeting DCs. We explored its efficacy in DCs mobilized by the cytokine Flt3-L for lung cancer immunotherapy.

Results: In vivo DC-targeted gene silencing of IDO by the novel man-GNR-siIDO nanocarrier resulted in robust IDO silencing with a higher DC-specificity. Combinatorial treatment with Flt3-L increased the DC populations and also enhanced tumor-specific $CD4^+$ T cell response and tumor killing by the cytotoxic $CD8^+$ T cells on Lewis lung cancer (LLC) cells. There was also a significant decrease in apoptosis in both $CD4^+$ and $CD8^+$ T cells in the spleen and lymph nodes. More importantly, man-GNR-siIDO treatment reduced LLC tumors, with a combinatorial treatment with Flt3-L achieving 9-fold reductions of tumors.

Conclusions: This study provides proof-of-principle of the man-GNR-siIDO nanosystem that targets mobilized DCs with specificity and achieves immunotherapy of lung cancer in vivo, highlighting a clinical potential for anti-cancer immunotherapy through simultaneous DC-mobilization and DC-targeted IDO gene silencing.

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DOI: 10.1002/eji.201970400

P1077

A CD19-targeting fusion protein combined with PD1 antibody, enhances antitumor immunity in mice models

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Immunotherapy has become the latest clinical therapeutic intervention in breast cancer treatment. In our previous studies, we have designed the B cell vaccine (scFv-HER2) and demonstrated that targeting tumor-associated Ag HER2 to B cells via anti-CD19 single chain variable fragment (scFv) could elicit anti-tumor immune response to inhibit the growth of breast cancer cells, however, the tumor was not eradicated by the immune system which indicated that there are still some tumor cells evading from immune surveillance, moreover, the mechanism of anti-tumor response is not very clear. In this study, we found that the B cell vaccine promoted the expression of PD1 (programmed death protein 1) and PDL1 (programmed death protein ligand 1) in the tumor microenvironment, although it could enhance the infiltration of CD8+T cells in tumor tissues. Therefore, we assessed whether targeting tumor-associated Ag HER2-D4 (scFv-D4) to B cell combined with PD1 antibody was more effective than either monotherapy. Our results revealed that combination therapy enhanced antitumor immunity, as evidenced by the increased expression of, augmented tumor-infiltrating CD4+T cells, CD8+ T cells, and reduced MDSC cells and B cells. Meanwhile, we also found that the number of IFN-g+ andgranzymeB+CD8+T cells in tumor-infiltrating lymphocytes and draining lymph nodes of tumor tissues was significantly increased in the combination therapy group. Moreover, the tumor size of the tumor-bearing mice in the combination therapy group was significantly reduced. Thus, our study established the combined treatment of novel B cell vaccine and PD1 antibody can significantly inhibit the growth of tumor cells.

P1078

A replication-deficient recombinant H9N2 influenza virus carrying H5 Hemagglutinin conferred protection against infection with H9N2 and H5N1 viruses in mice

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H5N1 and H9N2 avian influenza viruses (AIVs) consistently bring threat to public health. H9N2 AIVs are also the internal gene donor of H7N9 subtypes which caused several hundred deaths since 2013 in China. At present, vaccination is still the most important and effective measure to prevent infection by influenza viruses, however, the inactivated influenza vaccine showed low antigenicity resulting in low immune efficacy. The traditional live attenuated influenza vaccine (LAIV) is mainly the low temperature adapted strain, which has the risk of virulence reverting mutation. In this study, we generated a replication-deficient recombinant H9N2 influenza virus in which NA gene segment was replaced by H5N1 HA gene segment. This recombinant H9N2 influenza virus propagated well in the MDCK cells expressing NA of H9N2 influenza virus. Due to the lack of structural protein NA, the recombinant H9N2 influenza viruses is replication deficient in vitro and in vivo. In mice, intranasal

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DOI: 10.1002/eji.201970400

vaccination with the recombinant H9N2 influenza viruses provided immune protection against infection by H5N1 and H9N2 viruses.

P1079

Enhanced anti-melanoma efficacy of a Pim-3-targeting bifunctional shRNA via ssRNA-mediated activation of pDCs

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Background: The immunosuppressive tumor microenvironment and aberrant expression of some proto-oncogenes are the main cause of melanoma development. We investigated the therapeutic effects of a ssRNA-Pim-3-shRNA dual-function vector on melanoma.
Methods: Apoptosis and apoptosis-related molecules was examined. A subcutaneously B16F10 melanoma-bearing mouse model was established with C57BL/6 mice. The percentages and activation of immune cells in tumor sites and spleen were analyzed
Results: Therapy with ssRNA-Pim-3-shRNA dual-function vector not only promotes apoptosis and inhibits the proliferation of B16F10 melanoma cells by inhibiting Pim-3 expression, but also enhances activation of NK cells and $CD8^+$ T cells and simultaneously reduces the proportion of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. Together, these features effectively inhibit the growth of melanoma. Intriguingly, the bifunctional therapeutic effect that reverses the tumor immunosuppressive microenvironment is dependent on the activation of plasmacytoid dendritic cells (pDCs) and the production of a large amount of type I IFN.
Conclusions: The Pim-3-targeting bifunctional vector effectively inhibits the growth of melanoma via both shRNA-mediated Pim-3 silencing and ssRNA-mediated pDC activation. This therapy is expected to become a promising strategy for the treatment of melanoma and other solid tumors.

P1080

Study on differential expression proteins of HPV16 infection-related cervical cancer

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Objective: To explore the protein expression profile of all proteins in the cervical epithelial cells of the host cervix from the initial infection of HPV16 to cervical cancer and screen out the differentially expressed proteins between each group.
Methods: TMT (Tandem Mass Tag) combined with LC-MS/MS technology was applied to accurately identify and quantify all proteins in cervical exfoliated cells of HPV16 positive cervical cancer, HPV16 positive cervical normal and HPV negative cervical normal and search database. Based on the screened credible proteins, the FC(fold change) value and difference significance p-value of different groups were calculated by t-test test.
Results: 1. compared the HPV16(+)- cervical cancer with the HPV16(+)-cervical normal, A total of 276 differentially expressed proteins were identified, including 157 up-regulated proteins and 119 down-regulated proteins.
2. 351 differentially expressed proteins were identified in the HPV16(+)-cervical cancer and the HPV (-)-cervical normal, including 247 upregulated proteins and 104 down-regulated proteins.
3. Compared with the healthy control group, PRDX2 and G6PD protein levels were significantly up-regulate.
Conclusion: 1. With the progression of the disease,the more serious the protein expression

imbalance, the more the number of proteins expressed in the difference, indicating that the occurrence and development of HPV16 infection-related cervical cancer is closely related to the protein expression disorder.
2. The up-regulation or down-regulation of the protein expression level of these differential proteins may be closely related to the tumorigenesis of HPV16, and may promote the occurrence and development of tumors.
Keywords: TMT-LC-MS/MS; Proteomics; Cervical cancer

P1081

Enhanced efficacy of MUC1 mRNA nanovaccine against triple negative breast cancer by the combination treatment with anti-CTLA-4 monoclonal antibody

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The treatment of patients with triple negative breast cancer (TNBC) remains a great clinical challenge. The therapeutic induction of systemic anti-tumor immunity is a promising approach. In this study, a previously-constructed MUC1 mRNA nanovaccine targeting dendritic cells in lymph node was combined with anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) monoclonal antibody to enhance the immune response of the vaccine against TNBC through improvement of T cell function. *In vivo* studies demonstrated that therapeutic effects of the vaccine on TNBC in mice were greatly enhanced by additional treatment with anti-CTLA-4 monoclonal antibody, and that combination therapy could significantly increase the number of tumor-infiltrating $CD8^+$ T cells and reduced the levels of WBCs inside the normal range compared to treatment with either MUC1 vaccine or anti-CTLA-4 monoclonal antibody. An *in vivo* CTL assay and an IFN-γ production assay showed that mice treated with the vaccine and anti-CTLA-4 monoclonal antibody could induce the stronger CTL response and generate higher level of IFN-γ than those with single treatment. Taken together, the enhanced efficacy of the nanovaccine is associated with an increased anti-tumor cytotoxic T-lymphocyte activity by the additional immunization with anti-CTLA-4 monoclonal antibody.
Keywords: combination immunotherapy, CTLA-4 blockade, mRNA nanovaccine, triple negative breast cancer

P1082

Immunogenicity and protective efficacy of a subunit vaccine consisting of multi-stage antigens against *Mycobacterium tuberculosis* infection

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Objective: To construct a fusion protein SHR of subunit vaccine containing multi-stage antigens, and to evaluate the immunogenicity and protective efficacy of SHR.
Methods: *Rv0577*(replicating), *Rv2626*(dormant) and *RPFB*(resusciting) were selected and applied to construct SHR, WBIA was performed to detect the antigen-specific levels of IFN-γ from Chinese *M.tb* infections and non-infections. C57BL/6 mice were immunized with SHR/DMT. Nine weeks later, SHR-specific antibody titers (IgG, IgG1, IgG2a), the levels of Th1 cytokines by splenic lymphocytes and the

DOI: 10.1002/eji.201970400

mRNA levels from the lungs were detected. Four weeks after challenge by *M.tb* H37Rv, protective efficacy was evaluated.
Results: Recombinant plasmid pET30b-SHR and subgroups were constructed and expressed successfully, which stimulate statistically higher levels of IFN-γ in *M.tb* infected Chinese subjects than in healthy donors. SHR/DMT induced statistically higher levels of IgG, IgG2a and IgG1, as well as specific IFN-γ, TNF-α, IL-2 from splenic lymphocytes, the mRNA levels of IFN-γ, TNF-α and iNOS from the lung. The ratio of IgG2a/IgG1 indicated that SHR/DMT mainly induced the Th1-type cell-mediated immune response. Moreover, SHR/DMT resulted in the significant reduction of bacterial load in the lungs and spleens, as well as lessened inflammation in alveolar tissue.
Conclusion: Based on the predominant antigens expressed during multi stages which could be recognized by T cells of *M.tb* infections from China, a subunit vaccine SHR/DMT was constructed. SHR/DMT could provide significant protective efficacy against acute infection of *M.tb*, which is mainly attributed to the SHR-specific $CD4^+$ Th1-type cell immune response.
Keywords: *Mycobacterium tuberculosis*; Subunit vaccine; BCG; Immunogenicity; Protective efficacy

P1083

Intranasal administration of vaccines platform based on ca influenza virus elicit potent antibody and robust effector memory CD8+ T cells against respiratory syncytial virus

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Respiratory syncytial virus (RSV) is a world wide public health concern for which no vaccine is available. Cold-adapted attenuated influenza virus vector is a promising delivery vehicle for vaccine strategies that aim to elicit strong mucosal immunity. Here, we developed a series of cold-adapted attenuated influenza viruses, carrying 3-fold epitopes from RSV fusion (F) protein in the backbone of the ca influenza vaccine neuraminidase (NA) gene and hemagglutinin (HA) gene , using reverse genetics. Rg RSV-Flu ca viruses exhibited cold-adapted (ca) phenotype. Moreover, we immunized the adult and the aged BALB/c mice via the intranasal route and evaluated the HRSV-specific antibody and T cell response at different points. We found that intranasal vaccination generated potent HRSV-specific antibody for viral clearance in the adult and the aged mice respectively. Surprisingly , it induced robust and durable lung tissue-resident effector and effector memory CD8+T cell for interferon gamma secretion in the aged mice, but not the adult. Furthermore, the humoral, potent mucosal and durable cellular immune responses protected against subsequent RSV challenges, as determined by a significant decrease in viral titers and a noticeable alleviation of histopathological alterations in the lung tissue of challenged mice. Collectively, these findings validate the novel vaccines that emphasizes durable lung-tissue mucosal antibody and T cells response immune as key determinants of immune protection in the adult and the aged mice.

P1084

Oncolytic Herpes Simplex Virus can effectively make pancreatic cancer 'hot'

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DOI: 10.1002/eji.201970400

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The pancreatic tumor is characterized as immunologically 'cold' tumor due to high interstitial pressure, dense desmoplastic stroma, fibroblasts, and immunosuppressive cells in the tumor environment. Oncolytic viruses such as oncolytic Herpes Simplex Virus can not only kill tumor cells but can also evoke antitumor immune response.
In past, the antitumor effect of oncolytic virus on pancreatic cancer has been shown in an immunocompromised xenograft mouse model, which might not recapitulate clinical scenario. Therefore, we have established a syngeneic mouse model for pancreatic cancer to evaluate the tumor immune response elicited by oncolytic virus. Intra-tumoral treatment with HSV-1 derived oncolytic virus LMI-oV-001 in our syngeneic mouse model exhibited significant inhibitory effect on tumor growth. Further, immunoprofiling of tumor infiltrated immune cells by 10-color Flow Cytometry indicated that oncolytic virus LMI-oV-001 treatment dramatically increased $CD45^+$ leukocyte infiltration, increased numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, reduced the Tregs and $CD8^+PD1^+$ T cells, and stimulated M1-like macrophage polarization. At last, single cell transcriptomics analysis of tumor infiltrating cells provided valuable insights of immune landscape in cancers treated with oncolytic viruses.
Our immune competent mouse model of pancreatic cancer allowed for the first time to evaluate the immune response induced upon oncolytic virus treatment. This *in vivo* model will allow us to explore combination of oncolytic virus with checkpoint blockade immunotherapies and design next generation oncolytic virus.

P1085

Construction and immunogenicity analysis of multi-stage antigen fusion protein vaccine containing *Mycobacterium tuberculosis*

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Objective: To design and construct the fusion protein SHR3 to detect whether SHR3 and its sub-component proteins can be recognized by peripheral blood T lymphocytes in *M.tb*-infected patients. C57BL/6 mice were immunized with SHR3/DMT to evaluate their immunogenicity.
Methods: 1.Select Rv1626, Rv2866 and Rv1884 three target antigens to construct the recombinant plasmid of SHR3 and its sub-component proteins; 2.Use WBIA technology to detect SHR3 and its sub-component proteins to induce peripheral blood of *M.tb* infected and control subjects. IFN-γ level, and the total number of $CD4^+$ and $CD8^+$ cells of IFN-$γ^+$ and IL-2^+ single and double positive were detected by flow cytometry; 3.Preparation of subunit fusion vaccine SHR3/DMT, detection of antibody titer and levels such as IL-4 and IFN-γ.
Results: 1.Successfully constructed and expressed the fusion protein SHR3; 2.SHR3 and its sub-component proteins stimulated the level of IFN-γ produced by *M.tb* infection, and the number of induced $CD4^+$ T and $CD8^+$ T cells were significantly higher than the control group. SHR3/DMT induced high levels of IgG and its subclasses; BCG-SHR3/DMT mice induced the highest levels of IL-2, IFN-γ, and TNF-α were significantly higher than the PBS and DMT groups. ($P< 0.05$).
Conclusion: The fusion protein SHR3 constructed in this study can induce high levels of IL-2 and IFN-γ in peripheral blood lymphocytes and mice infected with *M.tb*, which is a basis for further evaluate the protective effect of SHR3 against *M.tb* infection.
Keywords: *Mycobacterium tuberculosis*; Fusion protein; SHR3; Immunogenicity

DOI: 10.1002/eji.201970400

P1086

Combining nanovaccine and radiotherapy to revert the immunosuppressive environment in tumor

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Generation of tumor-specific T cells is critically important for cancer immunotherapy. However, T cells alone are not adequate to eliminate well-established tumors due to the immune suppressive environment. We had previously reported a STING-activating nanovaccine by a simple physical mixture of an antigen with a polymeric nanoparticle, PC7A NP, which generated robust T cell response with low systemic cytokine expression. Increasing evidences showed that RT (radiation therapy) can augment adaptive T cell responses to tumors, thereby decreasing immunosuppression to mediate tumor eradication. But based on clinical evidence, RT alone is unlikely to induce or sustain an immune response that is therapeutically useful.
Here we demonstrate that combination of PC7A nanovaccine with local RT leads to a synergistic response with long-term regression of large established tumors in two mouse tumor models. The percentage of CD8+ T cells increased significantly in primary tumors after combination therapy. Mechanistically, the augmented T cell responses of radiotherapy and nanovaccine is STING pathway dependent. Furthermore, nanovaccine synergizes with radiotherapy to achieve a better therapeutic effect in distal tumors. These findings suggest that combination of local radiotherapy with systemic PC7A nanovaccine offers a useful strategy to improve the therapeutic outcome of late stage solid cancers.

P1087

Dengue and Zika virus domain III-flagellin fusion and glycan-masking E antigen for a prime-boost vaccine design

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The viral E proteins of dengue virus (DENV) and Zika virus (ZIKV) are the major viral proteins involved in receptor binding and fusion, and for the induction of protective antibodies against viral infections. E protein consists of the domain I, the domain II, and the domain III (DIII). DIII is an independent domain and stretches out on the virion surface that can elicit type-specific neutralizing antibodies and has been used for vaccine development. Prime-boost immunizations provide an advantage by more effectively recalling DIII antigens to elicit more potent serotype-specific neutralizing antibodies after DIII booster immunizations. Here, we reported on the use of prime-boost immunization strategies by the second-dose booster of four DENV serotype FliC-DIII proteins, but not ZIKV FliC-DIII proteins, significantly enhanced the neutralizing antibody titers in sera and protection against viral infections. Since the development of safe and effective vaccines is needed to abolish the elicitation of cross-reactive antibodies between DENV and ZIKV, we constructed the ij loop mutations on ZIKV and DENV2 E antigens using glycan-masking strategies to abolish the elicitation of cross-reactive antibodies for ADE activity. The results indicated that the glycan-masking mutation on the ZIKV E protein ij loop, but not on the DENV2 E protein ij loop, resulted in reducing the elicitation of cross-reactive antibodies for DENV and ZIKV infection enhancements. Our results may provide useful information for DENV and ZIKV vaccine development.

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DOI: 10.1002/eji.201970400

P1088

Evaluation of a novel fusion vaccine for Staphylococcus aureus

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Staphylococcus aureus is a major human pathogen and a notorious causative agent of opportunistic skin and soft tissue infections. Since 1995 the global incidence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) cases has been on the rise, with children, elderly, and immunocompromised individuals being the most susceptible to CA-MRSA. As a component of the microflora in 20-30% of the population and a pervasive member of hospital-associated pathogens, *S. aureus* is responsible for 20% of post-surgical infections often with high fatality. The decline in development of new antibiotics and rapid emergence of multi-drug resistance prompted WHO to list *S. aureus* as a pathogen for which high priority research is much needed. We have evidence that vaccination with a fusion construct could alleviate disease morbidity and enhance clearance of infection. Wild-type and attenuated proteins were expressed as two fusion vaccine constructs and evaluated with three formulations of adjuvants in an immunization study. Elevated humoral responses were found in vaccinated mice with one adjuvant in particular boosting T-cell proliferation and stimulating higher levels of IgG2a. Sera from mice vaccinated with attenuated and wildtype fusion proteins showed comparable immunogenicity and neutralizing responses. Antibodies from vaccinated mice showed a 30-50% neutralizing efficiency. Vaccinated mice will be challenged systemically with *S. aureus* to evaluate the fusion vaccine as a novel prophylactic for *S. aureus*. With the dearth of new antibiotics, prophylaxis in the form of vaccination is essential to curtail the rise of resistant strains and fill an unmet need for an effective solution to staphylococcal infection.

P1089

Use of viral proteins produced in insect expression system as vaccine candidates

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One of the greatest contributions science has made is the vaccine development, which enabled saving millions of lives globally. Traditional inactivated or live-attenuated vaccines have resulted in tremendous success in control and reduction of infectious diseases. Nevertheless, many pathogens specially viruses are still a dreadful source of ailments and cannot be dealt with traditional vaccine strategies. Fortunately, recent advances in the field of molecular biology, biochemistry, immunology, cell culture and virology have paved the way for new vaccine development approaches using viruses as a diverse source of protein antigens to target viruses for which no interventions currently exist. Viral proteins are good candidates as protein antigens since they are likely to be unique, hence they lower the possible issues with cross-reactivity of antibodies and prove excellent modulator of immune system. Using knowledge from the role of individual viral proteins, it is possible to map the function and domains within viral proteins and predict their structures, which can aid in devising a rational vaccine design. For many viruses, viral genes have been cloned, expressed and purified in a variety of expression system for vaccine development. Recently, utilization of Baculoviruses based insect expression system has widely gained acclaim as a platform to prepare antigen or subunit vaccines. CERVARIX® (Herpes Papilloma Virus), Porcilis® PCV (Porcine Circovirus) and FLUBLOK (Influenza Virus) are example of FDA approved viral vaccines produced in Baculoviruses based insect expression system. Future work in this field can help developing novel vaccines to address new emerging viral threats.

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DOI: 10.1002/eji.201970400

P1090

Th1 priming by vaccine dendritic cells depends on reprogramming of endogenous tolerogenic migratory XCR1⁺ dendritic cells towards IL-12 production

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Success of dendritic cell (DC) vaccines relies on the quality of antigen presentation, co-stimulation, lymph node migration, and the release of IL-12, in case of Th1 priming. Here, we provide evidence for a cooperation between the injected vaccine DCs with endogenous migratory DCs for Th1 induction. While migration of the injected DCs is essential for antigen delivery to the lymph node, they contribute only partially to Th0 priming and are unable instruct Th1 generation. Instead, our data indicate that the endogenous skin-derived migratory XCR1⁺ DCs undergo reprogramming from tolerogenic into immunogenic bystander DCs in the lymph node. The transcriptome of the bystander DCs shows a down-regulation of Treg and Th2/Th9 inducing genes and self-MHC molecules, and up-regulation of genes required for Th1 instruction, including IL-12p35 and p40. The kinetic of interactions in the draining lymph nodes appear step-wise as i) injected DCs with cognate T cells, ii) injected DCs with bystander DCs, and iii) bystander DCs with T cells. Together, these data show that injected mature lymph node-migratory DCs direct T cell priming and bystander DC activation, but not Th1 polarization which is mediated by endogenous IL-12p70⁺ XCR1⁺ bystander DCs. Our results are of importance for clinical DC-based vaccinations against tumors where endogenous DCs may be functionally impaired by chemotherapy.

P1091

Interleukin-15 in cancer immunotherapy: IL-15 receptor complex versus soluble IL-15 in a cancer cell-delivered murine leukemia model

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Cancer immunotherapy endeavors to stimulate the immune system to recognize, reject and destroy cancer cells. Previously we have demonstrated that IL-12 is an ideal candidate for leukemia immunotherapy. A Phase-1 human trial, underway at the Princess Margaret Cancer Center, is currently testing this approach in AML patients. In order to further our knowledge on immunomodulators we have expanded our studies to IL-15. IL-15, either in its soluble form (IL-15sol) or complexed with IL-15Ralpha (IL-15Rc), has been shown to exhibit potent anti-tumor-activities in various cancer studies.
In this study we describe the impact of intraperitoneal IL-15 in a cancer cell-delivered IL-15 immunotherapy approach using the 70Z/3-L leukemia mouse model. Whereas both forms of IL-15 led to significantly improved survival rates compared to the parent line, there were striking differences in the extent of the improved survival: mice receiving IL-15sol-secreting leukemia cells showed near 100% survival for up to 250 days, whereas mice receiving IL-15Rc-secreting leukemia cells started to perish around day 50.
Interestingly, cell-secreted IL-15sol and IL-15Rc activated different cell types, leading to different immune responses. IL-15Rc resulted in an expansion of intraperitoneal NK1.1⁺ cells, while injection of cells secreting IL-15sol led to a heightened expansion of CD4⁺ and CD8⁺ T-cells. Furthermore, IL-15Rc resulted in elevated PD-1-expression on T-cells, suggesting T-cell exhaustion.

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DOI: 10.1002/eji.201970400

To our knowledge, this is the first study detailing a significantly different biological effect of cell-delivered IL-15Rc vs IL-15sol in a mouse cancer immunotherapy study, suggesting a different mechanism of action of the two IL-15 forms.

P1092

Evaluating the impact of hepatitis B vaccination among young children in the Kassena Nankana district of Ghana

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Background: Ghana introduced the mass immunization program against hepatitis B infection in children in 2002 in her Expanded Programme on Immunization (EPI). Active immunization is the single most important and effective preventive measure against HBV infection. This study evaluated seroconversion and seroprotection status of children under this mass immunization program and measured their antibody levels five years after immunization.
Materials and method: 104 archived plasma samples of children between the ages of 1-10 years who have completed the EPI were retrieved and screened for HBsAg. Those found to be HBsAg-seronegative were stratified into three groups according to their age at which the last vaccine was administered. Their anti-HBsAg titer levels were estimated by enzyme linked immunosorbant assay (ELISA).
Results: The anti-HBs titers recorded ranged from 1.021 IU/L to 751.64 IU/L indicating a 100% seroconversion rate. In group one (0-6 months), 87.9%were seroprotected. Group two (2-3yrs) had 78.3% seroprotection and group three (3-5yrs) had 41.7% seroprotection. There was no significant difference between group 1 and 2. However, there was a significant difference between group 1 and 3 ($p = 0.0137$) and between group 2 and 3 ($p = 0.0390$) respectively. There was no significant difference between male and female children.
Conclusion: Significant seroversion was observed among all the children who received doses of hepatitis B vaccine at 6, 10 and 14 weeks in the immunization program, but their levels of protection decreased with increasing years. Booster doses are therefore recommended after 5 years.

P1671

Antitumor activities with a recombinant endoglin-MIP3a/Fc fusion protein as a model antigen

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Endoglin is a powerful marker of tumor angiogenesis,and MIP3α is the most important specific chemokine could effectively improve the antigens presenting ability of dendritic cells. In this study, we evaluated the anti-tumor activities with a recombinant endoglin-MIP3a/Fc fusion protein by intratumoral injection in two mouse cancer model. Remarkably, the endoglin-MIP3a/Fc fusion protein resulted in not only significant anti-angiogenic effects related-endoglin but also additional promotion of tumor cell apoptosis and inhibition of tumor cell proliferation in the treated tumor tissues. Moreover, the peripheral dendritic cells migrate into tumor site by intratumoral injection of the endoglin-MIP3a/Fc fusion protein, which can effectively induce specific antitumor immune response. **These findings**

DOI: 10.1002/eji.201970400

support that the endoglin-MIP3a/Fc fusion protein as a model antigen suppressed tumor growth may be to the synergistically inhibition tumor angiogenesis targeting endoglin and cell proliferation, promotion of tumor cell apoptosis and antitumor immune responses induce by MIP3a.

This work was financially supported by Hainan Provincial Key Research and Development Program (ZDYF2017165) and the Project of Hainan Province Innovative Team (2017CXTD008).

P1672

Immunotherapeutic effects of a novel vaccine (DNA-protein nanoparticle) on breast cancer via enhancing dendritic cell activation in mice

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It has always being a crucial impediment of cancer therapeutic vaccine, “how to break out of the immune tolerance for the tumor?” Single virus-based DNA vaccine or subunit antigen vaccine, could induce limited effective anti-tumor immunity.
However, cationic combination DNA with protein as a whole vaccine, whether or not, can generate more effectiveness, remains elusive. In the present study, we evaluated the functions of this novel therapeutic DNA-protein vaccine for breast cancer in mice. Firstly, DNA-protein vaccine was generated and identified as nanoparticles in vitro, as we had described previously. Secondly, this nanoparticle was administrated with intramuscular injection in mice. Finally, we evaluated the effectiveness of this novel vaccine. Results showed vaccination of this novel nanoparticle reduced tumor burden ($p< 0.05$), compared with either single DNA or protein vaccine. After vaccination, the expression levels of CD80, CD83, CD86 and MHC-Ⅱ of DC cells in blood and spleen were respectively elevated. GM-CSF and IL-12 in sera and culture supernatants of sorted DCs were significantly increased ($p< 0.05$). Meanwhile, vaccinated group showed higher sera IFN-γand IL-4 concentration, indicating both Th1-induced cellular immune response, and Th2-induced humoral immunity were activated by DC presentation and stimulation.
In conclusion, this novel nano-particle vaccine may serve as a high-performance treatment for anti-tumor immunotherapy, which may be mediated by DCs through presenting antigen peptide and activating CD4+ T lymphocytes.

P1673

Poly I:C-based rHBVvac therapeutic vaccine eliminates HBV via generation of HBV-specific $CD8^+$ effector memory T cells

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Objective: Chronic hepatitis B (CHB) virus infection is a global health problem. Finding a cure for CHB remains a challenging task.
Design: In this study, poly I:C was employed as an adjuvant for HBV therapeutic vaccine (referred to as pHBV-vaccine) and the feasibility and efficiency of pHBV-vaccine in CHB treatment were evaluated

DOI: 10.1002/eji.201970400

in HBV-carrier mice.
Results: We found that pHBV-vaccine decreased HBsAg and HBV DNA efficiently and safely in HBV-carrier mice. Further investigation showed that pHBV-vaccine promoted maturation and antigen presentation ability of dendritic cells *in vivo* and *in vitro*. This vaccine successfully restored the exhaustion of antigen-specific $CD8^+$ T cells, and partly broke the immune tolerance established in HBV-carrier mice. pHBV-vaccine also enhanced the proliferation and polyfunctionality of HBV-specific $CD11a^{hi}$ $CD8a^{lo}$ cells. Importantly, we observed that T cell activation molecule KLRG1 was only expressed on HBV specific $CD11a^{hi}$ $CD8a^{lo}$ cells. Furthermore, pHBV-vaccine reduced the expression of Eomes and increased the serum IL-12 levels, which in turn promoted the generation of effector memory short-lived effector cells (SLECs) to exhibit a critical role in HBV clearance. SLECs induced by pHBV-vaccine might play a crucial role in protecting from HBV reinfection.
Conclusions: Findings from this study provide a new basis for the development of therapeutic pHBV-vaccine, which might be a potential candidate for clinical CHB therapy.

P1674

Exploring precise prophylactic strategy of adult tuberculosis

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BCG vaccination can effectively prevent tuberculosis (TB) in infants and young children, while the protection period lasts only for 10-15 years. Currently, adolescents and adults are the leading population affected by TB epidemic and death. Developing an effective vaccine against adult TB is urgently needed. Adult TB is primarily caused by endogenous reactivation of latent TB infection (LTBI) secondarily by exogenous contagion or reinfection. BCG prime-heterologous boost strategy has been explored extensively to protect adults against primary TB infection, but the majority of experimental regimens have not improved the protection primed by the BCG vaccine. We assume that BCG prime-heterologous boost regimens have differential efficacy to prevent against LTBI or primary infection. Firstly, subunit vaccine CMFO in adjuvant of DMT was confirmed to provide a similar protection to that of BCG in the *M. tuberculosis* primary infection model. Further, BCG prime-boosted with CMFO-DMT provided more effective protection against both LTBI and reactivation in mice rather than with BCG, or other subunit vaccines such as A1D4-DMT and CTT3H-DMT. Finally, CTT3H-based heterologous subunit, DNA, and recombinant adenovirus boosters also could not enhance the protection conferred by the BCG vaccine alone against primary infection. Interestingly, these boosters provided stronger protection against LTBI than BCG alone. Therefore, BCG prime-boost strategy might be a promising measure for the prevention against LTBI rather than primary infection, and the efficacy could be affected by both antigen compositions of vaccines and the ability of vaccine-eliciting IFN-γ^+ T_{EM} and IL-2^+ T_{CM} cells in the lung.

DOI: 10.1002/eji.201970400

P1675

Sustained control of a highly pathogenic simian-human immunodeficiency virus infection by PD1-based DNA vaccine-induced CD8$^+$ T cells

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Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), has been a major health challenge globally. Developing effective vaccines remains one of the best strategies for controlling the HIV/AIDS epidemic. HIV-1-specific CD8$^+$ T lymphocytes are essential for suppressing viral replication, yet few vaccine candidates could elicit such cellular immunity. In this current study, a new program death-1 (PD1)-based DNA vaccine was developed and examined in rhesus macaques.
We designed a recombinant DNA vaccine that targets the simian immunodeficiency virus (SIV) capsid antigen to dendritic cells (DC) via a fused soluble rhesus PD1 domain. Homologous PD1-based DNA vaccination suppressed setpoint viremia to undetectable levels in all rhesus macaques tested following high-dose intravenous challenge with the highly pathogenic simian-human immunodeficiency virus $SHIV_{SF162P3}$. The vaccine induced high frequencies of long-tasting poly-functional effector-memory CD8$^+$ T cells, which were re-called potently upon viral challenge. A distinctive transcriptomic profile was detected in the CD8$^+$ T cells from the vaccinated macaques, suggesting the PD1-based vaccine strategy engages a unique mechanism for CD8$^+$ T cell priming. All vaccinated macaques under long-term observation showed sustained viremia control for 2 years. Depleting CD8$^+$ T cells resulted in transient viremia, highlighting the involvement of vaccine-induced CD8$^+$ T cells in $SHIV_{SF162P3}$ control. In summary, the PD1-based DNA vaccination shows potential as a straightforward approach towards a functional SIV/HIV cure. This strategy could be rapidly developed for human studies.

P1676

The cross activity of influenza mAb 9114 mutants targeting to different HA epitopes that is evaluated by Fluorescence lifetime imaging (FLIM)-FRET method

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We previously found 9114 show lower affinity and neutralizing activity for H5N1, H3N2, H7N9 strains than H1N1.Therefore, we modified the 9114H/mL, 9114mH/L, and 9114mH/mL by mutating partial acid-amino acid and tyrosine points in the WT9114H/L mAb in order to improve 9114's capacibility for crossing neutralization. Assessing the anibody-antigen interaction is important for predicting mAbs' binding activity. For analyzing such binding properties, Fluorescence lifetime imaging (FLIM)-FRET to detect the antibody-antigen interaction within the single cell was applied to screen the experimental 9114 mAb candidate possessed with high affinity. With FRET-type labeling, different fluorescence properties of labeled different HA1 and HA2 epitopes and modified-9114 mAbs are made visible.We can measure the proximity between HA1/HA2 epitope and influenza mAbs by calculating the FRET efficiency, revealing the binding kinetics of anibody-antigen interaction. Compared with WT9114H/L

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DOI: 10.1002/eji.201970400

9114H/mL, and 9114mH/L mAbs, The FRET efficiency between the 9114mH/mL mAb and HA2 epitope from different influenza strains were higher, demonstrating that 9114mH/mL had a better affinity for HA2 and possibly posessed the higher cross activity.We also verified the results in the Biacorre assay.The presented results indicate that 9114mH/mL had the better cross activity than WT 9114H/L, and our method using FLIM-FRET labeling could be useful in evaluating the binding properties of anibody-antigen within the cell.

P1677

CD83 is a new potential biomarker and therapeutic target for lymphoma

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Recent advances have significantly increased the overall survival of patients suffering from lymphoma. Despite these advances there remains a significant number of patients who relapse or are refractory to current treatment regimes. CD83 is a member of the Ig superfamily that is expressed as both membrane (mCD83) and soluble molecule (sCD83). CD83 plays a key role in the development of the immunosuppressive tumour microenvironment by reducing T cell proliferation and increasing their PD-1 expression. Our group first reported mCD83 expression on the Hodgkin and Reed-Sternberg cells of Hodgkin´s lymphoma (HL). High levels of sCD83 were detected in HL patient sera and these returned to normal in patients who had good clinical responses to chemotherapy confirmed by positron emission tomography scans. We generated a human anti-human CD83 antibody, 3C12C, which we conjugated to the toxin monomethyl auristatin E (MMAE) to produce 3C12C-MMAE. Both the unaltered antibody and its MMAE conjugate killed Hodgkin lymphoma cell lines but not CD83 negative cells. The native 3C12C antibody was tested in dose escalation studies of non-human primates. No toxicity was observed but there was evidence of *in vivo* efficacy with depletion of CD83 positive cells. These findings establish CD83 as potential biomarker and therapeutic target in HL.

P1678

Identification of neoantigen candidates in bladder cancer reveals negative correlation between antigen-specific immunoreactivity and inflammation

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Introduction and Objective: Bladder cancer is one of the most common malignancies in the urinary system. Neoantigen-based immunotherapy is one of the hotspots in the field of immunotherapy against cancer.In this study,we intended to identify mutant peptides with neoantigen property in bladder cancer.Correlations between the immunoreactivity of candidate neoantigens and clinical manifestations were further analyzed.

DOI: 10.1002/eji.201970400

Methods: HLA-A2 restricted mutant peptides (MT) and wild type (WT) counterparts were selected based on data from the TCGA database.Peptides with high affinity to HLA-A2 molecules which determined by T2 cell-based assay were subjected to the determination of immunoreactivity in bladder cancer patients by Elispot assay upon *in vitro* stimulation. Clinical relevance to immune responses was analyzed.
Results: 57 paired peptides that predicted from the TCGA database subjected to T2 cells based validation.Among them,12 MT peptides from 12 individual genes exhibited higher and stronger affinity to HLA-A2 molecules.Importantly,it was shown that bladder cancer patients exhibited higher responsiveness to MT peptides than to WT peptides.The clinical association analysis revealed that MT peptide specific T cell responsiveness was negatively correlated to C-reactive protein and thrombocytocrit. WT peptide-specific T cell responses,was more related to platelet count and thrombocytocrit.
Conclusion: The results illustrate that MT peptides can induce stronger immune reactivity than WT peptides in Chinese bladder cancer patients,making them neoantigen candidates for future immunotherapy against bladder cancer.The peptide-specific responses might be influenced by the inflammatory environment according to our study,providing potential strategy to enhance the efficacy of neoantigen-based vaccination against bladder cancer.

P1679

Immunogenicity investigation on recombinant BCG containing NY-ESO-1 upon vaccination

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Objectives: Bladder cancer is one of the most common malignant tumors in the urinary system. Intravesical instillation of BCG has been proved to reduce the recurrence of bladder cancer and prevents the progression of cancer.However,repetitive irrigation of large-dose BCG leads to various complications.In this study,we have introduced NY-ESO-1,a well-known tumor antigen, into BCG to construct recombinant BCG stains (rBCG::NY-ESO-1).The immunogenicities of the rBCG strains were evaluated in mouse vaccination model.
Methods: Human-derived *NY-ESO-1* cDNA was amplified and inserted into the shuttle expression vector pMV261.The recombinant plasmids pMV261-NY-ESO-1 were transformed into BCG by electroporation.The recombinant BCG strains were screened and the expression of NY-ESO-1 was verified by Western blot.The C57BL/6 mice were immunized by subcutaneous injection with rBCG and BCG at a dose of 5×10^6 CFU per 100μl.Immunological profiles were evaluated at 4 weeks by ELISPOT and flow cytometry.
Results: BCG vaccination induced comparable PPD-specific responses and less NY-ESO-1-specific responses compare to rBCG.In addition,rBCG vaccinated mice displayed more effect $CD8^+$ T cells and NY-ESO-1-specific $IFNg^+CD8^+$ T cells.Meanwhile,both NY-ESO-1 and PPD-specific IL-10 producing $CD8^+$T cells were decreased in rBCG vaccinated mice when compared to BCG vaccinated group.Mice vaccinated with rBCG showed less frequencies of Treg at 4 weeks post vaccination.
Conclusions: Vaccination of rBCG containing NY-ESO-1 can induce both PPD and NY-ESO-1 specific cellular responses where NY-ESO-1 specific CD8 T cells exhibit more functional effects.Introduction of NY-ESO-1 into BCG thus orchestrates NY-ESO-1-specific immune responses and immune modulator of BCG,which might increase the efficacy of intravesical instillation of BCG against bladder cancer.

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DOI: 10.1002/eji.201970400

P1680

Identification of antigenic epitopes of EGFR for lung cancer vaccine development

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It has been documented that approximately 85% of the patients with lung cancer are non-small cell lung cancer (NSCLC). The mutation rate of human epidermal growth factor receptor (EGFR) gene is about >60% in NSCLC patients. The mutation loci of EGFR are mostly concentrated in the regions of exons 18-21. In addition, it has been reported that EGFR exon 19 T790M mutation is responsible for drug resistance of more than 90% of NSCLC patients to tyrosine kinase inhibitors. Thus, EGFR is a good candidate for developing non-small cell lung cancer vaccines. In the current study, 40 peptides with 10 amino acids from EGFR protein were designed and synthesized for the prediction of T cell antigenic epitopes. Reverse immunology method was employed to screen T cell antigenic epitopes from 40 EGFR peptides. Three unique peptides of EGFR were identified to be T cell antigenic epitopes due to their ability in the stimulation of cell expansion and cytokine release. Further studies *in vivo* showed that three unique peptides of EGFR could stimulate anti-lung cancer immune responses in both wild-type mice and NUDE mouse tumor model. The clinical trials of three unique peptides of EGFR as NSCLC cancer vaccines are under way. Therefore, three unique peptides of EGFR may be used as cancer vaccines in the treatment of patients with NSCLC. (Supported by “Taishan Scholar” special fund (No. tshw20120718) from Shandong Government, China)

P1681

Incorporation of additional HIV env nanoparticle immunogen into sequential inoculation regimen enhances cross-reactive neutralization antibody immune responses

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An effective vaccine eliciting broadly neutralizing antibodies (bNAbs) against human immunodeficiency virus type 1 (HIV-1) remains elusive despite of almost thirty-year efforts. We have been testing a sequential vaccination strategy with heterologous HIV-1 envelop glycoprotein (Env) immunogens to raise bNAbs since last fifteen years. In this study, we first observed that the neutralizing antibodies (nAbs) induced by the heterologous gp140 and gp145 immunogens immunization neutralized all 8 tested HIV-1 B' and B'/C primary isolates in the guinea pig model. However, bNAbs in response to HIV-1 B, C and A/E recombinant clades panel were significantly decreased in nonhuman primates (NHPs). To further improve bNAbs responses, we employed a prime-boost regimen consisting of three different Env immunogens including DNA gp145, recombinant Tiantan vaccinia virus (rTV) gp145 and alum-adjuvanted native-like SOSIP.664 trimers on ferritin nanoparticles, and then tested in Chinese Rhesus macaques. The data should that bNAbs could be further enhanced after the incorporation of the nanoparticle immunogens. These observations suggested that a sequential vaccination strategy by incorporation of additional immunogen is able to further enhance the bNAbs responses, and enlighten the vaccine development.

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DOI: 10.1002/eji.201970400

P1682

Expression, significance and immunogenicity of reactivation-associated Mycobacterium marinum genes

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Tuberculosis remains a major health problem. Annually 10 million people are infected with *Mycobacterium tuberculosis*. Besides the active tuberculosis infection, it is estimated that 1.7 billion people have a latent infection and thus carry a 5-10 % lifetime risk of developing active disease. The current vaccine, Bacillus Calmette-Guérin, is inefficient and new immunization approaches are needed.
In this study, the fish pathogen *Mycobacterium marinum*, which is a close relative to *M. tuberculosis*, was used. Dormant *M. marinum* cultures were reactivated and samples were collected for mRNA sequencing at different time points. In total, 379 differentially expressed mycobacterial genes were identified from the mRNA sequencing data. Several genes were upregulated in reactivation; 27 genes when compared to dormancy, 165 genes compared to logarithmic growth and 7 genes compared to both dormancy and logarithmic growth. These seven genes were subjected to further studies. We have produced three mutant bacteria strains to investigate the significance of these mutated genes for growth *in vitro* and for pathogenicity *in vivo* in zebrafish (*Danio rerio*).
This study provides unique information about reactivation related mycobacterial genes and reveals new insight into reactivation mechanisms. Characterized genes are potential targets for vaccine and drug development against human tuberculosis.

P1683

Long-term immunogenicity and immune persistence of live attenuated and inactivated hepatitis A vaccines

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Background: Both live attenuated (HA-L) and inactivated (HA-I) hepatitis A vaccine were licensed by the CFDA for routine use in China. Although phase 1, 2 and 3 clinical studies of both vaccine have been completed, further systematic evaluation of their immunogenicity and immunological persistence under phase 4 clinical studies in a wide range of conditions and involving large populations is necessary.
Methods: A randomized, double-blind, parallel controlled phase IV clinical trial (NCT02601040) was performed in 9000 participants over 18 months of age in counties in Jiangsu Province, China. Geometric mean concentrations (GMCs) and seroconversion rates (SRs) were compared at 5 time points during 3 years.
Results: The GMCs and SRs were 986.28 mIU/mL(95% CI:846.98-1148.50 mIU/mL) and 98.08% (95% CI: 95.59-99.38%) 28 days after administration of HA-L. For HA-I, the values were 903.76mIU/mL(95% CI:800.56-1020.26 mIU/mL) and 99.64% (95% CI: 98.93-100.00%) 28 days after the first dose, 2571.04mIU/mL(95% CI:2248.23-2940.21 mIU/mL) and 100.00%(95% CI: 98.83-

DOI: 10.1002/eji.201970400

100.00%) after the booster dose. Three years after administration, the GMC and SR values reached 5319.93 mIU/mL(95% CI:4542.40-6230.56 mIU/mL) and 99.62%(95% CI: 97.92-99.99%) for HA-L, and 7707.92 mIU/mL(95% CI:6944.29-8555.52 mIU/mL) and 100.00%(95% CI: 98.75-100.00%) for HA-I. The GMCs between HA-I and HA-L showed no statistically significant differences(p>0.05) when only one dose of vaccine had been administered. SRs between HA-I and HA-L showed statistically significant differences(p< 0.01) only at 28 days after full vaccination course.
Conclusions: Both HA-I and HA-L vaccines could provide an excellent long-term protective effect, and supported the routine use of both vaccines.

P1684

Sequential vaccination with heterologous HIV env immunogens in different vaccine forms elicits broad cross-reactive neutralization antibody responses

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Broadly neutralizing antibodies (bNAbs) play a key role in protection from human immunodeficiency virus type 1(HIV-1) infection, but an effective vaccine that elicits bNAbshas not been achieved. Our previous exploration observed that a sequential vaccination with heterologous HIV Env is capable of elicit bNAbs in mice and guinea pig models, here we further attempted to optimize the sequential vaccination by employing different vaccine forms containing HIV-1 envelop glycoprotein (Env) immunogens derived from varied clades. We first identified that two vaccination sequences are optimal to raise bNAbs against tier 1 and tier 2 HIV viruses in mice model as “DNA AE gp145 - recombinant Tiantan vaccinia virus (rTV) AE gp145 - alum-adjuvanted native-like SOSIP.664 trimers on ferritin nanoparticles -recombinant Tiantan vaccinia virus (rTV) RL gp145” and “DNA AE gp145 - recombinant Tiantan vaccinia virus (rTV) AE gp145 - recombinant Tiantan vaccinia virus (rTV) RL gp145 - alum-adjuvanted native-like SOSIP.664 trimers on ferritin nanoparticles”, Importantly, rTV gp145 as a booster is critical to enhance the bNAbs titers. We then further tested them in non-human primate model of Chinese Rhesus macaques and vaccines were delivered sequentially with a 4-week interval. Interestingly, nAbs responses against tiers 2 HIV viruses were reproducibly induced. These data suggested that a sequential vaccination with heterologous HIV Env is able to elicit bNAbs against HIV, further incorporation of different vaccine forms could profoundly improve the bNAbs, this strategy is worth to be further explored in an HIV vaccine development pipelines towards clinical trials.

P1685

Production and characterisation of the first human monoclonal antibodies against *Plasmodium falciparum* transmission blocking antigen Pfs25

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Transmission-blocking vaccines (TBVs) targeting the sexual-stages of the malaria parasite are viewed as essential to achieve elimination and eradication of malaria. The *Plasmodium falciparum* Pfs25 protein (Pfs25) is the most clinically advanced TBV candidate antigen, important for ookinete

DOI: 10.1002/eji.201970400

maturation and survival in the mosquito midgut and capable of inducing transmission-blocking antibodies in animal models. Monoclonal antibodies (mAbs) can be used to inform immunogen design in the next generation of vaccines and potentially developed into therapeutic mAbs for transmission prevention of *Plasmodium falciparum* malaria. Due to the low level of Pfs25-specific B cell responses, an efficient high-throughput process is required for screening antigen-specificity of human mAbs (humAbs) generated. Here a method using Transcriptionally Active PCR to incorporate human immunoglobulin variable regions into linear expression cassettes for screening by ELISA was performed. Thirteen new anti-Pfs25 humAbs were isolated from antigen-specific B cells of volunteers vaccinated with Pfs25 encoded by viral vectors in our latest Phase Ia clinical trial, marking them the first reported anti-Pfs25 humAbs. Variable region DNA was sequenced and purified and humAbs were characterised by blocking ELISA. When the functional activity of the generated anti-Pfs25 IgG was tested using standard membrane feeding assay, tree of thirteen Pfs25-specific humAbs isolated, demonstrated significant reduction in transmission-reducing activity. Data will be presented to show the binding regions of isolated humAbs on Pfs25 and to correlate the location of their epitopes with their transmission-blocking activity which potentially can provide critical insights to guide the design of malaria transmission-blocking antibodies.

P1686

Application of CRISPR/Cas9-mediated editing of avian herpesvirus vectors for high efficient generation of recombinant vaccines

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Herpesvirus of turkeys (HVT) is successfully used as live vaccine against Marek´s disease (MD) worldwide for more than 40 years either alone or in combination with other serotypes. HVT is also widely used as a vector platform for generation of recombinant vaccines against a number of avian diseases such as infectious bursal disease (IBD) and avian influenza (AI) using conventional recombination methods or recombineering tools on cloned viral genomes. The CRISPR (clustered regularly interspaced short palindromic repeat)-Cas9 system can be used to edit genomic DNA with high precision and efficiency. CRISPR/Cas9-coupled NHEJ repair is a valuable homology-independent pathway for efficient knock-in of exogenous DNA. In the present study, we describe the application of CRISPR/Cas9-based genome editing as a rapid and efficient method of generating HVT recombinants harbouring VP2 gene of infectious bursal disease virus (IBDV). This approach offers an efficient method to introduce other viral antigen into HVT genome for rapid development of recombinant vaccines.

P1687

Regulation of programmed death-1 ligand (PD-L1) by oncolytic Newcastle disease virus (NDV) in non-small cell lung carcinoma (A549) and normal skin fibroblast (NB1RGB) cell lines

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The mechanisms of NDV oncolysis are through the direct killing and induction of antitumor immune responses. NDV induces the production of IFN type I and type III which give redundant stimulation of apoptosis in tumor cells. It has been reported that NDV treatment in combination with immune

DOI: 10.1002/eji.201970400

checkpoint inhibitor resulted an enhancement of antitumor effect. Given the numerous role of IFNs in antitumor response, we aimed to observe NDV or NDV-induced IFNs effect in the PD-L1 regulation in normal and tumor cells.
In this study, A549 and NB1RGB cell lines were infected with NDV LaSota strain; combination of IFN-α/β/λ; or IFN-λ only, for 24 hours. mRNA expressions of PD-L1 and MICA (MHC class I) were evaluated by choromogenic *in situ hybridization* (ISH), while protein expression of PD-L1 was analyzed by flow cytometry. By using chromogenic ISH we observed the downregulation of PD-L1 in both A549 and NB1RGB after NDV infection. MICA mRNA was upregulated which is confirming the efficient viral antigen processing in both infected cell types. Treatment with IFN-λ resulted in downregulation of PD-L1 and upregulation of MICA mRNAs in A549 cells but not in NB1RGB cells. Downregulation of PD-L1 protein on cell surface was also observed on both A549 and NB1RGB by flowcytometry after NDV infection and the result was dose-dependent. Meanwhile, IFN-λ treatment did not show any significant changes in both A549 and NB1RGB cells. This finding warrants a further study about the mechanism of NDV-driven PD-L1 downregulation and the role of IFN-λ in antitumor immune system.

P1688

Development of dengue virus vaccine by targeting at nonstructural protein 1 wing domain

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Current dengue virus (DENV) vaccine development is mostly based on the generation of antibodies against DENV envelop protein to neutralize DENV infection. However, it cannot avoid the potential danger that when the antibody titer induced is low, it may enhance DENV infection through the mechanism of antibody dependent enhancement. Nonstructural protein 1 (NS1) which can be expressed on DENV-infected cells and secreted into blood circulation. Secreted NS1 plays important pathogenic roles to cause vascular leakage and hemorrhage in dengue pathogenesis. Studies have shown that immunization with NS1 can induce protective immune responses against DENV infection. However, immunization with NS1 can also induce antibodies cross-react with host proteins. Therefore, to use NS1 as DENV vaccine candidate, we need to avoid this potential risk. To avoid this possible side-effect, a short modified NS1 peptide containing an 11-a.a. conserved wing domain (WD) region of NS1 (NS1-WD) was designed; this peptide modified the critical pathogenic amino acids to reduce cross reactivity but maintain immunogenicity. Both active immunization with NS1-WD peptide and passive transfer of polyclonal Abs against NS1-WD peptide provided protection against DENV in a hemorrhagic mouse model and a lethal infection mouse model. Therefore, this modified NS1-WD peptide can be a novel vaccine candidate against DENV infection.

P1689

Non-responsiveness to therapeutic tumor vaccination is overcome by functional modulation of myeloid derived suppressor cells

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DOI: 10.1002/eji.201970400

Therapeutic tumor vaccination represents a promising strategy to induce anti-tumor activity in cancer patients. However, usually only a subpopulation of patients benefits from tumor vaccines, whereas others display continuous tumor growth and metastatic spreading. Here we report a similar observation in a B16 melanoma model. Vaccinating these mice with a model tumor antigen and a combination of adjuvants, i.e. TLR-9-ligand CpG and NKT-cell ligand α-galactosylceramide, controlled the tumor in most animals (High-Responders), whereas others showed no clear benefit from the treatment (Non-Responders). No differences in numbers of B, NK or T cells were detected between both groups. However, Non-Responders showed significantly increased numbers of $CD11b^{+}Gr-1^{+}$myeloid-derived-suppressor-cells (MDSCs). Treatment with all-trans-retinoic-acid (atRA), which has been described to modulate MDSCs, effectively restored the tumor control in Non-responders. Frequencies neither of regulatory T cells nor MDSCs differed after treatment with atRA, but those cells from atRA-treated animals with a phenotype of monocytic MDSCs ($CD11b^{+}Ly6C^{high}Ly6G^{-}$-cells) had lost their suppressive capacity and displayed upregulation of MHC-Class-II, indicating acquisition of immune-stimulatory properties. Concomitantly, IFNγ production by $CD8^{+}$ T cells was significantly higher in atRA-treated mice.
Taken together, we demonstrate that atRA converts Non-Responders to tumor vaccination to responders, offering a novel opportunity for individualized tumor therapy.

P2265

The construction and evaluation of multi-stage tuberculosis subunit vaccine candidates

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To search for more effective tuberculosis (TB) subunit vaccines, antigens expressed in different growth stages of Mycobacterium tuberculosis (M. tuberculosis), such as Mtb10.4 (Rv0288), Mtb8.4 (Rv1174c), ESAT6 (Rv3875), Ag85B (Rv1886c) mainly secreted by replicating bacilli, and HspX (Rv2031c), Rv2626c highly expressed in dormant bacilli, were selected to construct several fusion proteins: Mtb10.4-HspX (MH), ESAT6-Ag85B-MPT64190-198-Mtb8.4 (EAMM), EAMM+MH, ESAT6-Ag85B-MPT64 (190-198)-Mtb8.4-HspX (LT69) and ESAT6-Ag85B-MPT64 (190-198)-Mtb8.4-Rv2626c (LT70). The fusion proteins were mixed with adjuvants being composed of N, N'-dimethyl- N, N'-dioctadecylammonium bromide (DDA), polyriboinosinic polyribocytidylic acid (PolyI:C) and cholesterol to construct subunit vaccines, whose immunogenicity and protective ability were evaluated in C57BL/6 mice. Among them, emulsified in adjuvant DDA-PolyI:C, EAMM induced stronger antigen specific immune responses in both humans and mice, and also provided more effective protection against M. tuberculosis infection in mice than MH; EAMM + MH vaccine showed even better protective efficacy than EAMM or MH alone; Furthermore, LT69 and LT70 had stronger immunogenicity and higher protective effect against M. tuberculosis H37Rv aerosol challenge than EAMM+MH, which was even higher than traditional vaccine BCG did at 30 weeks post the last vaccination. In addition, adjuvant composed of DDA, Poly I:C and cholesterol (DPC) with particle size of 400 nm and zeta potential of 40 mV was in better stability than the adjuvant composed of DDA and Poly I:C (DP) and had the ability to stimulate effective Th1 type cell-mediated immunity. In summary, multi-stage fusion proteins LT69 and LT70 in adjuvant DPC may serve as promising TB subunit vaccine candidates.

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DOI: 10.1002/eji.201970400

P2266

Oncolytic activity of a novel influenza A virus carrying PD1 antibody in hepatocellular carcinoma

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Oncolytic virotherapy is a promising strategy for the treatment of cancer. Influenza A virus (IAV) has shown potential as an oncolytic agent. In this study, a recombinant PR8 influenza viral vector, termed as IAV-PD1, were generated encoding the heavy chain of PD1 antibody on the PB1 segment and the light chain of PD1 antibody on the PA segment. The morphological characteristics of IAV-PD1 were examined. The IAV-PD1 virus replicated well in various cell lines, including MDCK, A549, SMCC7721, and HepG2 cells. Moreover, selective cytotoxicity of the virus was observed in various hepatocellular carcinoma (HCC) cell lines, while no effect was demonstrated in the normal liver cell line LO2, as indicated by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide and crystal violet assays. Importantly, using a model based on the growth of HepG2 cells in NSG mice reconstituted with human CD34+ cells, we found that a reassortant IAV-PD1 virus inhibited tumor growth significantly following intratumoral injection in a dose-dependent manner. *Ex vivo* results showed that the tumor inhibition efficacy of IAV-PD1 was observed in HCC clinical samples. Taken together, these results are the first to demonstrate that IAVs may have potential as oncolytic virotherapeutic agents against HCC.

P2267

Analysis of efficacy obtained with a trivalent inactivated *Haemophilus parasuis* serovars 4, 5, and 12 vaccine and commercial vaccines against Glässer's disease in piglets

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Haemophilus parasuis (HPS) has had a huge impact in the swine industry throughout the world. Inactivated bacterium for HPS is a traditional vaccine that can elicit efficient protection against homologous challenges. The objective of this study was to assess the minimum dose, antigen content, immunization duration, adjuvant and the efficacy of trivalent inactivated HPS serovars 4, 5 and 12 (prevalent serovars in China) vaccines against Glässer's disease. This vaccine was developed by our group, would quickly obtain a new veterinary drug certificate from government, and enter the veterinary market in China. Our results demonstrated that the minimum vaccine dose was 2 mL per pig and the optimal antigen content 2.0×10^9, 1.0×10^9, and 1.0×10^9 colony-forming units/mL of serovars 4, 5, and 12, respectively. The vaccine provided effective protection 14 d after the 2nd vaccination, and the period of immune protection was 180 d (6 mo) after the 2nd vaccination. Due to easy of injection, high safety, rapidly immune responses, high concentrations of antibody, and 100% of protective efficacy for piglets, new Montanide GEL 01 PR adjuvant can provide more homologous serovar protection than other domestically developed inactivated vaccines and should be used as a candidate adjuvant. Our results also demonstrated that the trivalent inactivated HPS serovars 4, 5, and 12 vaccine with GEL adjuvant can provide better protection against the 3 most common pathogenic serovars circulating in China than other commercial vaccines tested.

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DOI: 10.1002/eji.201970400

P2268

Recombinant adenovirus expressing granulysin is an effective adjunctive therapy for MDR-TB

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The major problems of tuberculosis (TB) chemotherapy require combination of 3 to 4 first-line anti-TB drugs and at least six-months duration treatments, which may result in poor compliance of patients and drug resistance. Recombinant replication-deficient adenoviruses intracellular expressing granulysin, namely rAdhGLi, showed a direct bactericidal effect on *Mycobacterium tuberculosis* in vivo. We assume that delivery of granulysin to the infected lung might enhance the clearance of TB. Combination therapy with both rAdhGLs (granulysin delivered as a secretory form) and rAdhGLi prolonged the survival time of SCID mice previously challenged with *M. tuberculosis* H37Rv, when compared to rAdhGLi or rAdhGLs alone. Importantly, immunotherapy with both rAdhGLs and rAdhGLi not only showed a sterilizing activity to shorten the chemotherapy duration from 6 months to 4 months in TB mouse model, but also provided a synergistic protective efficacy in BCG-primed mice to eliminate latent TB infection. More importantly, combination therapy with both rAdhGLs and rAdhGLi provided stronger immunotherapeutic efficacy as an adjunct to multidrug-resistant (MDR) chemotherapy regimen than chemotherapy alone. Therefore, granulysin mediated by recombinant adenovirus could enhance the clearance of TB in vivo and might be a promising adjunctive therapeutic vaccine for MDR-TB.

P2269

A DNA vaccine targeting the dengue virus envelope protein domain III elicits specific antibodies and CD4$^+$ T cells

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Introduction: DNA vaccines are safe, but currently induce relatively weak immune responses in humans. To improve immunogenicity, antigens may be targeted to dendritic cells (DCs) that are responsible for T and B cell activation. It was previously shown that a DNA vaccine encoding a fusion protein comprised of an antigen and a single-chain Fv antibody (scFv) specific for the DC endocytic receptor DEC205 induced strong immune responses to the targeted antigen.
Results: We evaluated this strategy to improve the immunogenicity of dengue virus (DENV) proteins. Plasmids encoding the scFv aDEC205, or an isotype control (scFv ISO), fused to the DENV2 envelope protein domain III (EDIII) were generated and used to immunize BALB/c mice. An analysis of the antibody responses indicated that EDIII fusion with scFv targeting the DEC205 receptor significantly enhanced serum anti-EDIII IgG titers that inhibited DENV2 infection. Similarly, mice immunized with the scDEC-EDIII plasmid developed a robust CD4$^+$ T cell response to EDIII that allowed the identification of two linear epitopes recognized by the BALB/c haplotype.
Conclusion: This strategy opens perspectives for the use of DNA vaccines that encode antigens targeted to DCs as a strategy to enhance their immunogenicity.

Supported by FAPESP (2014/50631-0 and 14/50890-5), CNPq (472509/2011-0) and CAPES (Finance Code 001).

DOI: 10.1002/eji.201970400

P2270

The mechanism of PfCSP-based malaria vaccines and its application to the viral vectored vaccine platform

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*Plasmodium falciparum*circumsporozoite protein (PfCSP) is one of the most intensively studied proteins as a vaccine candidate, whilst a PfCSP-based malaria vaccine candidate, RTS,S, conferred limited efficacy in the clinical trials in Africa. Identification of effective ways to induce strong protection by vaccine administration is key challenge to develop a novel malaria vaccine platform. The aims of this study are (1) to identify which region of PfCSP is most important for the protection against *Plasmodium*sporozoite challenge, and (2) to develop the novel malaria vaccine platform based on viral vectors expressing PfCSP. To this end, 3 types of truncated recombinant PfCSP as well as full-length PfCSP were produced. In a mice model, 3-dose vaccination of alum-adjuvanted full-length PfCSP conferred 100% protection against challenge with transgenic rodent sporozoites, whilst that of truncated proteins resulted in partial protection. Thus, the conformation of full-length PfCSP may have a dominant role to acquire the complete protection. Next, to develop a novel vaccine platform, viral vectors such as adenovirus and adeno-associated virus 1 (AAV1) that expressed full-length PfCSP were constructed. Prime vaccination with adenovirus and boost vaccination with AAV1 conferred stronger protection than other combinations such as homologous vaccination and reverse ordered vaccination did. The adenovirus-AAV1 vaccination not only induced strong anti-PfCSP antibody titer for long term, but also developed PfCSP-specific $CD8^+$ T cells in spleen, indicating that this regimen can induce both humoral and cellular immune responses and achieve a high level of protective immunity, which deserves further evaluation in clinical trials.

P2271

A new conditioned tumor lysate based melanoma vaccine inhibits in vivo tumor growth and potentiates anti PD1 treatment

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Clinical strategies using immune-checkpoint blockers, such as anti-CTLA4 or anti-PD1 antibodies, have demonstrated durable survival benefits in patients with melanoma and other tumors. Nevertheless, some patients treated with anti-checkpoint antibodies remain refractory, suggesting that combination with active immunization may improve responses rate. In this context, cancer vaccines become a complementary alternative as cancer treatment. Optimal delivery of antigens (Ags) and the use of adequate adjuvants are crucial for vaccine success. Here, a prototype of a generic therapeutic vaccine for the treatment of malignant melanoma named TRIMELVax™ was tested in an experimental model. This vaccine is based on conditioned melanoma allogeneic tumor lysates (TRIMEL) combined with *Concholepas Concholepas* hemocyanin as a specific adjuvant. The vaccine directly activates the immune response against tumor *in vivo*, inhibiting tumor growth. TRIMELVax was evaluated, in terms of safety and efficacy at pre-clinical level in C57Bl/6 murine melanoma model. In short, immunocompetent mice was vaccinated with three doses of TRIMELVax, and then challenged with B16F10 melanoma cells, or alternatively immunization was tested therapeutically in tumor bearing

DOI: 10.1002/eji.201970400

mice. Our results showed that vaccine reduced drastically the occurrence of tumor, inhibiting tumor growth in immunized mice. In contrast the separated use of tumor lysate, or adjuvant did not impact tumor growth. This response was associated to $CD8^+$ T cells enhanced tumor infiltration and increased antibody production. Additionally, anti-PD1 therapy was strongly potentiated by the combination with TRIMELVax, which encourage testing of the combined treatment in future clinical trials.

Financed by grants FONDECYT 1171213; FONDEF ID16I10148 and MIII P09/016-F.

P2272

Pan serotypic, highly conserved CD8 epitopes for a potential T-cell based vaccine against dengue infection

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An effective vaccine against DENV requires development of a long-lasting protective immune response against all four serotypes simultaneously. Accumulating evidence suggest that CD8 T cells may play a protective role against DENV infection. We set out to identify pan-serotypic conserved MHC class I restricted epitopes with their HLA coverage using an immunoinformatics-based approach. To establish conservation across four serotypes, a total of 3985 full-length DENV sequences were retrieved from VIPRBRC(https://www.viprbrc.org). Predicted HLA-I restricted epitopes were validated with experimentally known epitopes from the IEDB(https://www.iedb.org). We mapped the location of these epitopes in the DENV genome in relationship with the diversity of viral sequences using Shannon entropy (SE) calculated per site from the distribution of synonymous and non-synonymous single nucleotide variants (SNVs). We identified 10 epitopes which are at least 85% conserved between all 4 serotypes (C4 epitopes) and 15 epitopes conserved across 3 serotypes (C3 epitopes). These epitopes also showed the highest magnitude of IFN-γ responses from more than 400 epitopes. Six of the 10 C4 epitopes fall within the most immunodominant and highly conserved NS3 protein. The majority of the C3 and C4 epitopes are restricted within the HLA-I B alleles, with 6 of the epitopes HLA-I B*3501 restricted, which is known to be associated with protection. Phylogeographic analysis did not reveal a bias towards geographical regions with regards to prevalence of C3 and C4 epitopes. This analysis indicates that highly conserved epitopes exist, elicit a strong immune response and have the potential for a universal T-cell based vaccine candidate.

P2273

An effective multi-pathogen vaccine targeting filoviruses and an arenavirus

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Lassa fever (LF) is a viral haemorrhagic fever (VHF) whose aetiologic agent, Lassa virus, is widespread throughout most of West Africa, an area that overlaps with filovirus outbreaks, including Ebolavirus and Marburgvirus. Similar to the VHFs caused by Ebolavirus and Marburg virus, LF is associated with high levels of morbidity and mortality. In recent years, VHF outbreaks caused by filoviruses and arenaviruses have caused substantial morbidity and mortality in West and Central Africa, and there is a critical need to develop prophylactic treatments.

DOI: 10.1002/eji.201970400

In the absence of clearly defined correlates of protection against these VHF, any vaccine developed should elicit strong and preferably long-lasting humoral and cellular immunity. Ideally this immunity should cross-protect against viral variants, known to circulate in animal reservoirs.
We have made use of viral vectored vaccine platforms to develop a multi-pathogen vaccine against three filoviruses and an arenavirus. We demonstrate that a single vaccination with our multi-pathogen adenoviral vectored vaccine induces a strong cellular and humoral immune response, which confers protection in an EBOV challenge in guinea pigs. Importantly, no evidence of immune competition between vaccine-encoded antigens is found. The antigen-specific immune response can be further enhanced by a boost vaccination with a multi-pathogen Modified Vaccina Ankara (MVA) vaccine, resulting in a strong adaptive immune response and a diverse chemokine and cytokine profile.
This single dose, multi-pathogen adenoviral vectored vaccine could be rapidly deployed when a viral haemorrhagic fever outbreak occurs, with the advantage of having to manufacture and maintain stockpiles of only one vaccine.

P2274

Identification of novel mycobacterial antigens presented by MHC-I and -II by immunopeptidomics for vaccines against tuberculosis

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Mycobacterium tuberculosis (*M.tb*) is responsible for more deaths than any other pathogen. The only available vaccine, bacillus Calmette-Guérin (BCG) has variable efficacy. The immune response against tuberculosis relies on $CD4^+$ T-cells, therefore protective vaccines require induction of antigen-specific $CD4^+$ T-cells via mycobacterial peptides presented by MHC-II. Recent advances in immunopeptidomics based on mass spectrometry instrumentation and data analysis led to improvement in sensitivity. For the first time it is possible to precisely identify peptide sequences, bound to MHC molecules at the femtomolar scale. However, pathogenic mycobacteria downregulate antigen presentation limiting our ability to identify peptides. To overcome this, we have stimulated macrophages with a cytokine mix that prevented the mycobacterial downregulation of MHC class-II molecules. We have immunoprecipitated MHC-peptide bound complexes and analysed them by liquid chromatography tandem mass spectrometry. We have successfully identified 97 mycobacterial peptides presented by MHC-II and 54 by MHC-I from 76 and 41 antigens, respectively. The gene expression of all antigens were confirmed in infected macrophages and their functions assessed. The sequences of selected peptides were confirmed by spectral match validation and immunogenicity evaluated by IFN-gamma ELISpot against PBMCs from volunteers vaccinated with BCG, *M.tb* latently infected subjects or patients with tuberculosis disease. Three antigens were expressed in recombinant viral vectors, and evaluated as vaccine candidates alone or in combination in murine aerosol *M.tb* challenge model. When delivered in combination, these antigens conferred significant protection in the lungs and spleen compared with BCG alone. This demonstrates proof-of-concept for an unbiased approach to candidate antigen identification.

DOI: 10.1002/eji.201970400

P2275

Avirulent *Leishmania* priming elicits host-protective immune response

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Leishmaniasis, the diseases caused by protozoan parasite Leishmania, 150 million peoples world-wide, impose a major socio-economic burden, as a non-toxic drug or a vaccine is still not available. As prolonged use of a drug can drive the emergence of drug resistant parasites, drugs are not a permanent solution for eradicating the disease. Consequent upon this argument, deriving a *Leishmania* vaccine strain was imperative. Several virulent strains of *L. major* and *L. donovani* were continuously cultured in vitro for long time without any intermittent passage through a mammalian host. These long-term cultures of parasites lead to significant loss of virulence. These avirulent parasites were unable to impair the CD40- induced signaling in macrophages. The avirulent parasites were not able to survive in macrophages and were associated multiple changes in gene and protein expression- as determined by microarray and mass spectrometry. The avirulent parasites differed from their virulent counterparts in their ability to alter the host cell functions such as cytokine production, cell signaling and differentiation of T-cell subsets. As priming with the avirulent parasites resulted in the establishment of host-protective T cells that prevented the challenge infection with virulent parasites, the avirulent leishmania parasites can be of use in formulating an anti-leishmanial vaccine.

P2276

Haptoglobin enhances the *in vitro* migration capacity and the expression of immune related proteins in therapeutically used human dendritic cells

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Damage-associated molecular patterns (DAMPs) play a critical role in DCs' ability in triggering a specific and efficient adaptive immune response in different physiological and pathological scenarios. We have previously identified constitutive DAMPs (HMGB1 and Calreticulin) as well as new putative inducible DAMPs such as Haptoglobin (HP), from a therapeutically used heat-shocked cancer cell lysate (so called TRIMEL). Remarkably, HP was shown to be the most abundant protein in the proteomic profile of heat-treated TRIMEL samples. However, its relative contribution to the observed phenotype of the DCs has not been fully elucidated. Human DCs were generated from monocytes isolated from PBMC of healthy donors. DCs lineage was induced with rhIL-4 and rhGM-CSF. After additional stimulation with HP, DCs were phenotypically characterized by flow cytometry for canonical maturation markers, *in vitro* transmigration capacity was assessed using transwell plates, and the proteome of HP-stimulated DCs was analyzed. Our results showed that HP was associated with an *ex vivo* enhanced expression of specific DCs maturation markers, such as CD80, CD83, CD86 and the chemokine receptor CCR7, and *in vitro* transmigration of HP-stimulated primary human DCs. Importantly, HP was also associated with a higher relative abundance of specific immune-related proteins in DCs, such as Integrin aE and Dermcidin. Taken together, these data suggest that HP can be considered as a new iDAMP with an important role in DC activation during cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P2277

Tumor membrane vesicle-based vaccine inhibits growth of squamous cell carcinoma of head and neck in mice

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Head and neck cancer is a leading cause of cancer related deaths accounting for approximately 3% of all cancer related mortalities in the US. Currently, there is no cure for the advanced squamous cell carcinoma of the head and neck (SCCHN) thus development of efficacious therapies is urgently needed. To test whether vaccine-induced immunity inhibits tumor growth, we investigated efficacy of a tumor membrane-based vaccine (TMV) immunotherapy in murine SCCHN models. The MOC1, MOC2 and SCCVII tumors grown subcutaneously in syngeneic mice were harvested to generate TMVs. TMVs were then protein transferred with glycolipid-anchored immunostimulatory molecules GPI-B7.1 and GPI-IL-12 to generate the TMV vaccine. Mice were vaccinated with TMV vaccine after tumor cell challenge (therapeutic) and tumor growth was monitored every 3 days. Survival was then assessed using a Kaplan-Meier survival curve and significance determined using a Log-rank test for comparison analysis. MOC1, MOC2 (C57BL/6) and SCC VII cells (C3H/HeJ) respond to IFNg in vitro. The TMV vaccine inhibited tumor growth and improved the survival of mice challenged with MOC1 or SCCVII tumor cells. However, MOC2 (poorly immunogenic tumor) did not express MHC II, ICAM1 and did not respond to TMV vaccine, anti-CTLA-4 or their combination therapy. Interestingly, combination of anti-PD1 antibody with TMV vaccine decreased tumor growth. These observations suggest that tumor tissue-based vaccines can be harnessed to develop an effective immunotherapy for squamous cell carcinoma of the head and neck.
Funding: Supported by Head and Neck SPORE pilot funding from Emory Winship Cancer Institute.

P2278

Beneficial heterologous effects of a tetanus vaccination: the role of molecular mimicry and/or trained immunity

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Vaccines can have heterologous effects on the immune system i.e. effects other than triggering an immune response against the disease targeted by the vaccine. The underlying mechanisms are far from full understanding. The aim of this study has been to evaluate whether antibodies generated by immunization with tetanus toxoid (TTd) that are cross-reactive with chlamydial protein antigens (CPA) confer heterologous protection against ocular chlamydial infection.
Two murine monoclonal antibodies, MAb26 and MAb51, have been tested. MAb26 binds tetanus toxin (TTx) with low affinity and is not able to confer protection against TTx, while MAb51 possesses high affinity for TTx and protects mice from TTx intoxication. MAbs capability to prevent chlamydial infection has been assessed: i) *in vitro*, by using a neutralization assay based on human conjunctival epithelial (HCjE) cells infected with *Chlamydia trachomatis* (Ct) and ii) *in vivo*, by using a guinea pig model of chlamydial ocular infection.
MAb26 has been superior in comparison with MAb51 in prevention of Ct infection in HCjE cells. MAb26 has conferred ~40% inhibition of chlamydial infection, while in the presence of MAb51 the

DOI: 10.1002/eji.201970400

inhibition has been less than 5%. MAb26 has significantly diminished intensity of ocular pathology in guinea pigs infected with Chlamydia compared to either MAb51-treated guinea pigs or sham-control. TTd immunization generates antibodies that are improving immunity to heterologous CPA antigens. Better understanding the immune mechanisms underlying positive heterologous effects could promote protection beyond the intended target pathogen and improve vaccine outcome.

P2279

Molecular and immunological characterization of Plasmodium falciparum gametocyte-specific genes

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Plasmodium falciparum has an inherent ability to rapidly evolve resistance to drugs/vaccines. Therefore, the need for increased efforts towards drug/vaccine development. However, much of the parasite genome remains uncharacterized. This study sought to identify and characterize gametocyte-specific genes and determine their immunogenicity in malaria endemic regions. Analysis of transcriptome and proteomics data from laboratory strains was used to prioritize genes based on gametocyte-specific or uniform expression patterns across different stages of the erythrocytic cycle. Genetic diversity of selected genes was further assessed. Gene expression in field isolates was determined by RT q-PCR. Peptides with predicted B-cell epitopes were commercially acquired for naturally acquired humoral immune response investigations. Prioritization led to the identification of 2 genes: gametocyte-specific PF3D7_051300 and PF3D7_115800 with expression across all erythrocytic stages. Genomic sequence analysis revealed the genes were conserved and were found to be under directional selection based on the negative Tajima's D values, -2.35 and -2.33 respectively. Selection was stronger (lower Tajima's D values, 2.36 vs 1.47) in Africa compared to South East Asia. In general, genetic diversity was higher in Africa compared to South East Asia. RT-q-PCR with field isolate asexual and sexual stages confirmed that, PF3D7_051300 had a gametocyte-biased expression while PF3D7_115800 was expressed across stages. This study has demonstrated that PF3D7_051300 and PF3D7_115800 genes are relatively conserved in field isolates across continents and their expression patterns are similar to that of the 3D7 strain. Ongoing immunological investigations will further determine the potential of these genes as transmission blocking vaccine candidates.

P2280

Development of a vaccine against meningococcal meningitis using hepatitis B core protein virus-like particles to display heterologous neisseria meningitidis antigens

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DOI: 10.1002/eji.201970400

Meningococcal disease is a serious, life threatening illness caused by bacterium *Neisseria meningitidis* with high patient mortality (10%) and development of sequelae (50%). Efforts to develop a comprehensive vaccine against serogroup B meningococci (MenB) have focused on using surface-exposed outer membrane proteins, including NadA (Neisseria adhesion A) and fHbp (Factor H-binding protein). Here we report that the virus-like particle (VLP) Hepatitis B core protein (HBcWT) can be used as a carrier vehicle for presentation of MenB antigens. A high resolution cryoEM structure confirmed the successful assembly of a fusion of HBcWT with NadA and the C-terminal domain of fHbp, although electron density was only apparent for NadA and not for the fHbp antigen. Fusion of the antigens compromised thermal stability through a reduction in Tm of about 20°C. Introduction of multiple mutations into HBcWT (D4H/V13T; Y6F; S35M; V120R) led to only a modest increase in Tm of ~1ºC without compromising particle assembly.
IgG antibody responses were tested by inoculation of several HBcWT-meningococcal antigen fusions into two strains of mice (BALB/c and NIH-Swiss). IgG1, IgG2a and IgG2b antibodies were produced against both fHbp and NadA. However, only HBcAg fusions containing NadA elicited the production of antibodies with serum bactericidal activity in the presence of complement- the accepted correlate of protection for meningococcal disease. It is hypothesised that this observation is attributable to the adoption of a more native-like folding of crucial conformational epitopes of NadA within the chimeric VLP.

P2281

Protection against intrarectal SIV acquisition by live SHIV vaccine in the absence of anti-Env antibody responses

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Identifying the protective immune responses against HIV infection has been a major challenge in AIDS vaccine development. Anti-Envelope antibody responses have been proven to be the main protective mechanisms against viral acquisition in many of the SIV/HIV vaccine platforms. Here, 12 SHIV $_{SF162P4}$ -infected animals, which have cleared their plasma VLs, and had no significant $CD4^+$ T cell loss several months after infection, were used as a live SHIV vaccinated group. Since no SIV envelope antibody responses were detected in these animals, we tested the roles of other adaptive immunity, such as SIV-specific CTL responses, in mediating protection against viral acquisition. After 8 serial low-dose repeated rectal challenges with SIVmac251, 9 of 12 SHIV-exposed animals (75%) were still SIV virus-free; whereas 1 out of 6 (17%) naïve controls were not infected. Overall, significant protection was achieved in the absence of anti-env antibody responses. To further delineate the protective mechanisms, we continually challenged three protected macaques after CD8 depletion. None of them showed SIV viral loads with a sensitive envelope sequencing method. The fact that no CD8-depleted animals were infected by SIV suggested that $CD8^+$ cells may not essential for protection in this study or that sufficient $CD8^+$ T cells remained among tissue-resident immune cells. Using RNAseq analysis, we are currently identifying the protective signature genes of innate immunity, especially trained innate immunity, in reducing SIV viral acquisition. Regardless, we conclude that protection against rectal acquisition can be accomplished in the absence of anti-Env antibodies.

DOI: 10.1002/eji.201970400

P2282

LmjF_36_3850 is a novel, immunogenic hypothetical protein from Leishmania major

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Leishmania, an obligate intracellular pathogen residing inside macrophages, inflicts the disease Leishmaniases in 88 countries. However, an efficacious anti-leishmanial vaccine for human use remains elusive. While comparing the virulent and avirulent *L. major* transcriptomes by microarray, PCR and functional analyses for identifying a novel virulence-associated gene, we identified LmjF_36_3850, a hypothetical protein without any known function but significantly less expressed in the avirulent parasite. Sequence alignment and analysis revealed that LmjF_36_3850 protein shared similar motif and phosphorylation sites with sucrose non-fermenting protein (Snf7) that shuttles virulence factors. Predicted docked structure of LmjF_36_3850 with DAG yielded similar energy values as PKCα and PKCβ, implying that LmjF_36_3850 protein contributes to parasite virulence possibly by interfering with DAG-dependent anti-leishmanial PKC isoforms activation. BALB/c mice were primed with pcDNA6/HisA encoding LmjF_36_3850 or control vector followed by *L. major* challenge infection. We observed a higher parasite load, comparable antibody response and higher anti-inflammatory cytokines such as IL-4 and IL-10 while expression of major anti-leishmanial cytokine, IFN-γ, remained unchanged in LmjF_36_3850 vaccinated mice. CSA re-stimulated LN cells from vaccinated mice after challenge infection secreted comparable IL-4, IL-10 but reduced IFN-γ, as compared to controls. Our observations suggest a skewed T_H2 response, decreased IFN-γ secreting T_H1-T_{EM} cells and increased Central and Effector memory subtype of T_H2, T_H17 and Treg cells in the vaccinated mice. These data indicate that LmjF_36_3850 is a virulence factor that enhances disease-promoting response, possibly by interfering with PKCα/β activation and increases repertoire of disease-promoting T cells upon antigenic priming.

P2283

Chlamydia recombinant MOMP encapsulated within PLGA nanoparticles enhances mucosal Th1 antibodies, IFN-g and proliferating memory and effector T-cells via the subcutaneous prime-boost immunization of mice

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Biodegradable polymeric nanoparticulate-based vaccines are rapidly gaining attraction in the vaccine developmental field. We have developed a *Chlamydia* nanovaccine by encapsulating its recombinant MOMP (major outer membrane protein) in PLGA 85/15 (poly (lactic-co-glycolic acid) that induced robust adaptive immune responses and provided enhanced protection against *C. muridarum* (Cm) challenge in subcutaneously immunized mice. In the current study, we compared the immunogenicity and efficacy of priming routes; subcutaneous (sc) or intramuscular (im) followed by two sc-boosts to evaluate serum and mucosal antibodies [Th1 (IgG2a, IgG2b), Th2 (IgG1)], cytokine responses as well as memory and effector T-cells activation after immunization and Cm challenge of mice. We observed significantly high levels of MOMP-specific serum and mucosal antibodies with high avidity functional Th1 antibodies. A significant increase in Th1 serum and mucosal antibodies with even higher avidity was observed after genital tract Cm challenge in the sc-prime-boost immunized mice. Evaluation of the Th1/Th2 antibody titer ratios revealed that the nanovaccine evoked higher Th1 than Th2 responses, which skewed to a dominated Th1 antibody response after bacterial exposure to sc-prime mice. In addition, IFN-g along with CD4+ memory ($CD44^{high}$ $CD62L^{high}$) and effector ($CD44^{high}$

DOI: 10.1002/eji.201970400

CD62L^{low}) T-cells proliferation were about two-fold higher in the sc-prime mice. Herein, we reveal that the subcutaneous prime-boost nanovaccine immunization was more effective in enhancing Th1 antibodies, IFN-g and memory and effector T-cell responses against a chlamydial genital tract challenge of mice.

P2284

Active immunization with immune checkpoint inhibitors-mimotope elicits strong anti-tumor effect against Her-2/neu-expressing tumors

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Application of monoclonal antibodies (mAbs) as immune checkpoint inhibitors (ICIs) has demonstrated remarkable activity in cancer immunotherapy. Active immunization with mimotopes (B cell epitopes) of ICIs rather than application of the corresponding mAbs for passive immunotherapy may, however, provide advantages such as induction of antibodies by the patient's own immune system and overcoming the costly treatment of the mAbs.
By bacterial surface-display of overlapping peptides spanning the extracellular domains of human/mouse PD1, and in-vitro assays using T cells expressing h/mPD1 or PDL1, h/mPD1-derived mimotopes with capacity in blocking the respective PD1-PDL1 interactions were identified. Applying a syngeneic mouse model with human Her-2/neu-expressing tumors, active immunization with the mPD1-derived mimotope resulted in a significant tumor growth reduction, comparable to the corresponding mAb and associated with a marked increase of PD1 expressing immune cells in the tumors. Furthermore, a 68% homology between hPD1 and mPD1 sufficiently facilitated a significant induction of tumor growth reduction in the mice also by IgG against hPD1-derived mimotopes.
Our results demonstrate in vivo anti-tumor effect following active immunization with PD1-derived mimotope in syngeneic mice with tumors expressing human Her-2/neu, implying the use of ICIs mimotopes for cancer immunotherapy. Evaluation of efficacy and safety of our second generation of anti-Her-2/neu vaccine (HerVAXX; Tobias et al, 2017, BMC Cancer) in combination with mimotopes of ICIs as adjuvants is now being carried out. Use of ICI mimotopes may, thus, pave the way for construction of monovalent as well as polyvalent vaccines in combination with tumor specific antigens against different malignancies.

LB113

Immunogenicity comparison of neoantigen vaccines through different delivery platform

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Background: Neoantigens are promising cancer vaccine candidates because they're cancer-specific and can be predicted from the patient's genomic data. A major challenges for developing potent neoantigen vaccines is the low immunogenicity of neoepitopes. DNA vaccines can deliver multiple epitopes and cytokines as an adjuvant in a single plasmid, and its immunogenicity can be further

DOI: 10.1002/eji.201970400

enhanced through heterologous prime-boost strategy. Here, we hypothesized utilizing DNA prime, adenovirus boost (refer to the DDA approach) strategy can improve the immunogenicity of neoantigen vaccines.
Aim: To investigate whether vaccination through the DDA approach or DDA combine GM-CSF as adjuvant can improve the immunogenicity of neoantigen vaccines.
Methods: Using IEDB analysis resource to predict and rank the top 21 MHC class I epitopes (short peptide) for the B16F10 tumor while 27mer peptides (long peptide) were synthesized to cover both MHC class I and class II epitopes. The 27mer neoantigen sequences of the selected 21 MHC class I epitopes were cloned into one plasmid and adenoviral vector with or without GM-CSF sequence linked by p2a. Mice were immunized through the DDA approach or peptides with polyI:C.
Results: In the peptide vaccine, the majority of the mutations were recognized by $CD4^+$ T cells, less than 10% peptides can induce $CD8^+$ T cells response. For the DDA approach, 14% epitopes can induce $CD8^+$ T cell response in mice immunized with DNA vaccine encode neoantigen only. Moreover, 24% of epitopes can induce CD8+ T cell response in the group combined GM-CSF, which dramatically improved the immunogenicity of neoantigen vaccines.

P3113

The preliminary study of γδ T cell vaccine for cancer immunotherapy

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Background: DC vaccine has been widely used in cancer immunotherapy, while its application was restrained by the difficulty to obtain enough DCs. γδT cells was reported to be able to present antigen and could be expanded enormously ex vivo, therefore its potential for vaccination should be evaluated to replace DC vaccine.
Method: Ex vivo expansion of γδT cells was optimized, and the antigen-presenting function of expanded γδT cells was validated by CFSE dilution and ELISPOT assay. 14-day expanded γδT cells were pulsed with either oxidized whole-tumor cell lysate or predicted neoantigens, and their cytotoxicity and immunogenicity were tested respectively.
Result: PBMC extracted from blood collected with EDTA anticoagulant was preferential for γδT cell expansion when started with the concentration of 2x10e6/ml and cultured with our standard protocol. After 14 Days' expansion, the expansion fold could exceed 6000 with purity more than 90%. Expanded γδT cells pulsed with either oxidized whole-tumor cell lysate or predicted neoantigens were capable of presenting both antigens to cognate αβT cells purified from PBMC or tumor infiltrating lymphocytes, while retained its cytotoxicity against mutiple tumor cell lines.
Conclusion: The optimized expansion method pave the way for γδT cell vaccination. With the dual effects of both antigen presentation and directly killing, γδT cell vaccination represents a novel therapeutic option in treating cancer patients

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DOI: 10.1002/eji.201970400

P3114

Establishment of an efficient recombinant vaccinia virus Tiantan strain vector system

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In recent yeas, vaccinia virus was used as a vaccine vector against diseases such as HIV-1. It also can be used as the carrier of oncolytic virus to kill tumor cells, which is of great value. Due to technological limitations, recombinant virus was obtained mainly by homologous recombination, the efficiency of which was extremely low. With the successful application of CRISPR(clustered regularly interspaced short palindromic repeat)-Cas9 in a variety of animals, tissues and cells, this study intended to use the CRISPR-Cas9 to establish an efficient recombinant vaccinia vector system. Using three gRNA-Cas9 plasmids targeting TK region of the Tiantan strain, the recombinant viruses expressing EGFP were obtained by introducing gRNA-Cas9 plasmids, or using gRNA-Cas9 stable cell lines, together with repair donor pJ2R-EGFP and VTT infection. The recombinant efficiency was much higher than traditional homologous recombination. This system greatly improves the efficiency of recombination of vaccinia virus vector, and provides reference value for vaccine vector construction and tumor immunotherapy.

P3115

Anti-angiogenesis reversal resistance to advanced breast cancer

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Multidrug resistance (MDR) of tumor cells is the leading cause of chemotherapy failure and patient death. Angiogenesis plays an important role in the development and progression of malignant tumors. ATP-binding (ABC) transporter protein overexpression is an important cause of multidrug resistance. Basic research shows that anti-angiogenic agents inhibit the efflux of ABC transporters to chemotherapeutic drugs. To achieve the purpose of reversing drug resistance. There are three kinds of ABC transporters that play an important role in MDR, namely breast cancer resistance protein, multidrug resistance-related protein, and P glycoprotein. The efficacy of anti-angiogenic combination chemotherapy in the real world has been affirmed, especially in cancer populations without specific targeted therapies. However, the efficacy is inconsistent in different patients, so it is necessary to pay attention to the relevant gene detection and predict the efficacy, and achieve the goal of accurate anti-angiogenesis therapy.

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DOI: 10.1002/eji.201970400

P3116

Immune responses and protection induced by Brucella abortus BH1/Δbp26ΔwboA

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Brucella is an intracellular bacterial pathogen that infects humans and animals. Live attenuated Brucella abortus A19 vaccine is an effective means of brucellosis control and prevention with several drawbacks, such as an inability to distinguish between a natural infection and immunization and an insecurity for humans. Therefore, this study screened an attenuated Brucella abortus BH1 and constructed a Brucella BH1/Δbp26ΔwboA mutant and evaluated its virulence and immune responses. The virulence of the BH1/Δbp26ΔwboA mutant was less than the BH1 strain and attenuated in goats and in Kunming mice, with a high immunoprotectivity in mice. Additionally, a LPS-targeted slide agglutination test and BP26-targeted indirect ELISA screening were all shown to distinguish between a natural infection and BH1/Δbp26ΔwboA immunization. Together, the BH1/Δbp26ΔwboA mutant can be a potential vaccine candidate.

P3117

In vitro studies of IgY against fusion VacA-HpaA protein of Helicobacter pylori

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Objective: To study the physicochemical properties and biological activities of chicken yolk antibody (IgY) of VacA-HpaA fusion protein, which provide a solid experimental basis for the preparation of vaccine against H.pylori infection.
Methods: The purified antigen of fusion protein VacA-HpaA was produced and immunized hens, the VacA-HpaA IgY was extracted from the yolk by water dilution methods. (1)After the VacA-HpaA IgY was purified by deposition technique with anunonium sulfate, the purity of IgY was analyzed by SDS-PAGE, and the protein content of IgY was analyzed by Bradford method. (2)The indirect ELISA was used to detect its heat-stability, acid-stability and pepsin-stability.(3) The specificity of IgY was analyzed by Western blot. (4) The neutralization effect of different concentrations of VacA-HpaA IgY on cytotoxic activity was detected by MTT assay. (5) Scanning electron microscopy (SEM) was used to observe the neutralization effect of VacA-HpaA IgY on AGS cell adhesion.
Results: (1) The purity of IgY was 60%, the protein concentration was 22 g/L; (2)The IgY showed a good heat resistance, acid resistance and pepsin resistance; (3) Western blot analysis showed the specificity of IgY, It appears in the corresponding bands at a relative molecular weight of about 27,000 and 30,000, The titer of IgY to VacA and HpaA reach to 1:3200 and 1:6400; (4) the IgY has a neutralizing effect on the cytotoxic activity of VacA, and is dose- and time-sensitive; (5)VacA-HpaA IgY could block adhesion effect of H.pylori.
Conclusion: The VacA-HpaA IgY has good physical and chemical properties and can be used to prepare products resistant to H.pylori infection.

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DOI: 10.1002/eji.201970400

P3118

Several amino acid substitutions alter the replication and pathogenicity of an H5N1 influenza vaccine seed virus

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To evaluate the genetic stability of an H5N1 influenza virus vaccine strain (NIBRG-14) and its adaptability to mammals,we generated mouse-adapted strains of the H5N1 seed virus to identify adaptive changes that confer increased virulence toward mammals. At the 15th passage, we obtained a mouse-adapted H5N1 virus that contained two amino acid substitutions in polymerase protein 2 (PB2) (S360Y and P585S)、an amino acid substitution in the polymerase protein 1 (PB1) (G216S) and an amino acid substitution in nuclear protein (nucleocapsid. NP) (V353I). During the 15 generations, sequencing was performed every 5th generation, and deaths of the mice were recorded. The animal experiments showed that the mouse-adapted mutant viruses became highly pathogenic in mice after several passages. The animal experiments also revealed that the mouse-adapted mutant viruses could kill unvaccinated mice. This result suggests that these four mutations may be related to the pathogenicity and adaptability of the H5N1 virus toward mice. To test this hypothesis, we used reverse genetics to generate recombinant viruses of an H5N1 influenza virus vaccine strain (NIBRG-14) with four-point amino acid substitutions in genes PB1, PB2 and NP. We demonstrated that the reassortant H5N1 influenza virus with the same amino acid substitutions as the mouse-adapted mutant viruses, acquired greater virulence after the mutations. Our findings suggest that infection of mice can induce adaptive mutational changes in H5N1 viruses and increase their Virulence. This finding underlines the potential direct role of murine infection in an increase in the the pathogenicity and transmissibility of H5N1 influenza virus vaccine strains

P3119

Isolation and identification of gp96 associated peptides derived from bladder urothelial carcinoma

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Tumor-derived heat shock proteins (HSPs) such as gp96 (GRP94, 94 kDa glucose-regulated protein) can elicit protective antitumor immune responses, yielding a marked suppression of tumor growth and metastasis. The molecular basis for this response is proposed to reflect the association of tumor antigenic peptides with these HSPs. The identification of these peptides or their precursors stripped from the HSPs remained to be resolved. In this study, gp96-peptide complexes (HSPPC96) from bladder urothelial carcinoma (BUC) tissues were isolated by using a high-yield, one-step purification approach by combining antibody library technology with immunoaffinity chromatography technology, and associated peptides were stripped and analyzed by mass spectrometry (MS). A number of different peptides covering a mass range of ～990 Da - 3330 Da derived from known proteins of different cellular compartments or unknown proteins, were found and their molecular masses, physicochemical properties, origin and antigenicity were further determined. These results will help to understand the real entity of the peptides associated with gp96 that induce anti-tumor immunity and provide new insight into the development of peptide vaccine for bladder cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P3120

Adjuvanted-influenza vaccination in HIV-infected patients: A systematic review and meta-analysis of immunogenicity and safety

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Objective Adjuvanted-influenza vaccination is an efficient method to avoid the infection of influenza virus, but the medical community's understanding of its performance in HIV-infected patients remains limited. To ensure optimal knowledge about vaccine-induced clinical protection, the analysis mainly identify the advantages of adjuvanted-influenza vaccine's immunogenicity and safety in HIV-infected patients.
Methods: We have searched 6 different databases for articles published before January 2019. The research is conducted for qualified studies reporting the essential dates of geometric mean titer (GMT), seroconversion, seroprotection and adverse events.
Results: We have identified 11 studies that assess the immunogenicity and safety in vaccinated HIV infected patients. Involved a total of 1698 HIV-infected patients. Regarding immunogenicity, the pooled standardized mean differences (SMD) of geometric mean titer (GMT) is 0.61 (95%CI (0.40,0.82)) and the pooled relative risk (RR) of seroconversion and seroprotection are 1.34 (95%CI (0.91,1.98) and 1.61 (95%CI (1.00,2.58)). HIV-infected patients who had received adjuvanted-influenza vaccination were well tolerated in general. These studies indicated that injection only increased the occurrence rate of local pain at the injection site (RR = 2.03, 95%CI (1.06,3.86)), and none of the studies reported any serious adverse events related to adjuvanted vaccination.
Conclusion: In this research, all studies injecting adjuvanted influenza vaccination among HIV-infected patients showed good safety and immunogenicity reflecting on the higher levels of immunogenicity and provided superior protection. Adjuvanted influenza vaccination was well tolerated but only local pain at the injection site was higher.

P3121

Efficacy of DNA vaccine for protection against cyprinid herpesvirus 2 infection in the crucian carp

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Crucian carp (Carassius carassius) is an economically important fish in China, but herpesviral haematopoietic necrosis (HVHN) caused by Cyprinid herpesvirus 2 (CyHV-2) often causes high mortality and significant economic losses. Vaccination has been proven to be the most promising strategy for preventing a wide variety of infectious diseases, while the optimal antigen can play a critical role in the protective immunity elicited by vaccine. In this study, a fragment of the envelope protein encoding gene (ORF25) from an CYHV-2 was used to construct a DNA vaccine (pEGFP-ORF25). Crucian carp were immunized with the DNA vaccine by intramuscular injection before challenge with CYHV-2. After vaccination, the anterior kidney was collected aseptically by removing all tissues for detecting immune responses by real time quantitative RT-PCR assays. The results showed the DNA vaccine induced the cytokines of immune responses MHC I, IL-I, C3 and TF-A1 in the kidney of immunized crucian carp compared to control groups ($p< 0.05$). In addition, the highest antibody titer occurred at 4 weeks after vaccination, and the relative protection survival assay revealed high and significant protection efficacious of 70% in fish that received the DNA vaccine (pEGFP-ORF25). The

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DOI: 10.1002/eji.201970400

results of this study demonstrate that the plasmid pEGFP-ORF25 is therefore a promising vaccine candidate against herpesviral haematopoietic necrosis in crucian carp.

P3122

Seroprevalence of neutralizing antibodies against human adenovirus type-5 and chimpanzee adenovirus type-68 in cancer patients.

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Chimpanzee adenovirus serotype-68 (AdC 68) has high transduction efficiency, high gene expression, good genetic stability and low serological positive rate, which reminded us that AdC68 may be a suitable cancer vaccine vector. To evaluate neutralizing antibodies (NAbs) for AdC68 and human adenovirus serotype 5 (AdHu5), there are 440 cancer patients and 204 healthy adult volunteers in this study. In all human subjects, the seropositive rate of AdC68 NAb was much lower than that of AdHu5 NAbs. In cancer patients, the serum positive rate of Nabs for AdC68 was significantly lower than that of AdHu5 (43.64 vs 67.05%, P < 0.01). The serum positive rates of NAb and AdC68 in cancer patients were higher than those in healthy volunteers (43.64 vs 23.53%, P = 0.000). Compared with other types of cancer, the serum positive rate of AdC68 NAb was significantly lower in lung cancer (52.50%), laryngeal cancer (25.71%), esophageal cancer (32.89%) and cervical cancer (26.67%). Interestingly, the serum positive rate of NAb to AdC68 in lung squamous cell carcinoma samples was statistically higher than that in lung adenocarcinoma (70.00 vs 35.00%, P = 0.049). In stage I, II and III , the serum positive rate of AdC68 NAbs was significantly lower than that of AdHu5 Nab. The titer of AdC68 NAb in positive samples was much lower than that of AdHu5 NAb.Therefore, the serum positive rate of NAb for AdC68 is much lower than that of AdHu5. These results provide useful evidence that AdC-68 may be an appropriate cancer vaccine carrier.

P3123

Chimeric nanoparticle vaccine of Noroviruses and Enteroviruses

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Noroviruses and Enteroviruses are two major infectious agents leading to severe diseases in children and immunocompromised populations. Recently, although vaccines for serotype EV-A71 viruses came into use and have played important roles in preventing hand-and-mouth diseases (HMD), new serotypes of enteroviruses are emerging and giving new challenges for controlling HMD. There is no vaccine for noroviruses so far. Furthermore, noroviruses are non-cultivable pathogens. Therefore, recombinant expression of viral antigenic proteins is the only way to develop norovirus vaccines. In this study, we designed a chimeric antigen of noroviruses and EV-A71 viruses based on nanoparticles formed by p domain of norovirus VP1 protein via prokaryotic expression system. Selective 3 neutralizing epitopes of EV-A71 viruses were inserted into loop 1 of p domain of norovirus vp1 gene to form a fused gene. SWISS-MODEL analysis predicted that these 3 epitopes could be displayed on the surface of norovirus p domain-formed nanoparticle. Antigenicity of chimeric protein was determined by SDS-PAGE and immunoblotting. Percentage of 20nm nanoparticles was confirmed by fast protein liquid chromatography (FPLC) and electronic microscopy (EM). Immunization of Balb/c mice with this

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DOI: 10.1002/eji.201970400

chimeric antigen induced production of antibodies against both norovirus p domain of VP1 and each of neutralizing epitopes of EV-A71 viruses. In summary, a chimeric nanoparticle protein of Norovirus-Enterovirus has been developed and characterized. The findings in the present study not only lay foundation for developing bivalent vaccine of Norovirus and Enterovirus, but also pave the way for R&D of multivalent vaccines against other non-cultivatable virus and BSL3/4 pathogens.

P3124

Purification and immunogenic characterization of Epstein-Barr virus gH/gL

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Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus that infects more than 90% of the world's population and is responsible for a significant human health disease burden. No vaccine or antiviral therapy is available for EBV. EBV expresses a core set of highly conserved envelope glycoproteins among herpesvirus, gH/gL and gB-responsible for virus entry into both B lymphocytes and epithelial cells, which are major targets of neutralizing antibodies and protective immunity. However, the immunogenic properties of EBV gH/gL remain largely unexplored. In this study, we described the purification of full-length gH extracellular fragment in complex with gL expressed by drosophila melanogaster S2 cells with high yields. The purified recombinant gH/gL complex is homogenious and well-behaved. We immunized mice with purified gH/gL and high titers of anti-gH/gL antibodies were devoloped. The neutralizing activities that that block virus infection are being evaluated. Our data suggest that the S2-expressed gH/gL protein possess excellent immunogenicity and can be used as potential candidates for vaccine development to prevent EBV infection.

P3125

Preparation and immunogenicity analysis of swine type O foot-and-mouth disease virus VLPs

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Foot and mouth disease (FMD) is a kind of highly contagious infectious zoonosis caused by foot and mouth disease virus (FMDV) in pigs, cattle and sheep. FMD is listed as the member of List A infectious diseases by the Office International des Epizooties (OIE). FMD seriously endangers the development of animal husbandry and foreign trade of related products. Currently, the main vaccines for FMD in China are inactivated vaccines which require expensive high containment facilities for production and maintenance of the cold chain. Virus-like particles (VLPs), which have the similar structure to natural virus particles but do not contain viral genes, are self-assembled from the capsid proteins of viruses in vitro. VLPs vaccines are generally considered to be a very ideal and safe vaccine because of their unique advantages. Here we express and purificate simultaneously soluble structural proteins of FMDV, with SUMO tag fusion, in E.coli efficiently. To enhance the self-assembly efficiency of VLPs, we incorporated a designed mutation which can keep the same structure as intact virus while increase PH stability and thermostability of the recombinant empty capsids proteins.. Immunization of guinea pigs with the obtained FMDV VLPs can produce specific antibodies and neutralize antibodies, and thus induce cellular immune responses.

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DOI: 10.1002/eji.201970400

The study was partly supported by Wisdom Zhengzhou 1125 Talent Gathering Plan.
Key words foot and mouth disease; VLPs; vaccine

P3126

The effect of OMP31 T epitope, B epitope and T-B epitope polypeptipe in resisting Brucella infection in BALB/c mice

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Aims: Researching the effect of OMP31 T epitope, B epitope and T-B epitope polypeptipe in resisting Brucella infection in BALB/c mice.
Methods: In this study, we synthesized the T and B cell epitope proteins based on the analysis of the epitope of OMP31, and then, for verifying the effect of epitope proteins, BALB/c mice had been immunized by these proteins through respiratory tract.
Results: Compared with the control group, significantly increasing IgG1 and IgG2a in mice immunized with B epitope or T-B epitope polypeptide, higher IFN-γ levels in the lymph drained from their respiratory tract and spleen of the mice immunized with T epitope or T-B epitope polypeptide. And the bacterial load of the lungs of mice infected with Brucella 16M was also lower than that of the control group.
Conclusion: These results indicate that peptides containing B epitope and TB epitope could promote specific systemic humoral immunity and mucosal immunity of respiratory tract, peptides containing T epitope or TB epitope can induce specific T cell immunity, and T epitope, B epitope or TB epitope immunization could protect mice from B.melitensis infection in lung but cannot confer protection against its dissemination. In all, OMP31 is highly immunogenic as a candidate protein for Brucella vaccine, and its epitope polypeptide can help the host resist Brucella attack.

This report was funded by NSFC (81560322,81860375).

P3127

Induction of specific CD8$^+$T cells against hepatocellular carcinoma-associated neoantigens

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The tumor neoantigens derived from somatic mutations could serve as potential candidates of cancer vaccine. We aim to predict the tumor neoantigen peptides in hepatocellular carcinoma (HCC), and examine their specific immune effects against tumor cells. We analyzed the data of whole-genome sequencing and exome sequencing of HCC tumor and matched non-tumor liver tissues to confirmed the HCC-associated somatic mutations. The mutation frequency of tumor protein p53 (TP53) was 40%, and Laminin Subunit Alpha 3 (LAMA3) was 8% in the analyzed tissues. In GSE25097 database, TP53 and LAMA3 mRNA levels in tumors were 1.57±0.02 and 1.37±0.10, which significantly increased than those in matched no-tumor tissue [0.54±0.01 and 0.36±0.01; P< 0.001]. In 10 HCC tissues, the

DOI: 10.1002/eji.201970400

mRNA levels of TP53 and LAMA3 in tumors were 0.24±0.03 and 0.13±0.06 significantly elevated than those in normal tissues [0.11± 0.01and 0.01±0.01; P< 0.001]. Among the Chinese population, HLA-A2 and HLA-A11 and HLA-A24 accounted for 70%, representing the major MHC-I molecules. Based on the HLA phenotype of patients, we used NetMHC software to predict the neoantigen epitopes with high binding affinity to their MHC-I molecules. By using the predicted peptides, we induced the generation of antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) and confirmed their specific effects against tumor cells. **The HCC harbored relative higher mutation frequency in the genes of TP53 and LAMA3, and these gene products showed increased expression in tumor than in no-tumor tissues. The induced CTLs by predicted peptides derived from mutation-associated protein could kill the target cells without injury to normal cells.**

P3128

Inhibition of tumor miR-21-5p expression promoted the anti-tumor activity of the STAT3 knock down DC vaccine

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Objective: To observe the combination therapy of inhibiting tumor miR-21-5p combined with stat3-knock down DCs by using the tumor-bearing mouse model.
Methods: The mouse bone marrow DC cells were prepared and stat3 was down-regulated with fresh Lentiviral carrying stat3-shRNA. The DCs were loaded with proteins from X-ray-irradiated 4T1 cells Lysates and DC maturation was induced with LPS and IFN-γ. BALB/c mice tumor-bearing model was established by 4T1 cells inoculating breast fat pad and were randomly divided into 5 groups (PBS, sh-con/Len+sh-con DCs, sh-miR-21/Len+sh-con DCs, sh-con/Len+sh-stat3 DCs, sh-miR-21/Len+sh-stat3 DCs) and were injected intratumorally with 2×10^7 TU/100μL per mouse of the Lentiviral carrying sh-miR-21 or sh-con twice for interval of 2 weeks, then 1×10^6 per mouse of sh-stat3 DCs or sh-con DCs were inoculated intratumorally. The tumor growth and the lung metastasis of the tumor-bearing mice were observed. Tumor-infiltrating lymphocytes (TIL) were isolated from tumor tissues and CTL activity were detected by the standard microcytotoxicity assay. IFN-γ producing cells of $CD8^+$ or $CD4^+$ T cells and $CD4^+CD25^+$ Treg cells frequencies in spleen were analyzed by FCM.
Results: stat3 expression in DCs was down-regulated by 50%. The inhibition of miR-21-5p combined sh-stat3-DC vaccine could significantly slow the tumor growth and decrease the pulmonary metastasis. The combination therapy significantly increased CTL activity in tumor tissue and the IFN-γ production in spleen and was no significant effect on the distribution of Treg cells.
Conclusions: the inhibition of miR-21-5p expression in tumor cells could improve the anti-tumor activity of the stat3-shRNA DC vaccine.

P3129

FOXP3/miR139/MET axis inhibits gastric cancer metastasis by reversing the EMT

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Forkhead box P3 (FOXP3) is a member of Forkhead box (FOX) superfamily and was reported to be dysregulated in various types of cancers. However, its anti-invasion and anti-metastasis function and underlying mechanism remains largely unexplored, especially in gastric cancer (GC). In the current study, we found that restoration of FOXP3 induced inhibition of epithelial mesenchymal transition

DOI: 10.1002/eji.201970400

(EMT), invasiveness and metastasis of GC cells in vitro and in vivo. The mRNA microarray analysis found that upregulation of FOXP3 led to the transcript inhibition of oncogene kinase MET and repressed the PI3K/Akt and ERK1/2 signaling. Chromatin immunoprecipitation (ChIP) and luciferase assays revealed that FOXP3 transcriptionally downregulated the expression of MET through direct association with promoter locus of MET. In addition, the miRNA sequencing, ChIP and luciferase assay demonstrate that FOXP3 also promotes miR-139-5p, which specifically targets the 3'-UTR of MET, to further inhibited its expression. Furthermore, the data obtained from TCGA and our clinical GC samples also confirmed the expression correlation of FOXP3, miR-139-5p and MET. GC patients with FOXP3^{low}/miR-139-5p^{low}/MET^{high} expression in GC cells have a poor prognosis. Our data suggested that approaches aimed at restoring FOXP3 function and MET targeted miRNAs in GC cell could help inhibit GC metastasis and may approaches to target GC.

P3130

Decreased or deleted expression of FOXP3, via epigenetic and miRNA inhibition, contributes to the progression of gastric adenocarcinoma

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Background and aims: The expression pattern and underlying cellular function of Treg marker FOXP3 in gastric adenocarcinoma (GAC) remain largely unexplored. The purpose of this study was to investigate the expression and possible role of FOXP3 in GAC.
Methods: We used qPCR and IHC analyses to compare expression of FOXP3 between GAC samples and paired normal gastric tissues, and gene express chip to detect downstream genes of FOXP3 in GAC cell. Luciferase reporter and ChIP assay were used to study interactions between FOXP3 and target genes. We determined the effects of FOXP3 overexpression and knockdown in GAC cell lines and growth of GAC xenograft tumors in nude mice.
Results: FOXP3 is down-regulated or deleted in GC cell lines and GAC tissues. FOXP3 expression was negatively correlated with GAC stage. Low expression of FOXP3 predicts poor prognosis in GAC patients. Overexpression of FOXP3 in vitro inhibited GAC cell proliferation and clonal formation, induced cell cycle arrest and senescence, and its inhibitory function was attenuated when the carboxy-terminal FKH domain was deleted. At the molecular level, proliferation arrest and senescence induced by FOXP3 in GAC cells are mediated by FOXP3 directly targeted c-Myc and p21/Cip1. In addition, overexpression of FOXP3 in GAC cells reduced their growth as xenograft tumors in mice, particularly when mice were given cisplatin.
Conclusions: The study demonstrates the inhibitory function of FOXP3 in GAC development, which might involve the c-Myc and p21/Cip1 signaling, offering a prognostic biomarker for GAC outcome prediction and a therapeutic role for FOXP3 activators.

P3131

Chemotherapeutic tumor microparticles combining low dose irradiation reprogram tumor-promoting macrophages through a tumor-repopulating cell-curtailing pathway

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DOI: 10.1002/eji.201970400

Stem cell-like tumor-repopulating cells (TRCs) have a critical role in establishing a tumor immunosuppressive microenvironment. However, means to enhance antitumor immunity by disrupting TRCs are absent. Our previous studies have shown that tumor cell-derived microparticles (T-MPs) preferentially abrogate TRCs by delivering antitumor drugs into nuclei of TRCs. Here, we show that low doseirradiation (LDI) enhances the effect of cisplatin-packaging T-MPs (Cis-MPs) on TRCs, leading to inhibiting tumor growth in different tumor models. This antitumor effect is not due to the direct killing of tumor cells but is T cell-dependent and relies on macrophages for their efficacy. The underlying mechanism is involved in therapeutic reprograming macrophages from tumor-promotion to tumor-inhibition by disrupting TRCs and curtailing their vicious education on macrophages. These findings provide a novel strategy to reset macrophage polarization and confer their function more like M1 than M2 types with highly promising potential clinical applications.

P3132

Specific decrease in B-cell-derived extracellular vesicles enhances post-chemotherapeutic CD8+ T cell responses

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Systemic immunosuppression greatly affects the chemotherapeutic antitumor effect. Here, we showed that CD19+ extracellular vesicles (EVs) from B cells through CD39 and CD73 vesicle-incorporated proteins hydrolyzed ATP from chemotherapy-treated tumor cells into adenosine, thus impairing CD8+ T cell responses. Serum CD19+ EVs were increased in tumor-bearing mice and patients. Patients with fewer serum CD19+ EVs had a better prognosis after chemotherapy. Upregulated hypoxia-inducible factor-1α (HIF-1α) promoted B cells to release CD19+ EVs by inducing Rab27a mRNA transcription. Rab27a or HIF-1α deficiency in B cells inhibited CD19+ EV production and improved the chemotherapeutic antitumor effect. Silencing of Rab27a in B cells by inactivated Epstein-Barr viruses carrying Rab27a siRNA greatly improved chemotherapeutic efficacy in humanized immunocompromised NOD PrkdcscidIl2rg-/-mice. Thus, decreasing CD19+ EVs holds high potential to improve the chemotherapeutic antitumor effect.

P3133

Anti-tumour research of rBCG using fusion genes

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The random primer Oligo(dT)15 was used in RT-PCR to obtain cDNA from the human granulocyte macro-phage colony stimulating factor (hGM-CSF) gene and the Epstein-Barr virus (EBV) gene BZLF1. Then, the sequence splicing overlap extension method was used to obtain a GCBF fusion gene containing a linker sequence that encoded the polypeptide (Gly4Ser)3. The GCBF fusion gene was inserted into pMV261, which was then transformed into competent E. coli DH5 alpha cells, and positive cells were selected on LB plates. The recombinant plasmid was extracted from E. coli, and BCG was transformed into competent cells. Then, the influence of rBCG on tumour cells was analysed in C57BL/6 mice. We found that rBCG-secreting cytokines hybridized with hGM-CSF and BZLF1 antibodies and that the rBCG vaccine stimulated antibody production in C57BL/6 mice. The specific cytotoxic effects of the spleen cells from the rBCG group on EB virus-positive tumour cells was significantly different from the cytotoxic effects of the con-trol group cells ($P < 0.01$). CD8+ T and CD4+ T lymphocytes were detected in the tumour tissues of the rBCG group mice by flow cytometry, indicating that CD8+ T and CD4+ T lymphocytes infiltrated into the tumour tissue in the mice.

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DOI: 10.1002/eji.201970400

Morphological observations of the tumour sections from the rBCG-immunized mice showed the infiltration of lymphocytes into the tumour tissues. The average rBCG tumour volume was less than the average tumour volume of the control group. Thus, rBCG may inhibit the growth of EB virus-positive tumour cells in mice.

P3134

Genetic polymorphism in virB operon, flagella synthesis, and virulence regulating systems might result in the attenuation mechanism of Brucella. melitensis M5-10

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Brucellosis is a worldwide zoonosis. Vaccination is the most efficient means to prevent the disease. The current licensed attenuated vaccines were developed by sequential passage in non-natural hosts that decreased virulence in its original hosts. The attenuation mechanism of these strains remains largely unknown. In our study, the genome of *B. melitensis* vaccine strain M5-10 was sequenced and compared with that in the virulent 16M strain. Sequence analysis showed that numerous genetic changes occurred. A total of 2854 genetic polymorphic sites, including 2548 SNP, 241 INDEL and 65 MNV were identified. Of the 2074 SNPs in coding regions, 1310 (63.2%) were non-synonymous SNPs. Gene number, percent and N/S ratios were disproportionally distributed among the cog categories. Genetic polymorphic sites were identified in genes of the virB operon, flagella synthesis and virulence regulating systems. The data indicate that changes in some cog categories and virulence genes might result in the attenuation which prompt the development of new vaccines. The genetic changes represent candidate sites for differential diagnosis between the vaccine strains and virulence ones. Transcription analysis of virulence genes showed that expression of dnaK, vjbR were reduced in M5-10 strain. A duplex PCR targeting virB6 and dnaK was successfully used to differentiate between M5-10 and 16M.

P3135

Oral delivery of tumor microparticle vaccines activates NOD2 signaling pathway in ileac epithelium rendering potent antitumor T cell immunity

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Exploiting gut mucosal immunity to design new antitumor vaccination strategy remains unexplored.Tumor cell-derived microparticles (T-MP) are natural biomaterials that are capable of delivering tumor antigens and innate signals to dendritic cells (DC) for tumor-specific T cell immunity. Here, we show that TMPs by oral vaccination route effectively access and activate mucosal epithelium, leading to subsequent antitumor T cell responses. Oral vaccination of T-MPs generated potent inhibitory effect against the growth of B16 melanoma and CT26 colon cancer in mice, which required both T cell and DC activation. TMPs,once entering intestinal lumen, were mainly taken up by ileac intestinal epithelial cells (IEC), where TMPs activated NOD2 and its downstream MAPK and NF-kB, leading to chemokine releasing, including CCL2, from IECs to attract CD103C CD11cC DCs. Furthermore, ileac IECs could transcytose T-MPs to the basolateral site, where T-MPs were captured by those DCs for cross-presentation of loaded antigen contents. Elucidating these molecular and

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DOI: 10.1002/eji.201970400

cellular mechanisms highlights T-MPs as a novel antitumor oral vaccination strategy with great potential of clinical applications.

CAR Engineered Immune Cells

O238

A synthetic biology approach to control the *in-vitro* and *in-vivo* lifespan of CAR T-cells

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CAR T-cell therapy has become a revolutionary alternative in fight against cancer. Despite the remarkable results evidenced in hematologic malignancies, CAR T-cells therapy still shows limitations that threaten to it's mass production, like considerable variability and high production cost. Our group set out to face this problem through the development of a Turnable expression system to control the replicative lifespan or CAR T-cells, avoiding phenotypic alteration and maintaining its effector function. To develop this Turnable expression system, we built a dynamic network to represent the signaling pathway Interaction of CAR T-cell with different cell cycle related genes. Then, using computational tools based on the resolution of Ordinary Differential Equations, which allow us to to predict the dynamics of a reaction network of a CAR T-cell and our hypothetical synthetic network controlled by an inductor molecule. Our approach was validated in a CD38+ multiple myeloma model. $CD8^+$ T-cell population isolated from human PBMCs, was transduced to express an anti CD-38 CAR protein and our synthetic network. The responses that follow the activation of the synthetic network proposed in the model were validated in vitro trough Western Blot, and RT-PCR. The phenotype variation was evaluated using flow cytometry. The functionality of CAR T-cells was tested in a CD38+ myeloma multiple cell line. As result we demonstrate *in-vitro* antitumoral activity without evidence of functional alteration of CAR transduced cells. These results establish the basis for future studies of function and specific cytotoxicity of our model in xenograft models.

O239

TREM1/Dap12 based CAR-T cells show great antitumor activity *in vitro* and *in vivo*

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Chimeric antigen receptor T (CAR-T) cells have obtained huge success in lymphoma. To date, CAR-T cells reported in clinical trials are CD3ζ based CARs, which have demonstrated limitation in solid tumors. It is necessary to design novel form CARs that will be used in clinical therapy. Here, we design an alternative chimeric immunoreceptor design in which a single-chain variable fragment for antigen recognition to the transmembrane and cytoplasmic domains of TREM1, a myeloid cells immunoglobulinlike receptor (TREM). TREM1 along with DAP12 is transduced to human primary T cells. The TREM1/Dap12 based CAR-T cells show potent antitumor activity *in vitro* and in mouse models. Our results indicate that TREM1/Dap12 based CAR-T cells are potential for clinical immunotherapy in the future.

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DOI: 10.1002/eji.201970400

O240

Enhancing CAR T cell therapy by enabling their interaction with antigen-presenting cells

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Responses of solid tumors to chimeric antigen receptor (CAR) T cell therapy are often minimal. This is potentially due to a lack of sustained activation and proliferation of CAR T cells when encountering antigen in a profoundly immunosuppressive tumor microenvironment.
We previously demonstrated that adoptive transfer of CAR T cells in combination with a vaccine can eradicate large tumors in mice. In these studies, the extensive proliferation of CAR T cell was driven by their interaction with the major histocompatibility complex (MHC) on antigen-presenting cells (APCs), mediated by a vaccine-specific T cell receptor (TCR). However, major limitations for this approach include the need for a specific TCR and a specific vaccine for individual patients.
In the current study, we generated novel, yet safe, bispecific proteins to mediate the interaction between APCs and CAR T cells without the need for a specific TCR or vaccine. We termed these bispecifics "**B**ispecific **E**ngagers of **A**PCs and **T** cells (**BEATs**)". Various BEAT formats have been designed; all based on an ability to simultaneously bind the CAR T cells and APCs. CAR T cell proliferation and function was enhanced by BEATs *in vitro* and *in vivo*. Both murine syngeneic and human xenograft solid tumor growth was significantly inhibited when CAR T cells were administered in combination with BEATs. This study demonstrates that facilitating the interaction of CAR T cells with APCs can enhance their antitumor activity, and the great potential of using BEATs with CAR T cells in treating solid cancers.

P0500

Targeting and suppression of HER3-positive breast cancer by T cells expressing a heregulin-1β chimeric antigen receptor

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Chimeric antigen receptor modified T cells (CAR-T) therapy has become one of the hotspots for tumor immunotherapy due to its amazing clinical efficacy. The basic structure of CARs is composed of an extracellular antigen recognition domain, typically a single-chain variable fragment (scFv). Endogenous ligands for tumor antigen may outperform scFvs to serve as a component of CARs with high cancer recognition efficacy and minimized immunogenicity. As a heterodimer and signaling partner of human epidermal growth factor receptor 2 (HER2), HER3 has been involved in tumorigenic signaling and therapeutic resistance in breast cancer. In this study, we engineered T cells with a second-generation CAR consisting of the extracellular domain of heregulin-1β (HRG1β) that is a natural ligand for HER3/HER4, and evaluated the specific cytotoxicity of these CAR-T cells in cultured HER3 positive breast cancer cells and xenograft tumors. Our results showed that HRG1β-CAR was successfully constructed, and CAR-T cells were obtained by lentivirus infection. The HRG1β-CAR-T cells can specifcally recognize and kill HER3-positive breast cancer cell lines SK-BR-3 and BT-474 *in vitro*, and significantly suppress tumor growth and effectively prolong the survival on SK-BR-3 xenograft tumor models. Meanwhile, these CAR-T cells exerted no obvious toxic effect on HER3 knockdown target cells and normal breast epithelial cells. Therefore, Our findings suggest that HRG1β-based CAR-T cells effectively suppress breast cancer driven by HER family receptors, and may provide a novel strategy to overcome cancer resistance to HER2-targeted therapy.

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DOI: 10.1002/eji.201970400

P0501

Eradication of hepatocellular carcinomas by T cells redirected with an NKG2D-CAR

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Despite great success of chimeric antigen receptor T (CAR-T) cell therapy in hematological malignancy, similar clinical benefit against solid tumors is limited including hepatocellular carcinoma (HCC). NKG2D is a receptor located on NK cells and subsets of T cells. NKG2D ligands (NKG2DLs) are generally absent on the surface of normal cells but overexpressed on malignant cells, which suggests that it may be a good candidate in the field of CAR-T therapy. The Cancer Genome Altas (TCGA) results show that *MICA, MICB, ULBP1, ULBP2, ULBP4,* and *ULBP5* are elevated in tumors compared with normal tissues in transcriptional levels (N=50). Besides, MICA (23/32) and ULBP2 (31/32) are overexpressed in liver cancer specimens and some liver cancer cell lines such as SMMC-7721 and MHCC97H. So, we design a NKG2D-BBz CAR and the NKG2D-BBz CAR-T cells efficiently killed the HCC cells *in vitro*, SMMC-7721 and MHCC97H that express high levels of NKG2DLs, but is much less efficient in case of the Hep3B cells with low expression of NKG2DLs. Over-expression of NKG2DL such as MICA or ULBP2 in Hep3B cells can facilitate their efficient targeting by NKG2D-BBz CAR-T cells. In addition, T cells expressing NKG2D-BBz CAR could eradicate HCC xenografts made from SMMC-7721 cells (N=6). Therefore, these results suggest that **NKG2D-BBz CAR-T cells can potently eliminate NKG2DL-high HCC cells both *in vitro* and *in vivo*** and thereby provide a promising therapeutic intervention for NKG2DL-positive HCC.

P0502

T cells expressing an NKG2D chimeric antigen receptor efficiently eliminate glioblastoma cells and cancer stem cells

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Traditional therapies failed to cure most glioblastoma patients with the 5-year survival rate of less than 10%, suggesting the pressing need for new therapeutic approaches. The NKG2D ligands (NKG2DLs) were reported to be highly expressed in the glioblastomas and considered as promising targets for the Chimeric Antigen Receptor (CAR) T-Cell therapy. So, we constructed the NKG2D-BBz CAR expressing extracellular domain of NKG2D into lentiviral expression vector and infected T cells at an multiplicity of infection (MOI) of 10. The CAR expression was approximately 40% by FACS analysis on day 3. We confirmed the high expression of NKG2DLs in human glioblastoma cells, cancer stem cells and tumor samples by flow cytometry and immunohistochemistry. Consistent with this, the NKG2D-BBz CAR-T cells were proved to lyse glioblastoma cells and cancer stem cells efficiently *in vitro* and produce high levels of cytokines like IL-2, IL-10, TNF-α and IFN-γ. Additionally, the CAR-T cells remarkably eliminated xenograft tumors *in vivo* and had no significant treatment-related toxicity in the treated mice. The expression of the CAR delivered by lentiviral particles didn't have any obvious effects on cell proliferation, apoptosis and genomic stability, which suggested the biosafety of our NKG2D-BBz CAR. **Conclusion:** Our findings demonstrated NKG2D CAR T-cells targeted

DOI: 10.1002/eji.201970400

glioblastoma cells and cancer stem cells in an NKG2D-dependent manner, supporting the use of the CAR T-cells for glioblastoma therapeutic.

P0503

"Ectopic antigens" prime "kill me" programmed cytotoxicity by γδ T cells and CARγδ-αβT cell

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Tumor immunotherapy ascribed in part to T cell immunity directed against certain tumor antigens. Different from αβT cells, innate-like γδ T cells recognize a series of damage-associated molecular patterns by means of non MHC-restricted antigen presentation. Thus, γδ T cells have significant advantages in tumor immunotherapy.
In this study we constructed a novel CARγδ molecule with a single chain Vγ-Vδ as its extracellular domain. The Vδ segment was modified by CDR3δ sequence (GTM or OT3) derived from tumor-infiltrated γδT cells, both of which could specifically bind to a variety of different tumor tissues, tumor cell lines and stress-induced proteins. We found that CARγδ-αβT cells could possess strong cytotoxic activities against a broad-spectrum of tumor cells *in vitro* through a Granzyme B dependent pathway. A high throughput screening using 20K human ProteinChip was use to further identify the potential targets of CARγδ-αβT cells. To our surprise, some intracellular proteins, including NCL and AGR2, were identified with specific binding activities to GTM or OT3 peptides. The results showed that these intracellular proteins served as "ectopic antigens", which could ectopically express on the surface of cancer cells or DNA damaged cells, and trigger the activation and "kill me" programmed cytotoxicity of γδT cells.
Taken together, combination of CAR-T cells and γδTCR, the novel CARγδ-αβT cells demonstrate strong cytotoxic activities against a broad-spectrum of solid tumors by targeting a series of "ectopic antigens" on tumor cells. CARγδ-αβT cell and ectopic antigens will provide promising strategies for γδT cell-based multi-targets cytotoxicity to solid tumors.

P0504

Target-decorating of tumors potentiates chimeric antigen receptor-T cell therapy

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Chimeric antigen receptor (CAR)-T cell therapy has been noticeable successful in the treatment of hematologic cancers and shows a promising potential to treat solid tumors. However, one challenge for CAR-T in solid tumor treatment is the tumor target problem resulting from the heterogeneous antigen expression and antigen escape. In this study, we constructed tumor target-decorating system in which tumor cell membrane modified with exogenous antigens and followed by adoptive transfer of CAR-T cells recognizing the exogenous targets decorated to tumor cell membrane. EGFRvIII peptide was delivered into the membranes of human gastric carcinoma cell lines MKN45 and AGS by fusogenic liposomes in vitro, as was detected by anti-EGFRvIII antibody. Laser scanning confocal microscopy showed that EGFRvIII peptide was located to the tumor cell membranes. EGFRvIII peptide decorated to the cell membranes demonstrated the high stability with a half-life period of 8 days. IFN-γ production of CAR-T cells stimulated with tumor cells modified by EGFRvIII peptide increased in a peptide dose-dependent manner. Increased cell surface expression of the CD25 and

DOI: 10.1002/eji.201970400

CD69 proteins occurred after T cell activated by target-decorated tumor cells. Tumor cells decorated with EGFRvIII peptide were dose-dependedly killed by CAR-T cells. The tumor target-decorating strategy enables CAR-T cell therapy to treat all kinds of tumors regardless of the expression of tumor antigens, indicating a great potential in the treatment of tumors with heterogeneous antigen expression and antigen escape.

P0505

Immunomodulation of EAE using car-T cells that target autoreactive epitopes

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Autoimmune diseases are often mediated by abnormal activation of autoreactive CD4+ T cells. These cells interact with antigen presenting cells (APCs) that express disease associated autoantigens on MHC class II molecules in a pro-inflammatory context, thereby inducing an inflammatory response against self. Multiple sclerosis (MS) is a CD4+ mediated demyelinating autoimmune disease that is associated with HLA-DR2 MHC class II allele. Presentation of myelin associated epitopes, such as myelin oligodendrocyte glycoprotein (MOG) on HLA-DR2 by APCs in a pro-inflammatory context, results in autoreactive T cell activation and consequently nerve cells damage. Current MS treatments are based on immune response suppression and therefore development of antigen-specific treatments is highly desirable. The APC:T cell interactions are the core drivers of immune responses and also exhibit a specific check-point in the inflammatory process. Thus, we suggest that neutralization of myelin-associated epitopes presentation by APCs should decrease myelin-specific CD4+ T cell activation and consequently reduce inflammation during MS. Accordingly; we have isolated a T cell receptor like (TCRL) antibody that specifically targets DR2/MOG35-55 and constructed second-generation chimeric antigen receptor (CAR) molecules based on its variable regions. DR2/MOG35-55 specific CAR-CD8+ T cells successfully induced DR2/MOG35-55 APCs death *in vitro*. We are currently conducting *in-vivo* experiments in experimental autoimmune encephalomyelitis diseased HLA-DR2 transgenic mice to assess the function of CAR-CD8+ T cells. These data may provide a proof of concept for the notion that elimination of disease-specific APCs by TCRL-based CAR-CD8+ T cells may serve as an antigen-specific therapeutic approach for autoimmune disease.

P0506

Combination therapy of CAR-NK-cells and anti-PD-1 antibody results in high efficacy against advanced-stage glioblastoma in syngeneic mouse model and induces protective anti-tumor immunity *in vivo*

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Introduction: Checkpoint inhibitors as well as adoptive cell therapy hold great promise for cancer treatment and encouraging treatment responses have already been demonstrated in different cancer indications. Glioblastoma (GBM) is the most common and aggressive primary brain tumor. Standard therapy has very limited efficacy in the majority of patients. Analysis of the GBM tumor

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DOI: 10.1002/eji.201970400

microenvironment (TME) has shown prominent immunosuppressive features including expression of PD-L1 on tumor cells and increased frequency of FOX-P3 positive regulatory T cells. While the surrounding brain is HER2-negative, GBM tumors are frequently HER2-positive, suggesting HER2 as a promising target for adoptive immunotherapy.
Materials and methods: The murine glioma cell line GL261 was transfected with HER2. Tumor cells were orthotopically implanted into C57BL/6 mice and treated either with HER2-specific NK-92/5.28.z cells alone or in combination with an anti-PD-1 antibody. Effects on tumor growth and survival were determined, and lymphocyte infiltration and immunosuppressive TME were characterized.
Results: Combined treatment with NK-92/5.28.z cells and anti-PD-1 antibody resulted in synergistic tumor regression and long-term survival of advanced-stage tumor bearing mice. Analysis of TME showed enhanced cytotoxic lymphocyte infiltration and altered profiles of exhaustion markers in tumor and immune cells.
Conclusion: These data demonstrate that efficacy of NK-92/5.28.z cells can be enhanced by co-therapy with checkpoint inhibitors, resulting in successful treatment of advanced tumors refractory to mono-therapy. Furthermore, it induces a cytotoxic rather than immunosuppressive TME, leading to a primed immune system. To address this question in a clinical setting, we are planning a phase I clinical study (CAR2BRAIN-CHECK).

P1093

Potent antitumor efficacy of anti-EpCAM CAR T cells in relapse acute myeloid leukemia

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Patients with acute myeloid leukemia (AML) have a dismal prognosis that 70% face relapse and drug resistance. Chimeric antigen receptor T (CAR T) cell therapy has produced exciting results in $CD19^+$ malignancies and may overcome many of the limitations of conventional leukemia therapies. Our previous research found that EpCAM is frequently overexpressed in patients with AML, with EpCAM leukemic cells exhibiting enhanced chemoresistance and oncogenesis, suggesting that EpCAM exhibits promise as a novel target for the treatment of relapse AML. Therefore, we developed CAR T cells to target EpCAM using the anti-EpCAM single chain variable fragment, in combination with a CD28/41BB costimulatory domain and CD3-ζ signaling domain. Then we tested the activity of these cells against $EpCAM^+$ cell lines. Anti-EpCAM CAR T exhibited significant effector functions in antigen-dependent cytokine generation and killing $EpCAM^+$ K562 and HL60 cells in vitro,and EpCAM CAR T resulted in eradication of leukemia in immunodeficient xenograft mice of AML.These results suggest that EpCAM CAR T cells are a promising immunotherapy for the treatment of relapse AML.

P1094

Potent antitumor efficacy of anti-LunX CAR-T cells in lung cancer

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Nowadays, many breakthroughs have been made in the targeting of hematologic tumors, while the research on solid tumors is very limited. Genetically modified T cells expressing chimeric antigen receptors demonstrate robust responses against lineage restricted, non-essential targets in

DOI: 10.1002/eji.201970400

hematologic cancers. LunX belongs to the family of PLUNC, our previous studies have found that LunX can be used as specific biomarker to diagnose lung cancer. It only expression in lung cancer tissue, and no expression in normal lung tissue, and LunX is related to the growth and metastasis. Therefore, using LunX protein as the target of CAR-T therapy to treat lung cancer showing the great significance and value. We developed a CAR that recognized LunX protein, which demonstrated target-specific cytotoxicity and successfully controlled lung cancer cells growth in vitro. In addition, we find that when co-culturing CAR-LunX T cells and lung cancer cells can stimulate CAR-LunX-T cells secrete series of cytokines to kill lung cancer cells. These findings demonstrate the therapeutic efficacy of CAR T cells directed against LunX and present LunX protein as a novel class of targets for tumor therapy with engineered T cells.

P1095

In vivo and in vitro studies and molecular mechanisms of chimeric antigen receptor T cells against CD19

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Background: CD19-positive B cell derived lymphocytic leukaemia and lymphomas remain the significant causes of cancer-associated mortality in hematological malignancies. The genetic engineered T-cells with chimeric antigen receptors targeting CD19 is one of potential immunotherapeutic modalities.
Methods: *PiggyBac*, a transposon/transposase system was used to express the CAR in PBMC. CAR-T cells were selectively propagated by artificial antigen presenting cells. After 21 days, the positive rate of CAR and the memory phenotype of CD44+CD62L+ was analyzed by flow cytometry. Meanwhile, the cytotoxicities of CAR-T on CD19 expressing cell lines (NAMALWA, Raji, $K562^{CD19+}$) were evaluated. The *in vivo* effeciency of CAR-T was detected in murine xenografts model bearing NAMALWA and Raji cells. We used *in vivo* bioluminescence and fluorescence imaging system to detect tumor process. The molecular mechanisms involved in above effects were explored by ELISA and western blot.
Results: The positive expression rate of CAR was 36.33±4.53% on average, and the memory phenotype of CD44+CD62L+ was 53.50±6.98%. *In vivo* and *in vitro* studies showed that CAR-T significantly eliminated CD19-positive cancer cells compared with control. The mechanism studies found that CAR-T could eradicate cancer cells mainly by releasing IFN-γ, perforin and grazyme-B. Moreover, the secretion of GM-CSF and IL-2 was also up-regulated after CAR-T treatment, while the secretion level of inflammatory factors TNF-α and IL-6 was no obviously changed.
Conclusion: The CAR-T cells targeting CD19-positive hematological malignancies had been successfully constructed with significant anti-tumor effects, which provides a new therapeutic regimen for this kind of cancer.

DOI: 10.1002/eji.201970400

P1096

Efficacy and influencing factors of CD19 CAR-T cell therapy for children and adults with r/r B-ALL

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Background: Chimeric antigen receptor T (CAR-T) cells have shown exciting response rate in children and young adults with r/r B-ALL, but how to obtain long-term survival? Which clinical factors were related with this new immunotherapy? More clinical trials may be needed.
Method: We conducted a phase 2 clinical trial in which child and adult patients with r/r B-ALL received autologous T cells expressing a CD19-specific chimeric antigen receptor, assess the safety and efficacy, especially the factors which may affect the response rate and long-term survival.
Results: A total of 40 patients with r/r B-ALL were enrolled. Of 32 evaluable patients (12 pediatric and 20 adult patients), grade ≥3 cytokine release syndrome (CRS) occurred in 7 patients (21.8%). Overall complete remission rate with or without hematologic recovery was 78.1%. Complete remission rate in pediatric and adult patients was 75.0% and 80.0% respectively. The rates of overall (OS) and event-free survival (EFS) were 65.3% and 28.8% respectively at 1 year. Patients with extramedullary disease had lower remission and shorter survive. No proliferation *in vivo*, higher regulatory T cells and high risk cytogenetic factor significantly also related to lower remission or poor survive.
Conclusion: The CD19 CAR-T cell can induce high remission rate in r/r B-ALL with good safety profile. Durable remission observed in some patients. Extramedullary disease, no proliferation, higher Tregs and high risk cytogenetic factors may hinder the efficacy.

P1097

EGFR-specific switchable CAR-T cells for targeted therapy of gastric cancer

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Chimeric antigen receptor（CAR）has achieved a great success in clinical trials against hematological cancers, but has experienced challenges in solid tumors, because CAR-T cells can exhibit excessive activity that is difficult to control and can cause severe toxicity. To control these side effects, we designed EGFR-specific CARs that enable small-molecule control over T cell therapeutic functions while still retaining antigen specificity. The small molecule is proved a rapamycin analog that can heterodimerize domain FKBP/FRB, resulting in the activation of T cells. Quantitation of IL-2 and IFN-γ production significantly increased when switchable CAR-T cells were mixed with gastric cancer cells MKN45 (EGFR+) and the small molecule ($p<0.05$), while stayed the same without the small molecule, compared with the CAR-T cells alone. Our data demonstrate that switchable CAR-Ts can kill target cell population only in the presence of the small molecule, which could allow physicians to precisely control the timing, location, and dosage of T cell activity, there by mitigating toxicity.

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DOI: 10.1002/eji.201970400

P1098

Selective elimination of autoreactive B cells in patients with neutralizing anti-interferon-γ autoantibodies by reengineering chimeric autoantibody receptor T cell

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The emerging roles of neutralizing anti-interferon-γ autoantibodies (nAIGAs) in severe intracellular pathogen infection is gaining recognition. nAIGAs diminish the interferon-γ (IFN-γ) immunity, predisposing patients to mycobacteria, salmonellosis and other intramacrophagic bacterial infection. Therefore, elimination of nAIGAs is considered the ultimate approach for treating nAIGAs-associated diseases. Recently, a chimeric autoantibody receptor (CAAR) T cell was developed and successfully eliminated autoreactive B cell derived from pemphigus vulgaris patients, presenting a promising scheme to treat nAIGAs diseases. In the current study, we would like to generate an IFN-γ CAAR T cell to annihilate nAIGAs-producing B cells but cause no off-target toxicity. Previously we have identified a major epitope of IFN-γ recognized by nAIGAs from patients with mycobacterial diseases. Several AIGAs have been cloned from those patients and their physical and biological properties have been characterized. Moreover, it´s well described that the residues in AB loops and H111 of IFN-γ are critical for IFN-γ-IFN-γ receptor (IFN-γR) complex formation. Collectively, the recombinant IFN-γ will be designed as the CAAR to recognize AIGA on autoreactive B cell but ignore IFN-γR on almost every cell. The specificity of the recombinant IFN-γ and CAAR with nAIGAs will be assessed in molecular and cell-based experiment respectively. Furthermore, to mimic actual condition in patients, the cytotoxicity of CAAR T cell will be determined in the presence of nAIGAs as well. Taken together, we hope the reengineering IFN-γ CAAR T cell can eradicate the pathological nAIGAs B cells, providing a new strategy for anti-cytokine autoantibody diseases.

P1099

Human CD8$^+$ Treg expressing a MHC-specific chimeric antigen receptor display enhanced suppression of human skin rejection and GVHD in NSG mice

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Polyclonal CD8$^+$CD45RC$^{low/-}$ Tregs are potent regulatory cells able to control solid organ transplantation rejection and even induce tolerance. However, donor-specific Treg are more potent than polyclonal Tregs to prevent acute as well as chronic rejection in rodent models. The difficulty to identify disease relevant antigens able to stimulate Tregs has dampered the possibility to obtain antigen-specific Tregs. To bypass this requirement, we stably introduced a chimeric antigen receptor derived from a HLA-A*02 antigen-specific antibody (A2-CAR) in human CD8$^+$Tregs and expanded them. We demonstrated that A2-CAR specific CD8$^+$Tregs were not phenotypically altered by the process while they were more potent suppressor of HLA-A*02 mismatch induced immune responses both in vitro and in vivo in models of human skin rejection and GVHD in NSG mice. We showed that skin graft integrity was preserved >100 days in vivo after administration of A2-CAR CD8$^+$Tregs. Interaction between the A2-CAR CD8$^+$ Tregs and HLA-A*02$^+$ kidney endothelial cells (EC) resulted in a fine tuned and pro-tolerogenic activation of the EC cells without cytotoxicity. Together our results

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DOI: 10.1002/eji.201970400

demonstrated the relevance of the CAR engineering approach to develop CAR-CD8$^+$ Tregs for clinical trials in transplantation.

P1100

Optimization of human T cell activation and expansion protocols improves efficiency of genetic modification and overall cell yield

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Cancer immunotherapy using CAR T-cells is a rapidly progressing field, and manufacturing these cells is a complex process that requires multiple optimization steps. We have developed reagents for the isolation, activation, and expansion of human T cells that will be available for clinical cell therapy manufacturing. Soluble ImmunoCult™ Human T Cell Activator-stimulated T cells can be genetically modified and expanded in ImmunoCult™-XF, a serum- and xeno-free T cell expansion medium. Here, we present several optimization strategies with ImmunoCult™ products in order to obtain high gene-editing efficiency and maximum cell yield. By evaluating the activation dynamics of T cells and determining the optimal transfection time points, the transfection efficiency can be substantially improved in both CRISPR/Cas9- and lentiviral-mediated gene-modification methods. Our study also suggests that maintaining T cells at lower cell density after the third day following activation greatly improves cell viability and cumulative cell growth, resulting in a more than 1000-fold expansion of total human T cells with >85% viability over 10-12 days of culture. Expanded T cells co-express CD45RO and CD62L, with low expression of PD-1. The workflow described here has generated T cell receptor alpha beta (TCRαβ) KO T cells from healthy donors with up to 90% knockout efficiency. The purity of TCRαβ KO T cells can be further increased with the use of an EasySep™ Human TCRαβ depletion kit. Taken together, the processes outlined in this study can be easily and rapidly implemented to improve T cell manufacturing efficacy.

P1690

Potent anti-leukemia activities of humanized CD19-targeted CAR-T cells in patients with relapsed/refractory acute lymphoblastic leukemia

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Chimeric antigen receptor T (CAR-T) cell therapy has showed promising results for treating relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). However, immune response induced by murine single-chain variable fragment (scFv) of the CAR leads to its premature elimination, and may be overcome by humanized CAR designs. A pilot phase 1 study of CAR-modified T cells containing a humanized anti-CD19 scFv domain (hCART19s) was performed with recruitment of children and adults with R/R B-ALL with or without prior exposure to a murine CD19 CAR-T cell product. After lymphodepletion chemotherapy with cyclophosphamide and fludarabine, patients received a single dose (1×10^6/kg) of autologous hCART19s infusion. The post-infusion responses, toxicities, expansion and persistence of hCART19s in patients were observed and monitored. 18 R/R B-ALL patients were treated with hCART19s. Among 15 patients without previous CAR-T therapy, 13 achieved complete remission (CR) or CR with incomplete count recovery (CRi) on day 30, while 1 of 3 patients, who failed to second murine CAR-T infusion, achieved CR after hCART19s infusion. At day

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DOI: 10.1002/eji.201970400

180, the overall survival rate and leukemia-free survival rate was 65.8% and 71.4%, respectively. The cumulative incidence of relapse and non-relapse mortality rate was 22.6% and 7.1%. During treatment, 13 patients developed grade 1-2 cytokine release syndrome (CRS), 4 patients developed grade 3-5 CRS, and 1 patient experienced reversible neurotoxicity. Our results indicated that hCART19s could induce remissions in patients with R/R B-ALL, especially in patients who received reinfusion of murine CAR-T previously.

P1691

IL-6 trans-signaling robustly promotes the expansion and antitumor activity of CAR T cells

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Chimeric antigen receptor T (CAR T) cell immunotherapy targeting CD19 positive B cells malignancies induces promising clinical remissions. However, the treatments are often accompanied with high levels of IL-6 characterized cytokine release syndrome (CRS). The IL-6 trans-signaling plays important roles in regulating immune responses, cell survival, apoptosis, and proliferation. To our knowledge, comprehensive assessment of the IL-6 trans-signaling in biologic functions of CAR T has not been well explored. We thus tested whether the IL-6 trans-signaling promotes expansion and anti-tumor activity of CAR T. For simulating the functions of IL-6 trans-signaling, we next constructed a constitutively expression of Hyper IL-6 (HIL-6) with various CAR T cells. Anti-tumor efficacy of HIL-6/CAR T cells were confirmed in leukemia and solid tumors. Our results demonstrate that HIL-6-CAR T cells are more effective and proliferative after targeting tumors than CAR T alone in vitro and in pre-clinical models. Transcriptomic profiling revealed that the expression of genes that facilitate T-cell migration, early memory differentiation and the IL-6/GP130/STAT3 signaling was upregulated in HIL-6 simulated CAR T cells compared to the Mock T cells. IL-6 trans-signaling activates downstream signaling via GP130, mechanistically constituted GP130 activation demonstrate superior anti-tumor activity with delayed xGVHD in pre-clinical model of leukemia and solid tumor. Taken together, these results showed that IL-6 trans-signaling can significantly enhanced expansion anti-tumor activity of CAR T cells via GP130/STAT3 activation and GP130/CAR T may have the potential to promote anti-tumor effects with reduced toxicity in vivo.

P1692

Therapy of fourth-generation CD19-specific CAR T cells achieves good response with no severe CRES or CRS in adult patients with relapsed and refractory ALL

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Chimeric antigen receptor (CAR) targeting CD19 T-cell therapy has been reported to induce high response rate in patients with refractory and relapsed(R/R) B-cell acute lymphoblastic leukaemia (ALL). However, severe adverse reactions including cytokine release syndrome (CRS) and CAR T-cell-related encephalopathy syndrome (CRES), were significant challenges to executing this strategy. We conducted a phase 1, single-cohort clinical trial to explore the safety and feasibility of fourth-generation CAR T-cell in adult patients with R/R B-cell ALL. T cells were transduced with lentiviral vectors encoding a fourth-generation anti-CD19 CAR containing a chimeric intracellular signalling element (CD28/CD27/CD3ζ-iCasp9). Safety, efficacy and long-term outcomes were assessed.25 patients enrolled the clinical trial.Under a median CAR T-cell dose of 7.133×10^5/kg, the objective

DOI: 10.1002/eji.201970400

response rate was 92%, and 80% of patients obtained minimal residual disease (MRD) -negative complete remission (CR). Patients with lower disease burden have more opportunity to achieve MRD-negative remission. A total of 12 patients (48%) experienced CRS in our clinical trial. Patients with higher than 5×10^5/kgof CAR T-cell infusion had a higher incidence of CRS than patients infused with lower doses. Notably, no grade 3 or higher CRS occurred in the 25 patients, neither with any grade of CRES. With a median follow-up of 291 days, the median disease-free survival (DFS)was 257 days, and the median overall survival (OS)was 267 days. Our study showed that this simple cost-efficient, time-saving procedure of producing fourth-generation CAR T-cell is a promising strategy, which remains potent anti-leukaemia efficacy while without CRES or severe CRS.

P1693

Antagonism of IAPs enhances CAR T cell efficacy

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Chimeric antigen receptor (CAR) T cell therapy has proven highly successful in the treatment of haematological malignancies, notably acute lymphoblastic leukaemia and B cell lymphoma. However, the efficacy of CAR T cells against solid tumours is poor, likely due to tumour-associated immunosuppression. Here, we demonstrate that antagonising Inhibitor of Apoptosis Proteins (IAPs) with the clinical smac-mimetic, birinapant, significantly enhanced the anti-tumour activity of CAR T cells in a TNF-dependent manner. Enhanced tumour cell death occurred independently of the perforin-mediated granule exocytosis pathway, underscoring the cytotoxic potential of CAR T cell-derived TNF. Importantly, combining CAR T cell therapy with birinapant dramatically reduced established tumour growth *in vivo,* where either therapy alone was relatively ineffective. Using patient biopsy-derived tumouroids, we demonstrate the synergistic potential of combining CAR T cell therapy with smac-mimetics. Taken together, we identify CAR T cell-derived TNF as a potent anti-tumour effector, which can be further harnessed by smac-mimetics.

P1694

High expansion level and long term persistence of BCMA CAR-T cells contribute to potent anti-tumor activity against heavily treated multiple myeloma patients

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BCMA CAR-T cells have demonstrated substantial preclinical and clinical activity for relapsed/refractory multiple myeloma (RRMM) patients. Previous studies indicated higher CAR-T cell expansion in vivo achieved better remission. Our study was to evaluate the safety and efficacy of autologous BCMA CAR-T treatment for RRMM. Patients were subjected to a lymphodepleting regimen with FC chemotherapy before CAR-T infusion. 17 patients were infused with BCMA CAR-Ts. Patients

DOI: 10.1002/eji.201970400

had a median of 14 prior regimens. All the 17 patients were eligible for initial evaluation of early clinical response. ORR was 100%. All the patients achieved MRD negative in bone marrow by flow cytometry. Partial response (1 PR, 5.9%), very good PR (6 VGPR, 35.3%), and complete response (10 CR, 58.9%) within 12 weeks post CAR-T infusion were achieved. Durable responses were found in 15/17 patients (88.2%). One patient died of severe infection by Day 9. All patients had detectable CAR-T expansion by flow cytometry from Day 3 post CAR-T cell infusion. Expansion peaks were found between Day 6 to Day 17. The peak CAR-T cell expansion in CD3+ lymphocytes of peripheral blood (PB) varied from 35% to 95% with a median percentage of 83.5%. Cytokine release syndrome (CRS) was reported in all the 17 patients, including 2 with Grade 1, 4 with Grade 2 and 11 with Grade 3. CAR-T -related AEs were 9 pancytopenia, 14 fever , 8 nausea, 1 heart failure. We observed high expansion level and long term persistence of BCMA CAR-T cells contribute to potent anti-myeloma activity.

P2286

TCR-T immunotherapy targeting EBV-associated malignancies

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In the most recent years, CAR-T therapy has been successfully applied to hematopoietic malignancies with significant clinical outcomes, while **TCR-T** is still in preclinical even though it is complementary to CAR-T and may have greater potential for solid tumors. In this study, **we identified a set of TCRs targeting EBV latent membrane protein LMP2A**, which contributes to multiple malignancies including lymphoma and nasopharyngeal carcinoma. Interestingly, response to different pMHC epitopes varies greatly. **TCR pairs share high similarity and even identical for both α chain and β chain among individuals** when using a pMHC-A*1101 tetramer. While for the generated A*0201 andA*2402 tetramers, the identified TCRs are private. By expressing the paired TCR genes in $\alpha^-\beta^-$ Jurkat cell line, we **measured TCR structure affinity with micropipette technique, which in accordance with functional activity evaluated by signaling cascade**. Furthermore, we generated primary TCR-T cells for therapeutic application. Notably, **engineered TCR-T cells expressing high affinity TCRs, especially our public TCR E141, could efficiently eliminate LMP2A-expressing cells with superior cytokine release and toxicity in vitro**. Besides, the **TCR-T cells could significantly suppress tumor progression and prolong mice survival in vivo.** Together, these data provide the evidence for clinical trials and pave the way to TCR-T cell immunotherapy against EBV-associated diseases and malignancies. And **public TCRs might elicit a better immune response and lower cytotoxicity**, which could **broaden the source** of TCR-T immunotherapy and benefit the refractory patients losing high affinity TCRs during the course of diseases.

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DOI: 10.1002/eji.201970400

P2287

CD22 CAR T-cell therapy in refractory or relapsed B acute lymphoblastic leukemia

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Background: Despite worldwide promising clinical outcome of CD19 CAR T-cell therapy for B-ALL, relapse after this therapy is associated with poor prognosis and has become an urgent problem to be solved.
Methods: We conducted a phase 1 trial of anti-CD22 CAR T-cell therapy in 34 pediatric and adult B-ALL patients that are refractory or relapsed after previous chemotherapy, stem cell transplantation, and anti-CD19 CAR T-cell therapy. Patients were monitored for remission rate, safety and long-term outcome.
Results: A total of 34 pediatric and adult patients received anti-CD22 CAR T cells. CR/CRi was achieved in 70.5% of all 34 enrolled patients. Most patients only experienced mild CRS and neurotoxicity. Seven CR patients received no further treatment, and 3 of them remained in remission at 6, 6.6, and 14 months after infusion. Eleven CR patients were promptly bridged to transplantation, and resulted in 1-year LFS of 71.6%. Tumor cell CD22 antigen loss or mutation was not observed in relapsed patients.Though normal B cells were absent in all patients with continued remission, normal B cells were present in all the relapsed patients evaluated, suggesting persistence of CD22 CAR T-cell surveillance is required for preventing relapse.
Conclusions: Anti-CD22 CAR T-cell therapy can be effective in treating B-ALL patients who failed previous CD19 CAR T-cell therapy, with a low incidence of severe CRS. Subsequent bridging to allogeneic hematopoietic stem cell transplantation may further reduce the relapse rate. Relapse was not associated with CD22 antigen loss.

P2288

Improved efficacy and safety of a dual-target CAR-T cell therapy targeting BCMA and CD38 for relapsed/refractory multiple myeloma from a phase I study

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Background: Encouraging results are seen from several early phase clinical trials on the cellular immunotherapy for relapsed/refractory (RR) multiple myeloma (MM), including anti-B cell maturation antigen (BCMA) CAR-T cell therapy and anti-CD38 daratumumab (DARA) monotherapy. **We conducted an anti-BCMA and anti-CD38 dual-target CAR-T cell in order to avoid negative relapse of single-target loss. Here we report a promising results on 12 patients about the efficacy and safety in the ongoing phase I study(ChiCTR1800018143).**
Results: As of January 31, 2019, 12 patients consisting of 66.67%(8/12) with genetic abnormalities,16.7%(2/12) with ASCT and 16.7%(2/12) with extrandal disease, have been infused with the dual-target CAR-T cell product. The ORR is 83.3%(10/12), including 5 sCR, 2 VGPR and 3 PR. 60%(3/5) have still maintained sCR over 2 month and the longest one is over 26 weeks, and allextramedullary lesions have been eliminated. The 8-week PFS is 0.90 and 12-week PFS is 0.77.

DOI: 10.1002/eji.201970400

The CAR-T cells still exist in the patients' peripheral blood by flow cytometry and q- PCR. 83.3%(10/12) were observed with CRS and severe CRS(Grade 3 and 4) accounted for 33.33%(4/12). No neurotoxicity was observed. Almost all the patients were observed with hematological toxicities. **Conclusion: Our study demonstrates an improved efficacy with dual-target CAR-T therapy targeting BCMA and CD38 for RRMM with a high ORR, especially a higher rate and a longer duration of sCR. CRS and hematological toxicities are observed but manageable.**These initial data provide strong evidence to support the further development of the dual-target CAR-T therapy for RRMM.

P2290

Peripheral eosinophil counts predict efficacy of anti-CD19 CAR-T cell therapy against B-lineage non-Hodgkin lymphoma

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The onset of cytokine release syndrome (CRS) and the in vivo persistence of anti-CD19 chimeric antigen receptor T-cells (CAR-T) after infusion are known to correlate with clinical responsiveness. However, baseline biomarkers capable of predicting the prognosis of patients with B-lineage non-Hodgkin lymphoma (B-NHL) remain elusive. Here, we aimed to identify blood cell populations associated with beneficial outcomes in CAR-T-treated B-NHL patients. Specifically, we quantified peripheral blood counts and numbers of CAR-T cells by retrospectively analyzing an anti-CD19 CAR-T trial including 17 B-NHL patients. Following an up to 30 month observation period, B-NHL patients with higher baseline eosinophils showed an increased objective response rate compared with the eosinophil-low subgroup. A greater baseline eosinophil number was also significantly associated with durable progression-free survival. In a pre-clinical model, eosinophil depletion impairs the intratumoral infiltration of subsequently transferred CAR-T cells, resulting in reduced CAR-T antitumor efficacy. This study suggests that peripheral eosinophils may serve as both a stratification biomarker and recruitment machinery to facilitate anti-CD19 CAR-T therapy in B-NHL patients.

P2291

A novel Epstein-Barr virus-latent membrane protein-1-specific T-cell receptor for TCR gene therapy

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Adoptive transfer of genetically engineered T-cells to express antigen-specific T-cell-receptor (TCR) is a feasible and effective therapeutic approach for numerous types of cancers, including Epstein-Barr virus (EBV)-associated malignancies. Here, we describe a TCR gene transfer regimen to rapidly and reliably generate T-cells specific to EBV-encoded latent membrane protein-1 (LMP1), which is a

DOI: 10.1002/eji.201970400

potential target for T-cell-based immunotherapy. A novel TCR specific to LMP1 (LMP1-TCR) was isolated from HLA-A*0201 transgenic mice that were immunized with the minimal epitope $LMP1_{166}$ (TLLVDLLWL), and LMP1-TCR-transduced peripheral blood lymphocytes were evaluated for functional specificities. Both human CD8 and CD4 T-cells expressing the LMP1-TCR provoked high levels of cytokine secretion and cytolytic activity towards peptide-pulsed and LMP1-expressing tumor cells. Notably, recognition of these T-cells to peptide-pulsed cells was maintained at low concentration of peptide, implying that the LMP1-TCR has high avidity. Infusion of these engineered T-cells revealed remarkable therapeutic effects and inhibition of tumor growth in a preclinical xenogeneic model. We observed explosive *ex vivo* proliferation of functional TCR-transduced T-cells with artificial antigen-presenting cells that express co-stimulatory molecules CD80 and 4-1BBL. These data suggest that the novel TCR-targeting LMP1 might allow the potential design of T-cell-based immunotherapeutic strategies against EBV-positive malignancies.

P2292

LCK-independent chimeric antigen receptor T cell signaling improves specificity and function

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By merging antibody technology and TCR signaling, T cells equipped with a chimeric antigen receptor (CAR) can be redirected to the target in a non-MHC restricted fashion. Unprecedented response rates and durability of response has been reported in acute and chronic leukaemia. However, severe toxicity has been observed due to massive, and to some extent nonspecific, T cell activation. Although CARs have been improved in design, and investigated heavily, how a CAR transduces its signaling, and whether critical elements involved in normal TCR signaling are kept or rewired in CAR signaling, are unclear. A better understanding of these questions would greatly facilitate improvements in CAR-T technology in clinical practice. A CAR targeting a peptide-MHC complex has been used to mimic the process of TCR recognition and to take advantage of CAR technologies developed so far. We discovered that CAR signal transduction occurs without the presence of the normally crucial LCK kinase, demonstrating an important, previously unknown characteristic. Using CRIPSPR/Cas9 deletion, we demonstrated that another SFK, FYN, can not only substitute for LCK, but is in fact crucial for CAR signalling. We generated an LCK-deficient CAR-T cell, finding that it improves the specificity of CAR-T cells, reducing the risk of autoimmune side effects by quenching TCR signaling, thus providing a new approach for allogeneic CAR-T therapy. The LCK-deficient CAR-T cells also showed a less exhausted phenotype after encountering antigen. These findings about the non-canonical signaling of CAR-T cells have important clinical implications, and renewing our perceptions of TCR signaling.

P3136

MicroRNA-374b inhibits liver cancer progression via down regulating programmed cell death-1 expression on cytokine-induced killer cells

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Programmed cell death-1 (PD-1) is an oncogene associated with suppressing proliferation and cytokine production of T cells in the progression of liver cancer. microRNAs (miRs) regulate gene expression via specific binding to the target 3´untranslated region of mRNA. In the present study, miR-

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DOI: 10.1002/eji.201970400

374b was indicated to interact with PD-1 and affect the tumor-targeting capacity of cytokine-induced killer (CIK) cells. miR-374b inhibitor significantly increased PD-1 expression in CIK cells. A synthetic small interfering (si)RNA targeting PD-1 was employed to silence the expression level of PD-1 in CIK cells. Then, the antitumor effect of siPD-1 in CIK cells was investigated. *In vitro* study demonstrated that IFN-γ secretion and the concentration of lactate dehydrogenase were significantly increased in the PD-1 knockdown group; however, the viability of HepG2 cells in the PD-1 knockdown group had significantly decreased, compared with the HepG2 cells in the negative control group. *In vivo* study indicated that mice inoculated with HepG2 cells and CIK cells with PD-1 knocked down had a significantly smaller tumor volume, compared with the control group. To conclude, human CIK cells transfected with siPD-1 can target liver cancer cells and enhance immunotherapy efficacy, and therefore they have potential in the immunotherapy of liver cancer.
Funding: This work was supported by grants from Hainan Key Program of Science &Technology (ZDYF2019148), and Hainan Provincial Natural Science and Technology Foundation of China (819QN369).

P3137

Photoswitchable CAR T cells function in vitro and in vivo via a cleavable mediator

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Chimeric antigen receptor (CAR) T cell (CAR-T)-based therapeutics are a major breakthrough in cancer treatment; however, they are hampered by constitutive activation, which leads to worrisome side effects. Engineering CAR-Ts to be as tightly controllable as possible remains a topic of ongoing investigation in cancer research. Here, we report a photoswitchable approach that uses a mediator for at will regulation of CAR-Ts. This mediator carries dual folate and fluorescein isothiocyanate moieties tethered by an ortho-nitrobenzyl ester photocleavable linker (folate-O-FITC). CAR-Ts were shown to be highly cytotoxic to folate receptor (FR)-positive cells but not FR-negative cells only in the presence of the mediator. Additionally, CAR-T activation and proliferation were strictly dependent on FR-positive cells and were dose titratable with the mediator both *in vitro* and *in vivo*. Furthermore, CAR-T cytotoxicity rapidly decreased upon photoirradiation and was reactivated by supplementation with the mediator, addressing the persistence issue inherent to this type of immunotherapy. The versatile approach described here provides a direction for photoswitchable immunotherapies and may help to increase the safety of CAR-T clinical applications in cancer patients.

P3138

Co-transplantation of haploidentical CD19-targeted chimeric antigen receptor-modified T cells and high-dose peripheral hematopoietic stem cells for advanced acute lymphoblastic leukemia

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Autologous CD19-targeted chimeric antigen receptor-modified T cells (CD19-CART) remarkably improved outcome of patients with advanced acute lymphoblastic leukemia. However, there are limitations for those lacking autologous T cell harvest window or failing to manipulate CAR T cells from

DOI: 10.1002/eji.201970400

patients´ own T cells. Herein, a novel co-infusion model of HLA-haplo-identical CD19-targeted CAR T cells and high-dose donor-derived G-CSF mobilized peripheral blood mononuclear cells (GPBMCs) were developed, and three patients with advanced B-ALL were treated following a reduced-dose induction chemotherapy. The infused CAR^+ T and GPBMC numbers were 8.44-22.19x10^6/kg and 19.04-25.34´10^8/kg, respectively. All three patients achieved complete remission (CR) within 4 weeks after cell infusion, and two of them had an ongoing 4- and 14-month CR accompanying with a persistent full donor chimerism (FDC) and detectable donor CART cells in peripheral blood, bone marrow and cerebrospinal fluid. However, leukemia reoccurred in another patient 50 days after cell infusion, accompanying with the loss of donor chimerism and subsequently CART cells undetectable. All three patients had Grade 2-3 cytokine release syndromes (CRS), and two patients with consistent CR developed controllable Grade II acute intestinal or limited chronic oral graft-versus-host disease (GVHD). These findings may provided a effective complement therapy for advanced ALL

P3139

Efficiency of cytokine-induced killer cells in combination with chemotherapy for triple-negative breast cancer

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Purpose: The treatment of triple-negative breast cancer (TNBC) remains challenging, due to the absence of estrogen, progesterone, and human epidermal growth factor receptors. This study was designed to evaluate the efficiency and safety of cytokine-induced killer (CIK) cell immunotherapy, following regular chemotherapy, for patients with TNBC.
Methods: A total of 340 patients with postmastectomy TNBC, from January 1, 2010 to June 30, 2014, were included in this retrospective study. Seventy-seven patients received CIK cell immunotherapy, following regular chemotherapy (arm 1), and 263 patients received regular chemotherapy alone (arm 2). The primary aim was overall survival (OS) and disease-free survival (DFS), and the treatment responses and adverse events were also evaluated.
Results: The 5-year DFS and OS rates in arm 1 were 77.9% and 94.3%, compared with 69.8% and 85.6% in arm 2, respectively (p=0.159 and p=0.035, respectively). This clearly shows that there was no statistical difference in the 5-year DFS between the two groups. Multivariate analyses of arm 1 indicated that a Karnofsky performance score (KPS) ≥90 and stage I/IIA disease were significantly associated with a prolonged DFS period (hazard ratio [HR], 0.25; 95% confidence interval [CI], 0.09-0.74; p=0.012; and HR 0.21; 95% CI, 0.06-0.82; p=0.024, respectively), but a KPS ≥90 and stage I/IIA disease were not independent prognostic factors for OS. Toxicity was mild in patients who received the CIK therapy.
Conclusion: The data suggested that CIK cell immunotherapy improved the efficiency of regular chemotherapy in patients with TNBC, and the side effects of CIK cell immunotherapy were mild.

DOI: 10.1002/eji.201970400

P3140

Correlative study on the efficacy and complications of CD19 chimeric antigen receptor T cells in the treatment of recurrent/refractory acute lymphoblastic leukemia

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Objective: Efficacy and complications of CD19 chimeric antigen receptor T cells in the treatment of recurrent/refractory acute lymphoblastic leukemia.
Methods: Retrospective analysis was performed on the efficacy and complications of 14 cases of refractory recurrent acute lymphoblastic leukemia treated with CD19 CAR-T cells in the hematology department of the second hospital of hebei medical university from January 2017 to January 2019.
Results: 1. Among the 14 patients, 13 patients could be evaluated for efficacy for 28 days.The total response rate of 28d was 9/14, in which 7 cases were complete remission, 2 cases were partial remission, and 4 cases were invalid.
2. All 14 patients (100%) had symptoms attributable to CRS: 2 had grade 1 (14%), 6 had grade 2 (43%), 3 had grade 3 (21%), 2 had grade 4 (14%), and 1 had grade 5 (8%).
3. Fever occurred in all 14 patients.Nine patients (64%) developed different degrees of liver dysfunction.
Conclusions: 1. To determine the efficacy of CD19-CAR-T cells in the treatment of recurrent/refractory acute lymphocytic leukemia.
2. CAR-T cells were maintained in vivo for up to 3 months.
3. IL-6, IFN-γ and IL-10 were significantly increased in CRS.The highest levels of IL-6, IFN-γ and IL-10 in the grade 3, 4 and 5 CRS group were significantly higher than those in the level 1 and 2 CRS grade.
Keywords: Acute lymphocytic leukemia, CAR-T cells, Cytokine releasing syndrome, Immunotherapy

P3141

Development of immunotherapy targeting TRAIL-R1

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Tumor necrosis factor-related apoptosis-inducing ligand receptor-1/2 (TRAIL-R1/R2) are expressed in a wide variety of tumor cells. Their ligand TRAIL or monoclonal antibodies (mAbs) can induce cell apoptosis by engaging its cognate TRAIL-R1/R2. We developed 10 human mAbs against TRAIL-receptor 1(TR1-mAbs). We found that IgM type TR1-mAbs strongly induced cell apoptosis even in a low concentration. Moreover, we identified novel antigenic epitopes on the extracellular domain of TRAIL-R1 using the TR1-mAbs. Among these mAbs, binding epitopes of TR1-422 and TR1-419 mAbs are in same binding region but they require different essential amino acid residues. Antigenic epitope of TR1-419 mAb is ^{144}ACNRCTE GVGYTNAS1^{58}, and that of TR1-422 mAb consists of only 5 amino acid residues (^{149}TEGVG^{153}) that were located in a central site of the TR1-419 epitope. These two antigenic epitopes could play key roles for TRAIL-R1-mediated apoptosis. Finally, we constructed second and third generation chimeric antigen receptors targeting TTRAIL-R1(TR1-scFv-CAR) using TR1-419 and -422 mAbs. Our results showed that TR1-scFv-CAR T cells could induce apoptosis in TRAIL-R1-expressing tumor cells. TR1-scFv-CAR T cells killed tumor cells by activated T cells, including CD8^{+}T cells and CD4^{+}T cells. TR1-scFv-CAR T cells cocultured with TRAIL-R1 expressing tumor cells could secret IFN-γ. **We proposed that TR1-scFv-CAR T cells could directly contact with target cells and plays dual killing effects by cytotoxic T cells and**

DOI: 10.1002/eji.201970400

TRAIL-R1-mediated apoptosis. TRAIL-R1 might be a potential target for tumor immunotherapy. These still require further experimental evidence.

P3142

Treatment of relapsed and refractory acute myeloid leukemia (MDS transformation) with CD123 chimeric antigen receptor T cell and literature review

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Objective: The gene express anti-CD123 chimeric antigen receptor (CD123-CART) was modified to the patient T cells ,then the CD123-CART cells were assessed with the safety and efficacy in a patients with relapsed refractory acute myeloid leukemia (MDS transformation).
Methods: A 48-year-old man with relapsed refractory acute myeloid leukemia (transformation of myelodysplastic syndrome) received a single infusion of anti-CD123-CART cells after pretreatment with cyclophosphamide and fludarabine. The clinical response of the infusion and post-infusion was assessed by monitoring the patient´s temperature, blood pressure, blood routine,inflammatory factor,peripheral blood flow and the bone marrow flow.
Results: The patient was given a single infusion of CD123-CART cells, which showed a significant anti-tumor effect, with 48% of the original cells in the bone marrow before infusion and 2% on the 14th day after the cell infusion. After the infusion, serum cytokines and CRP levels were observed to rise sharply. The patient developed severe fever, capillary leakage and other CRS reactions, central nervous system toxicity.After the infusion, the patient experienced a bone marrow suppression period of up to 3 months but eventually relapsed.
Conclusions:
1. The results demonstrated the feasibility and effectiveness of CD123-CART cells in the treatment of relapsed and refractory acute myeloid leukemia with MDS transformation. This case was the first reported at home.
2. Long-term myelosuppression may be related to the CD123-CART.
3. Leukemia cells no longer express CD123 when the patient relapses, and are no longer recognized by CD123-CART cells as an important cause of recurrence.
Keywords: Acute myeloid leukemia, CD123-CART

P3143

Split glypican-3 specific chimeric antigen receptor-modified T cells control hepatocellular carcinoma with decreased cytokine release

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Background aims: Glypican-3 (GPC3) is a protein highly expressed on hepatocellular carcinoma (HCC) but limited on normal tissues, making it an ideal target for immunotherapy. The adoptive transfer of GPC3-specific chimeric antigen receptor T (CAR-T) cells for HCC treatment has been conducted in clinical trials. Due to the rigid construction, conventional CAR-T cells have some intrinsic limitations like uncontrollable over-activation and inducing severe cytokine release syndrome.
Methods: Here we redesigned the GPC3-specific CAR by splitting the conventional CAR to two parts: the antigen-recognition part and the signaling part. By using co-culturing assays and a xenograft

DOI: 10.1002/eji.201970400

mouse model, the *in vitro* and *in vivo* cytotoxicity and cytokine release of the split GPC3 CAR were evaluated against various HCC cell lines and compared with conventional CAR.
Results: The *in vitro* data demonstrated that split GPC3 CAR-T cells could recognize and lysis GPC3+ HepG2 and Huh7 cells in a dose-dependant manner. Impressively, split GPC3 CAR-T cells produced and released significantly lower amount of proinflammatory cytokines including IFN-γ, TNF-α, IL-6 and GM-CSF than conventional CAR-T cells. When injected into immunodeficient mice inoculated subcutaneously with HepG2 cells, our split GPC3 CAR-T cells could control the tumor growth with much less cytokines release and rectal temperature arising compared to conventional CAR-T cells.
Conclusions: We described here the first time using split GPC3 CAR-T cells to treat HCC with decreased cytokine release, which is a more versatile and safer application than conventional CAR-T cells treatment.

Checkpoint Blockade and Antibody Therapy

O225

Gene expression profiling identified $TP53^{Mut}PIK3CA^{Wild}$ as a potential biomarker for triple-negative breast cancer patients treated with immune checkpoint inhibitors

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Triple-negative breast cancer (TNBC) accounts for 15-30% of all breast cancer types and is clinically difficult to treat due to the lack of hormone or HER2 receptors that are usually targeted by the most successful therapeutic approaches. Immune checkpoint inhibitors (ICIs) have offered long-term survival benefit in many types of solid tumors but with low response rates. Thus, there is an urgent need to develop feasible biomarkers for identifying responsive TNBC patients. Here, we found that the immune microenvironment of TNBC has the highest expression of immunoregulatory molecules among all pathologic types. Tumor mutation burden (TMB) of TNBC did not have a strong correlation with cytolytic activity and showed no significant differences between different degrees of immune cell infiltration and TMB. The machine learning method divided TNBC patients into two groups characterized by “hot” and “cold” tumors, according to whether immune-associated genes were highly expressed, which suggested different responses to immunotherapy. Further, patients with $TP53^{Mut}PIK3CA^{Wild}$ showed favorable immunotherapy-responsive signatures and might have a better outcome to ICIs. In conclusion, we found that TP53 and PIK3CA might be appropriate biomarkers to screen for patients who would benefit most from ICIs, which could guide precise immunotherapy for patients with TNBC.

O226

A novel HPV16 E7-affitoxin for targeted therapy of HPV16-induced human cervical cancer

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Background: Cervical cancer, the second most common cause of cancer death in women worldwide, is significantly associated with infection of high-risk human papillomaviruses (HPVs), especially the most common genotype, HPV 16. To date, there is no established noninvasive therapy to treat

DOI: 10.1002/eji.201970400

cervical cancer.
Methods: Here, we report a novel affitoxin that targets HPV16 E7 protein, one of the primary target proteins in molecular targeted therapy for HPV-induced cervical cancer. The affitoxin, $Z_{HPV16E7}$ affitoxin384 was generated by fusing the modified *Pseudomonas* Exotoxin A (PE38KDEL) to the HPV16 E7-specific affibody. The expressed and purified, $Z_{HPV16E7}$ affitoxin384 was characterized using numerous methods. SPR assay, indirect immunofluorescence assay, and near-infrared (NIR) optical imaging were respectively performed to assess the targeting ability of $Z_{HPV16E7}$ affitoxin384 to HPV16 E7 protein both *in vitro* and *in vivo*. Cell viability assays and SiHa tumor-bearing nude mice were used to evaluate the efficacy of $Z_{HPV16\ E7}$ affitoxin384 *in vitro* and *in vivo*, respectively.
Results: Using *in vitro* methods the SPR assay and indirect immunofluorescence assay, $Z_{HPV16E7}$ affitoxin384 targeted HPV16 E7 with high binding affinity and specificity. Significant reduction in cell viability in HPV16 positive cells was observed in the presence of $Z_{HPV16\ E7}$ affitoxin384. By NIR optical imaging, $Z_{HPV16\ E7}$ affitoxin384 specifically targeted HPV16 positive tumors *in vivo*. $Z_{HPV16E7}$ affitoxin384 showed significant *in vivo* antitumor efficacy in two kinds of tumor-bearing nude mouse models.
Conclusions: $Z_{HPV16E7}$ affitoxin384 is a potent anti-cervical cancer therapeutic agent that could be effective against HPV16 positive tumors in humans.

O227

PVRL1/TIGIT pathway is responsible for PD1 blockade resistance in liver cancer

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Mortality rate of hepatocellular carcinoma (HCC) patients is high due to late symptom presentation and lack of curative treatments. Immune checkpoint inhibitor, Nivolumab (anti-PD1 monoclonal antibody, anti-PD1 Mab), has been recently approved by FDA as HCC treatment. A recent clinical trial demonstrated that Nivolumab has a response rate of 20% in HCC patients. Our study aims to dissect the molecular mechanisms that contribute to anti-PD1 Mab resistance in HCC. Mass cytometry and high dimensional analysis revealed that anti-PD1 Mab resulted in the expansion of $KLGR1^{int}$ effector memory CD8 T cells in a resistant HCC mouse model. Interestingly, another immune checkpoint, TIGIT, was elevated. TIGIT is an inhibitory T cell receptor. When TIGIT binds to ligands in the PVR family, TIGIT inactivates T cells. We found that PVRL1 was significantly over-expressed in human HCC and was closely associated with poor survival in HCC patients. Knockdown or knockout of PVRL1 in mouse HCC cells repressed HCC growth and reinstated T cell infiltration in mouse HCC. In a T and HCC cell co-culture experiment, mouse HCC cells with PVRL1 knockdown could restore T cell proliferation. Interestingly, PVRL1 allowed HCC cells to evade immune surveillance by preventing PVR from PVRL3-mediated endocytosis. With PVRL1, PVR was stabilized on HCC cell surface and bound to TIGIT of T cells to silence T cells. Blockade of TIGIT was able to unleash T cells in HCC in vivo. Excitingly, combination treatment of anti-TIGIT Mab and anti-PD1 Mab synergistically extended the survival of HCC-bearing mice.

O228

Soluble immune checkpoints as circulating immune regulators in health and disease

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DOI: 10.1002/eji.201970400

Immune checkpoints (ICPs) exist in both membrane-associated and soluble forms *in vivo*. Current ICP blockade for cancer therapy solely focuses on development of blockers against membrane-associated inhibitory ICPs, which has emerged as a new paradigm in treating advanced cancer. Such ICP blockade therapy is faced with many challenges including (1) a large portion of cancer patients are unresponsive, (2) the lack of biomarkers to evaluate clinical applications, and (3) immune-related adverse events (irAEs)ranging from mild to life-threatening complications. It is currently unknown whether these challenges are related to the presence of soluble ICPs that have been found in normal physiological conditions. We used HIV infection as a disease model to study the levels, profiles, function, and clinical relevance of soluble ICPs in the peripheral circulation of HIV patients versus healthy individuals.We measured the plasma levels of 16 soluble ICPs from 23 healthy individualsand 102 HIV patients (46 ART-naïve and 56 ART-treated) and found that all 16 soluble ICPs were detected in the plasma from healthy individuals, and were highly elevated in ART-naïve HIV patients. ART profoundly suppressed the production of soluble ICPs as only 3 soluble ICPs (HVEM, CD40, and CTLA-4) remained significantly higher in the plasma of HIV patients on ART. Thus, soluble ICPs are present in the peripheral circulation of healthy individuals, dysregulated in HIV patients, and differentially affected by ART. The soluble ICP abnormalities were associated with viral load, inflammation, immune activation, and microbial translocation, and were significantly, but not fully, reversed by ART.

O229

Titin mutation associated with responsiveness to checkpoint blockades in solid tumors

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Immune checkpoint blockade (ICB) immunotherapy induces potent anti-tumor immunity across multiple solid tumors, although few patients respond well to this therapy. An emerging biomarker for predicting the responsiveness to ICB immunotherapy is the tumor mutational burden (TMB). Although several surrogate biomarkers, including deficient mismatch repair, *TP53/KRAS* mutations, or co-mutations in DNA damage response pathways, have been shown to be effective for predicting the response to checkpoint blockade immunotherapy, each is positive for only a small cohort of candidates, and many potential responders to ICB are inevitably missed. Here, we found that Titin (*TTN*), which is frequently detected in solid tumors, is associated with increased TMB and correlated with the objective response to ICB. In seven public clinical cohorts, all patients with mutated *TTN* showed longer progression-free survival or overall survival than those with wild-type status. Furthermore, an improved objective response rate and higher TMB were identified in cohorts with accessible information. Identification of *TTN* mutation as a predictor of improved outcomes in response to ICBs provides a clinically feasible assessment for estimating TMB and ICB therapy outcomes.

DOI: 10.1002/eji.201970400

O230

Combined blockade of TGF-β and PD-1 restores the function of tumor-infiltrating CD8$^+$ T cells in glioblastoma

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Transforming growth factor (TGF)-β is a potent immunosuppressive cytokine and a major target for anti-cancer therapeutics. However, it has not been elucidated how TGF-β affects CD8$^+$ T cell immune responses in glioblastoma. In the present study, we examined the expression of TGF-β receptor 1 (TGF-βR1) and immune checkpoint receptors in peripheral blood (PB) and tumor-infiltrating CD8$^+$ T cells from glioblastoma patients and investigated a role of TGF-β on functional deterioration of tumor-infiltrating CD8$^+$ T cells. We found that tumor-infiltrating CD8$^+$ T cells highly expressed TGF-βR1 and immune checkpoint receptors such as PD-1 compared to PB CD8$^+$ T cells. Interestingly, tumor-infiltrating TGF-βR1$^+$CD8$^+$ T cells expressed a higher level of T-bet compared to the TGF-βR1$^-$ counterpart, indicating that tumor-infiltrating TGF-βR1$^+$CD8$^+$ T cells possess a capacity of T-bet-dependent effector functions despite their exhausted phenotypes. Inhibition of TGF-β signaling by a specific inhibitor, galunisertib upregulated the expression of T-bet and Ki-67, a proliferation marker, in tumor-infiltrating CD8+ T cells and increased their effector functions. Furthermore, the combination of galunisertib with anti-PD-1 blocking antibody resulted in enhanced functional restoration of tumor-infiltrating CD8$^+$ T cells, demonstrating a synergy of co-targeting TGF-β and PD-1/PD-L1 pathways. These data suggest that TGF-β can be targeted to enhance the efficacy of the PD-1 blockade therapy in glioblastoma patients.

O231

In anti-PD-L1 immunotherapy FcγR interaction can add additional benefit depending on the tumor model

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Immunomodulatory antibodies blocking CTLA-4, PD1 and PD-L1 on immune cells show strong therapeutic efficacy. Their therapeutic effect is believed to be mediated by re-activating antitumor T cell immune responses. However, detailed analysis of anti-CTLA4 antibody therapy revealed that an optimal therapeutic efficacy also requires binding to IgG Fc Receptors (FcγR), mediating depletion of intratumoral regulatory T cells. Therefore, we investigated the role of FcγR binding in anti-PD-L1 antibody therapy in the MC38 C57BL/6 and CT26 BALB/c colon adenocarcinoma tumor models. In the MC38 tumor model, the therapeutic efficacy was similar for anti-PD-L1 of all IgG subclasses and unaffected in mice deficient for all FcγR. In contrast, anti-PD-L1 IgG2a, the IgG subclass with the highest affinity for activating FcγR, showed stronger therapeutic efficacy than other IgG subclasses in the CT26 tumor model. This was associated with a reduction of a myeloid cell subset with high expression of PD-L1 in the tumor microenvironment. The higher therapeutic efficacy of IgG2a was lost

DOI: 10.1002/eji.201970400

in C57BL/6 x BALB/c F1 mice, indicating that the genetic background of the host may determine the additional clinical benefit of antibody subclasses with high affinity binding for FcγR. Based on these data, we conclude that FcγR are not crucial for PD-L1 antibody therapy but may contribute to further therapy improvement most likely by mediating depletion of intratumoral PD-L1-positive myeloid cells.

LB031

Targeting tumors with IL-10 prevents dendritic cell-mediated CD8⁺T cell apoptosis

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Summary: Increasing evidence demonstrates that interleukin-10 (IL-10), known as an immunosuppressive cytokine, induces antitumor effects depending on $CD8^+$ T cells. However, it remains elusive how immunosuppressive effects of IL-10 contribute to $CD8^+$ T cell-mediated antitumor immunity. We generated Cetuximab-based IL-10
fusion protein (CmAb-$(IL10)_2$) to prolong its half-life and allow tumor-targeted delivery of IL-10. Our results demonstrated potent antitumor effects of CmAb-$(IL10)_2$ with reduced toxicity. Moreover, we revealed a mechanism of CmAb-$(IL10)_2$ preventing dendritic cell (DC)-mediated $CD8^+$ tumor-infiltrating lymphocyte apoptosis through regulating IFN-γ production. When combined with immune checkpoint blockade, CmAb-$(IL10)_2$ significantly improves antitumor effects in mice with advanced tumors. Our findings reveal a DC-regulating role of IL-10 to potentiate $CD8^+$ T cell-mediated antitumor immunity and provide a potential strategy to improve cancer immunotherapy.
Significance: The severe adverse effects of nontumor-targeted delivery and short in *vivo* half-life have greatly limited the use of immunostimulatory cytokines as cancer therapeutics. The short half-life of IL-10 significantly limits its clinical application. Here, we generated an antibody-based CmAb-$(IL10)_2$ that not only prolongs the half-life but also demonstrates tumor-targeted delivery of IL-10 to induce potent antitumor effects. In contrast to immunostimulatory cytokines, CmAb-$(IL10)_2$ has significantly reduced toxicity. Moreover, we revealed a mechanism for CmAb-$(IL10)_2$ of preventing DC-mediated $CD8^+$ TIL apoptosis. In the context of immune checkpoint blockade, CmAb-$(IL10)_2$ further prevents tumor-specific $CD8^+$ T cell apoptosis and achieves significantly improved antitumor effects. Taken together, we have developed the next generation of IL-10-based therapeutic to improve immunotherapies with reduced toxicity.

LB032

Antitumor effects of liposomal anti CTLA-4 combined with Doxil chemotherapy in the B16 large established tumors of melanoma

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Immunosuppressive networks play an important role in the evasion of anti-tumor immunity, and as such could restrain the immunopotentiating effect of chemotherapy. Chemotherapy leads upregulation of immune checkpoint receptors such as CTLA-4, PD1, PDL1and CD73 could potentially deplete immune effector cells. One of the potentially relevant restraining pathways is mediated by the immune inhibitory molecule CTLA-4. Although anti-CTLA-4 treatment had low to moderate antitumor activity as single agents, there are significant difference between monotherapy of CTLA-4 blockade antibody and combination with other chemotherapy or immunotherapeutic agents. One of the possible ways to

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DOI: 10.1002/eji.201970400

improve the efficacy beside using anti CTLA-4 it in combining therapy regimen of is to encapsulate it to a nanocarrier. It this study we encapsulated Anti CTLA-4 in PEGylated liposome, and used it as monotherapy or combining therapy with Doxil (liposomal form of Doxorubicin) in B16 mouse model of melanoma. Encapsulation of anti CTLA-4 improved its efficiency both on monotherapy and combining therapy. Survival of tumor bearing mouse increased and their Tumor growth significantly decreased after encapsulation of anti CTLA-4 in PEGylated liposome. Encapsulation of CTLA-4 leads to decrease in Tregs in both tumor microenvironment and tumor draining lymph nodes especially in combining therapy receiving mice. Increase in CD8 T cells were seen only in Doxil plus PEGylated anti CTLA-4 therapy receiving group. Our finding suggests a new approach to improve immune check point therapy by encapsulation in nanocarriers.

LB069

Binding characteristics of therapeutic monoclonal antibodies, avelumab and durvalumab, to PD-L1

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Programmed cell death1 (PD-1) and its ligand programmed cell death 1 ligand 1 (PD-L1) are two of the most important targets for antibody-based cancer immunotherapy. Three monoclonal antibodies (MAbs), avelumab, durvalumab and atezolizumab, have been approved by FDA for the treatment of multiple advanced cancers. Herein, we investigated the molecular mechanisms of anti-PD-L1 antibodies, avelumab and durvalumab in blocking PD-1/PD-L1 signaling pathway for cancer immunotherapy. The blockade of avelumab to the interaction of PD-1/PD-L1 is mainly contributed by VH domain while both VH and VL of durvalumab are involved in blockade of PD-1/PD-L1 interaction. The binding of these two MAbs provide stereo clash to that of PD-1, and thus abrogate the binding of PD-1 to PD-L1. Moreover, the binding of MAbs to PD-L1 has stereo clash with each other, which suggests that co-administration of these MAbs would not achieve additive efficacy. Moreover, a comparative study of the binding characteristics of anti-PD-L1 MAbs currently available in clinics and small-molecule inhibitors indicated that the binding sites of the VH domain of durvalumab may serve as hot spots for small inhibitory biologics development. These findings would facilitate the development of future biologics by targeting PD-L1 for cancer immunotherapy.

LB070

Synergistic antitumor effect of anti-PDL1 combined with oxaliplatin on a mouse tumor model

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Oxaliplatin (OXP) can change tumor microenvironment from immune-suppressive toward the immune-favorable condition. Almost all of the antitumor agents cannot totally cure cancer as monotherapy. Sothe current focus of cancer research became combining therapy using different treatment regimen,especially chemotherapy with checkpoint blockers. In this study, we assessed the activity of combiningregimen using anti-PD-L1 with OXP in CT26 tumor-bearing BALB/c mice. We further analyzed theimmune cell phenotypes in tumor site, lymph nodes, and spleen by flow cytometry

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DOI: 10.1002/eji.201970400

analysis. Our studyshowed that combination therapy with OXP and anti-PD-L1 significantly increased survival in vivo andinhibited tumor growth of tumor-bearing mice. Inconsistent with better antitumor activity, ourcombination therapy led to an increase in tumor-infiltrating activated CD8+ T cells. In draining lymphnodes and spleen, regulatory T cells decreased significantly. Mice receiving either anti-PD-L1 or OXPalone had a larger tumor and lower survival rate in comparison with combination therapy receivinggroup. The time and order of administration of each component of the combination therapy affectedantitumor response.

LB106

Prolonged blood circulation M13 bacteriophage displaying an immunoregulatory peptide for cancer immunotherapy

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Background: Blockade of the protein-protein interaction between the transmembrane protein programmed cell death protein 1 (PD-1) and its ligand PD-L1 has emerged as a promising immunotherapy for treating cancers but also faces many challenges. **One of the issues hampering PD1/PD-L1 immunotherapy is the short blood residence time for antibody drugs.**
Objective: To explore a strategy of long-circulating cancer immunotherapy drugs.
Methods: Briefly, phage library was injected into SD rats through tail vein for in vivo phage display screening following by as much blood as possible being collected from the heart. After three rounds of enrichment, the phage capable of extending the maximum blood retention time was selected and engineered to display one more peptide to block checkpoint protein PD-L1 for cancer immunotherapy.
Results: Here we show, in a proof-of-concept study in rats, that ***in vivo*** **phage display is an effective approach to discover peptides with the capability of substantially extending blood circulation time for M13 phage**. Intriguingly, the majority of the isolated phages contained RGD within their displayed sequences. We further demonstrated that the RGD-mediated specific binding of long-circulating phage to platelets was primarily responsible for the enhanced blood retention. **The engineered M13 phage displaying peptide inhibitor and long-circulating peptide exhibited significantly prolonged anti-tumor action *in vivo*.**
Conclusions: Our results demonstrated an innovative yet simple strategy, **with the aid of phage display, to engineer phages with improved anti-tumor performance and may have implications for phage therapy.**
Keywords: PD1/PD-L1 immunotherapy; Blood circulation-prolonging; Peptide; Phage display

LB107

Clever-1 as a therapeutic switch to stop immunosuppression and tumor spread

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Clever-1 (also known as Stabilin-1) is a scavenger and adhesion receptor expressed by a subset of monocytes, macrophages and lymphatic endothelial cells. Clever-1 mediates both immune and tumor cell trafficking within lymphatics and promotes immunosuppression in the tumor microenvironment. High Clever-1 in various human cancers associates with poor overall survival. Both genetic and immunotherapeutic targeting of Clever-1 in several preclinical cancer models promotes anti-tumor

DOI: 10.1002/eji.201970400

immune responses via macrophage re-programming and recruitment of CD8 T cells. Characterization of several Clever-1 antibodies has led to the humanization of FP-1305, a function blocking antibody that recognizes a discontinuous epitope on Clever-1 and selectively recapitulates the effects seen in mouse. The potential clinical use of FP-1305 will be directed to cancers with a high number of Clever-1^{+} macrophages. In future, eligible patients benefiting from FP-1305 can possibly be identified by a liquid biopsy since peripheral monocyte expressed Clever-1 has significant correlation with Clever-1 expression on tumor-associated macrophages. With high enthusiasm, a phase I/II clinical trial with FP-1305 for cutaneous melanoma, hepatobiliary, pancreatic, ovarian and colorectal cancer was initiated in December 2018. Remarkably, a consistent increase in Th1 polarized peripheral CD8 T and NK cells was observed in patients receiving FP-1305 with a partial response seen in one patient with metastatic disease after 10 weeks on treatment. Thus, FP-1305 has the ability to function as a novel macrophage checkpoint immunotherapy for patients with currently untreatable solid cancers.

P0507

Construction and affinity improvement of human scFv-Fc against TSLP

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Thymic stromal lymphopoietin (TSLP) is an important target for asthma and tumors. However, there is no available full-human single-chain antibody that can be successfully applied in the clinic. In this study, human anti-TSLP-scFv was selected from a constructed human naïve scFv library by phage display and then we developed a computer simulation method by using DS 4.5 software to increase the affinity of anti-TSLP antibodies. The specific primers were designed and the mutated DNA sequences of anti-TSLP-scFv were obtained by overlap PCR. The mutant scFvs were expressed in the prokaryotic expression vector pLZ16 and the affinity-enhanced anti-TSLP-scFv bacterial strain M4 was screened by ELISA. However, scFv has low stability, short half-life in vivo. So, the scFv-M4 was inserted into the eukaryotic expression vector pcDNA3.1 and PMH3^{EN}, and then transfected into HEK-293F cells to express the anti-TSLP-scFv-Fc. ELISA and Western Blot results showed that the size of anti-TSLP-scFv-Fc was about 65 kDa, the binding ability of anti-TSLP-scFv-Fc-M4 to TSLP was enhanced than pre-mutated. Finally, the affinity of mutated recombinant antibody was determined by BIAcore technique, and the affinity of mutated M4 was about 10 times higher than pre-mutated.

P0508

The potent HER2 targeted tumor suppression by the reconstituted immunoapoptotin husFv-Fdt-tBid with humanized anti-HER2 single-chain variable fragments (husFvs)

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As HER2 is frequently overexpressed in various malignancies, HER2-targeted anti-tumor theraputics are proved to be efficient and highly selective in clinic practice. HER2-targeted antibodies and conjugates are being developed continuously in recent years. We developed series of HER2-targeted immunoapoptotins which are able to induce persistent remission of HER2-overexpressing tumors in previous studies. However, the targeting moiety we used, which is single chain variable region (scFv), namely e23sFv, is of murine origin and may increase the risk of severe adverse events like immunogenicity reaction. Here, to the end of improving the therapeutic efficacy of immunoapoptotin,

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DOI: 10.1002/eji.201970400

we humanized the HER2-targeting e23sFv by human antibody consensus sequence engraftment. Afterwards, the affinity of the initially humanized e23sFv was improved by selective mutagenesis and phage-display-based affinity panning of the mutant pool. The resulting humanized e23sFv candidates (husFvs) exhibited up-to-94-fold increased affinity to recombinant HER2. And the immunogenicity of e23sFv was dramatically alleviated after humanization, as indicated by the impaired production of cytokines by husFv-stimulated human PBMCs. Two internalizable husFvs with optimal affinity were applied to generate humanized immunoapoptotins by infusion with the translocation domain Fdt and the proapoptotic domain truncated Bid. The husFv-immunoapoptotins demonstrated improved HER2-targeting and tumor-killing capacities both in vitro and in vivo compared with the e23sFv-immunoapoptotins and would enable the administration of multiple treatment cycles to patients, resulting in significantly improved antitumor efficacy. Furthermore, the husFvs recognized distinct HER2 epitopes and could thus be used in combination with trastuzumab or pertuzumab to achieve robust synergistic antitumor effects in HER2-positive malignancies.

P0509

Study on the mechanism of PD-1 McAb combined with cytokine IL-33 in antitumor immune response

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Objective: To investigate whether immune checkpoint molecule PD-1 blockage combined with cytokine IL-33 can inhibit tumor growth, prolong the survival of tumor-bearing mice and its possible mechanisms.
Methods: B16 and B16-IL33 melanoma cells were injected intradermally into humanized PD-1 KI/KO mice. Each group of mice were injected intraperitoneally with humanized PD-1 mAb or control IgG. Tumor growth and survival of the mice were monitored every two days. The infiltration of immunocyte populations and the expression of IFN-γ, Ki-67 and Foxp3 were detected by flow cytometry in the early stage (after the second treatment). The expression of IFN-γ, GZ-B, Perforin were detected by real-time PCR.
Results: PD-1 mAb combining with cytokine IL-33 significantly inhibited tumor progression and improved the survival of tumor-bearing mice. The infiltration of $CD4^+$and $CD8^+$ T lymphocytes of the combined treatment group increased significantly by flow cytometry, and secretion of IFN-γ and Ki-67 were enhanced. Real-time PCR confirmed, the secretion of IFN-γ, GZ-B, and Perforin with combined effect were significantly higher than that in the control group. The percentages of $Foxp3^+CD4^+$Treg cells and myeloid-derived suppressor cells ($CD11b^+Gr\text{-}1^{high}$) increased of the combined treatment group.
Conclusion: PD-1 immune checkpoint blockage therapy combined with cytokine IL-33 enhanced the expression and secretion of effector molecules by promoting the infiltration of $CD4^+$ T and $CD8^+$ T lymphocytes, thereby enhancing the anti-tumor immune response. This combined effect significantly inhibited tumor progression and prolonged survival of tumor-bearing mice.
Keywords: PD-1; IL-33; Humanized PD-1 KI/KO mice

P0510

Targeting CD24 anti-hepatocellular carcinoma treatment

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DOI: 10.1002/eji.201970400

Liver cancer is the sixth most commonly diagnosed cancer. Antibody treatment for tumor cell surface targets has achieved significant clinical results, but HCC (Hepatocellular carcinoma) has not yet found targets. CD24 is a glycosylphosphatidylinositol-anchored membrane protein with a small protein core and a high level of glycosylation. We found the expression of CD24 in human liver cancer tissues was significantly higher than that in adjacent tissues from 365 patients. The high levels of CD24 are associated with lower survival rates and the malignancy of liver cancer. We found that CD24 is overexpressed in fresh tissue samples from HCC patients in nucleic acid and protein levels. The same results were obtained in human hepatoma cell line Huh-7. The results of immunohistochemistry showed that CD24 and PCNA were co-localized in cancer tissues which indicates those cells with high expression of CD24 are proliferating. We extracted and purified the CD24 antibody, which can be applied to image and FACS for CD24 detection. This antibody is a potential drug for the treatment of liver cancer.

P0511

Lower dose paclitaxel augments the efficacy of PD1/PDL1 blockade therapy in ovarian cancer by reprogramming tumor microenvironment

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As the first line chemotherapy drug for ovarian cancer, paclitaxel can inhibit tumor progression, but, the dose-limiting toxicity of paclitaxel need to decrease the concentration. Blockade of immune-checkpoint programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 can enhance effector T-cell responses. However, the lack of response in many patients to checkpoint-inhibitor therapies emphasizes the need for combination immunotherapies to pursue maximal antitumor efficacy. Here, we evaluated the antitumor efficacy of lower dose paclitaxel combined anti-PD-1 (αPD-1) antibody in an immunocompetent syngeneic mouse model of ovarian cancer. The results showed that combination of lower dose paclitaxel and αPD-1 significantly inhibited tumor growth and metastasis, retard ascites production and prolonged the survival of tumor-bearing mice compared with single-agent administration. Benefits of tumor control and animal survival were associated with immunomodulation mediated by these two agents, which were characterized by enhanced the percentage and number of $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and $CD8^+Perforin^+$ T cells in lymph node and tumor tissues, reduces the percentage of Treg and TAM in tumor tissues, decreases the proportion of MDSCs in spleen and tumor tissue. Moreover, the expression of CRT induced by lower dose paclitaxel on tumor cells maybe contributed to increasing anti-tumor function of effector T cells. Taken together, this results suggest that combination of lower dose paclitaxel and αPD-1 can overcome the dose-limiting toxicity of paclitaxel and enhance the anti-tumor efficacy of immunotherapy, which could be clinically benefit to patients with this difficult-to-treat disease.

P0512

Discovery and structural characterization of a therapeutic antibody against coxsackievirus A10

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DOI: 10.1002/eji.201970400

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Coxsackievirus A10 (CVA10) recently emerged as a major pathogen of hand, foot, and mouth disease and herpangina in children worldwide, and lack of a vaccine or a cure against CVA10 infections has made therapeutic antibody identification a public health priority. By targeting a local isolate, CVA10-FJ-01, we obtained a potent antibody, 2G8, against all three capsid forms of CVA10. We show that 2G8 exhibited both 100% preventive and 100% therapeutic efficacy against CVA10 infection in mice. Comparisons of the near-atomic cryo-electron microscopy structures of the three forms of CVA10 capsid and their complexes with 2G8 Fab reveal that a single Fab binds a border region across the three capsid proteins (VP1 to VP3) and explain 2G8´s remarkable cross-reactivities against all three capsid forms. The atomic structures of this first neutralizing antibody of CVA10 should inform strategies for designing vaccines and therapeutics against CVA10 infections.

P0513

Therapeutic effects of human anti-prostate-specific membrane antigen antibody mediated TRIM24 siRNA delivery in CRPC

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Background: Prostate specific membrane antigen (PSMA) is specifically expressed on prostate epithelial cells and markedly overexpressed in almost all prostate cancers. TRIM24 is also up-regulated from localized prostate cancer to metastatic castration-resistant prostate cancer (CRPC).
Methods: The therapeutic complexes were constructed by conjugating PSMAb and sulfo-SMCC-protamine, and encapsulating TRIM24 siRNA. Flow cytometry, immunofluorescence, and fluorescence imaging were performed to detect the receptor-binding, internalization, and targeted delivery of PSMAb-sulfo-SMCC-protamine (PSP)-FAM-siRNA complex (PSPS) *in vitro* and *in vivo*. CCK-8, plate-colony formation, apoptosis, cell cycle, and Transwell assays were performed to evaluate the therapeutic potential of the PSP-TRIM24 siRNA complex *in vitro*, whereas the *in vivo* therapeutic efficacy was monitored by small animal imaging, radiography, and micro CT.
Results: We confirmed that PSP could efficiently protect siRNA from enzymatic digestion, enable targeted delivery of siRNA, and internalize and release siRNA into PSMA-positive (PSMA+) prostate cancer cells *in vitro* and *in vivo*. Silencing TRIM24 expression by the PSP-TRIM24 siRNA complex could dramatically suppress proliferation, colony-formation, and invasion of PSMA+ CRPC cells *in vitro*, and inhibit tumor growth of PSMA+ CRPC xenografts and bone loss in PSMA+ CRPC bone metastasis model without obvious toxicity at therapeutic doses *in vivo*.
Conclusion: PSMAb mediated TRIM24 siRNA delivery platform could significantly inhibit cell proliferation, colony-formation, and invasion in PSMA+ CRPC *in vitro* and suppressed tumor growth and bone loss in PSMA+ CRPC xenograft and bone metastasis model.

P0514

A novel fully human antibody targeting extracellular domain of PSMA inhibits tumor growth in prostate cancer

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DOI: 10.1002/eji.201970400

Prostate cancer (PCa) is the most commonly diagnosed malignancy in men and the second leading cause of cancer-related death. It is of vital importance to develop new strategies for PCa therapy. PSMA (Prostate specific membrane antigen) is specifically expressed in PCa and the neo-vasculature of certain cancer types, thus is considered to be an ideal target for cancer therapy. In our previous study, we have obtained an PSMA specific single chain variable fragment (scFv), named gy1, from a large yeast-display naive human scFv library. In this study, we reconstructed the PSMA scFv into a fully human antibody (named PSMAb) and evaluated its characterization both in vitro and in vivo. We showed that PSMAb can specifically bind with and internalize into $PSMA^+$ cells. The binding affinity of PSMAb is measured to be at nanomolar level, and PSMAb has very good thermostability. In vivo study showed that near infrared dye labeled PSMAb can specifically localize at $PSMA^+$ tumors, and the application of PSMAb in vivo significantly inhibited the growth of $PSMA^+$ tumors, but not PSMA- tumors. At the studied doses, no obvious toxicity was observed when applied in vivo, as shown by the relative liver and kidney function and normal structure of important organs, shown by HE staining. In addition, PSMAb may inhibit tumor growth through ADCC and CDC mechanisms. Our results indicated that the novel fully human antibody PSMAb deserve further study for PSMA targeted diagnosis and therapy for PCa and other cancer types with vascular PSMA expression.

P0515

PH-dependent antigen-binding antibody accelerates the clearance of antigen in vivo

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A new modality in antibody engineering is to design pH-dependent antigen-binding antibod, combining high affinity binding at neutral pH with low affinity binding at acidic pH, which can more effectively neutralize the target antigen while avoid antibody-mediated antigen accumulation. The antigen-antibody complex will be internalized to the endosome (pH 5~6) and then lysosome (pH 4~5) to be cleared, however, pH-sensitive antibody will be detached to the antigen in the endosome and recycled outside of the cell to be reused efficiently, leaving only the antigen to be degradated in the lysosome. Here, based on the complex structure, especially the key site messages in the antibody-antigen interface, we tried to summarize the universal principle between the pH value and the affinity of antibody. We then established a smaller-content, epitope-constant synthetic library called “epitope-targeted pH-sensitive antibody library” for in vitro evolution of antibodies, from which we obtained an anti-SEB antibody and an anti-HER2 antibody with pH-dependent antigen binding, respectively. In in vivo experiments, all antibodies were effective in neutralizing the toxin and reducing the toxin-induced cytokine production. However, the pH-dependent antibody led to faster elimination of SEB from the circulation than the parent antibody. The area under the curve of SEB PK profile correlated well with the pH-dependent affinity ratio value of antibody, indicating the importance of pH dependency in antigen binding. These results suggested that a pH-dependent antibody may be optimal with accelerated clearance of toxic antigens to show better PK parameter in vivo.

P0516

The antitumor effect of small molecule inhibitor PSdis

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PDL-1 expressed on the tumor cells can specifically bind to PD-1 receptor of T cells, which can inhibit the activation of T cells and enhance the immune escape of tumor cells. Anti-PD-1 or PDL-1

DOI: 10.1002/eji.201970400

monoclonal antibodies have achieved great success in tumor immunotherapy, but the reality of the PD-1 inhibitory signaling pathway of T cells is still unclear. In this study, we find that PD-1 can be phosphorylated by the tyrosine kinase Src, recruiting negative signal regulator protein SHP-2 to inhibit the positive TCR signaling pathway adjacent to PD-1. Through the large scale of computer simulation screening based on SHP-2 protein structure, we developed a small molecule compound named PSdis, which can disrupt the interaction between PD-1 and SHP-2 without affecting SHP-2 enzyme activity. In vitro assay results, PSdis could block the interaction between PD-1 and SHP-2, preventing the immunosuppression of PD-1 on the TCR signaling pathway in $CD8^+$ T cell. It could promote $CD8^+$ T cell activation, enhanced IFN-γ, IL-2 and TNF-α cytokines secretion. It predicted that PSdis can be effectively used in tumor immune checkpoint blockade therapy. Moreover, we firstly use the PLGA nanoparticles as drug carrier for targeted delivering PSdis to explore the antitumor effects of PSdis in vivo. In animal tumor suppression experiment, we find that PSdis loaded on the nano-carrier can significantly activate tumor infiltration $CD8^+$ T cell and inhibit the tumor growth. Taken together, our study will provide a novel clinical tumor immunotherapeutic potential drug.

P0517

Eukaryotic expression and identification of extracellular domain of recombinant human lymphocyte activation gene-3 protein

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Objective: The recombinant human lymphocyte activation gene 3 (LAG-3, CD223) eukaryotic expression vector was constructed and the expressed protein was identified.
Method: In this study, Jurkat cells were stimulated by phytohaemagg lutinin (PHA). Extracting total mRNA from Jurkat cells , the extracellular segment of human LAG-3 protein was amplified by RT-PCR. The His tag was introduced at the C-terminus of the protein and cloned into the pcDNA3.1+ expression vector, transformed into E. coli, picked single colony, expanded culture, extracted plasmid and double enzyme digestion and sequencing verification, transfected into Expi293F eukaryotic cells. When the cell viability was less than 50%, the cell supernatant was harvested and purified by nickel column affinity chromatography. The purified protein was sodium dodecyl sulfate. Purification analysis was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC), and Western Blot (WB) was used for specific analysis. Concentration determination was performed using a Micro BCA Protein Assay Kit.
Result: The plasmid double digestion and sequencing results showed that the plasmid was constructed correctly. After purification of the transfected supernatant, more than 95% purity of LAG-3 protein was obtained. The purified protein WB analysis showed that the specificity appeared at the molecular weight of 60Kd. The band has a concentration of 2.4 mg/ml.
Conclusion: The LAG-3/pcDNA3.1+ expression vector was successfully constructed and protein expression was successfully performed in Expi293F cells. This study laid the foundation for the preparation of late LAG-3 monoclonal antibody.

P0518

Novel human monoclonal antibodies targeting the F subunit of leukocidins reduce disease progression and mortality caused by Staphylococcus aureus

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DOI: 10.1002/eji.201970400

Staphylococcus aureus is a leading cause of Gram-positive bacterial infections worldwide; however, the treatment of *S. aureus* infection has become increasingly difficult due to the prevalence of methicillin-resistant *S. aureus* strains, highlighting the urgent need for the development of novel strategies. The complexity of *S. aureus* pathogenesis relies on virulence factors. Recent studies have demonstrated that leukocidins expressed by the majority of clinical isolates play important roles in the pathogenesis of *S. aureus*. In this study, we developed three human monoclonal antibodies against all F-components of leukocidins HlgABC, LukSF, and LukED with high affinity. These antibodies were found to be capable of blocking leukocidin-mediated cell lysis in vitro. Furthermore, the antibodies dramatically reduced disease progression and mortality after *S. aureus* infection in vivo. Our findings revealed that targeting of F components using the neutralizing antibodies generated herein may be an effective therapeutic strategy for the treatment of infectious diseases caused by *S. aureus.*

P0519

Construction, expression and identification of bispecific antibody for targeting CD3 and B7-H3.

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Objective: To construct and express bispecific antibodies targeting CD3/B7-H3 and analyze their physicochemical properties and biological activity.
Methods: HuM291 and MGA271 were used as parental antibodies**,** bispecific antibodies with Dual-Variable Domain Immunoglobulin (DVD-Ig) format were engineered and constructed by gene recombination technology. Expression plasmids successfully constructed were transfected into Expi293F cells for expression, and the supernatants were purified by Protein A. The purified antibodies were characterized by SDS-PAGE, SEC-HPLC, LC-MS and other methods. The cytotoxicity of T lymphocytes on MCF-7 cells and A549 cells was detected by cell assay.
Results: Two bispecific antibodies were successfully constructed and named CD3/B7-H3 DVD-Ig and B7-H3/CD3 DVD-Ig. The SEC-HPLC purity of bispecific antibodies purified by Protein A was 90.425% and 96.674%, respectively. The intact molecular weight determined by LC-MS was consistent with the theoretical value. The binding activity of the bispecific antibodies to the antigen were comparable to that of the monoclonal antibody. Compared with the parental monoclonal antibody MGA271, the CD3/B7-H3 DVD-Ig and B7-H3/CD3 DVD-Ig binding activity is similar, and the relative binding activity is 98.4 % and 103.5%, respectively. Both can mediate the killing effect of PBMC cells on target cells MCF-7 cells and A549 cells, and there is a dose-dependent effect in the range of 0.1 ng/ml - 1000 ng/ml.
Conclusion: CD3/B7-H3 DVD-Ig and B7-H3/CD3 DVD-Ig bispecific antibodies were successfully designed and constructed, and their physicochemical properties and biological activities were analyzed. Initially demonstrated that they have tumor killing activity, which laid a foundation for subsequent experiments.
Key word：Bispecific antibody；DVD-Ig；CD3；B7-H3

DOI: 10.1002/eji.201970400

P0520

A novel protein CMTM1-v5 specifically induced human lymphoma cells apoptosis in vitro and in vivo

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Commonly seen lymphoma are among the top 10 male leading causes of cancer-related morbidity and mortality around the world, but the treatment has not been satisfied. Recently, we identified a spliced isoform CMTM1-v5 which is significantly associated with tumor development and could be a potential therapeutic drug of lymphoma. Here, we show that overexpression of CMTM1-v5 or addition of the polypeptide CMTM1-v5 in Raji cells obviously induced apoptosis in *vitro*. For the *in vivo* experiments, most of the fluorescent CMTM1-v5 polypeptide converged on the tumor cells of Raji xenografts after 24hr and the injection of the polypeptide by vein tails significantly extended the survival of the mice bearing Raji cell. Mechanistically, the interaction between CMTM1-v5 and CAML negatively regulated the $Ca2^{+}$ response in ER, which allowed the activation of caspases and release of cytochrome c in mitochondria and resulted in cell apoptosis. Thus, our study provides proof-to-concept for CMTM1-v5 to treat lymphoma.

P0521

Identification of transcriptomic markers predictive of clonal response to checkpoint inhibitor treatment

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Background: Immune checkpoint blockers (ICBs) have revolutionised the treatment of a wide range of tumours, but show marked variation in response between patients. CD8+ T-cells form the primary target of ICBs yet insights into the clonal behaviour of these cells are limited. We sought to characterize the clonal composition of the peripheral T-cell repertoire in patients with metastatic melanoma, defining how this changes following ICB therapy.
Methods: We used single cell transcriptomic and VDJ sequencing to characterise peripheral $CD8^{+}$ T cells pre and post one cycle of treatment with single-agent ICB (sICB; n=4) or combination ICB (cICB; n=4) in eight patients with metastatic melanoma.
Results: We find expansion of peripheral $CD8^{+}$ T cell clones shows high variability between individuals receiving ICB with numerous clones identified that expand and contract post treatment. Within individuals we observe that different clusters of CD8 cells show divergent propensity to expansion. The gene expression profiles of enlarging subsets are characterised by over-expression of metabolic pathways. We further identify gene expression profiles present at baseline that correlate with subsequent expansion or contraction of clones as well as transcriptomic signatures of clones that have undergone expansion or contraction.
Conclusion: We provide novel insights into the function and organisation of peripheral $CD8^{+}$ T-cell repertoires at a single cell level. We identify variable clonal changes in circulating $CD8^{+}$ T-cells following ICB and uncover underlying transcriptional correlates of these. This work furthers our

DOI: 10.1002/eji.201970400

understanding of T-cell clonality - an immunological phenomenon common to a wide range of pathological states.

P0522

Target cell-dependent T cell engaging and activation by the asymmetric heterodimeric Fc-scFv fusion antibody for cancer therapy

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Development Center for Biotechnology (DCB) has developed a novel asymmetric IgG-like bispecific antibody (BsAb) platform for cancer therapy. The BsAb composes the Fc-scFv fusion. In the case of IgG-scFv BsAb, the full length IgG targets the tumor associated antigen (TAA) and the scFv which linked to the C-terminus of one of the heavy chain targets the T cell for engaging and activation. We have demonstrated that the TAAxCD3 BsAb has great potency to eradicate the tumor cells. To minimize the side effect when the BsAb encounters the T cell prior to the target cancer cells, the Fc of the BsAb was engineered to reduce the effector function. In the cytokine secretion study, the TAAxCD3 BsAb with wildtype Fc induces IL-2 production in the absence of target cancer cells, whereas the Fc-engineered TAAxCD3 BsAb induces the cytokines production only in the presence of target cancer cells. This property reduces the safety concern when used in the clinics. In conclusion, the asymmetric IgG-like BsAb possesses various advantages comparing to other formats, including (1) simple construction without labor intensive screening; (2) high productivity comparable to its parental mAb; and (3) target-dependent T cell activation.

P0523

Sipa1 which controls stromal activation and T-cell recruitment in cancer tissue may be a new checkpoint component for cancer immunity

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Sipa1 is a negative regulator of Rap1 signaling, which plays a key role in controlling cell adhesion and migration for many cell types in tissues. Sipa1 deficient mice showed a remarkable resistance to Chronic myelogenous leukemia (CML) development upon transfer of Bcr-Abl^{+} hematopoietic progenitors cells (HPCs) from wild type (Wt) mice, which cause lethal CML disease in Wt recipients. Both T cells and non-hematopoietic cells in Sipa1 deficient hosts are required for the CML resistance. Mesenchymal stroma cells (MSCs) in Sipa1 deficient mice showed accelerated directed migration toward Bcr-Abl^{+} CML cells and produced Cxcl5/9, while Sipa1$^{-/-}$ memory T cells exhibited markedly enhanced chemotactic migration in response to the chemokine, leading to the increased accumulation of T cells within the tumor tissues. Sipa1 deficient mice also showed resistance to the HPCs expressing drug-resistant mutant Bcr-Abl as well. And this CML resistance diminished in aged Sipa1 deficient mice, which suggested T cell senescence may be a major factor in CML resistant sensitivity. In other cancer models with possible cancer stem cells, our preliminary results indicated that Sipa1-deficient mice also showed significant resistance to colon cancer cells (MC38) in an orthotopic model. These results indicate that Sipa1 deficiency unleashes a potential host immune mechanism against certain cancer cells and suggest that Sipa1 may be a promising target for cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P0524

Effects of constant regions of igg1 and igg3 on the binding of murine monoclonal antibodies to Cryptococcus neoformans capsule

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Cryptococcosis is a disease with more than 223,000 new cases per year, resulting in the death of approximately 181,000 people. Currently used antifungal drugs are limited, so monoclonal antibodies (mAbs) are being created for its treatment and diagnosis. Studies show that mAbs (IgGs-immunoglobulinG) against glycuroxylomannan (GXM) from the capsule of *C. neoformans* may protect against infection in mice, but that isotypic variants IgG3 may aggravate the disease. In addition, it has already been described that differences in the constant region of IgGs with identical paratopes may lead to changes in antigen binding pattern. In order to produce recombinant antibody variants to elucidate which regions are responsible for the change in *C. neoformans* capsule binding pattern, seven vectors encoding hybrids between IgG1 and IgG3 isotypes derived from the 2H1 antibody (a secretory hybridoma of IgG against GXM). Following production of the above mAbs, indirect immunofluorescence assay was performed to observe its binding pattern to the *C. neoformans* capsule. The 2H1 recombinant antibodies produced were functional and the changes made in 2H1 CH1 generated punctate (IgG3) and annular (IgG1) binding patterns. However, when the hinge of the 2H1 IgG3 antibody was replaced by the IgG1 hinge, the capsule binding pattern changed from punctate to annular. When the reverse is done by replacing the IgG3 hinge in an IgG1 antibody, the pattern continues annular. These results indicate that the hinge is a necessary region, but not sufficient to determine the binding pattern of an antibody.

P0525

Antibody-mediated recruitment of viral-specific effector cells via recombinant antibody-MHC T cells engagers

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Our lab has developed a novel T cell engager (TCE) format whereby a recombinant antibody fragment specific for a tumor-associated antigen (TAA) of choice is genetically fused to HLA-A2/peptide complex that presents a viral T cell epitope. The HLA-A2/peptide complex is designed to engage the TCR of a $CD8^+$ T cell while the scFV/Fab redirects specificity towards a TAA, thus recruiting potent antiviral memory CTLs to attack tumor cells. We used the highly immunogenic cytomegalovirus (CMV) pp65-derived peptide as a target peptide for CTLs, taking into consideration the CMV prevalence of seropositive humans. Mesothelin was used as a TAA.
We monitored the binding of TCEs to target cells demonstrating that their binding is dependent exclusively on the interaction of the targeting domain with mesothelin. Potency of TCEs was determined *in vitro* using co-cultures of CMV-specific CTLs and mesothelin-expressing human tumor

DOI: 10.1002/eji.201970400

cell lines. The TCEs specifically mediated the killing of mesothelin-positive cells in TCE-dose-dependent matter. A significant increase in CD25 expression and dose-dependent secretion of IL2 and IFNγ were observed in CTLs exposed to mesothelin-positive cells. *In-vivo* studies using mice models are planned.
We present a powerful new approach for immunotherapy. The MHC-based TCEs can mediate efficient recruitment of viral-specific CTLs to kill tumor cells, adding an approach by which specific population of T cells can be recruited to the tumor site in a controllable manner, in contrast to current CD3-based TCEs that cannot selectively target the specificity of interest. Such treatment can be very potent but with decreased toxicities involved.

P1101

Screening of high-affinity PD-1 receptor antagonistic by computer-aided design combined with mammalian cell library

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The native PD-1/PD-L1 has an intermolecular low affinity of about 10^{-6} M. Wild-type PD-1 as an antagonist molecule can't effectively compete to reverse the PD-1/PD-L1 inhibition signals in the tumor microenvironment. Similar to the antibody drugs, PD-1 mutants can effectively block PD-1/PD-L1 signals if they are artificially modified with higher affinity (e.g. up to 10^{-9} -10^{-10} M). The engineered high-affinity receptor or ligand possesses overlapped epitope with that of the natural PD-1/PD-L1, so its blocking effect might be superior to that of antibodies theoretically. In this study, based on the structure of the wild-type PD-1 molecule, we used the computer-aided molecular analysis and design methods to simulate and dock the PD-1/PD-L1 structure, following the principle of keeping the epitopes fixed. At the same time, the key pharmaceutical characters (e.g. affinity, stability, etc.) were considered to obtain the virtual library of PD-1 mutants. With the help of mammalian cell display technology, the mutated PD-1 library were displayed on the mammalian cell surface. Then the high-throughput flow cytometry method was used to screen and enrich positive clones to improve the maturity of PD-1 molecule in vitro. Finally, a PD-1 high-affinity candidate molecule 463 was obtained（KD:2.06×10^{-9}M）, The candidate can effectively competed the signal axis for PD-1/PD-L1, PD-1/PD-L2, CD80/PD-L1.The results of in vitro and in vivo showed that 463 can effectively activate peripheral blood mononuclear cell to release IFN-γ and inhibit tumor proliferation. The data currently available showed that 463 was non-inferior to the listed anti-PD-L1 antibody atezolizumab.

P1102

Gastric cancer mesenchymal stem cells facilitate the disease progression of gastric cancer and resistance to AntiPD-1Therapy

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Gastric cancer associated mesenchymal stem cells (GCMSCs) derived IL-8 inhibits anti-tumor immunity via up-regulating the expression of PD-L1 in gastric cancer. However, the effect of GCMSCs on treatment of gastric cancer with antiPD-1 is not clear. In this study, we constructed human immune

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DOI: 10.1002/eji.201970400

system in NPG mice through adoptive human umbilical cord blood hematopoietic stem cells. AntiPD-1 therapeutic antibodies could inhibit the growth of subcutaneous tumor in mice while injection of GCMSCs decreased the anti-tumor effect of the therapeutic antibody. In mice tumor tissues, GCMSCs restrained the anti-tumor immunity via reducing the infiltration of CD4+T cell, cytotoxic T cell, NK cells and increasing the proportion of Treg cells. In the presence of GCMSCs, antiPD-1 therapeutic antibody could not effectively enhance the anti-tumor immune function of those immune cells. Furthermore, GCMSCs facilitated the expression of PD-L1 and the production of lactate in gastric cancer via regulating the glycolysis. Increased glycolysis of gastric cancer cells not only promotes the proliferation of the tumor cells itself, but also inhibits the anti-tumor immunity in tumor microenvironment. These results indicate that GCMSCs decreased the therapeutic effect of antiPD-1 antibody via maintaining the growth of gastric cancer cells and inhibiting anti-tumor immunity. Blocking the paracrine signal of GCMSCs may be a new target for the treatment of gastric cancer.
This study was funded by Jiangsu Province's Project of Key Research and Development Plan (Social Development) (Grant no. BE2017694)

P1103

Immunogenic chemotherapy sensitizes lung cancer to LunX antibody therapy

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Inducing the immunogenicity of tumor cells is an important means of initiating an anti-tumor immune response. However, it has rarely been reported that new tumor-specific immunogens are induced in vivo to induce stronger immunogenicity. We have previously found that lung-specific X protein (LunX) is closely related to the malignancy of lung cancer, and it is proved that monoclonal antibodies targeting LunX can inhibit the growth of human lung cancer. We found that combination of antineoplastics and anti-LunX antibody efficiently repressed tumor growth and extended the overall survival of tumor-bearing mice in the xenograft model and the patient-derived-xenograft model. Mechanismly, pretreatment of the tumor cells using low does of antineoplastics drived these cells to express more LunX protein on their membrane surface with the help of CRT and subsequently administration of anti-LunX antibody killed these LunX-high-expressing cells by CDC effect both in vitro and in vivo. We have demonstrated a new valid treatment for lung cancer patients which promote tumor regression and improve the patients' quality of life.

P1104

Anti-PD-L1 immunotherapy inhibits the progression of sorafenib acquired resistant hepatocellular carcinoma by inducing immune activation

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Background: Sorafenib is widely used as the first-line treatment of advanced hepatocellular carcinoma (HCC). However, due to frequent drug resistance, the efficacy of sorafenib treatment is still limited. Recent advances in immunotherapy provide a promising approach for the treatment of HCC. To develop promising strategies, we investigated the feasibility of targeting PD-L1 to overcome sorafenib resistance.

DOI: 10.1002/eji.201970400

Methods: In this study, we first established a sorafenib acquired resistant mouse model. In vitro experiments were used to explore the characteristics of sorafenib resistant cells and immune microenvironment of resistant tumors, so as to further uncover the mechanism of sorafenib resistance. We subsequently used the subcutaneous and orthotopic tumor models to verify the feasibility of anti-PD-L1 in the treatment of acquired resistant liver cancer.
Results: Compared with the control group, the proportion of immunosuppressive cells, such as TAM and MDSC, significantly increased in tumor tissues from acquired resistant mice. Moreover, the expression of classical immune effector genes in sorafenib acquired resistant HCC tumors was significantly higher than that in the control group. In addition, we observed a significant elevation of PD-L1 in sorafenib acquired resistant HCC tumors, while other immunotherapy targets such as CTLA-4 and PD-1 had no significant changes. Anti-PD-L1 can significantly inhibit the tumor growth of sorafenib acquired drug-resistant HCC both in subcutaneous and orthotopic mouse models.
Conclusions: Our results reveal that sorafenib acquired resistant HCC leads to an immunosuppressive microenvironment characterized by elevated PD-L1 expression. **Anti-PD-L1 can activate the immune system and inhibit the progression of sorafenib acquired resistant HCC.**

P1105

Anti-PD1 antibodies plus chemotherapy for first-line treatment of advanced oesophageal squamous cell carcinoma

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Objective: To compare with the effect of **anti-PD1 antibodies plus chemotherapy** and standard chemotherapy for **the fist-line treatment** of **advanced oesophageal squamous cell carcinoma.**
Methods: A total of 11 patients with advanced oesophageal squamous cell carcinoma who cannot undergo radical surgery or radiotherapy were assigned to receive BGB-A317, CS1001 or nivolumab (all are anti-PD1 antibodies) plus cisplatin (CDDP)/5-fluorouracil (5-FU) chemotherapy every 3 weeks for five or six cycles, followed by maintenance therapy with BGB-A317, CS1001 or nivolumab respectively. The two primary end points were progression-free survival (PFS) and overall survival (OS) assessed by investigators. Results were compared with previous data of standard CDDP/5-FU chemotherapy for advanced oesophageal squamous cell carcinoma.
Results: Of the 11 patients, **8 (72.7%) responded** to treatment: 7 partial and 1 complete response. Other 3 patients had stable disease. **The study is still in process:** 2 patients had diseases progression with their **PFS of 471 days and 314 days**, respectively; 1 patient reached end point with its OS of 181 days; other 9 patients without disease progression are still under observation. **The minimum disease progression free period** of these subjects was **326 days so far. The PFS of** 90.9% (10 of 11) of patients with **anti-PD1 antibodies plus chemotherapy** was **longer than** the **median OS** of patients with **standard CDDP/5-FU chemotherapy** (**231 days**, as previous studies showed)
Conclusions: When compared to standard CDDP/5-FU chemotherapy for advanced oesophageal squamous cell carcinoma, **anti-PD1 antibodies plus chemotherapy as the first-line treatment of advanced oesophageal squamous cell shows tremendously positive potential.**

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DOI: 10.1002/eji.201970400

P1106

CD103+CD8+ T lymphocytes in non-small cell lung cancer are phenotypically and functionally primed to respond to PD-1 blockade

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CD103+CD8+ tumor infiltrating lymphocytes (TILs) have been linked to prolonged survival in various types of cancer including non-small cell lung cancer (NSCLC). However, the factors associated with the retention of CD103+CD8+ TILs in lung cancer tissues remain largely unknown. Additionally, the contribution of CD103+CD8+ TILs to effective PD-1 based immunotherapy has not been fully elucidated. In this study, we identified that the expression levels of E-cadherin and TGF-β were significantly correlated with the distribution and the density of CD103+ TILs in lung cancer tumor tissues. Unexpectedly, we observed that CD103+CD8+ TILs that expressed higher levels of PD-1 co-express Ki-67. Moreover, CD103+CD8+ TILs expressed an increased level of T-bet compared to their counterparts, indicating these cells may be better armed for immunotherapy. Lastly, PD-1 pathway blockade led to a significantly increased production of IFN-γ by CD103+CD8+ TILs, suggesting CD103+CD8+ TILs could serve as a predictive biomarker for PD-1 based immunotherapy

P1107

Development of C-based single domain antibodies as therapeutic candidates against emerging viruses

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Monoclonal antibodies have been successfully used for the therapy of many diseases. However, because of their large size (~150 kD), many limitations have also been found during their development and manufacture. The use of antibody fragments with smaller sizes is one of the attractive strategies to overcome these limitations. Antibody constant CH2 domain (~12 kD) was proposed as scaffold for library construction and selection of specific binders as novel therapeutic candidates termed C-based single domain antibodies (C-sdAbs). In our previous work, we engineered the wildtype CH2 scaffold (m01s) for improve its stability and extension of its serum half-life to about 10 hours. We constructed a phage display library based on m01s which has complexities in excess of 10^{10} independent clones. Panning of this library against the antigens from envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1),Ebola virus (EBOV), and Middle East respiratory syndrome coronavirus (MERS-CoV) resulted in selection of several specific neutralizing C-sdAbs with EC_{50} at nM range against these viruses respectively. Epitope analysis revealed that these C-sdAbs bind to membrane proximal external region (MPER) in HIV-1 GP160, glycan cap in EBOV Gp, and receptor binding domain (RBD) in MERS-CoV spike protein respectively. These C-sdAbs also showed neutralizing activities in pseudotype and live virus assay. Our results suggest that these C-sdAbs have potential for being developed as novel antiviral therapeutic candidates, and the C-sdAb technical platform could be very useful in screening of new candidate drugs for prophylaxis and treatment of infectious diseases.

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DOI: 10.1002/eji.201970400

P1108

The expression and clinical significance of costimulatory molecule PD-L1 on cervical cancer

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Immunohistochemical assay (streptavidin-perosidase) was applied to study PD-L1 expression in chronic inflammation and cervical intraepithelial neoplasia (CIN). 111 patients（June 2001 to April 2017）were enrolled and paraffin-embedded specimens were collected by surgical resection or biopsy including in cervical chronic inflammation and cervical intraepithelial neoplasia patients. PD-L1 expression and clinicopathological features was analyzed with individual patient data. Our results showed that the positive staining PD-L1 was detected among 66.67% in chronic inflammation patients , 96.43% in the CINⅠ group ,and 97.14% in the CINⅡ-Ⅲ group. PD-L1 expression in CIN group was significantly higher than that in chronic inflammation group (66.67%) , which showed statistical significance (p< 0.05). Cervical cytology with histopathological examination is one of the major methods for screening and diagnosing cervical cancer and precancerous lesions, which can improve the diagnosis of cervical intraepithelial neoplasia and reduce misdiagnosis rate. No PD-L1 expression was detected in normal cervical tissues, but over-expressed in high-grade lesions, indicating the increased PD-L1 espression accompanying the increased severity of cervical lesions. In this study, PD-L1 expression analysis by immunohistochemical SP could provided a new biomarker for the examination of cervical lesions.

P1109

Inhibitory role of the intracellular antibody against CDK4 in breast cancer progression

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Cyclin-dependent kinase (CDK) 4 is a key regulator of cell cycle progression. Increasing evidence has shown CDK4 activity is required for tumorigenesis and has become a potential target for tumor therapy. Intracellular antibodies (intrabodies) are antibody molecules which are expressed intracellularly and directed to intracellular compartments to neutralize and block the function of target proteins. This study is designed to investigate the inhibitory role of an intrabody against CDK4 in breast cancer progression. A single-chain fragment of antibody variable region against CDK4 (AK) was prepared by antibody phage display. Anti-CDK4 intrabody (NLS-AK) was generated by introducing nuclear localization signal sequence (NLS) and E-tag sequence to AK. A tumor-specific expression plasmid pBg-NLS-AK was constructed by inserting NLS-AK directly into the tumor-specific vector plpBg. Then pBg-NLS-AK was stably transfected into MDA-MB-231 and SK-BR-3 breast cancer cells to investigate the role NLS-AK. The results showed that NLS-AK could recognize and bind its target protein CDK4 in breast cancer cells. Compared with the control, the expression of NLS-AK significantly inhibited the growth and proliferation of breast cancer cells, resulted in cell cycle G1 phase arrest and induced more cell apoptosis. NLS-AK significantly lowered cell migrating rate and obviously decreased the wound healing closure rate of breast cancer cells. Results from transwell assay showed that NLS-AK inhibited invasion and metastasis of breast cancer cells. In summary, the intrabody against CDK4 inhibits proliferation and migration of breast cancer cells. It suggests that anti-CDK4 intrabody may offer an effective strategy for breast cancer therapy.

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DOI: 10.1002/eji.201970400

P1110

A recombinant fusion protein with soluble PD-1 and TIM-3 domains effectively binds to cancer cells

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Programmed cell death-1 (PD-1) and T-cell immunoglobulin and mucin-3 (TIM-3) are two important inhibitory immune checkpoint members that mediate potent immunosuppressive signaling in T cells. Many cancer cells express high levels of the ligands for these receptors which lead to the evasion of immunosurveillance. Blockade of these signaling pathways may achieve effective therapy of cancer, while simultaneous activation of different immunosuppressive signaling pathways may lead to resistance to single checkpoint inhibition therapy. We designed and synthesized a DNA sequence by joining the coding regions for soluble PD-1 and TIM-3 domains in this work, and cloned the whole open reading frame into a lentiviral expression vector. Recombinant lentivirus was obtained after packaging and used to infect HEK293T cells. Expression of the recombinant fusion protein was successfully detected by Western blotting. PD-1 ligand PD-L1 and TIM-3 ligand Galectin 9 expressing cancer cells SW620 (colorectal adenocarcinoma), MDA-MB-231 (breast adenocarcinoma) and PC-3 (prostate adenocarcinoma), as determined by reverse-transcription PCR, were used for binding assay. After incubating the recombinant fusion protein-containing cell lysate with cancer cells and subsequent fluorescent staining for the fusion protein, strong membranous signals were observed as compared to negative controls using cell lysate without the fusion protein, suggesting specific binding of the recombinant fusion protein with soluble PD-1 and TIM-3 domains to these ligand-expressing cancer cells. The recombinant fusion protein provides a useful tool for studying the effect of dual-blockade of immune checkpoint, its potential in targeted immunotherapy of cancer needs to be further investigated.

P1111

Expression of CMTM6 in gastric cancer and its correlation with immune checkpoint blockade therapy

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Gastric cancer (GC) is the fourth most common cancer worldwide and in urgent need of improved therapy in consideration of the difficulty of early diagnosis, frequent relapse and limited treatment options. Immune checkpoint blockade(ICB) emerges as a promising therapy by “normalizing” the anti-tumor immune response. Despite that Pembrolizumab is approved by FDA for the third line treatment of GC, the effectiveness of PD-L1 as a biomarker is still undefined. Researches have shown that CMTM6 is a critical regulator of PD-L1 on post-translational level and inhibition of CMTM6 promotes the degradation of PD-L1 in lysosome dependent manner. Therefore, we investigated the function of CMTM6 in GC cell lines and found that knockdown of CMTM6 had no obvious effects on cell proliferation, but inhibited PD-L1 expression in SNU-1 and MKN28 cell lines. Polychromatic staining of tissue microarray confirms the positive correlation of CMTM6 and PD-L1. The expression level of CMTM6 on epithelial cells is significantly higher in tumor compared with adjacent normal tissue and is positively correlated with tumor burden, while PD-L1 is slightly higher in cancerous tissue, both of

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DOI: 10.1002/eji.201970400

which are associated with poor prognosis. The number of CMTM6+PD-L1+ cells is slightly higher in the epithelial and mesenchymal region of tumor tissue than in adjacent normal tissue. In tumor tissue sections, CMTM6 co-localizes with PD-L1 in epithelial and mesenchymal region, although heterogeneity is seen among cases. These results provide basis for further investigation of CMTM6 in GC and the application of CMTM6 as a biomarker in patient-selection of ICB.

P1112

Exosomal PD-L1 and T lymphocyte status predict the effect of anti-PD-1 therapy

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Background: Anti-PD-1 therapy is approved to be remarkable for cancer treatment. However, the response rate is dissatisfactory. PD-L1 expression in tumor tissue is unreliable to predict treatment response. Recent studies suggest that exosomal PD-L1 plays a significant role in development of tumor microenvironment.
Method: We enrolled 28 patients with advanced tumor who were treated by anti-PD-1 therapy followed the standard regimens. Exosomes were collected and purified from plasma with exosome isolation kit. Exosomal PD-L1 was detected by ELISA. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll gradient. Exhausted T lymphocyte were stained for cytometry.
Result: Compared with responder, exosomal PD-L1 of non-responder is significantly higher than responder (P=0.036) before treatment. Moreover, the CD4+ and CD8+ T lymphocyte count is higher and the percentage of exhausted T lymphocyte rate is lower in responder than the non-responder cohort on baseline. Although there is no statistical difference, the level of exosomal PD-L1 decreased in responder cohort on 6 to 8 weeks after treatment, on the contrary, increased in non-responder cohort after anti-PD-1 therapy. Concurrently, exosomal PD-L1 and tumor burden were decreased when the therapy was effective.
Conclusion: Low levels of exosomal PD-L1 and exhausted T lymphocyte rate on baseline and decreasing expression of exosomal PD-L1 after treatment could screen out the potential benefit populations of anti-PD-1 therapy in a variety of solid tumors. These findings suggest that expression of exosomal PD-L1 and exhausted T lymphocyte cell rate could be attractive and predictive biomarkers for clinical responses to anti-PD-1 treatment.

P1113

Periplocin induces apoptosis of ESCC cell lines, downregulates the protein level of CDK4/6 and upregulates PD-L1 level

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Cyclin-dependent kinases 4 and 6 (CDK4/6) are earthshaking drivers of the cell cycle and are required for the initiation and progression of various malignancies. Pharmacological inhibitors of CDK4/6 have shown significant activity against several solid tumours and it has been reported the protein level of PD-L1 could be raised under the influence of CDK4/6 inhibitors. Here, we demonstrate that the protein level of CDK4/6 and cyclin D1 are descended under the treatment of periplocin extracted from cortex periplocae while the mRNA is impregnability. Periplocin monotherapy inhibits proliferation of esophageal squamous cell carcinoma (ESCC) cell lines Eca-109 and Yes-2, and cell cycle is retarded at G2/S. In this experiment, we verify treatment with periplocin inhibits ESCC cell proliferation, induces apoptosis but do not promote apoptosis of human T lymphocytes. IL-2 secretion of T lymphocytes

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DOI: 10.1002/eji.201970400

could also be facilitated and expression of PD-L1 could be upregulated under influence of periplocin in ESCC cell lines. These data collectively support periplocin could be used as a novel CDK 4/6 inhibitor and the combination of periplocin with agents such as anti-PD-L1 might modulate T cell anti-tumor immunity in vivo.

P1114

Anti-Rituximab antibody in patients with NMOSDs treated with low dose Rituximab

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Background: Rituximab is a mouse-human chimeric anti-CD20 monoclonal antibody and has been increasingly used for preventing relapses in NMOSDs. The clinical relevance of anti-Rituximab antibodies (ARA) against Rituximab in NMOSDs is unknown.
Methods: Nineteen NMOSDs patients receiving repeated 100mg Rituximab treatment were recruited. The ARA was quantitatively analyzed by enzyme linked immunoassay. Annualized relapse rate (ARR) and Expanded Disability Status Scale (EDSS) were analyzed concurrently.
Results: ARR was reduced markedly since starting Rituximab therapy in the majority (78.9%) of NMOSDs patients. 36.9% (7/19) patients were ARA positive. There was no significant difference in the improvement of ARR and EDSS after treatment with Rituximab in either ARA positive or negative groups. The frequency of Rituximab reinfusion was higher in patients with ARA, suggesting that the presence of ARA led to an increased requirement of Rituximab to maintain B cell depletion.
Conclusion: The majority of (78.9%) patients with NMOSDs were responsive to low dose Rituximab. The presence of ARA is associated with short lived B cell depletion and the requirement for increased frequency of Rituximab reinfusion to maintain treatment response in NMOSDs. In patients with ARA it might be necessary to detect ARA levels and monitor B cell depletion closely, or even attempt other treatments.

P1115

Nano-liposomal anthrax lethal factor armed with anti-CD19 VHH effectively blocked MAPK pathway in B cells

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Mitogen-activated protein kinases (MAPKs) are among the most highlighted current discussions in diagnosis and treatment of various diseases. MAPK activity can be regulated by p38 MAP kinase inhibitors and *Bacillus Anthracis* lethal factor (LF). Lethal factor requires PA to bind different cell types and block MAPK pathway. Several syndromes including lymphoma (NHL) and autoimmune diseases are caused by disturbance in the differentiation and efficiency of B cells. In the present study, we specifically targeted MAPK pathway using a novel anti-CD19 Nanobody(VHH) to transfer Nano-liposomal anthrax lethal factor into the Raji B cells. BrdU lympho-proliferation assay on immune-liposomal LF nanoparticles confirmed a significant cytotoxic response similar to anthrax lethal toxin. Our results indicating that the cell death response induced by the LF nanoparticles may be mediated

DOI: 10.1002/eji.201970400

by the MEK1/2-extracellular signal-regulated kinase (ERK1/2) branch of the MAPK pathway. Western blotting analysis of the MAPK pathway activation revealed the presence of phospho-MEK1/2, P38 and JNK, only in the negative control group. Real-time PCR showed an increase in caspase gene expression in positive cells, and an absence of caspase gene expression in control cells, suggesting that LF-induced cell death is caspase-dependent, proapoptotic and apoptotic. These data was subsequently confirmed by flow cytometry assay. In conclusion, the designed nanoparticles can be used as an efficient targeted therapy for regulation of B cell proliferation and elimination of these cells by blocking the MAPK pathway.

P1116

Establishment of a new strategy to eradicate SIV-latently infected cells for a cure treatment

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To achieve the functional cure of AIDS, it is important to eliminate HIV-1 latently infected cells from the body. For the purpose, it is strongly suggested that not only reactivation of HIV-1-latently infected cells but also induction of HIV-1-specific CTL are required simultaneously. In addition, there have been some reports that HIV-latently infected cells are mainly accumulated in the T_{FH}cell area of lymphoid germinal centers (GCs). Therefore, it has been suggested that elimination of HIV-1-latently infected cells in GCs is essentially required.
We previously reported that STING ligand, an adjuvant used to stimulate innate immunity, has the ability to reactivate SIV-latently infected cells and also boosting CTL responses with PBMCs derived from natural controller of SIV-infected cynomolgus monkeys. Recently, we have established our cART regiment to lower the viral load to the undetectable level during chronic SIVmac239 infection. Thus, we further investigated whether STING ligand could be useful for eliminating SIV-latently infected cells even from lymphoid tissues in the cART-treated cynomolgus monkeys. Furthermore, to maximize SIV-specific CTL responses, we made a chimeric anti-PD-1 antibody whose Fc region was replaced to cynomolgus monkey's Fc region. We checked the cross-reactivity of our new chimeric anti-PD-1 antibody with both human and monkey PBMCs and confirmed that it actually reacted with both of them. So, we have been examining the potential usages of anti-PD-1 antibody together with this STING ligand to eradicate latently infected cells in the GCs.

P1117

PD-1 modulates immune cell functions in prostate tumor microenvironment

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Regulatory T (Treg) suppresses the immune response and maintain tolerance. Tregs immunosuppressive mechanism(s) role in context to programmed death 1 (PD-1) are not completely understood. The aim of this study is to explore the role of PD-1 on Treg cells and their impact on $CD8^+$

DOI: 10.1002/eji.201970400

T cell function in prostatic tumor microenvironment using transgenic adenocarcinoma of the mouse prostate (TRAMP) cells (TRAMP C1, C2, and C3) as a model system. We performed tumor induction studies to test this aim. Briefly, the C57BL/6 mice were administered with serial log concentrations of TRAMP (C1-3) cells. Interestingly, the TRAMP-C1 and TRAMP-C2 are tumorigenic while the TRAMP-C3 cells do not form a tumor. Mice were sacrificed by cervical dislocation; tumor, lung, spleen, and draining lymph nodes were harvested when the tumor size reached approximately 20mm. Single cell suspensions were prepared from different organs, cells were then stained with specific antibodies, and flow analyzed for the expression of different immune markers. Our preliminary findings demonstrated that PD-1 expression on $Foxp3^+$ Treg cells displayed greater suppressive capacity against $CD8^+$ T cell function in tumor, lung, spleen, and draining lymph node when compared to the control. More importantly, $Foxp3^+$ $Treg^{(high)}$ $PD\text{-}1^{(high)}$ interaction with PDL-1 induced immunosuppression by blocking $CD8^+$ T cells function in the prostatic tumor microenvironment. Our data suggest that the expression of Foxp3 and PD-1 may enhance tumor progression; thus, targeting the PD-1 on Treg cells may be a possible therapy to treat prostate cancer.

P1118

Developing an enhanced 7-color multiplex IHC protocol to dissect immune infiltration in human cancers

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In the past years, multiple inhibitors of the immune checkpoint receptor PD-1 or its ligand PD-L1 have been approved in the U.S. in a selected, yet growing number of indications including non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), renal cell carcinoma (RCC), and certain subsets of breast cancer (BCa). In some patients with advanced malignancies, checkpoint inhibition can significantly prolong overall survival. However, there is still a critical need for novel immunotherapeutic combinations to improve clinical outcomes and overall survival. The rational design of future combination treatments with immunomodulatory agents like PD-1 inhibitors will greatly benefit from a detailed understanding of the immune contexture and the functional state of tumor infiltrating lymphocytes (TILs).
To this end, we characterized the frequency and spatial localization of TILs in cohorts of primary NSCLC, HNSCC, RCC, and BCa resections using 7-color multiplex immunohistochemistry (mIHC) analysis of formalin-fixed paraffin embedded (FFPE) tissue sections. The combined use of Opal mIHC and conventional IHC knowledge and techniques makes it possible to more accurately evaluate changes in the spatial distribution and expression pattern of immune-related biomarkers. The protocol developed in this study allowed us to stain sequentially 7 biomarkers on one single FFPE tissue section and then, subsequently, custom algorithms were applied to the imaging data to interrogate the expression and spatial arrangement of immune infiltration within the tumor and stromal compartments. This optimized technique may further characterize the tumor microenvironment across different tumor types and elucidate indication-specific differences.

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DOI: 10.1002/eji.201970400

P1119

Differences of tumor-recruiting myeloid cells in murine squamous cell carcinoma influence the efficacy of immunotherapy combined with a TLR7 agonist and PD-L1 blockade

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Objectives: The immune status of the tumor microenvironment has a marked impact on clinical outcomes. Here we examined the immune environments of tumor-infiltrating leukocytes (TILs) in two murine models of squamous cell carcinoma and compared the effects of immunotherapeutic agents, including a TLR7 agonist and an immune checkpoint inhibitor, and a chemotherapeutic agent, gemcitabine.
Materials and methods: TILs from NR-S1- and SCCVII-grafted mice were analyzed by flow cytometry. NR-S1-inoculated mice received resiquimod (a synthetic TLR7 agonist), an anti-PD-L1 antibody, or both, and tumor growth and TILs were examined. Gemcitabine was administered to deplete $CD11b^+$cells.
Results: More than 50% of TILs from NR-S1- and SCCVII-inoculated mice were $CD11b^+Gr\text{-}1^+$cells. A major fraction of NR-S1 $CD11b^+$cells was $Ly6G^{high}Ly6C^{low\text{-}nega}F4/80^-$tumor-associated neutrophils (TANs) and the majority of SCCVII $CD11b^+$cells were $Ly6G^{low}Ly6C^-F4/80^+$ tumor-associated macrophages. NR-S1 TANs did not express MHC class II and CD86, but did express reactive oxygen species and PD-L1. Resiquimod, alone and in combination with an anti-PD-L1 antibody, did not regress NR-S1 tumors, but the combination increased the CD8/regulatory T cell-ratio, and IFN-g and PD-1 expression in $CD8^+$TILs. Pre-administration of low-dose gemcitabine prior to the combination treatment suppressed the progression of NR-S1 tumors.
Conclusions: NR-S1 tumors with abundant recruitment of TANs were resistant to treatments with a TLR7 agonist, alone and in combination with PD-1 blockade, and required an additional gemcitabine treatment. The phenotype and status of tumor-infiltrating $CD11b^+$myeloid cells may influence the efficacy of immunotherapeutic agents.

P1695

Clinical outcomes of a new anti-PD-1 antibody in the treatment of solid tumors in one center

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In China, the incidence of malignant tumors is increasing, but currently there are no satisfactory treatments, especially for those with refractory/relapsed tumors, who lack effective therapeutic strategies after the failure of standard treatment. The programmed death-1 (PD-1) receptor blockade plays a vital role in the immune regulation of tumor progression. We used a new humanized monoclonal antibody against PD-1 for the treatment of 27 patients with solid tumor, including lung cancer, urothelial cancer, colorectal cancer, gastric cancer, melanoma, liver cancer, esophageal cancer, pancreatic cancer, nasopharyngeal cancer and of breast cancer. The response was evaluated every 9 weeks after treatment. So far, 8 patients (29.6%) have partial response and stable disease ,

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DOI: 10.1002/eji.201970400

17 patients (63.0%) have progressive disease, and 2 patients withdrew from the experiment for some reasons. During the course of treatment, 4 patients showed pseudoprogression. The common immune-related adverse reactions included pruritus (2 cases), abnormal liver function (2 cases), and thrombocytopenia (1 case), which were generally grade I/II. There were no serious adverse reactions. For immunotherapy, different solid tumors have different curative effects, so it is crucial to further screen out the dominant beneficiaries. In addition, given the occurrence of adverse immune events, oncologists have to keep a balance between efficacy and life quality, ensuring maximum benefits for patients.

P1696

Generation of TIM-3 domain antibodies and evaluation of antitumor activities

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Objective: To generate VH domain antibodies targeting TIM-3 and to evaluate their anti-tumor activities in combination with CAR-T therapy.
Methods: Rabbit domain antibodies against TIM-3 were generated via rabbit immunization and phage display technology. These single domain antibodies were expressed and purified in E. coli and mammalian cells. Cell binding affinity and anti-tumor activity were evaluated both in vitro and in xenograft mouse model.
Results: We generated 5 domain antibodies. ELISA and flowcytometry analysis showed that two of the domain antibodies bind both recombinant and cell membrane TIM-3 protein, and can block the interaction of Galectin-9 and TIM-3. Further, one domain antibody, R53, can boost the killing activity of CAR-T cells at concentrations above 10 ng/mL in vitro, and combination therapy with CAR-T can further suppress tumor growth in xenograft mouse model.
Conclusion: We successfully generated TIM-3 domain antibodies that can block the interaction of Galectin-9 and TIM-3, therefore being able to boost the anti-tumor activity of CAR-T cells.

P1697

Refractory or relapsed ALK-positive anaplastic T large cell lymphoma treated with crizotinib

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Refractory or relapsed ALK-positive anaplastic T large cell lymphoma do not respond to traditional chemotherapy, and the prognosis is poor. Systemic toxicity of targeted therapy is smaller than traditional chemotherapy, with better curative effect. Most patients have NPM-ALK fusion gene, and crizotinib is the first generation of ALK inhibitors. Four refractory or relapsed ALK-positive anaplastic T large cell lymphoma patients were treated with crizotinib. Patients can benefit from crizotinib even with myeloid invasion, hematophagocytic syndrome and brain invasion .We conducted a literature review and found that crizotinib had good curative effect and mild side effects, but the disease may recur quickly after drug discontinuation. Crizotinib can be used not only as a bridging therapy for hematopoietic stem cell transplantation but also as a maintenance therapy after transplantation. Crizotinib is expected to be the first line treatment of ALK+ALCL.

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DOI: 10.1002/eji.201970400

P1698

Contributions of T cell dysfunction to the resistance against anti-PD-1 therapy in oral carcinogenesis

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Background: Programmed death 1 (PD-1) blockade has great effect in the prevention of oral precancerous lesions, but the drug resistance has also been observed. The determinants of immune resistance during the malignant transformation are poorly understood.
Methods: Anti-PD-1 antibody was administered in the 4NQO-induced carcinogenesis mouse models. The mice were then subdivided into PD-1 resistance(PD-1R) group and PD-1 sensitive(PD-1S) group according to the efficacy. The expression of PD-1 and PD-L1, and the abundance of CD3+ T cells in tumor microenvironment between the two groups was tested by immunohistochemistry. In addition, the activation and effector functions, as well as the accumulation of immunosuppressive cells and expression of immune checkpoints of T cells in the draining lymph nodes and spleen between PD-1R and PD-1S group were analyzed by flow cytometry.
Results: Our results showed that T cell infiltration in tumor microenvironment, effector T cell cytokine secretion and central memory T cell accumulation in peripheral lymphoid organs were all inhibited in the anti-PD-1 resistance group. Furthermore, we found that an increase of regulatory T cell (Treg) population contributed to the resistance of the anti-PD-1 therapy. Notably, TIM-3 was found to be the only immunosuppressive molecule that mediated the resistance to anti-PD-1 therapy in the oral malignant transformation model.
Conclusions: Our findings identified a novel mechanism that T cell dysfunction contributes to the immune resistance during the malignant transformation of the oral mucosa. This study provides new targets for improving the efficacy of immunotherapy for early stage of tumorigenesis.

P1699

Efficacy and safety of sintilimab in combination with XELOX in first-line gastric or Gastroesophageal Junction Carcinoma (GC/GEJC)

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Background: Immune checkpoint inhibitors have shown benefit in advanced GC/GEJC. This phase 1b study evaluates the efficacy and safety of sintilimab, an anti-programmed cell death-1 antibody (PD-1 Ab) with XELOX for GC/GEJC in first-line setting (NCT02937116).
Methods: Patients with treatment-naïve unresectable locally advanced or metastatic GC/GEJC patients without HER2 amplification were enrolled. Patients received sintilimab 200mg IV q3w until disease progression, unacceptable toxicity or death, in combination with XELOX (oxaliplatin 130mg/m^2 IV D1 and capecitabine 1000mg/m^2 PO BID D1-14) for up to 6 cycles. The primary objective was to evaluate the efficacy per RECIST v1.1.
Results: Twenty patients were enrolled. As data cutoff (15 Jan 2019), median follow up was 5.8 months. The median dose of sintilimab was 6.5. ORR was 85.0% (95%CI, 62.1 to 96.8) and DCR was 100.0% (95%CI, 83.2 to 100.0). Among 17 patients with BOR of PR, two patients achieved a complete

DOI: 10.1002/eji.201970400

response (CR) of the target lesion. The median duration of response (DOR) and median progression free survival (PFS) had not been reached. Treatment-related AEs (TRAEs) occurred in 14 (70.0%) patients. The incidence of TRAE ≥ Grade 3 was 15%. AEs of immune-related etiology, occurred in 6 patients (30.0%). There were no AEs that resulted in death.
Conclusions: Sintilimab in combination with XELOX in first-line GC/GEJC shows promising anti-tumor efficacy and a tolerable safety profile. The further randomized, phase 3 study of Sintilimab in combination with XELOX in this setting is ongoing (NCT03745170).

P1700

Dual blockade of both LAG-3 and PD-1 produce a restorative immune response in a murine colon adenocarcinoma model

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LAG-3(CD223) is one potential immune checkpoint whose synergy with PD-1 has been observed in multiple tumor models. We have characterized a novel anti-LAG-3 fully-human antibody in previous study, which has high specificity and affinity to human LAG-3. We revealed that dual targeting of immune checkpoints PD-1 and LAG-3 significantly controlled tumor development of MC38 colon adenocarcinoma cells inoculated s.c. in transgenic C57BL/6 models. In order to explore *in vivo* function of anti-LAG3 antibody and its synergistic mechanism with PD1, we identified the immune cell populations and cytokines production in the peripheral blood, spleen and tumor microenvironment. Dual PD-1/LAG-3 blockade displays specific cytokines and spleen volumes changes compared with the control. T-cell phenotypes in the peripheral blood and spleen plasma show strong diversity among different treatment groups. Increased percentage of activated T cells and more production of cytokines, such as IL2, IFN-γ,TNFα were detected, which indicate a restorative immune response. We demonstrate that PD-1 and LAG-3 can synergistically interact to regulate T cell function, which expands the way how LAG3 interplay with other immune checkpoints. All these results represent a promising antitumor regimen for immunotherapy of solid tumors.

P1701

Assessment of programmed cell death ligand-1 expression with multiple immunohistochemistry antibody clones in non-small-cell lung cancer

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Objective: We demonstrated programmed death ligand 1 (PD-L1) protein expression in non-small-cell lung cancer (NSCLC) using three different primary antibodies. The concordance among antibodies was examined by analytical comparison, and the association between PD-L1 expression and clinicopathological characteristics was assessed.
Methods: The study utilized three anti-PD-L1 IHC antibodies, SP142, SP263 and UMAB228 to test PD-L1 status on 84 NSCLC specimens, and analyzed the concordance using Kappa coefficient.
Results: The samples from 40(47.6%), 51(60.7%) and 50(59.5%) patients were positive for PD-L1 expression evaluated by SP142, SP263 and UMAB228 assay, respectively. Kappa coefficient was 0.53, 0.58 and 0.46 for SP263 vs SP142, SP263 vs UMAB228 and SP142 vs UMAB228. On the other hand, univariate analysis showed that PD-L1 expression was associated with tobacco use.
Conclusion: The analytical comparison showed moderate concordance between SP142, SP263 and

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DOI: 10.1002/eji.201970400

UMAB228, whereas SP263 exhibited higher overall positive rate. Moreover, PD-L1 positive rate was significantly higher in patients with smoking history, which might help in identifying patients who would benefit from PD-1/PD-L1 checkpoint inhibitors.

P1702

Novel CD3ε humanized mouse models for preclinical pharmacology study of therapeutic bispecific antibody

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T cell-mediated immunotherapy has taken a prominent area for various diseases in immune-oncology. In this therapeutic approach, the CD3 complex is one of the key targets to modulate T cell activation; however, researches on therapeutic T cell-redirecting antibodies are often hampered by a lack of appropriate *in vivo* models, when the monoclonal antibodies specific to human CD3 can't cross-react with mouse endogenous CD3. Here we have generated a CD3ε-extracellular domain KI mouse, in which the mouse CD3ε extracellular domain was replaced by human CD3ε. We found human CD3ε was detected in the homozygous B-hCD3ε mice, while the mouse CD3ε was knocked out. The T cell development and subtypes in the spleen, thymus, lymph nodes, and blood in B-hCD3ε homozygous mice were comparable to that in wildtype C57BL/6 mice. After anti-hCD3ε antibody stimulation, normal *in vitro* T cell proliferation and cytokine production were found in humanized B-hCD3ε mice. Furthermore, we have demonstrated that the efficacy of bispecific antibodies that simultaneously bind to human CD3 and a tumor associated antigen (e.g. CD19 or EpCAM) can be evaluated in humanized CD3ε mice engrafted with tumor cells.
Taken together, our novel humanized CD3ε mouse is a useful model for *in vivo* efficacy study of CD3-mediated therapy, especially CD3ε based bispecific antibodies.

P1703

The biological effect of novel antibody against transferrin receptor in vitro

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Transferrin receptor(TfR) is a type II transmembrane glycoprotein on the cell membrane.TfR is an important marker for tumor targeted imaging and anti-tumor therapy because of its characteristics of extracellular segment binding, endocytosis and the role of human tumor cells pathology. However, it is a key point that how to obtain a large amount of antibody with high affinity. This research aims to obtain a new tetravalent antibody against TfR (TfR-TeAb).We had successfully constructed a new tetravalent antibody TfR-TeAb against TfR. We have obtained DG44-TfR-TeAb stable cell line by DHFR system. Purified TfR-TeAbs howed correct molecular weight, good purity, high specificity and appropriate affinity. TfR-TeAb can bond to TfR and be inernalized into cells, which hinted that it could act as a carrier. TfR-TeAb mediated the ADCC effect to kill tumor cell. The CHO-GRP78 stable cell line was established. Then the TfR-TeAb was transfected into CHO-GRP78 and CHO cells. GRP78 could enhance the viability of CHO cells in serum-free culture, thus increasing the antibody production. Furthermore, we discovered that GRP78 can enhance the quality of produced TfR-TeAb to improve their binding ability with TfR which hinted that GRP78 has potential application value in biopharmaceutical industry. GRP78 may inhibit the apoptosis induced by serum deprivation

DOI: 10.1002/eji.201970400

viainhibiting the activation of caspase-3.The expression of cleaved-caspase-3 was detected by Western Blot in CHO-GRP78 cells and CHO cells cultured in serum-free medium. The results showed that the cleaved-caspase-3 decreased expression in CHO- GRP78 cells was lower than that in CHO.

P1704

Vincristine-loaded platelets coated with anti-CD41 mAbs: a new proposal for the treatment of immune thrombocytopenia

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Immune thrombocytopenia (ITP) is an autoimmune disorder in which platelet-reactive autoantibodies accelerate the destruction of platelets. Macrophages play an important role in ITP through Fc receptor (FcR)-mediated antigen presenting and platelet clearance. In this study, a novel drug delivery system of vincristine-loaded platelets coated with anti-CD41 mAbs (CD41-VCR-PLT, CD41-VLP) was successfully established. The therapeutic effects and safety of CD41-VLP *in vitro* and *in vivo* were evaluated, and the possible mechanism was also explored. The results showed that PLT-CD41 could load VCR with high drug loading (DL) and encapsulation efficiency (EE), which were up to 41.16±1.92% and 60.73±2.79%, respectively, where platelets had no obvious morphological or functional changes. CD41-VLP could facilitate vincristine accumulation in macrophages, where the intracellular VCR concentrations were 30.72±3.11% at 72 h, which was significantly increased compared with the other groups ($P < 0.01$), thus inhibiting macrophage cell viability and inducing apoptosis. The cell viability inhibition rate and total apoptosis rate were 73.06±5.26% and 69.70±4.26%, respectively, both much higher than those of the other groups ($P < 0.05$). In the ITP mouse model, CD41-VLP increased platelet count in peripheral blood, which was 720±197.98 $\times 10^9$/L, and improved the platelet count significantly compared with that in the VCR group ($P < 0.05$); moreover, it reduced the systemic toxicity and peripheral neurotoxicity of vincristine. The possible mechanism was that CD41-VLP could precisely target M1 macrophages in spleen tissues through FcγR, thus reducing the platelet destruction caused by M1 macrophages. Therefore, CD41-VLP provides a new targeted therapy for ITP treatment.

P1705

βklotho is essential for the anti-endothelial mesenchymal transition effects of N-acetyl-seryl-aspartyl-lysyl-proline

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Endothelial dysfunction has been involved in different immunopathological processes. Endothelial-mesenchymal transition (EndMT) has emerged as an essential bioprocess responsible for the development of organ fibrosis. We have previously reported that fibroblast growth factor receptor 1 (FGFR1) is involved in the anti-EndMT effect of N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). FGFR1 is expressed on the cell membrane and performs its biological function through interaction with co-receptors, including βklotho (KLB). However, it remains unknown whether KLB is involved in the anti-EndMT effects of AcSDKP. Here, we demonstrated that AcSDKP increased KLB expression in an FGFR1-dependent manner and that KLB-deficiency induced AcSDKP-resistant EndMT via the induction of the mitogen-activated protein kinase (MAPK) pathway. In cultured endothelial cells,

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DOI: 10.1002/eji.201970400

AcSDKP increased KLB protein level in an FGFR1-dependent manner through induction of the FGFR1-KLB complex. KLB suppression by small interfering RNA (siRNA) transfection did not affect FGFR1 levels and resulted in the induction of EndMT. In contrast to the EndMT observed under FGFR1 deficiency, the EndMT induced by KLB suppression was not accompanied by the induction of Smad3 phosphorylation; instead, KLB-deficient cells exhibited induced activation of the MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK) and ERK pathways. Treatment with the specific MEK inhibitor U0126 diminished KLB deficiency-induced EndMT. Consistent with this finding, AcSDKP did not suppress either EndMT or MEK/ERK activation induced by KLB deficiency. Application of either FGF19 or FGF21 synergistically augmented the anti-EndMT effects of AcSDKP. Taken together, these results indicate that endogenous peptide AcSDKP exerts its activity through induction of the FGFR1-KLB complex in vascular endothelial cells.

P1706

B7-H1 blockade shapes a focused T cell response to tumor antigens

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Background: Polyclonal T-cell responses against a broad spectrum of antigens (Ag) and antigenic epitopes are desirable because they can prevent the emergence of resistant bacteria, viral, and tumor variants. However, due to immunodominance, few T-cell clones are actively generated to select epitopes during T-cell response to multiple antigens. B7-H1/PD-1 interaction blockade enhances T-cell responses and suppresses tumor growth. In addition to cancer cells, B7-H1 is expressed on dendritic cells (DCs), but its role in DC functions, especially when DC present more than one antigen, is less understood.
Methods: DCs can present multiple antigens (Ags) to stimulate dominant or subdominant T cell responses. Hence, tumor established mice were immunized with DCs pre-loaded with dominant or subdominant peptides and then treated with anti-B7-H1 mAb. Immune responses were analyzed by multiple T cell function assays in vitro or in vivo.
Results: Immunization with multiple tumor Ag-loaded DCs, in the absence of B7-H1, vastly enhances cytotoxic T lymphocyte (CTL) responses to dominant Ag. In sharp contrast, CTL responses to subdominant Ag were paradoxically suppressed, facilitating outgrowth of tumor variants carrying only subdominant Ag. Suppressed CTL responses to subdominant Ag are largely due to the loss of B7-H1-mediated protection of DCs from the lysis of CTL against dominant Ag.
Conclusions: B7-H1 expression on DCs may help maintain the diversity of CTL responses to multiple tumor Ags. This is a possible mechanism of tumor escape from therapy caused by biased stimulation of T cells to dominant antigen.

P1707

PAPPA2 mutation were related with superior response to PD-1/PD-L1 blockade in solid tumors

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Background: In one retrospective analysis, the PAPPA2 mutation was found over-presented in patients who diagnosed as non-small cell lung cancer (NSCLC) and experienced durable clinic benefit

DOI: 10.1002/eji.201970400

from anti-PD-1 immune checkpoint blockade (ICB). However, the robustness and underlying mechanism of PAPPA2 mutation in guiding ICB immunotherapies remain largely unknown.
Methods: The whole exome sequencing data, RNA sequencing and clinic characteristics was retrieved from TCGA database via cBioportal CGDS-R package. For the survival analysis, genomic data, non-synonymous mutation counts and response evaluation of the 5 cohorts were downloaded from publicly accessible publications reporting trials administrated with anti-PD-1PD-L1 or anti-CTLA-4 antibodies.
Results: In 5 cohorts, three in melanoma and 2 in NSCLC, the PAPPA2 mutant patients demonstrated higher objective response rate (ORR), prolonged progression free survival (PFS) and overall survival (OS) after ICIs treatment but not the conventional therapy. In these cohorts, significantly higher level of nonsynonymous mutation count were found in PAPPA2 mutate patients. In NSCLC and melanoma, elevated number of clonal neo-antigen was found in the mutant patients compared with PAPPA2 wild type patients. The functional profiling of $CD8^+$T cells (cytolytic activity, GZMB/CD8A, IFN-γ signal and PD-1) were significantly increased in PAPPA2 mutate patients compared with wild type ones.
Conclusion: The PAPPA2 mutation were closed to an inflamed immune microenvironment which featured as higher level of cytolytic activity, nonsynonymous mutation count and number of clonal neo-antigen. Patients with mutated PAPPA2 showed improved response rate and prolonged survival benefit to ICB immunotherapies

P1708

CASP8 deficiency leads to down-regulated tumoral dendritic cell infiltrating and B7/H1-PD-L1 blocking resistance

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Induction of immunogenic cell death were regard as a potential therapy to robust response to immune therapy. Caspase 8 play an important role in necroptosis which was regarded as more immunogenic form of cell death. However, the role of caspase 8 in anti-PD-1/PD-L1 antibody immunotherapy have not been well recognized. With Transcriptome data and mutate profile of CASP8 retrieved from TCGA database via cBioportal CGDS-R package, we found that the loss-of-function mutation of CASP8 occurs in multi-solid tumor types in clinical patients. Surprisingly, by performing CIBERSORT, we found that dendritic cell infiltration in CASP8 loss-of-function mutate melanoma patients was much less than that in CASP8 wild type ones. These findings were validated in CASP8 knock out B16F10-bearing mice model. Furthermore, the CASP8 deficiency in B16F10 leads to PD-1/PD-L1 blocking resistance in B16F10-bearing mice model. Our present results indicate CASP8 mutate as a potential non-beneficial biomarker for anti-PD-1/PD-L1 antibody immunotherapy.

P1709

Synergistic effects of low dose radiation and immune checkpoint therapy in cancer treatment

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Introduction: Immune checkpoint therapy (ICT), which removes the “brake” of the immune system to fight cancer, is considered as one of the major breakthroughs in cancer treatments. However, the major obstacle of ICT is its low response rate (only 20-30%). To tackle this problem, one approach is to combine traditional cancer therapies, especially radiation therapy (RT), with immune checkpoint therapy. It has been reported that low-dose irradiation (LDI) activates the immune system by altering

DOI: 10.1002/eji.201970400

tumor and immune cell surface molecule expression, promoting T-cell-stimulatory capacities of DC, or an anti-tumor macrophage phenotype. We are proposing that LDI is an adjunct therapy for ICT, and the combined treatment enhances the anti-tumor effects of ICT.
Methods: CT26 (colon adenocarcinoma) cell line were injected s.c. into the right flank of six-week old female BALB/c mice. Both PD-L1 and gamma beam whole body radiation treatments were started on the first day after the tumor injection (Day1).
Results and Discussion: LDR significantly improved the anti-tumour effects of PD-L1 therapy in mice for both preventive and established models, where mice treated with both ICT and LDR showed a significant reduction in tumour growth over a post-injection period of 18 days. However, animal survival rates did not differ with the addition of LDR, suggesting that further dose optimization is required.

P1710

Natural killer T cell agonist and PD-1 blockade cooperate to reduce intestinal tumor development

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Murine and human invariant natural killer T (iNKT) cells are activated by α-galactosylceramide (α-GalCer) presented on CD1d. We have shown that α-GalCer treatment leads to reduction in polyp size in the *APC*$^{min/+}$ model for human colon cancer, however this treatment induced anergy in iNKT cells characterized by upregulation of the inhibitory receptor PD-1. We used the *APC*$^{min/+}$ model to investigate whether combined treatment with α-GalCer together with PD-1 blockade results in improved effects on polyp development. We find that PD-1 expression was highly increased on T cells in lamina propria (LP) of *APC*$^{min/+}$ mice compared to polyp free *APC*$^{+/+}$ littermates. Anti-PD-1 treatment alone reduced polyp numbers and size. Moreover, the combined treatment with anti-PD-1 and α-GalCer had an additive effect on polyp size resulting in a highly significant reduction of large polyps. Analysis of immune cells in treated mice revealed that addition of PD-1 blockade to α-GalCer treatment prevented anergy induction and loss of iNKT cells, and resulted in increased activation of T cells, most notably in the polyps. Further, PD-1 blockade prevented α-GalCer induced accumulation of polymorphonuclear myeloid-derived suppressor cells in spleen and LP, while α-GalCer treatment, regardless of PD-1 blockade, skewed the macrophage phenotype from M2 towards M1 in the spleen. Taken together, we have shown that the combined treatment with α-GalCer and PD-1 blockade cooperate to reduce polyp formation, accompanied by increased T cell activation in polyps, and a less immunosuppressive environment.

P1711

Thymosin alpha1 mitigates intestinal immunopathology on immune checkpoint inhibition

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Immune checkpoint inhibitors targeting CTLA-4 and PD-1/PD-L1 have represented a major breakthrough in cancer therapy. However, a broad application in clinical practice is still limited by two major shortcomings: first, the efficacy is limited as the majority of patients do not respond to the therapy; second, the safety is compromised by side effects most commonly associated with colitis. Current efforts are devoted to make the tumor responsive to therapy by turning a non-T-cell-inflamed

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DOI: 10.1002/eji.201970400

("cold") into an infiltrated inflamed ("hot") tumor while preserving gastrointestinal integrity. Thymosin alpha1 (Tα1) is used worldwide as an immunomodulator in viral infections, immunodeficiencies, and malignancies. By modulating signals delivered through innate immune receptors, Tα1 regulates innate and T cell effector and regulatory functions. Indeed, it activates NK cells and increases infiltrating CD4+ and CD8+ T cells and the expression of MHC class I in tumor cells. This may potentially create a suitable background for the activity of checkpoint inhibitors, a hypothesis strengthen by a retrospective study demonstrating a higher overall survival of melanoma patients treated sequentially with Tα1 and ipilimumab vs ipilimumab alone. However, its ability to promote immune tolerance would anticipate an unforeseen capacity to prevent immune checkpoint inhibitor-induced colitis. In the present study, we have assessed the effect of Tα1 in different models of intestinal damage, including a checkpoint blockade-related colitis mouse model. Tα1 proved to be effective in preventing and curing pathological gut immune reaction, while preserving the antitumor activity of checkpoint inhibitors. Thus, Tα1 may exert multiple beneficial effects during tumor immunotherapy.

P1712

An emerging checkpoint for cancer immunotherapy: from preclinical models to the clinic

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The field of cancer immunotherapy is evolving rapidly, and CD73-targeted therapy serve as a valuable example for fundamental new therapeutic approaches. CD73 is an emerging immune checkpoint associated with adenosine metabolism that promotes tumor progression by suppressing antitumor immune response and promoting angiogenesis. The inhibition of CD73, in combination with immune checkpoint blockade, targeted therapy, or conventional therapy, improves antitumor effects in numerous pre-clinical models of cancer. CD73 blockade also has demonstrated clinical activity in patients with advanced solid tumors when combined with additional immune checkpoint inhibitors. We have intensively investigated the differential effects of CD73 expression by tumor versus non-tumor cells, and its relationship to suppression of the antitumor immune response, in addition to therapeutic targeting approaches evaluated in both preclinical models and patients with cancer settings. Deep characterization of CD73-mediated adenosinergic effect in the tumor microenvironment during ongoing clinical trials, holds the key to improve current immunotherapies for human subjects with aggressive malignancies

P1713

Lenalidomide and ILT2 blockade enhance NK cell function in chronic lymphocytic Leukemia

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Chronic lymphocytic leukemia (CLL) is associated with a profound suppression of the immune system affecting to practically all immune cells, but especially to NK cells. The overexpression of inhibitory receptors (best known as checkpoint proteins) on NK cells has been described in CLL and it has been involved in the suppression of NK cell activity. Here, we analyzed whether the checkpoint ILT2 (also termed LILRB1) is involved in the immunosuppression of NK cells in CLL. We observed that ILT2 expression was significantly decreased on leukemic cells, but increased on NK cells of CLL patients. ILT2 expression was more profoundly dysregulated in advanced patients and those patients with bad prognostic features, including those carrying chromosome del(11q). A significant dysregulation of ILT2

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DOI: 10.1002/eji.201970400

ligands on leukemic cells was also observed. The treatment with the immunomodulatory drug lenalidomide restored the expression of ILT2 on immune cells and partially reestablished the expression of its ligands on leukemic cells. Lenalidomide also increased the activation, proliferation and cytotoxic activity of NK cells resulting in an increased elimination of leukemic cells. This immune stimulatory activity of lenalidomide was strongly enhanced by the blockade of ILT2 using a monoclonal antibody. Overall, our results show that ILT2 is involved in the suppression of NK cell activity in CLL. Further, the anti-leukemic activity of NK cells may be restored by the combination of ILT2 blockade and lenalidomide, indicating that this combination could be a novel therapeutic strategy to be explored in this disease.

P2293

The diversity of gut microbiome is associated with favorable responses to anti-PD-1 immunotherapy in Chinese non-small cell lung cancer patients

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Gut microbiome affecting the responses to immune checkpoint inhibitors (ICIs) against advanced non-small cell lung cancer (NSCLC) has been investigated in western population. However, considering preexisting genetic and gut microbiota variation, the relevance remains unknown in east-Asian NSCLC population. In this study, 37 advanced NSCLC patients receiving the treatment of nivolumab were enrolled in the study from the clinical trials CheckMate 078 and CheckMate 870. Fecal samples were collected at the starting point, every time point receiving nivolumab as well as clinical evaluation and that with disease progression for 16S ribosome RNA gene sequencing. When subgrouping patients into responder (R) and non-responder (NR) according to the clinical response assessed by RECIST1.1, R patients harbored higher diversity of gut microbiome at the starting point with stable composition during the treatment. Patients with high microbiome diversity had significantly prolonged progression-free survival when compared to those with low diversity. Compositional difference was observed between two groups as well with the enrichment of *Alistipes putredinis, Bifidobacterium longum, Prevotella copri* in R whereas *Ruminococcus_unclassified* in NR. Strong associations were observed between high abundance of microbiome diversity in the gut and more frequencies of unique memory $CD8^+$ T cell and NK cell subsets in the periphery in response to anti-PD-1 therapy. These findings provide important implications for the prediction and the evaluation of anti-PD-1 immunotherapy against NSCLC in Chinese population.

P2294

Therapeutic effects of TeAb-Anti-CCP-ScFv-p53 against inflammatory reaction in DNaseII-/-IFN-IR-/- rats with rheumatoic arthritis

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Objective: To investigate whether TeAb-Anti-CCP-ScFv-p53 could be used as a blocking antibody on the treatment of DNaseII-/-IFN-IR-/- RA rat model.
Methods: DNaseII-/-IFN-IR-/- RA rats were treated with TeAb-Anti-CCP-ScFv-p53. The therapeutic effects were evaluated by a series of analyses concerning morphology, pathology, imaging and cellular immunology.

DOI: 10.1002/eji.201970400

Results: After 21 weeks of TeAb treatment, the average joint pad thickness of the rats in blocking group (BLK) was decreased from 11.03±1.14mm to 4.70±0.95mm (T=12.09, P< 0.001); Arthritis index of BLK group was decreased from 3.83±0.35 to 1.37±0.74 (T=8.45, P< 0.01). Pathological score of synovitis in BLK group was markedly improved, reflected by the reduction of synovitis T/NT ratio (from 1.27±0.08 to 0.99±0.10, T=5.89, P< 0.001) measured by 18F-FDG labeling, and the reduction of Synovial pannus T/NT ratio (from 11.02±1.34 to 4.70±0.95, T=12.08, P< 0.001) measured by 99mTc-3P4-RGD2 labeling. The levels of pro-inflammatory cytokines (IL-6, TNFa and IL-17a) and VEGF, as well as the ratio of Th17/Treg were lower, whereas the ratio of CD19+CD24hiCD38hi Breg cells was higher in BLK group than those in control group. A significant difference in the ratio of CCP secreting B cells/CD19+ B cells was observed between BLK and control group (7.76±3.29 vs 20.56±4.87, T=-5.45, P< 0.001).
Conclusion: TeAb-Anti-CCP-ScFv-p53 acts as a blocking antibody for the treatment of DNaseII-/-IFN-IR-/- RA rat model by interfering anti-CCP antibody mediated pathogenetic loop and repressing the autoimmune inflammatory reaction in RA.

P2295

Therapeutic bispecific T-cell engager antibody targeting the transferrin receptor

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Bispecific T-cell engager antibodies (BiTE) have been explored as a means to recruit cytolytic T cells to kill tumor cells. The transferrin receptor (TfR) is highly expressed on the surface of rapidly proliferating tumor cells. Therefore, it holds great potential in T cell redirecting therapies. In this paper, we developed a BiTE targeting TfR and CD3 (TfR-BiTE) and studied its therapeutic impact on TfR-positive cancer. TfR-BiTE had the ability to induce the selective lysis of various TfR-positive cancer cells through the activation of T cells, the release of cytokines, and then the coming proliferation of T cells, whereas TfR-negative cells were not affected. In a subcutaneous HepG2 xenograft model, low concentrations of TfR-BiTE inhibited tumor growth. Taken together, these results reveal that TfR-BiTE can selectively deplete TfR-positive HepG2 cells; hence, it represents a novel immunotherapeutic approach for the treatment of hepatocellular carcinoma.

P2297

Meta-analysis: nivolumab, ipilimumab and pembrolizumab *versus* chemotherapy for treatment of malignant melanoma

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This study aimed to analyze the efficacy of monoclonal antibodies ipilimumab, nivolumab and pembrolizumab for treatment of malignant melanoma. Data sources: MEDLINE, CENTRAL and LILACS. Articles were selected from categories: clinical study, clinical trials, comparative study, controlled clinical trial and randomized controlled trial, and should had compare the use of at least one monoclonal antibody with chemotherapy in the treatment of advanced melanoma. Three authors independently made the search at the databases and articles that had match the eligibility criteria (n=5) were selected. After, an expert researcher confirmed their quality (κ=1). For the ´progression-free survival´ and ´toxicity cessation´ variables, all five studies (n=1937) were included. One of these

DOI: 10.1002/eji.201970400

studies did not evaluate the ´overall survival´ variable in patients undergoing immunotherapy, resulting in four studies considered for it (n=1519). Both therapies had similar overall survival rates (OR: 0.868, 95% CI: 0.554-1.359, p: 0.535) and progression-free survival rates (OR: 0.794, 95% CI: 0.525-1.200, p: 0.273). However, when the toxicity interruption was evaluated, immunotherapy showed a higher index (OR: 0.385, 95% CI: 0.263-0.564, p< 0.001). Some studies have used monoclonal antibodies in monotherapy or in combination and some groups of participants showed heterogeneity, which made the analysis difficult. As conclusion, treatment interruption due to toxicity was lower in patients submitted to chemotherapy, suggesting that the current degree of aggressiveness of immunotherapy compromises the success of its therapeutic action. Taking into account the still higher costs of monoclonal antibodies, the evidence of its benefits is limited when considering the replacement of conventional therapy.

P2298

The effect of anemia on immunity in cancer patients and anti-PD-L1 efficacy in tumor-bearing mouse model

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Anemia is one of the common complications among patients with cancer. However, the impact of anemia on patient's immunity and anti-PD-L1 efficacy remains elusive. In this retrospective study, we found that non-small-cell lung cancer (NSCLC) patients with anemia (Hemolobin < 110 g/L) have poor overall survival (OS) and progression-free survival (PFS), and are susceptible to pneumonia and Epstein-Barr virus infections. Moreover, absolute numbers of lymphocytes, CD3+, CD4+T, CD8+T, and NK cells in blood the blood of NSCLC patients with anemia were substantially reduced. CEA-specific CD8+ T cell responses were dramatically suppressed in patients with anemia. As the patients' anemia statuses improved, lymphocytes, CD3+, CD4+ T, CD8+ T, NK, and B cells were all considerably increased, and the CD8+ T cell responses were also augmented. Tumor growth of anemia mice, induced by intraperitoneal injection of phenylhydrazine, could not be controlled after anti-PD-L1 therapy, both in B16F10- and MC38-bearing mouse model. Percentages of IFN-γ+ TNF-α+ and CD107a+ cells in CD4+T and CD8+ T cells were significantly lower in tumor tissue of anemia group compared to that without anemia. This study indicates that anemia might be unfavourable prognosis for cancer patients treated with immunotherapy.

P2299

Human immunoglobulin G hinge regulates agonistic anti-CD40 immunostimulatory and antitumor activities through biophysical flexibility

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Human immunoglobulin G (IgG) agonistic antibodies targeting costimulatory immunoreceptors represent promising cancer immunotherapies yet to be developed. Whether and how human IgG hinge and Fc impact on their agonistic functions have been disputed. Here, we show that different natural human IgGs confer divergent agonistic anti-CD40 immunostimulatory and antitumor activities in FcγR-humanized mice, including inactive IgG3 and superior IgG2. This divergence is primarily due to their CH1-hinges despite all human IgGs requiring Fc-FcγR binding for optimal agonistic activities.

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DOI: 10.1002/eji.201970400

Unexpectedly, biophysical flexibility of these CH1-hinges inversely correlates with and can modulate their agonistic potency. Furthermore, IgG Fc's optimized for selective FcγR binding synergize with and still require IgG hinge selected for rigidity to confer improved anti-CD40 immunostimulatory and antitumor activities. These findings highlight the importance of both hinge rigidity and selective FcγR binding in antibody agonistic function, and the need for newer strategies to modulate antibody agonism for improved clinical application.

P2301

Efficacy of potent neutralizing nanobody against SFTSV in humanized mice

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel bunyavirus that recently emerged in China. Infection with SFTSV is associated with case-fatality rates of of 6.3%-30%, and neither antivirals nor vaccines are available at present. Our data also indicated that neutralizing antibodies specific for SFTSV envelope glycoprotein (GN) played a vital role in the survival of SFTS patient. Compared to traditional antibody, nanobody from camel presented unique features like high stability, high affinity and low immunogenicity, which contributed to its widely application in antibody drug development. Here, we plan to develop humanized nanobody drug in the therapy of SFTS with lack of effective drug and vaccine. GN protein was expressed to immunize camel, which was applied to screen nanobody via phage display library. Accordingly, humanized nanobody (Nb-huFc) and AAV-delivered Nb-huFc (AAV-NB-huFc) will be constructed and generated, which will be evaluated in SFTSV infected humanized mice model. One potential nanobody drug against SFTSV infection will be provided.

P2302

Impaired STAT1 activation results elevated STAT3 activation and accelerated lung cancer growth

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The balance between STAT1 and STAT3 activation is critical for host immunity. In this study, we examined the role of their balance in lung cancer growth using the mouse orthotopic lung tumor cell implant model with wild type and partial STAT1 deficient ($STAT1^{+/-}$) recipients. Our data showed that reduced STAT1 activation by partial STAT1 deficiency led to increased STAT3 activation, elevated inflammatory responses, high levels of IL-6 and IL-17 expression, exhausted $CD8^+$ T cell generation, and accelerated lung tumor growth. These results support the notion of the opposing effects of STAT1 and STAT3 and further demonstrate that their balance plays critical roles in the tumorigenesis. Whether and how the altered balance between STAT1 and STAT3 activation may affect checkpoint blockade-based immunotherapy is currently under investigation.

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DOI: 10.1002/eji.201970400

P2303

Balancing anti-tumor efficacy and liver toxicity of 4-1BB antibodies through modulating agonistic strength and Fc-FcγR interactions

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Although agonist antibodies against co-stimulatory receptor 4-1BB show robust anti-tumor activity in preclinical models, their clinical development as a cancer therapeutic has been hampered by severe liver toxicity. We present distinct epitope- and isotype-dependent activation mechanisms responsible for the differential efficacy and toxicity of therapeutic anti-4-1BB antibodies. While ligand-mimicking anti-4-1BB can activate 4-1BB in the absence of Fc**γ**Rs with potential liver toxicity, non-ligand-mimicking Ab rely on Fc**γ**Rs to activate 4-1BB, do not induce liver toxicity, and maintain strong anti-tumor efficacy. While all Fc**γ**Rs can cross-link anti-41BB antibodies for co-stimulation, activating Fc**γ**R-induced antibody-dependent cell-mediated cytotoxicity (ADCC) compromises anti-tumor efficacy by deleting 4-1BB$^+$ effector cells. Taken together, binding-epitope and isotype co-determine the efficacy and toxicity of anti-4-1BB Abs. Manipulation of these characteristics may generate antibody candidates with favorable therapeutic profiles for cancer therapy.

P2304

Immune landscape-orchestrating PD-L1 heterogeneity controls cancer hallmarks and therapeutic efficacy

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Immune landscapes and cancer hallmarks of human PD-L1$^+$ tumors, as well as their roles in determining therapeutic efficacies are unknown. Here we identify, in detailed studies of gene data regarding 9769 patients of 32 types of human cancers, that PD-L1 cannot exclusively represent IFN-γ signature and potentially signifies pro-inflammatory myeloid responses in a tumor. PD-L1 heterogeneity endowed by local immune landscapes controls cancer hallmarks and clinical outcomes of patients. Mechanically, NF-κB signal elicited by macrophage inflammatory responses generates PD-L1$^+$ cancer cells exhibiting capabilities to aggressively survive, support angiogenesis, and metastasize, whereas STAT1 signal triggered by activated T cells induces PD-L1$^+$ cancer cells susceptive to apoptosis. Importantly, PD-L1$^+$ cancer cells generated by macrophages establish great resistance to conventional chemotherapy, cytotoxicity of tumor-specific effector T cells, and therapy of immune checkpoint blockade. Therapeutic strategy combining immune checkpoint blockade with macrophage depletion or NF-κB inhibition *in vivo* effectively and successfully elicits caner regression. Our findings imply that cancer patients may be treated with immune checkpoint blockade combining with macrophage depletion or NF-κB inhibition.

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DOI: 10.1002/eji.201970400

P2305

Immunological therapy: a novel thriving area for triple-negative breast cancer treatment

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Triple-negative breast cancer (TNBC) refers to cancers that are low in expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2. TNBC tends to behave more aggressively than other types of breast cancer. Unlike other breast cancer subtypes (ie, ER-positive, HER2- positive subtypes), there are no approved targeted treatments available, other than the administration of chemotherapy. Immunotherapy is a new kind of treatment approach for TNBC when compared with the surgical treatment, chemotherapy, endocrine therapy, and molecular targeting therapy. The present article reviews the research progresses of immunotherapy for TNBC in recent years. The full text structure covers molecular classification of TNBC, active immunotherapy of TNBC, passive immunotherapy of TNBC, oncolytic immunotherapy and the prospect of immunotherapy for TNBC. Considering the limitations of traditional treatment methods, immunotherapy might improve the survival rate and prognosis of TNBC patients based on its high specificity and immune memory, hopefully providing a new turning point for the treatment of TNBC. At present, there have been few studies on immunotherapy for TNBC patients. Most studies are still at the basic research stage, and many problems remain to be solved. Researchers need to further explore and carry out clinical validation studies. Nevertheless, we are convinced that immunotherapy will create a new era for TNBC treatment on the basis of basic research, the reform of science and technology, the discovery and screening of highly specific antigens isolated from tumors, and combined immunotherapy. This will also be an era of “individualized medicine” and “precision medicine”.

P2306

Application of CTLA-4 mutants with altered ligand affinities in HeLa cell populations for improving autoimmune drug efficiency and checkpoint blockade immunotherapy

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The initiation and maintenance of immune responses are controlled by both co-stimulatory and co-inhibitory signaling through T cell co-receptors. These receptors have both stimulatory (CD28, ICOS) as well as inhibitory roles (CTLA-4, PD-1, BTLAand TIGIT) in T-cell function. These pathways are targeted as part of immunomodulatory strategies to treat cancers, referred to as checkpoint blockade, and conversely to treat autoimmunity and CTLA-4 deficiency.
While the exact mechanism of CTLA-4 functioning remains unclear, pathway dissection has led to increased understanding of CTLA-4 functions, which has helped develop tests of human T cell function and Treg-cell suppression. CTLA- 4, predominantly a membrane protein, preserves its immunosuppression as a soluble protein. This finding has clinical potential, as affinity and stability engineered variants of CTLA- 4-Ig fusion molecules with enhanced pharmacokinetic profiles could yield improved immunotherapies. CTLA4-Ig fusion proteins Abatacept (approved for the treatment of rheumatoid arthritis) and Belatacept (approved for prophylactic-transplant-rejection) comprise human CTLA-4 fused to a modified human IgG1 Fc region and CTLA-4-Ig fusion protein respectively, containing mutations that modestly improve binding to the ligands CD80 and CD86. The clinical

DOI: 10.1002/eji.201970400

success of anti-CTLA-4 has ushered in the era of immune checkpoint therapies that have revolutionized cancer treatment.
In this study, we have used CTLA-4 variants expressing HeLa cells to characterize mutant populations with altered ligand affinities, suggesting that an increased affinity of CTLA-4 toward CD80 and CD86 correspondingly improves its affinity for the uptake of CD80-Ig and CD86-Ig. This has implications for improving drugs and targeted therapies against immune-regulatory disorders and cancer.

P2307

Generation of anti TNF therapeutic antibodies by implementing ALTHEA Gold Libraries™: nobel antibody libraries for therapeutic antibody discovery

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ALTHEA Gold Libraries™ are scFv semisynthetic libraries built on synthetic human well-known IGHV and IGKV germline genes combined with natural human CDR-H3/J_H (H3J) fragments. One IGHV gene provided a universal V_H scaffold and was paired with two IGKV scaffolds to furnish different topographies for binding distinct epitopes. Previous validation of of the libraries with seven targets yielded specific antibodies in all the cases. Further characterization of the isolated antibodies indicated KD values as human IgG1 molecules in the single-digit and sub-nM range and thermal stability (Tm) of all the Fabs was 75°C - 80°C (Valadon et al, mAbs, 2019) . In the present work, we have isolated several promising anti-TNF-α antibodies by panning and screening the libraries with recombinant Tumor Necrosis Factor alpha (TNF-α), a cytokine involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, psoriasis and Crohn´s disease. These antibodies are fully human, built with well-known and developable VH and VL scaffolds and highly stable. Currently, we are working on physicochemical characterization of the lead candidates and evaluating the biologic properties of these antibodies. Our results along with previous work demonstrate that ALTHEA Gold Libraries™ are a valuable source of high affinity and highly stable antibodies specific to well validated therapeutic targets.

P2308

Immunosuppressive, extracellular adenosine - a potential treatment target for multiple myeloma

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Although new drugs for multiple myeloma have improved survival, anti-PD1/PDL1 checkpoint therapies have been unsuccessful. Adenosine has emerged as a potent immunosuppressive molecule and an attractive new therapeutic target for cancer therapy. Two ectoenzymes, CD39 (ENTPD1) and CD73 (NT5E), catalyse formation of adenosine from extracellular ATP. CD39 converts ATP to AMP

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DOI: 10.1002/eji.201970400

and CD73 converts AMP to adenosine. When investigating the possible role of adenosine in the immune response to multiple myeloma, we found increased concentration of adenosine in bone marrow plasma from myeloma patients compared with healthy controls. CD39 was expressed by myeloma cells from patients and on myeloma cell lines, whereas CD73 was found on leukocytes and stromal cells in the bone marrow. Importantly, co-cultures of CD39+ myeloma cells and CD73+ stromal cells catalysed conversion of extracellular ATP to adenosine. Supernatants from such co-cultured cells reduced the proliferation of T cells stimulated with anti-CD3/CD28 beads. This proliferation blockade was reversed by inhibitors of the adenosine receptor A2AR, further indicating that the suppression was mediated by adenosine. A CD39 inhibitor, POM-1, and an anti-CD73 antibody inhibited adenosine generation in the co-culture system and prevented the blockade of T cell proliferation *in vitro*. Blocking of the adenosine pathway *in vivo* with a combination of POM-1, anti-CD73, and the orally-delivered A2AR antagonist AZD4635 reduced the tumor load in a murine model of multiple myeloma. This suggests that the adenosine pathway is involved in immune suppression in multiple myeloma and that this pathway is a potential therapeutic target.

P2309

Immune cell phenotype and cytokine secretion in mouse breast cancer responding to anti-CD47 antibody treatment

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Anti-CD47 antibodies have shown to inhibit the progression of several types of cancer by attenuating the “don't eat me” signals to macrophage through CD47/SIRPα interaction and cause phagocytosis of tumor cell. However, specificity in the response to the cancer cells was still a challenge. In this study, we investigated different cytokine level in 4T1 mammary carcinoma induced mouse breast cancer model with or without anti-mouse CD47 monoclonal antibody treatment. CD43, CD74, CD80 and CD86 protein expression were also upregulated after 1 week of 4T1 cells injection. Furthermore, 4T1 induced breast cancer mouse secreted higher amount of IL-1α in the tumor. After 4 doses of anti-mouse CD47 antibody (IP injection per 2 days started from one week after 4T1 challenge) treatment, anti-CD47 antibody not only significantly reduced tumor size but also inhibited IL-1 α secretion in spleen and elevated IL-1α secretion in tumor respectively. Interestingly, fresh isolated 4T1 induced breast cancer mouse spleen and bone marrow cells appeared 5%-10% CD11b+/Sca-1+ population whereas in vitro culture of 4T1 cells have 5% of cells of Sca-1+/CD11b+. If we continuously cultured freshly isolated 4T1 induced breast cancer mouse spleen and bone marrow cells for 2-3 weeks 10% - 20% of population showed Sca-1/CD11b double positive and 50-60% of the cells were CD117/CD11b double positive. This was the first study thoroughly investigated the response of cytokines and 4T1 induce mouse mammary carcinoma. Our data may further discover the mechanism for tumor growth and metastasis.

P3144

Preliminary observation of efficacy and safety of 15 patients with neuromyelitis optic spectrum disorder treated with rituximab

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Objective: This article is aimed to observe the efficacy, safety of rituximab in patients with neuromyelitis optica spectrum disorder (NMOSD).

DOI: 10.1002/eji.201970400

Methods: In this study, retrospective analysis of 15 patients with anti-aquaporin-4 antibody (AQP4-Ig-G) positive for rituximab and poor of treated with glucocorticoid therapy.The patient´s medication and clinical conditions were compared, and the differences in annual recurrence rate (ARR) and extended disability status scale (EDSS) before and after treatment were compared to evaluate the efficacy, safety of rituximab.
Results: 15 NMOSD patients (2 males and 13 females) received RTX treatment,The age of onset was 16-55 years old, with an average of (31.8±13.2) years, the course of disease was 1-144 months, and RTX treatment was 1-6 times.The annual recurrence rate (ARR) decreased from 1.83±2.30% before treatment to 0.07±0.26% after treatment ($P < 0.05$).The Extended Disability Status Scale (EDSS) score decreased from 3.27 ± 1.97 to 2.37 ± 1.85 ($P < 0.05$).The difference in EDSS and ARR between the patients before and after treatment was statistically significant.Only 3 patients developed mild adverse reactions during the treatment period, and no serious adverse reactions occurred. Common adverse reactions were: reversible liver enzyme elevation (changes in serum gamma glutamyltransferase) and infusion reactions.
Conclusions: Rituximab can reduce clinical recurrence and MRI lesion activity in NMOSD patients, improve the degree of disability in NMOSD patients, and is safe. It may be an effective drug for the treatment of NMOSD.
Keywords: Rituximab; neuromyelitis optica spectrum disorder (NMOSD).efficacy; safety; retrospective study

P3145

Antitumor activities of recombinant FGFR1-PD1-MIP3a/Fc fusion protein vaccine in mice model

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Tumor immunotherapy against programmed cell death protein 1(PD1) has been shown to effectively eliminate immunosuppression and inducing a persistent immune response. In this study, we evaluated the anti-tumor efficacy with recombinant FGFR1-PD1-MIP3a/Fc (rFPM) fusion protein as a hepatocelluar carcinoma model antigen. Remarkably, the rFPM fusion protein by injected intratumorally resulted in not only significant anti-angiogenic effects targeting FGFR1 but also additional promotion of tumor cell apoptosis and inhibition of tumor cell proliferation in the treated tumor tissues. Moreover, the peripheral dendritic cells migrate into tumor site by intratumoral injection of the rFPM fusion protein, which can effectively induce specific antitumor immune response. These findings support that the rFPM fusion protein suppressed tumor growth may be to the synergistically inhibition of tumor angiogenesis and cell proliferation, promotion of tumor cell apoptosis and immune effects significantly which could prevents exhaustion of antitumor T cells.

This work was financially supported by grant from Hainan Provincial Natural Science and Technology Foundation of China (818QN245; 2017CXTD008) and Hainan Key Research and Development Program (ZDYF2016159).

DOI: 10.1002/eji.201970400

P3146

Targeted antitumor activities of recombinant adeno-associated virus secreting PD1 in a mouse hepatocelluar carcinoma model

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Cancer immunotherapy with the PD1-blocking antibodies could prevents exhaustion of antitumor T cells and significantly enhances the efficacy of existing antitumor immune responses. In this study, we evaluated the antitumor efficacy of recombinant adeno-associated virus 8 (AAV8-sPD1), which inhibits the PD1/PDL1 pathway specifically within the tumor microenvironment by secreting a soluble form of PD1 from infected cells in a mice hepatocellular carcinoma model. Compared with the control groups, the tumor volume was significantly decreased and the survival time was significantly prolonged in AAV8-sPD1 group. Moreover, this recombinant virus can target the liver, over-express and secrete PD1 extracellular protein in the liver. Remarkablely, the recombinant virus both induced and maintained antitumor $CD8^+$ T cells responses within directly treated tumors and proved safer and more effective than control groups. These results demonstrate that **the recombinant virus could effectively suppressed tumor growth via reducing the immuno-suppression and improving antitumor immune responses without systemic toxicity.**

This work was financially supported by the Project of Hainan Province Innovative Team (2017CXTD008) and the National Science Foundations of China (81760553).

P3147

Hyperprogressive disease in cervical small cell carcinoma treated by immune checkpoint inhibitor

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A new progression pattern, hyperprogressive disease (HPD), has been recently acknowledged in cancer patients accepted immune checkpoint inhibitors (ICIs). We report a unique case of cervical small cell carcinoma which showed primary resistance to pembrolizumab, and was with a rapid radiological progression after the initiate of ICIs treatment. However, the detection results of multiple predictive biomarkers suggested that the patient was eligible for ICIs treatment. The whole exome sequencing showed that AKT1 E17K mutation was high (26.316%) in tumor tissue, and dynamic monitoring of circulating tumor DNA indicated that AKT1 E17K mutation rate was increasing successively and highly consistent with tumor growth in peripheral blood. Therefore, the correlation between AKT1 E17K mutation and HPD, and the role of AKT1 E17K mutation in identifying patients who might not benefit from ICIs treatment need to be further studied.

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DOI: 10.1002/eji.201970400

P3148

Predictive markers for efficacy in malignancies treated with immune checkpoint inhibitors

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Background: Predictive markers for tumor response and efficacy of immune checkpoint inhibitors (ICIs) are still controversial. Measurements of the widespread biomarkers, such as PD-L1 expression or tumor mutational burden (TMB), are invasive and costly. Therefore, we investigated several accessible factors to predict prognosis.
Methods: Clinicopathologic features and previous treatment records were collected from 64 patients with diverse malignancies between 2016 and 2018 in oncology department of Xinqiao hospital. Endpoints were progression free survival (PFS) and best overall response (bOR). The best cut-off points of continuous variables were determined by R. Kaplan-Meier was applied to analyze survival. The correlations between bOR and biomarkers were analyzed by Chi-square test.
Results: After a median follow-up of 5.5 months, a significant improvement in PFS was observed in 38 patients with higher body mass index (BMI, cut-off=21.45kg/m^2) compared to the other 26 patients (10.0 months vs 4.6 months, p=0.015), also the former experienced a tendency of higher bOR rate (28.9% vs 11.5%, p=0.178). Moreover, patients who had radiotherapy records experienced better PFS (12.3 months vs 4.5 months, p=0.019) and higher bOR rate (26.1% vs 19.5%, p=0.542) than the others. Further, receiving antibiotics during immunotherapy was a negative factor in the prognosis of ICIs, which led to worse PFS (4.2 months vs 9.7 months, p=0.019) and lower bOR rate (8.3% vs 25.0%, p=0.383) than its counterparts.
Conclusion: Higher BMI and radiotherapy records are associated with better clinical outcomes of immunotherapy, while receiving antibiotics is negatively correlated with efficacy of ICIs.

P3149

Dynamic changes of plasma PD-L1 mRNA expression is associated with anti-PD-1/anti-PD-L1 response in malignancies

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Background: PD-L1 expression in malignant tumor tissues is a rational biomarker to predict the efficacy and prognosis of anti-PD-1/anti-PD-L1 treatment, but few studies focus on the role of blood PD-L1 expression.
Methods: Fifty-one paired tissue samples and blood samples were collected from patients with diverse malignancies to investigate the correlation among tissue PD-L1 (tPD-L1) expression, plasma PD-L1 mRNA expression and soluble PD-L1 (sPD-L1) expression. Tissue PD-L1 were measured by immunohistochemistry. PD-L1 mRNA and self-designed plasma inner reference PLACON were measured by quantitative real-time PCR. Soluble PD-L1 were detected by ELISA kit. Besides, blood of 24 patients with malignancies (at baseline and within 2 months) who received anti-PD-1/anti-PD-L1 treatment were obtained to evaluate dynamic changes of blood PD-L1 expression and their correlation with efficacy and survival.
Results: Moderate correlation between tPD-L1 and PD-L1 mRNA (r=0.62, P< 0.001), weak correlation between tPD-L1 and sPD-L1 (r=0.37, P=0.007) and weak correlation between PD-L1 mRNA and sPD-L1 (r=0.32, P=0.02) were found. Further, patients who had over 2.03-fold PD-L1 mRNA increase (n=11) within 2 months experienced better progression-free survival (PFS) than those with less than 2.03-fold increase (n=13), these patients also had higher best overall response (bOR) rate (45.45% vs. 7.69%). By comparison, the dynamic changes of sPD-L1 expression had no significant correlation with PFS and bOR.

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DOI: 10.1002/eji.201970400

Conclusions: Our study demonstrates that plasma PD-L1 mRNA expression was significantly correlated with tissue PD-L1 expression, and provides proof for the application of plasma PD-L1 mRNA as a predictor for anti-PD-1/anti-PD-L1 treatment.

P3150

Inhibitory effect of apatinib combined with cisplatin on colon cancer cells and its molecular mechanism analysis

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Objective: To investigate the clinical efficacy of apatinib combined with cisplatin in the treatment of colon cancer and its effect
and molecular mechanism on colon cancer cells.
Methods: Eighty patients diagnosed with colon cancer in our hospital from October 2016 to October 2017 were randomly divided into two groups. The study group was treated with apatinib in combination with cisplatin, and the control group was only treated with cisplatinApatinib for treatment , and then the clinical efficacy of two groups of patients, adverse reactions and cancer cell proliferation rate, apoptosis rate and other indicators were compared.
Results: After the corresponding treatment, the efficacy of the study group was significantly higher than that of the control group (P ＜ 0. 05). After the treatment of colon cancer cells in both groups, the larger the drug concentration, the higher the inhibition rate of the cells, and at different concentrations (P ＜ 0. 05) . After treatment with different drugs, the apoptosis rate of both groups increased with time, and the apoptotic rate of the study group at 24 h, 48 h, 72 h (P ＜ 0. 05) . The incidence of adverse reactions in the study group was significantly lower than that in the control group (P ＜ 0. 05).
Conclusion: Apatinib combined with cisplatin is effective in treating colon cancer. It can effectively inhibit the proliferation of cancer cells and induce the apoptosis of cancer cells. In addition, there are few adverse reactions and should be widely used clinically.
Key words: Colon cancer; Apatinib; Cisplatin; Molecular mechanism; Clinical effect

P3151

Targeted therapy with PD-1 blockade and novel stimulatory signaling in colon cancer

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Immune checkpoints were widely study against tumor in medicine practice, but in solid tumor, the immunotherapy has little therapeutic effect. And the immune escape was the most important reason, which reported as common occurrence due to the complicated tumor microenvironment. Many evidences suggesting that the tumor infiltrating macrophages mainly play immunosuppressive function, escaping attack of T killer cells and facilitating the metastasis oftumor.Herein we report the PSG-1, one ganoderma lucidum polysaccharide, as immune regulator against the tumor infiltrating macrophages. The PSG-1 significantly reprogrammed tumor infiltrating macrophages from pro-tumor M2-like phenotype to anti-tumor M1-like phenotype. Furthermore, PSG-1 could promote the NO release and ROS generation. The functional mechanism analysis showed the Toll like receptor 4 (TLR4) was mediated in the process of PSG-1 induced the activation of macrophages. Besides, the PSG-1 also enhanced the proliferative activity of T-lymphocyte. Meanwhile, combined with PD-1

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DOI: 10.1002/eji.201970400

antibody therapy in CT26-bearing colon cancer model. The combined therapy could significantly enhance the levels of IL2, IL-8, TNF-α and IFN-γ. After the treatment of PD-1 inhibitor and PSG-1, the ratio of CD4+ positive and CD8+ positive T cells was remarkably increased. With the combined therapy, the bearing tumor growth was inhibited, and the survival analysis demonstrate the characteristics of high security of PSG-1 combined PD-1 antibody treatment. In conclusion, the strategy could prevent the immunosuppression through promoting the macrophage polarization, and further enhance the anti-tumor activity of T killer cells, it is of great significance to develop a new generation of immune checkpoint treatment.

P3152

Neutralizing potency of bispecific tetravalent antibody against BoNT/B and its mechanism of action

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Botulinum neurotoxins (BoNTs), known as the most toxic substance, are responsible for botulism. Currently, there is only horse or human antisera available against botulism, however, administration of antiserum can result in adverse effects. Within the past decade, efforts have been successfully made to develop neutralizing mAbs as substitutes for antisera, providing avenues to treat botulism with lesser side effects. But the potencies of mAbs against BoNTs are usually less than antisera. MAb has been intensely explored as inhibitor of BoNTs. Anyway, the two-receptor model of BoNTs makes it difficult to develop single mAb against botulism. Combination of two or three mAbs recognizing non-overlapping epitopes can neutralize BoNTs very potently. Because the Syt II and ganglioside receptor are essential for the entrance of BoNTs into neurons, two mAbs, recognizing Syt II and ganglioside receptor respectively, may neutralize synergistically against BoNTs. We have obtained three neutralizing mAbs, which recognize non-overlapping epitopes of BoNT/B, 8E10, responses to gangliosides receptor binding site, could synergistically collaborate with 5G10 or 2F4, recognizing Syt II binding sites. Our results implied the relationship between function of mAbs and structure of BoNT/BHc, which provided a potential universal partner for the synergistical combination with other mAb against Syt II binding sites in other BoNTs. Furthermore, we successfully developed an BoNT/B bispecific tetravalent antibody molecule using 8E10 and 5G10 as parental mAbs, which retained the antigen-binding activity of parental mAbs. The bispecific tetravalent antibody can remarkably protect mice from botulism. It may be a promising therapeutic agent for treatment of botulium

P3153

Immune checkpoints blockade by four tandem shRNAs embedded in homo- or hetero-miRNA backbones

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The functional influence of miRNA backbone selection remains unclear with respect to multiplexing miRNA-based shRNAs (shRNAmiR), due to a lack of comparative studies. To this end, we designed a pair of shRNAmiR tetramers targeting four different genes with a shared miR30a backbone (homo-BB) or four different miRNA backbones (hetero-BB). We established a PBLT$^+$ 293A cell line overexpressing four immune checkpoint molecules (PD1, BTLA, LAG3 and TIM3), which permitted

DOI: 10.1002/eji.201970400

simultaneous dissection of individual gene knockdown. Multi-target inhibition was confirmed by a decrease in positive cell populations of the relative gene and mean fluorescence intensities, with almost comparable activities of homo- and hetero-BB tetramers. Of note, this multi-inhibition was sustained over a one-month period, with no obvious difference especially in late-phased inhibitory effects between homo- and hetero-BB tetra-shRNAmiRs. These preliminary data may indicate little influence of scaffold substitution in functionalities of multiplexed shRNAmiRs and little recombination-depleted risk of repetitively adopting the same miRNA backbone in this artificial *in vitro* system. More comparative studies are further required to explore extended repertoires of scaffold-paralleled multi-shRNAmiRs in more physiologically relevant models.

P3154

Tumor associate antigen MUC1 combined with PD1 antibody, enhances antitumor immunity in mice models

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The MUC1 (Mucin 1) tumor associated antigen is aberrantly overexpressed in colorectal cancer and has generated considerable interest as an attractive target for immunotherapy. However, tumor vaccines are often difficult to elicit tumor antigen-specific T cells, the important reason is that T cell is exhausted by PDL1-PD1 (programmed death protein 1) signaling in the tumor microenvironment. In this article, we assessed whether tumor-associated antigen MUC1 combined with PD1 antibody could enhance the anti-tumor immune response and inhibit MUC1-positive colon cancer progression in a murine model. Our study revealed that the number of CD4+T cells and CD8+Tcells, and the amount of IFN-g+ andgranzymeB+CD8+T cells in tumor-infiltrating lymphocytes and draining lymph nodes of tumor tissues was significantly increased in the combined treatment group of MUC1 antigen and PD1Ab. Meanwhile, our results also suggested that the tumor size of the tumor-bearing mice in the combined treatment group was significantly reduced and the survival time of tumor-bearing mice was prolonged. Thus, our study established the combined treatment of MUC1 antigen and PD1 antibody can enhance the cytotoxicity of CD8+cells and transform the tumor microenvironment, and inhibit the growth of tumor cells.

P3155

Targeting either tumor or tumor stromal cells expressing TEM1 by a novel human antibody is highly effective against multiple cancers

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The tumor endothelial marker 1 (TEM1 / endosialin / CD248) is a tumor-restricted cell-surface protein expressed by certain tumor cells and tumor stromal cells. Here, we demonstrate that TEM1 is broadly overexpressed in various tumor tissues and its expression is associated with clinicopathological

DOI: 10.1002/eji.201970400

features and prognosis, making it a potential ideal target for antibody-based cancer therapy. We identified a fully human TEM1-specific antibody, Lh217, which has high binding affinity to both human and mouse TEM1 positive cells and can mediate ADCC and CDC. We also generated an antibody-drug conjugate (ADC), Lh217-DM1, by conjugation of Lh217 with maytansine (DM1) through an uncleavable SMCC linker. Both Lh217 and Lh217-DM1 specifically killed TEM1 positive tumor cells. Lh217 was specifically enriched in tumors and inhibited their growth as well as synergistically promoted the anti-tumor effect of chemotherapeutic drugs. Lh217-DM1 induced remarkable tumoricidal responses in human bone osteosarcoma, colon, pancreatic and prostate cancer xenograft models. Importantly, their administration didn't affect normal physiological function, such as liver and kidney function, and angiogenesis in wound healing and gestation. These results demonstrate that TEM1 is a good target for antibody-based cancer therapy, and that the fully human antibody Lh217 and its ADC are promising candidate therapeutics for various tumors.

P3156

Discovery and design of peptides as MMP9 inhibitors through structure-based molecular docking for targeted mantle cell lymphoma therapy

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Objective: To find potential peptide inhibitor against MMP9 which in turn, can inhibit MCL progression.
Method: MMP9 expression and its correlation with clinicopathological characteristics were analyzed using tumor tissue from MCL patients. CCK8 assay, Western blot and transwell assays were carried out to determine Jeko-1 cells which silenced of MMP9 by RNAi activity. MMP9 and peptides were docked using Molecular Operating Environment (MOE) software after structural optimization. Confocal microscopy and gelatin zymography were used to confirm the peptides can inhibit MMP9 activity in Jeko-1 cells. CCK8 assay, transwell assay and flow cytometry were performed to evaluate cell proliferation and metastasis.
Results: Patients with high MMP9 expression were more likely to present with high stage (P=0.010), bone marrow invasion (P=0.033) and high level LDH (P=0.000). Silencing of MMP9 in Jeko-1 cells by RNAi suppresses cells migration and invasion in vitro. According to the docking results, peptide M3 bound deeply in the binding pocket of MMP9 and had interaction with the active-site Zn^{2+} ion in the catalytic domain. In contrast to the other peptides, M3 had higher affinity with Jeko-1 cells highly expressing MMP9 and CHO cells transfected with MMP9 plasmid. M3 was not only compatible with MMP9, but also inhibited its activity. M3 inhibited Jeko-1 cells proliferation, metastasis and cell cycle progression, and promoted cell apoptosis rate.
Conclusion: The peptide M3 designed through structure-based molecular docking has potential use as an antagonist in the treatment of MMP9-overexpressed MCL.
Keywords: Mantle cell lymphoma; Metalloproteinase-9; Peptide; Molecular docking;

P3157

TIM-3: an emerging target in the liver diseases

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T cell immunoglobulin domain and mucin domain containing molecu le 3 (TIM-3) is found expression in the surface of terminally differentiated T cells and belongs to the TIM family of type Ⅰ transmembrane proteins. It binds to the ligand Galectin-9 and mediates T cell apoptosis. As the

DOI: 10.1002/eji.201970400

research progresses, TIM-3 is also expressed in Th17, NK, Monocyte, which binds to ligand, and induce immune peripheral tolerance in both mice and man. Numerous researches have demonstrated that TIM-3 influences liver diseases, including liver-associated chronic viral infection, liver fibrosis, liver cancer et al and suggest new approaches to intervention. Currently, targeted therapy of TIM-3 is a new treatment in the field of immunization. Although many studies have proven that TIM-3 has an inhibitory effect in vivo, the specific mechanism is not clear. Herein, we summarize the important role of TIM-3 in the regulation of liver disease and prospects for future clinical research. TIM-3 will provide new targets for improving clinical liver disease.

P3158

Blocking of the PD-1/PD-L1 interaction by an affinity peptide for cancer immunotherapy

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Although great success has been obtained in the clinic, the current immune checkpoint inhibitors still face two challenging problems: low response rate and immune-related adverse effects (irAEs). Many tumors can upregulated expression of PD-L1, inhibiting antitumor T-cell responses and avoiding immune surveillance and elimination. Blockade of the PD-L1 and its ligand PD-1 has emerged as a promising immunotherapy for treating cancers. Here we screened out the PD-L1 specific affinity peptides (PPL-C) with an affinity of 0.75μM by using the technology of mirror-image phage display. PD-1/PD-L1 interaction was effectively interfered by PPL-C that resulted in reverses PD-L1-mediated inhibition of T-cell activation. Subcutaneous or intraperitoneal administration for the treatment of early mouse CT26 colon cancer is effective, with a tumor inhibition rate of 77% and prolonging the survival time of the animal about 50%. The PPL-C demonstrated completely opposite effects of conventional PD-L1 axis by promoting T-cell proliferation, inhibiting Tregs differentiation, regulating cytokine production, inducing activation and cytotoxicity of lymphocyte in tumor microenvironment, especially the proliferation of CTL, $CD3^+CD8^+IFN\text{-}\gamma^+$, $CD3^+CD4^+IFN\text{-}\gamma^+$, $CD4^+$Teff and $CD8^+$Teff is extremely significant. Stimulation of PD-L1 antibody and PPL-C in vivo or in vitro indicates that PPL-C has less immunological side effects on cell subsets, as well as study of PPL-C toxicity, pharmacokinetics, in vivo half-life, tissue distribution and specificities, which suggest the potential of developing peptides as low molecular weight drug candidates for corresponding cancer immunotherapy.

P3159

Therapeutic efficacy, pharmacokinetic profiles, and toxicological activities of humanized antibody-drug conjugate Zt/g4-MMAE targeting RON receptor tyrosine kinase for cancer therapy

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DOI: 10.1002/eji.201970400

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Background: Aberrant expression of the RON receptor tyrosine kinase is a pathogenic feature and a validated drug target in various types of cancers. Here we report the development and validation of a novel humanized anti-RON antibody-drug conjugate for cancer therapy.
Methods: The humanized anti-RON antibody H-Zt/g4 was conjugated with monomethyl auristatin E using a dipeptide linker to form H-Zt/g4-MMAE. Pharmacokinetic analysis and toxicological activities of H-Zt/g4-MMAE were determined in mouse and cynomolgus monkey. Biochemical and biological assays were used for measuring RON expression, internalization, cell viability and death. Therapeutic efficacies of H-Zt/g4-MMAE were validated *in vivo* using three pancreatic cancer xenograft models.
Results: H-Zt/g4-MMAE had a drug to antibody ratio of 3.77:1, and displayed a favorable pharmacokinetic profile in both mouse and cynomolgus monkey. *In vitro*, H-Zt/g4-MMAE induced RON internalization, which results in killing of pancreatic cancer cells with IC_{50} values at 10-20 nM. *In vivo,* H-Zt/g4-MMAE inhibited pancreatic cancer xenograft growth with tumoristatic concentrations at 1~3 mg/kg bodyweight. Significantly, H-Zt/g4-MMAE eradicated tumors across multiple xenograft models regardless their chemoresistant and metastatic statuses. Moreover, H-Zt/g4-MMAE inhibited and eradicated xenografts mediated by pancreatic cancer stem-like cells and by primary cells from patient-derived tumors. Toxicologically, H-Zt/g4-MMAE is well tolerated in mice up to 60 mg/kg. In cynomolgus monkey, H-Zt/g4-MMAE up to 30 mg/kg had a manageable and reversible toxicity profile.
Conclusions: H-Zt/g4-MMAE is superior in eradication of pancreatic cancer xenografts with favorable pharmacokinetic profiles and manageable toxicological activities. These findings warrant the transition of H-Zt/g4-MMAE into clinical trials in the future.

P3160

Clinicopathological significance and prognostic value of programmed cell death ligand 1 (PD-L1) expression in resected gastric cancer: a meta-analysis

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Background: PD-L1 expression has become a promising biomarker in predicting the efficacy of immune checkpoint inhibitors in various advanced cancers, including gastric cancer (GC). However, the prognostic value and clinicopathological significance of PD-L1 in GC is still not clearly identified. Hence, we carried out a meta-analysis to investigate the potential role of PD-L1 in GC, expecting to provide new clues for the judgement of prognosis in resected gastric cancer.
Methods: A literature search strategy was performed from the PubMed, Web of Science, EMBASE, and the Cochrane Library database as of December 1, 2018. Relevant data was rigorously extracted from the included literatures. HRs and ORs along with 95% CIs were used to evaluate the prognostic value or clinicopathological significance of PD-L1 expression in GC.
Results: A total of 21 studies containing 6021 patients fitted into our meta-analysis. The pooled HRs indicated that PD-L1 high-expression accompanied with a worse OS(HR=1.28, 95%CI:1.01-1.62, *P*=0.04) in GC, while no correlation with DFS(HR=0.88, 95%CI:0.53-1.45, *P*=0.61). Subgroup analysis showed that PD-L1 high-expression was negatively associated with distant metastasis groups (OR=0.66, 95%CI:0.48-0.89, P=0.01), but was positively related to elderly(OR = 1.19, 95%CI: 1.01-1.40, P=0.04), lymph-node metastasis(OR=1.63, 95%CI:1.08-2.46, P=0.02), deeper tumor infiltration(OR=1.73, 95%CI:1.08-2.79, P=0.02), EBV infection positive (OR=8.01, 95%CI:3.10-20.72, P< 0.0001), and MET positive groups (OR=1.78, 95%CI:1.05-3.00, P=0.03).
Conclusions: Our meta-analysis found that the high-expression of PD-L1 accompanied with a poor OS. Moreover, PD-L1 high-expression was related to age, lymph-node metastasis, depth of infiltration, distant metastasis, EBV infection, and MET status.

DOI: 10.1002/eji.201970400

P3161

Pancreatic cancer therapy with PD-1 blockade and anti-tumor immunity of a novel oncolytic vaccinia virus expressing immunoregulatory protein LZ-8 in murine model

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Pancreatic ductal adenocarcinoma (PDA) is characterized by its highly immunosuppressive tumor microenvironment (TME) that limits T cell iniltration and induces T cell hypofunction. Current therapeutic interventions have limited effects on patients with late-stage disease. Although immune checkpoint inhibitors are currently approved for several types of cancers, the response rate is often limited by the lack of tumor specific T-cells within PDA. Therefore, new combinatorial strategies are needed to enhance the clinical benefit of immune checkpoint inhibitors. One emerging approach is genetically modified oncolytic viruses. We have previously developed oVV-gene, an oncolytic vaccinia virus platform capable of directing the immune response toward tumor epitopes. In this study, we evaluated whether the oVV-LZ8 could be used to increase the response rate to PD-1 blockade in PDA. LZ-8, an immunomodulatory protein isolated from Reishi, has been shown to promote cell proliferation and IL-2 production in T cells. Here, we provided evidence that oVV-LZ8 induced apoptosis in the infected PDA cell lines *in vitro* as well as promoted Th1 phenotype in $CD4^+$ T cells and M1 polarization in THP-1 cells. Using KPC syngeneic mouse PDA model, we demonstrated oVV-LZ8 infection potently suppressed KPC tumorigenesis. More importantly, oVV-LZ8 enhanced the tumor inhibitory effects of PD-1 antibody *in vivo* and was associated with increased tumor-infiltrating lymphocytes, number of NK, $CD8^+$ and $CD4^+$ cells as well as increased secretion of anti-tumor cytokines, IFN-α, IFN-γ and IL-2. Consistent to our previous results, improved cytotoxicity was observed with combination of oVV-LZ8 and anti-PD-1 in murine PDA model.

P3162

EGCG mediated tumor killing through PD-L1/L2 immune checkpoints blockade and autoinflammation inhibition

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Sustained autoinflammation in tumor microenvironment promotes tumor growth and disease progression. Here we investigated how tumor autoinflammation/inflammasome activation affects the expression of immune checkpoints (ICP) molecules (PD-L1/L2) in melanoma. Compared to early-stage melanoma, late-stage cells expressed higher ICP molecules, and this trend was more evident with PD-L2 expression. The expression of ICP molecules greatly increased upon the stimulation of pro-inflammatory cytokines IL-1, whereas the inhibition of autoinflammation, especially the NLRP3-IL-1 pathway, significantly decreased the expression of ICP molecules. This inhibitory effect was more evident with PD-L2 expression, suggesting PD-L2 expression is more related with autoinflammation activation in melanoma. Further analysis revealed that the activation of inflammasome augmented IFNγ-induced ICP molecules through STAT-IRF and NLRP3-IL1B pathways. Therefore, we used Epigallocatechin gallate (EGCG), the most abundant catechin in tea, to inhibit both STAT-IRF and NLRP3-IL1B pathway, and found EGCG significantly deceased the auto-inflammation and ICP expression both *in vitro* and *in vivo*. To investigate anti-tumor immune responses, mouse melanoma B16F10 was used. Cytotoxic T lymphocyte assay showed that EGCG increased CD8+ T cells killing against B16F10 cells *in vitro*. *In vivo* study showed EGCG inhibited not only tumor PD-L1 expression but also tumor progression of B16F10 in mice, and this tumor suppressive effect was equivalent to that

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DOI: 10.1002/eji.201970400

from PD-1 antibody. In summary, our findings for the first time associate the autoinflammation/inflammasome and ICP molecules expression. Inhibition of both autoinflammation and ICP molecules expression might serve as a novel cancer therapy strategy, which EGCG could be a potential candidate.

Adoptive Cell Therapy

O113

The stem cell-derived viral antigen-specific T cells reduce HBV replication through production of IFN-γ and TNF-α

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The viral antigen (Ag)-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) derived from pluripotent stem cells (PSCs), i.e., PSC-CTLs, have the ability to suppress the hepatitis B virus (HBV) infection. After adoptive transfer, PSC-CTLs can be programmed to be HBV-specific and to infiltrate into the liver to suppress HBV replication. Nevertheless, the mechanisms by which the viral Ag-specific PSC-CTLs provoke the antiviral response remain to be fully elucidated. In this study, we generated the functional HBV surface Ag-specific CTLs from the induced PSC (iPSCs), i.e., iPSC-CTLs, and investigated the underlying mechanisms of the CTL-mediated antiviral replication in a murine model of HBV infection. A hydrodynamic injection of an HBV expression plasmid, pAAV/HBV1.2, into the liver induced HBV replication efficiently in mice. We show that adoptive transfer of HBV surface Ag-specific iPSC-CTLs greatly suppressed the HBV replication in the liver and blood in the animal model and prevented the HBV surface Ag expression on hepatocytes. Furthermore, we demonstrate that the adoptive transfer significantly increased the migration of $CD8^+$ T cells and the production of the antiviral cytokines (IFN-g, TNF-a) in the liver. These results indicate that the stem cell-derived tissue-associated CTLs can robustly accumulate in the liver and suppress the HBV replication through producing antiviral cytokine.

O114

Study of stem cell niche favoring drug resistance, immune suppression and maintenance of leukemia supportive microenvironment in acute myeloid leukemia

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Acute Myeloid Leukemia (AML) is the most common form of leukemia in adults. Immune-inflammatory cells and mesenchymal stromal cells (MSC) which form part of tumor microenvironment, play a critical role in cancer initiation and progression. The study was aimed at profiling of leukemic blasts, immune subtypes, MSC and soluble factors in AML bone marrow microenvironment and evaluating sensitivity of leukemic blasts to chemotherapeutic drugs and immune cells. MSC were successfully cultured from bone marrow of high-risk AML patients. AML-MSC and leukemic blasts were characterized for ultrastructure by transmission electron microscopy and surface markers by flow cytometry. AML-MSC exhibited characteristic profile of MSC Type-II $CD90^+CD45^{lo}$ expressing high percent of TLR3 than

DOI: 10.1002/eji.201970400

TLR4 receptors, indicating pro-tumorigenic nature. At ultrastructural level, MSC demonstrated increased mitochondria and vesicles near leading edges. MSC and leukemic blasts exhibited increased secretion of immunosuppressor adenosine and adenosine receptors A1R and A2BR, and varying expression of immune checkpoints Tim3 and PDL1. MSC were found to be resistant to anti-cancer drugs as compared to HL-60 and OCI-AML-2 cells. Laser confocal microscopy revealed increased survival of HL-60 in co-cultures due to transfer of mitochondria from MSC to leukemic blasts, suggesting transfer of cellular contents through extracellular vesicles. It was interesting to note that MSC and vesicle-rich conditioned media demonstrated significant efficacy against AML xenograft in NOD-SCID mice. Understanding crosstalk between MSC-AML blasts, and landscape of soluble factors involved in chemoresistance and immune suppression may give us leads to manipulate microenvironmental niche to control abnormal myelopoiesis in AML and develop targeted therapy.

O115

Double negative T cells mediate Lag3-dependent antigen-specific protection in OVA-induced allergic asthma

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Allergic asthma is a chronic inflammatory disorder of the airways. Traditional therapies have been unsatisfactory in controlling the underlying pathology. New approaches that overcome the detrimental effects of immune dysregulation are required. We have identified a new differentiation pathway for **CD4−CD8− (double negative, DN) T cells** that exhibits potent immunosuppressive function *in vitro* and *in vivo*, however, without CD4 molecule, the critical coreceptor of the TCR that assists the TCR in communicating with MHC-II; **what allows DN T cells to recognize MHC-II and maintain antigen specificity is still unknown**.
In this study, we adoptively transferred ovalbumin (OVA) peptide 323-339 primed DN T cells intravenously into a mouse model of OVA-induced allergic asthma. OVA-induced airway hyperresponsiveness, lung inflammation, mucus production, granulocyte accumulation and OVA-specific IgG/IgE production were significantly suppressed after DN T cell therapy. **The immunosuppressive function of the OVA-specific DN T cells was dependent on inhibiting CD11b+ dendritic cells, T follicular helper cell proliferation and IL-21 production.** Compared with CD4+ T cells, DN T cells markedly upregulated Lag3 expression. A significantly lower frequency of I-Ab $OVA_{323-339}$ tetramer-positive cells and decreased inhibition function were observed in the OVA-primed $Lag3^{-/-}$ DN T cells compared with OVA-primed DN T cells.
In conclusion, **elevated Lag3 expression and trogocytosis contribute to MHC-II antigen recognition by DN T cells**. The effectiveness of *ex vivo* generated allergen-specific DN T cells in alleviating airway inflammation supports the **feasibility of potentially utilizing this novel cell-based therapeutic approach** clinically for the treatment of allergic asthma with antigen specificity.

DOI: 10.1002/eji.201970400

O116

Dissecting the T cell receptor interactions governing recognition of peptide-MHC complexes

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The promiscuous nature of T-cell receptors (TCRs) is fundamental for our ability to recognize and fight a large range of pathogens; however, this feature makes it challenging to fully understand and control T-cell recognition. Existing technologies provide limited information about the key requirements for T-cell recognition and the ability of TCRs to cross-recognize structurally related elements. Herein we present a proof-of-concept of a novel strategy to establish TCR recognition patterns that will contribute to our fundamental understanding of TCR interactions with the peptide-major histocompatibility complex (pMHC) and can serve as an important tool to assess potential TCR cross-recognition prior to clinical development. We examined the requirements for TCR interaction with pMHC by the use of libraries of peptide variants, which are generated from sequentially substituting each position of the originally identified target peptide with all naturally occurring amino acids. We assembled these onto MHC multimers labeled with DNA barcodes, which allows the simultaneous assessment of the interaction between a single TCR and multiple pMHC variations. With this approach, we established an affinity-based hierarchy of pMHC interactions, which enabled us to describe the TCR recognition motif, here termed the TCR fingerprint. We describe the TCR fingerprints of 16 different TCRs that target cancer-specific epitopes and use these to predict and validate cross-recognized peptides. The fingerprints differed even among TCRs recognizing the same epitope and hence demonstrated the value of this strategy for understanding the T-cell recognition profile and potential cross-recognition, which is crucial for selecting TCRs intended for clinical applications.

O117

The culture dish surface influences the phenotype and cytokine production of immunogenic and tolerogenic monocyte-derived dendritic cells

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Monocyte-derived dendritic cells (moDC) are an important scientific and clinical source of functional dendritic cells (DC). However, the optimization of the generation process has to date mainly been limited to the variation of soluble factors. In this study, we investigated the impact of the cell culture surface on the DC phenotype and cytokine profile. We compared a standard cell culture dish to a non-adherent culture dish for two immunogenic maturation conditions, two tolerogenic conditions and an unstimulated control. Phenotype and cytokine profile were determined after a 3-day culture. Light microscopy revealed an increase in homotypic cluster formation correlated with the use of non-adherent surfaces which could be reduced by using blocking antibodies against CD18. All surface markers analyzed showed moderate to strong differences depending on the culture dish surface. Significant differences in the secretion of many cytokines were observed, especially for cells stimulated with LPS. These results provide evidence that the DC phenotype crucially depends on the

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DOI: 10.1002/eji.201970400

culture dish surface used during moDC generation. This might have an important application in the optimization of DC-based immunotherapy development and underlines that the local surrounding can interfere with the final DC population beyond the soluble factors.

O118

Enhanced stealth property on iPSC to generate universal T cell source for allogeneic cancer immunotherapy

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A key to success of allogeneic iPSC-derived T-cell immunotherapy should be immunosuppressant-independent control of the T-cell graft rejection by host immunity. One approach to escape from the host immunity is to “stealth” the T-cell graft by antigen-modification at iPSC. Here we successfully generated cancer antigen-specific functional T cells derived from a *B2MG*, *CIITA*, and a NK-cell activating ligand triple knockout and single chain *HLA-E* transduced iPSCs. Such HLA Class I and II-null and NK-mediated killing resistant T-cell grafts were not recognized by CD8 T cells, CD4 T cells and NKG2A+ and/or DNAM+ NK cells of allogeneic recipient in vitro. Importantly, these T cells showed better survival than HLA-expressing parent iPSC-T cells in vivo model. These results suggest the feasibility of the generation of iPSC-derived T cells with a reduced allo-reactivity using CRISPR/Cas9 technology.

O119

Human gingiva-derived mesenchymal stem cells treat murine model with aplastic anemia through regulating the balance between Treg and Th17 cell

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Severe aplastic anemia (AA) is a rare autoimmune disease characterized by severe pancytopenia and bone marrow failure. The recent finding of an abnormal quantity and function of T helper (Th) and regulatory T cells (Tregs) in peripheral blood of patients has emerged as the major cause of the condition. Accumulating evidence has revealed that human gingiva-derived mesenchymal stem cells (GMSCs) are emerging as a new line of mesenchymal stem cells and may have the potential to control or even treat autoimmune diseases through correcting the balance between Th and Tregs. Given that GMSCs have a robust immune regulatory function and regenerative ability, we investigated the effect of GMSCs on preventing T cell-mediated bone marrow failure (BMF) in an AA mouse model. We observed that infusion of GMSCs markedly attenuated histological BM damage, controlled immunologically-mediated disorders in AA mice, significantly improved AA mice survival by significantly reducing cell infiltration of both CD8+ cells and CD4+ T cells (including Th1 and Th17 cells) in lymph nodes and decreased pro-inflammatory cytokines levels in lymph nodes and plasma.

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DOI: 10.1002/eji.201970400

Moreover, GMSCs up-regulated the levels of Tregs in lymph nodes. Our results demonstrate that the predominant Th1 and Th17 cell infiltration in the AA model could be significantly reduced while Tregs are increased by GMSCs treatment. Our data demonstrate that GMSCs can attenuate T cell-mediated bone marrow failure through targeting the balance of Th17 and Treg cells, implicating that the use of GMSCs many have potential clinical application in prevention and treatment of patients with AA.

O120

Establishment of a genetic manipulation-free method for long-term culture of murine lymphoid progenitors with differentiation potential

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Common lymphoid progenitors (CLP) can differentiate to a variety of lymphoid-lineages such as T- and B-lymphocytes, dendritic cells, natural killer cells and innate lymphoid cells. Here, we describe culture conditions which, for the first time, allow the establishment of uncommitted, long-term proliferating CLP cell lines and clones from a small pool of these cells from normal mouse bone marrow, without any genetic manipulation. The cultured CLP (cCLP) had the differentiation potential to lymphoid and myeloid cells in vitro and in vivo at a single clone level. This simple method to obtain robust numbers of cultured non-committed CLP will contribute not only to clarify the yet-unknown immunological mechanisms, but also to build a basis for the development of new immune cell therapies in humans.

LB033

Induction of cytomegalovirus pp65-specific CD8+T Cells *in vivo f*ollowing administration of autologous tumor lysate-pulsed dendritic cells, co-pulsed with zoledronate and a-galactosylceramide in glioblastoma multiforme patient

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Vg9gdT cells, innate immune cells activated by zoledronate (Z), and Va24 natural killer T (NKT) cells, innate/adaptive immune cells activated by a-galactosylceramide (G) can link innate and adaptive immunities through cross talk with IFNa-derived DCs (IFN-DCs) from patients with GBM in a way that can amplify the activation and proliferation of CMVpp65-specific CD8+T cells and that can reduce proliferation of CD4+CD25+FoxP3+regulatory T cells (Treg cells) *in vitro*. In this study, we demonstrate for a patient with GBM that multiple administrations of IFN-DCs pulsed with autologous tumor lysates and Z plus G to the patient result in the enhancement of CMVpp65-specific CD8+T cells and in the reduction of the Treg cells *in vivo.* This leads to the higher induction of CMVpp65-specific CD8+T cells by IFN-DCs pulsed with CMVpp65-peptide and Z plus G derived from PBMCs after DC therapy, compared with that by IFN-DCs pulsed with the peptide and Z plus G derived from PBMCs before DC therapy in *vitro.* Our results indicate that administration of IFN-DCs pulsed with autologous tumor lysates and Z plus G may lead to a good clinical outcome for Pts with GBM.

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DOI: 10.1002/eji.201970400

LB071

Dendritic cells cross-talk with tumour antigen-specific CD8+ T cells, Vg9gdT cells and Va24NKT cells in patients with Glioblastoma multiforme *in vitro*

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The finding that dendritic cells (DCs) orchestrate innate and adaptive immune responses has stimulated research on harnessing DCs for developing more effective vaccines for DC therapy. The expression of cytomegalovirus (CMV) antigens in glioblastoma multiforme (GBM) presents a unique opportunity to target these viral proteins for patients with GBM. Here, we demonstrate that Vg9gdT cells, innate immune cells activated by zoledronate (Z) and Va24 natural killer T (Va24NKT) cells, innate/adaptive immune cells activated a-galactosylceramide (G) can link innate and adaptive immunities through cross-talk with interferon (IFN) DCs from patients with glioblastoma multiforme (GBM) in a manner that can amplify the activation and proliferation of CMVpp65-specific CD8+T cells. The IFN-DCs derived from patients with GBM used in this study express lower levels of programmed cell death ligand (PD)-L1 and PD-L2 and higher levels of C-C receptor 7 (CCR7) than the most commonly used mature interleukin (IL)-4 DCs. The expression level of a cell death 1 (PD-1) on CD8+ T cells, including CMVpp65-specific CD8+ T cells, expanded by IFN-DCs pulsed with the CMVpp65-peptide and Z plus G (IFN-DCs/P+Z+G), was lower than that expanded by IFN-DCs pulsed with the peptide alone (IFN-DCs/P). Multifunctional T cells, including HLA-A*0201-restricted CMVpp65-specific CD8+T cells, Vg9gdT cells and Va24NKT cells, efficiently kill the HLA*0201-positive GBM cell line expressing CMVpp65 protein (T98G). These findings indicate that DC therapy using IFN DCs/P+Z+G and/or CTL therapy using CMVpp65-specific CD8+T cells expanded by IFN-DCs/P+Z+G may lead to a good clinical outcome for patients with GBM.

P0526

Anion exchange chromatographic isolation of iPSC-MSC derived extracellular vesicles ameliorated allergic asthma in mice

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Background: The extracellular vesicles derived from mesenchymal stem cells (MSC-EVs) elicits similar therapeutic effects to MSCs, but the difficulties in the large-scale preparation of high-purity MSC-EVs and the therapy of MSC-EVs in asthma remained poorly studied.
Objectives: We sought to improve anion chromatography for the isolation of iPSC-MSC-EVs and explored the effects and mechanisms of iPSC-MSC-EVs in asthma.
Methods: The morphologies and markers of iPSC-MSC-EVs were characterized by transmission electron microscope and western blot respectively. The anti-inflammatory effects of the iPSC-MSC-EVs were determined by macrophage assay. The uptake activities of macrophages on iPSC-MSC-EVs were also determined. The iPSC-MSC-EVs were administrated intravenously or intratracheally to a murine asthma model and the lung pathology, inflammatory cytokines and the polarization of macrophages were evaluated.
Results: We successfully obtained concentrated iPSC-MSC-EVs and the final concentration of EVs was about 10-15*10^{11}/mL (200 µg/mL). The iPSC-MSC-EVs were morphologically intact and were positive for CD9, CD63, CD81, Alix and TSG101. The iPSC-MSC-EVs could significantly decreased the level of IL-6 in macrophage assay. The macrophages began to uptake iPSC-MSC-EVs at 4h, which peaked at 12h and then receded at 24h both in vivo and in vitro. Additionally, iPSC-MSC-EVs

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DOI: 10.1002/eji.201970400

significantly reduced the infiltration of inflammatory cells, the numbers of epithelial goblet cell and the levels of inflammatory cytokines in BALF. Specifically, the polarization of pulmonary macrophages was also regulated by iPSC-MSC-EVs.
Conclusion: Our results showed that anion exchange chromatography was a promising method for the large preparation of iPSC-MSC-EVs, which could be an alternative therapy for asthma in future.

P0527

Cytokine-treated natural killer cells exhibit enhanced responses against cancer

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NK cells can kill different kinds of tumor without prior direct sensitization or causing graft-versus-host disease (GVHD), thus NK cells have a promising potential in cell immunotherapy. However, the insufficient number of NK cells in human peripheral blood limits it application in clinical therapy, which leads to the development of methods to promote NK cell proliferation. In addition, boosting anti-tumor function of NK cell possesses another effective method for the NK cells immunotherapy. In this study, the expended NK cells from PBMC was treat with a panel of cytokines for 12 hours, *in vitro* killing assay and animal imaging assay shown that the boosted NK cells have a stronger killing efficacy against tumor cells in the mouse model comparing to non-treated NK cells and have stronger proliferative activity *in vivo* compare to the untreated NK cells Our findings revealed that boosting NK cells before infusion offers the significant advantage on anti-tumor immunotherapy.

P0528

Identification of critical immunogenic molecules during allogeneic transplantation of Mesenchymal stem cells

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Mesenchymal stem cells (MSCs), a type of multipotent stem cell with immune regulatory property and low immunogenicity, has a potential to be used in “off the shelf” cell therapy. However, the findings of allo-rejections of MSCs by specific antibodies or T cells indicate that MSCs are not fully immune privileged. In this study, we tried to investigate the immunogenicity of human MSC and identify important immunogenic molecules. MSCs were transplanted into humanized mice, which were constructed by intravenously injection of human peripheral blood mononuclear cells (PBMCs) into NOD/SCID/γ null (NSG) mice, to observe the T cell infiltration and clearance of MSCs. The results showed that allogeneic MSCs recruited human T cells in humanized mice and caused a faster clearance than those in NSG mice. The proliferation of PBMCs could be significantly enhanced by MSCs and the expression level of HLA-II on MSCs was significantly increased after co-culture with each other *in vitro*. High throughput sequencing was applied to analyze the TCR repertoire of MSCs recruited T cells to identify dominant TCR CDR3 sequences. With synthesized TCR CDR3 peptides, we identified some possible immunogenic candidates including alpha-enolase (ENO1). The expression level of ENO1 was very low on the surface of fresh isolated MSCs but significantly elevated after co-cultured with PBMCs. After treated with the inhibitor of ENO1, MSCs-triggered proliferation of T cells was significantly decreased.
Our findings provide a better understanding of the immunogenicity of human MSCs and a theoretical basis for clinical application and safety of allogeneic MSC therapy.

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DOI: 10.1002/eji.201970400

P0529

Metformin enhances the long-term persistence and antitumor ability of CD8$^+$T lymphocytes through AMPK-miR-107-Eomes-PD-1 pathway

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Metformin has been studied for its anti-cancer effects by regulating T cell functions. However, the underlying mechanisms involved in improving the memory differentiation of T cells by metformin are not well defined. The effect of metformin on overall survival of diabetic patients with lung cancerwere determined by a Kaplan-Meier analysis.The subsets and function of CD8$^+$T cells derived from both peripheral blood and tumor tissues were analyzed by flow cytometry. Further, the metformin mediated pathway on memory T cell generation were determined by qRT-PCR, western blotting and chip assay. Anti-tumor activity was evaluated after adoptive transfer of the metformin treated HER2 CAR-T cells into HER2$^+$tumor-bearing immunodeficient mice. We found that the frequencies of memory stem (Tscm) and central memory (Tcm) T cells increased for both peripheral and tumor-infiltrating CD8$^+$T cells in metformin-treated lung cancer patients compared to those not taking the medication. An *in vitro*assay showed that metformin promoted the formation of memory CD8$^+$T cells and enhanced their anti-apoptotic abilities. In addition, AMP-activated protein kinase (AMPK) activation decreased microRNA-107 expression, thereby promoting enhanced Eomes expression and the subsequent suppression of PDCD1 transcription in metformin-treated CD8$^+$T cells. In the CAR-T cell therapy model, metformin also exhibited persistence- and cytotoxicity-promoting effects that led to decreased tumor growth. Metformin could reprogram the differentiation of CD8$^+$T cells, which may benefit the clinical therapy of cancer patients by facilitating long-lasting cytotoxic functions.

P0530

Autologous cytokine-induced killer cell therapy in lung cancer patients: a retrospective study

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Cytokine-induced killer (CIK) cells have the ability to kill tumor cells in vitro and in vivo. This study aimed to evaluate the clinical effect of adjuvant immunotherapy with CIK cells on the prognosis of lung cancer patients. In the present study, we investigated the clinical outcomes of autologous CIK cell immunotherapy for patients with lung cancer in a case-control study. Our study included 60 patients who received chemotherapy combined with autologous CIK cell adoptive immunotherapy in CIK treatment group and 60 patients who received chemotherapy alone in the control group. Progression-free survival (PFS) and overall survival (OS) of these two groups were evaluated. After 14 days of incubation in vitro, the percentages of CD3(+), CD3(+)CD8(+), CD3(+)CD56(+) and CD3(-)CD56(+) were significantly increased ($P< 0.05$). The clinical symptoms of 60 patients were apparently improved. No severe toxicity and side effects were observed in the CIK treatment group. The 3-year, 5-year PFS rates were 44.7% and 26.8% and the 3-year, 5-year OS rates were 74% and 62% in the CIK group, respectively, which were significantly improved compared to that in the control group. The median PFS and OS in the CIK group were significantly improved than those in the control group (PFS, 24 months vs. 14 months, $P=0.014$; OS, 72 months

DOI: 10.1002/eji.201970400

vs. 44 months, P=0.006). Our results indicated that autologous CIK cells can efficiently improve the immunological status and prolong PFS and OS in patients with lung cancer.

P0531

Human gingival tissue-derived MSC suppress osteoclastogenesis and bone erosion *via* CD39-adenosine signal pathway in autoimmune arthritis

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Background: Bone destruction is one of many severe complications that occurs in patients with rheumatoid arthritis (RA) and current therapies are unable to cure this manifestation. This study here aims to determine whether GMSC can directly inhibit osteoclast formation and eventually attenuate osteoclastogenesis and bone erosion in an inflammatory milieu.
Method: GMSC were co-cultured with osteoclast precursors with or without CD39 inhibitor, CD73 inhibitor or adenosine receptors inhibitors and osteoclast formation was evaluated in vitro. 2*10^6 GMSC per mouse were transferred to CIA mice and pathology scores, the frequency of osteoclasts, bone erosion in joints were assessed in vivo.
Finding: GMSC but not fibroblasts, markedly suppressed human or mice osteoclastogenesis *in vitro*. GMSC treatment also resulted in a dramatically decreased level of NF-κB p65/p50 in osteoclasts *in vitro*. Infusion of GMSC to CIA significantly attenuated the severity of arthritis, pathology scores, frequency of osteoclasts, particularly bone erosion, as well as a decreased expression of RANKL in synovial tissues *in vivo*. Blockade of CD39/CD73 or adenosine receptors has significantly abrogated the suppressive ability of GMSC *in vitro* and therapeutic effect of GMSC on bone erosion during CIA *in vivo*.
Conclusion: GMSC inhibit osteoclast formation *in vitro* and *in vivo* partly *via* CD39-CD73-adenosine signals. Manipulation of GMSC may have a therapeutic implication on rheumatoid arthritis and other bone erosion related diseases.

P1120

Clinical observation of treatment of cytokine - induced killer (CIK) cells in postoperative patients with colorectal cancer

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Purpose: CIK cells are heterogeneous natural killer cell like T-lymphocytes produced in-vitro, which show MHC-unrestricted antitumor activity. It was reported that CIK cells displayed broad cytotoxic activity against many types of tumors. This study intended to investigate the efficacy of CIK cells on postoperative patients with colorectal cancer compared with those with postoperative adjuvant chemotherapy.
Methods: 124 postoperative patients with stage II/III colorectal cancer were recruited into the study from 2007 to 2014. These patients were divided into two groups: 1) CIK group, n=51, 2) chemotherapy group (FORFOX), n=73. CIK group were received at least 3 times of CIK cells intravenously (1×10^9~3×10^9 for each infusion). The assessment of the efficacy for each group lasted for 120 months.

DOI: 10.1002/eji.201970400

The follow-up deadline was 2018. T-test and x^2-test were used to validate the difference of the Disease-free survival (DFS) and Overall survival (OS) between two groups.
Results: The 5-year OS of CIK group were significantly higher than those of the chemotherapy group (62.7% vs 45.6%, $p< 0.05$). The median OS of CIK group was significantly longer than that of chemotherapy group (77.2 months vs 43.4 months, $p< 0.05$). The 5-year DFS of CIK group were significantly higher than those of chemotherapy group (56.9% vs 42.6%,$p< 0.05$). The median DFS of CIK group was longer than that of chemotherapy group (77.7 months vs 33.5 months, $p< 0.05$).
Conclusion: The postoperative treatment of cytokine-induced killer cells can significantly improve the prognosis of patients with colorectal cancer.

P1121

CTLs induced by rAAV-DC is effective and well-tolerated for postoperative patients with digestive tract malignant tumors - a phase II clinical trial

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Background: Adoptive infusion of lymphocytes expanded and activated *in vitro* is a promising immunotherapy which may achieve survival benefits.
Methods: We developed a method to prime cytotoxic lymphocytes (CTLs) *in vitro* using engineered dendritic cells and conducted a prospective, nonrandomized controlled trial to test its efficacy and safety. We used dendritic cells loaded tumor specific antigens by recombinant adeno-associated virus (rAAV) to prime CTLs obtained from autologous or allogeneic peripheral blood mononuclear cells. Then these CTLs were transfused to the postoperative patients with digestive tract malignant tumors. The total survival time, progression-free survival time, incidence of adverse reactions were analyzed. 42 postoperative patients with various malignant digestive tract tumors received CTLs infusion including colorectal cancer, gastric cancer, pancreatic cancer and cholangiocarcinoma. 50 postoperative patients received chemotherapy were included as control.
Results: There was no significant difference in total survival and progression-free survival between the two cohorts. Analysis by disease showed the outcome of patients who received CTLs treatment was better than those who received chemotherapy for gastric cancer. In CTLs cohort, 2 cases (4.76%) had fever, 1 case had fatigue and 2 cases (4.76%) had leukopenia. In chemotherapy cohort, 48 cases (96%) had vasculitis, 43 cases (86%) had fatigue, 10 cases (20%) had leukopenia, and 12 cases (24%) had liver dysfunction.
Conclusion: These results show that CTLs induced by rAAV-DC is as effective as chemotherapy and well-toleratedbetter tolerance for postoperative patients with digestive tract malignant tumors.

P1122

TGF-β-induced CD4+CD25+regulatory T cells show potential therapeutic effect on autoimmune ovarian disease mice by suppressing B cells

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DOI: 10.1002/eji.201970400

Objective: To evaluate the role of TGF-β-Induced CD4+ CD25+Regulatory (iTreg) T cells in autoimmune ovarian disease and possible potential molecular mechanisms.
Method of study: AOD model was set up by thymectomy of BALB/C mice on day 3 (d3tx). iTreg cells were transfused into mice before 5 weeks and after 5 weeks of D3tx operation, Histopathological changes of ovary by HE staining, and Immunohistochemical observation of ovarian antibody expression. Magnetic bead sorting of iTreg, CD4+CD25− Med, and nTreg, Flow sorting B cells, Coculture to examine the suppressive effect of iTreg on B cell in vitro, Including whether to add Transwell chamber and anti-TGF-β, anti-IL-10 inhibitors.
Results: In d3tx mice, Infusion of iTreg Cells Signicantly improving levels of hormone , reduced ovarian pathological lesions. iTreg Cells Suppress B Cell Responses in vitro, Cell Contact and TGF-β/IL-10 Signals Are All Needed for Suppressive Effect of iTreg Cells on B Cell Responses.
Conclusion: CD4+CD25+ iTreg induced ex vivo significantly control the appearance and development of autoimmune ovarian disease; therefore, use of CD4+CD25+ iTregs may have a potential promise for the treatment of autoimmune ovarian disease and other autoimmune and in inflammatory diseases.

P1123

HLA class I restricted epitopes prediction of common tumor antigens in Caucasoid and Oriental ethnics population: implication for TCR-T immunotherapy and cancer vaccine design

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Tumor antigens processed and presented by HLA Class I alleles are important targets in tumor immunotherapies. Clinical trials showed that CD8+ T cells specific to tumor associated antigens (TAAs) or tumor neoantigens are the main factors resulting tumor regression. In this study, we selected 6 CTAs commonly used in cancer immunotherapy and top 96 hot mutations from the Cancer Genome Atlas to analyze potential epitopes which may be presented by the common HLA class I molecules in Caucasoid and Oriental ethnics population respectively. The results showed that the overall difference of CTAs epitopes is small between the two populations. Meanwhile, the longer sequence the CTA, the higher the overall epitope score. With the antigen length increases, the possibility of finding epitope for an HLA allele increases. However, epitopes prediction score of substitution mutations is significantly different between the two populations. It's worth noting that the single point mutations with the highest incidence have the lowest prediction scores while the mutations with the highest prediction scores are of low mutation incidence. This may be explained by the immune selective pressure that hot mutations need to be low immunogenic to escape the host immunosurveillance. Epitopes analyzed in this study provide clues for target and peptide selection in tumor immunotherapy involving CD8+ T cell.

P1124

Changes of T lymphocyte subsets after CAR-T cell therapy and its clinical significance

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DOI: 10.1002/eji.201970400

In tumor cell immunity, T cells play a central regulatory role. T cells are divided into several major subsets based on different cell surface molecules to maintain the body´s immune balance. In our study, we detect the changes of T lymphocyte subsets after CAR-T cell infusion, to explore the effect of CAR-T cell therapy on body immune system and its possible mechanism. 10 patients with good clinical response and complete data were analyzed and summarized. The proportion of CD8+ cells in CAR+ cell population was (61.4±32.5) % at the initial of CRS, and went up to (74.1±24.5) % as CAR-T cells proliferated to a peak level. After that, CD8+ cells began to decline as CAR-T cells decreased (F=0.647, P=0.531). The changes of CD4+ cells went the opposite way (F=2.678, P=0.087). The same change patterns of CD8+ and CD4+ cells were shared in CAR- cell population. In CAR+, CAR+CD4+, CAR+CD8+ cell populations, CD45RA+CCR7- cells (effector T cells) have decreased before CAR-T cell peak level. We assumed that effector T cells began to decrease as tumor cells were completely cleared. CD45RA-CCR7-, CD45RA-CCR7+, CD45RA+CCR7+ cells (effector memory T cells, central memory T cells, naïve T cells) gradually increased as the body immune system began to recover. ALL the change patterns were shared in CAR- cells, suggesting that CAR-T cells have certain optimization effects on the body immune system.

P1125

Passive transfer of Grp78-treated DC to prevent type 1 diabetes in NOD mice

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The 78-kDa glucose-regulated protein (Grp78) has been described to be released at times of cellular stress and as having extracellular properties that are anti-inflammatory or aid the resolution of inflammation. We previously reported that Grp78 could induce myeloid $CD11c^+$ cells to differentiate into a distinct tolerogenic cells, and these cells could retain tolerogenic signature after lipopolysaccharide (LPS) stimulation. This durable tolerance induction makes Grp78 a potential therapeutic agent for transplanted allogeneic grafts and autoimmune diseases such as type I diabetes. In this paper, $CD11c^+$ cells derived from NOD mice bone marrow were induced in vitro by rmGrp78. These Grp78-treated $CD11c^+$ cells which highly expressed B7-H4, had a potent deactivating effect on proinflammatory LPS-stimulated DCs. Clinical therapeutic efficacy was assessed after adoptive transfer in NOD T1D mice. Fluorescent imaging analyses revealed that adoptively transferred DC_{Grp78} cells specifically homed to the inflamed pancreas, promoting β-cell survival. And the passive transferred DCs help reduce the amount and rate of CTL, suppress the inflammatory cytokine production. The above work suggests that passive transfer of Grp78-treated DC helps the resolution of inflammation in NOD mice and may have relevance in a clinical setting.

P1126

Cytokine pre-activated γδT cells in cancer adoptive immunotherapy

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Adoptive cell therapy harnesses the power of immune system by stimulating and expanding immune cells to combat cancer. The application of tumour-specific αβT cell-based therapy may be hindered by limited tumour antigens. γδT cells are promising candidates for cellular immunotherapy because of their abundant IFN-γ or IL-17 production and MHC-independent cytotoxicity against a broad spectrum of tumors. Numerous attempts to use γδT cells in cancer immunotherapy have been made and resulted in variable efficacy and an overall good safety profile. Better expansion strategies are needed

DOI: 10.1002/eji.201970400

to improve the efficacy of the clinical trials using γδT cell-based immunotherapies. In the current project, we aim to explore the efficacy of cytokine pre-activated γδT cells in cancer immunotherapy. Pre-activated γδT cells by a combination of cytokines were characterised for their surface activation marker expression, cytokine production, and cytotoxicity against tumour cells. We found that IL-12/18, IL-12/15/18, IL-12/18/21 and IL-12/15/18/21 are the most effective cytokine combinations for in vitro expansion of γδT cells in terms of activation and cytokine production. IL-21 upregulated their cytotoxicity against tumour cells in murine systems. All four combinations showed upregulated cytotoxicity of human γδT cells. These findings will be extended to *in vivo* studies using murine hepatocellular carcinoma model and xenograft models.

P1127

Type I interferon-delivery by iPSC-derived myeloid cells elicits antitumor immunity via XCR1$^+$dendritic cells

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Immunotherapy is an effective treatment modality for many cancer types, although the lack of baseline T cell infiltration after immune checkpoint blockade is linked to the absence of tumor response. The presence of type I interferon (IFN) in the “sterile” tumor microenvironment is a crucial step for T cell infiltration. We generated IFN-α-producing cells by genetically engineering induced pluripotent stem cell (iPSC)-derived proliferating myeloid cells (iPSC-pMCs). Local administration of IFN-α-producing iPSC-pMCs (IFN-α-iPSC-pMCs) alters the tumor microenvironment and propagates the molecular signature associated with type I IFN. The gene-modified cell actively influences host XCR1$^+$dendritic cells to enhance CD8$^+$T cell priming, resulting in CXCR3-dependent and STING-IRF3 pathway-independent systemic tumor control. Administration of IFN-α-iPSC-pMCs in combination with immune checkpoint blockade overcomes resistance to single-treatment modalities and generates long-lasting antitumor immunity. These preclinical data suggest that IFN-α-iPSC-pMCs might constitute effective immune-stimulating agents for cancer that are refractory to checkpoint blockade.

P1714

Safety and efficacy of HBV specific TCR-redirected T cell therapy in patients with HBV-related hepatocellular carcinoma

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Adoptive transfer of T cells engineered with HBV-specific T cell receptors (HBV TCR-T) can supplement the defective antiviral T cell response that characterizes chronic hepatitis B and HBV-related hepatocellular carcinoma (HCC) patients.
HBV TCR-T cells can not only inhibit HBV replication but also directly lyse HBV-infected hepatocytes in HCC patients.
In this study, we analyzed the minimum dose of T cells transiently expressing HLA-A0201-restricted HBV-specific TCRs that can be safely adoptively transferred to non-operable HBV-associated HCC patients.

DOI: 10.1002/eji.201970400

The patient was scheduled to receive an escalating dose of HBV TCR-T cells ($1x10^4$/kg, $1x10^5$/kg,$1x10^6$/kg, $5x10^6$/kg) to understand whether the anti-HCC therapeutic dose ($5x10^6$/kg) could be performed safely. However, the second dose (1x 10^5/kg, 6 ´10^6 HBV TCR-T cells) infusion triggered an elevation of ALT and total bilirubin at day 3 after infusion. The ALT reached a peak on day 23 (506 IU/L) and return to normal 1 month later. Then the patients received the rest infusion (up to $5x10^6$/kg) without experiencing any severe adverse effects. With a follow-up of 756 days, the patients remained in partial response.
In conclusion we demonstrated that **as little as 6 million HBV TCR-T cells (frequency in total T cells~ 0.000003%) can trigger a robust liver inflammation. But it was tolerable and fully recovered. These results highlight the importance of carefully dosing the quantity of HBV-TCR T cells for T cell adoptive transfer in HBV infected liver and advocate this strategy as a novel therapeutic opportunity in HBV-related HCC.**

P1715

Galectin-9 serves as a prognostic biomarker for adoptive cell therapy using autologous tumor-infiltrating T lymphocytes in nasopharyngeal carcinoma

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It has been evidence that the adoptive cell therapy（ACT）using *ex vivo* activated autologous tumor-infiltrating T lymphocytes (TIL) is safe and effective in phase I and II study in cancers including melanoma and nasopharyngeal carcinoma (NPC). We have reported a phase I clinical trial study of TIL treatment following concurrent chemoradiotherapy (CCRT) in 23 patients with locoregionally advanced NPC (ClinicalTrials.gov number, NCT 01462903) in 2016. Here, we re-evaluate the survival rate of these patients and screen the immune biomarkers related to the survival of NPC patients accepted ACT after over 5 years of follow-up. Twenty of 23 NPC patients accepted ACT, 18 of them are still in CR, 2 patients have been in PR and 2 of them have been lost to follow-up. The disease-free survival (DFS) is 90% and higher than DFS (70%~80%) in the reported clinical trials of CCRT for patients with locoregionally advanced NPC. Moreover, we found that the levels of tumor PD-L1, Galectin-9 and Ki67 in CD8+ infiltrating cells have no association with the DFS, while the serum level of Galectin-9 is increased in one week of all patients after ACT, and negatively associated with the patient DFS. In addition, the level of tumor PD-L1 is positive correlated with tumor stage and Galectin-9 serum level after 1 week of ACT is positive correlated with lymphoid node transfer of patients. Overall, our finding suggests that TIL therapy maybe benefit to survival of NPC patients and the change of serum Galectin-9 level maybe a biomarker for ACT treatment.

P1716

Central role of memory regulatory T cells in adoptive Treg therapy for kidney transplantation patients

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DOI: 10.1002/eji.201970400

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Adoptive immunotherapy with regulatory T cells (Treg) is a promising option to promote immune tolerance following solid organ transplantation (SOT). However, Treg from elderly patients awaiting transplantation are dominated by the $CD45RA^{-}CD62L^{+}$central memory type Treg subset (TregCM), and the yield of well characterized and stable naïve Treg (TregN) is low. It is therefore, important to determine whether these TregCM are derived from the thymus and express high stability, suppressive capacity and a broad antigen repertoire like TregN. In this project, we showed that TregCM use a different T cell receptor (TCR) repertoire from conventional T cells (Tconv), using next-generation sequencing of all 24 Vß families, with an average depth of 534 677 sequences. This showed almost no contamination with induced Treg. Furthermore, TregCM showed enhanced suppressive activity on Tconv at early checkpoints of immune activation controlling activation markers expression and cytokine secretion, but comparable inhibition of proliferation. Following in vitro expansion under mTOR inhibition, TregCM expanded equally as well as TregN without losing their function. Despite relatively limited TCR repertoire, TregCM also showed specific alloresponse, although slightly reduced compared to TregN. In addition, we also observed that operationally tolerant (TOL) kidney transplantation patients had a higher proportion of $CD45RA^{-}FoxP3^{hi}$ memory Treg with a Treg-specific-demethylation-region (TSDR) demethylation pattern, which may contribute to the maintenance of graft tolerance. These results support the significance of memory Treg in kidney transplantation tolerance induction and the therapeutic usefulness of manufacturing Treg products from $CD45RA^{-}CD62L^{+}$Treg-enriched starting material to be applied for adoptive Treg therapy.

P1717

Targeting macrophage modified by Fasudil as a novel therapy for EAE

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Background: Macrophage polarization is considered to be an important factor affecting the progression of central nervous system (CNS) diseases. Although Fasudil has shown good therapeutic potential in experimental autoimmune encephalomyelitis (EAE), certain side effects may limit its clinical application.
Objective: To explore the possibility and mechanism of Fasudil-modified macrophage in the treatment of EAE.
Methods: On day 9 postimmunization, encephalitogenic macrophage was prepared from splenocyte of EAE mice by magnetic bead and treated with/without Fasudil in vitro for 72 hours. Macrophage was resuspended and transferred by intraperitoneal injection with 2×10^7 on day 3 and 11 postimmunization to EAE mice, which were divided into PBS- macrophage group and Fasudil-macrophage group.
Results: Using flow cytometry, western blot and chemical/enzymatic determination, we found Fasudil converted macrophages from M1 to M2 phenotype, decreased the expression of iNOS/NO, CD16/32, IL-12 and increased the expression of Arg-1, CD206, IL-10 and CD14, which were linked to the inhibition of ROCK-II activity, TLRs/Myd88/MAPK signaling pathway expression and inflammatory cytokines IL-1β, IL-6 and TNF-α production. Importantly, Fasudil-modified macrophages have a better therapeutic effect on EAE mice, showing reduced symptom score, improved demyelination and

DOI: 10.1002/eji.201970400

suppressed inflammatory cell infiltration of CNS.
Conclusion: Fasudil-mediated immunocytotherapy, inhibiting inflammatory response by controlling a helpful polarization of macrophages, will become a novel therapeutic strategy for EAE (NNSF of China 81501032).
Keywords: Experimental autoimmune encephalomyelitis, Fasudil, Macrophage polarization, inflammation

P1718

The optimal timing of immunotherapy in unexplained recurrent spontaneous abortion(URSA) : a perspective cohort study in Northern China

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Objective: Immunotherapy including lymphocyte immunotherapy (LIT) and intravenous immunoglobulin (IVIG) is extensively used in URSA. Current trials on the timing of immunotherapy remain ambiguous. We conducted a perspective cohort study to investigate the optimal timing of immunotherapy among URSA.
Methods: 217 preconception URSA women were enrolled after rigorous etiology screening,127 accepted preconception LIT(pre-LIT Group)and 90 refused (Not-pre-LIT Group), which could choose preconception LIT and post-conception LIT or IVIG independently. A total of 85 of 127 in pre-LIT group(66.9%) and 55 of 90 in not-pre-LIT group(61.1%) became pregnant within 6 months by natural fertilization, which were suggested immunotherapy immediately after hCG increasing. Among the 140 natural fertilized patients, 35 refused post-conception immunotherapy, the other 105 accepted. Autoimmune antibodies (AIA) and blocking antibodies (BA) were detected before and after preconception LIT. Success outcome was pregnancy beyond 14 weeks of gestation.
Results: The success rate was 75.29% (64/85) in pre-LIT group and 80.00% (44/55) in not-pre-LIT group(P>0.05).AIA [30.59%(26/85), p=0.000] and BA[31.76%(27/85), p=0.000] significantly shifted to positive induced by preconception LIT. Patients with post-conception immunotherapy were 17.17 times [95% (CI)=3.90-91.26,p=0.000] not likely to have biochemical pregnancy loss than without patients,while post-conception immunotherapy cannot reduce pregnancy losses with clear location (p>0.05).
Conclusions: Preconception LIT cannot improve pregnancy outcome and may lead to iatrogenic turbulence of autoimmune system manifesting as autoimmne antibodies shifting to positive. Post-conception Immunotherapy initiated from elevated serum hCG as early as possible can reduce the occurrence of biochemical pregnancy losses, but it cannot relieve pregnancy loss with clear location.

P1719

Human gingiva-derived mesenchymal stem cells treat osteoporosis via regulating the balance of osteoclasts and osteoblasts through CD39 signal

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DOI: 10.1002/eji.201970400

The pathogenesis of osteoporosis is closely related to immune cells and inflammatory factors. Existing treatments failed to reduce the risk of fractures in patients, so it is urgent to search more effective treatments. We evaluated the therapeutic potential of GMSC infusion in an animal model of osteoporosis that was induced through the ovariectomy (OVX). To explore therapeutic potential, OVX was performed in adult C57BL/6J mice after complete skeletal remodeling, and GMSC therapy was given 2 weeks after OVX. After 8 weeks of treatment, the distal femoral trabecular bone was sparse and discontinuous in OVX group, and GMSC treatment showed a clear therapeutic effect in bone density, trabecular BV/TV, trabecular numbers. GMSC treatment also resulted in a dramatically decreased frequency of osteoclasts and increased osteogenic index in vivo. To identify the mechanism of GMSC function in osteoporosis in vivo, we analyzed the role of CD39 signals, since we found that GMSC inhibited osteoclast differentiation through CD39 signals and CD39 inhibitors (POM-1) inhibited the osteogenic potential of GMSC in vitro. Interestingly, POM-1 almost completely abolished the therapeutic effect of GMSC on osteoporosis. These results highlight the key role of CD39 expressed on GMSC to regulate the balance between osteoclasts and osteoblasts in osteoporosis.

P1720

Adoptive transfer of cytokines-preactivated NK cells provides reduction of established tumour

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Introduction: Cancer represents one of the major causes of mortality, despite huge research efforts for deciphering the molecular mechanisms of disease and for developing new targeted and personalized therapeutic approaches with acceptable side-effects. We investigated whether adoptive transfer of IL-12/15/21-preactivated NK cells reduces growth of established mouse tumors. Our results raise the possibilities for the development of novel NK cell-based therapeutic strategies for clinical application.
Materials and methods: We used C57BL/6 mice, 8-10 weeks old. NK cells were isolated by negative selection from spleens and treated with 10 ng/ml IL-12, 10 ng/ml IL-15, and 100 ng/ml IL-21 or 10 ng/ml IL-15 for 20h. Mice were s.c. injected with 10^5 B16F10 cells and received adoptive transfer of NK cells at day 7 after tumor cell inoculation. Tumor diameters were measured by a caliper, once on week, for three weeks.
Results: IL-12/15/21-preactivated NK cells persisted in spleen for 3 weeks after adoptive transfer with high effector function. Adoptive transfer of IL-12/15/20-preactivated NK cells in mice significantly reduced tumor growth of recipient mice.
Conclusions: Based on results from experimental animal model, adoptive transfer of cytokines-preactivated NK cells seems to be promising for cancer therapy. There is no doubt that NK cells have major role in cancer treatments, and in the future, NK cell immunotherapy from "a new hope" may become "a reality" for malignant diseases.
Acknowledgement: This work was partially supported by Core Program, implemented with the support NASR, projects nos. 19.29.02.03 and 7PFE/16.10.2018.

DOI: 10.1002/eji.201970400

P1721

Effects of IL-2 and IL-15 on the human natural killer cell line NK-92 - implications for clinical trials

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The human natural killer cell line NK-92 is increasingly being used in adoptive cellular immunotherapies, either in vitro or in animal models transduced with different chimeric antigen receptor (CAR) constructs. Herein, NK-92 cells were analyzed with respect to their proliferation and cytotoxicity. A time-resolved fluorometric assay (TDA-labeled K562 target cells) was used for measuring the cytotoxic activity of NK-92 cells treated with IL-2, IL-4, IL-7 and/or IL-15, whereas their proliferation, in the presence of these common cytokine receptor γ chain (γc)-dependent cytokines, was measured by traditional tritiated thymidine ([^{3}H]-TdR) incorporation. IL-2 and IL-15, but not IL-4 or IL-7, were able to induce dose-dependent proliferation of NK-92 cells. IL-15 was, depending on the dose and culture time, up to 10 times more potent compared to corresponding concentrations of IL-2, whereas both of them combined could potentiate the NK-activity almost equally well. No synergistic effects could be noticed with respect to the cytotoxicity and the proliferation of these cells. Data presented here indicate that of the common gamma chain receptor-dependent cytokines tested here, IL-15 alone is able to cultivate and trigger NK-92 cells to such an extent so that they can be used for immune-based cancer therapies. Implications with respect to CAR - transduced NK-92 cells are also discussed.

P2310

Combination therapy for treating advanced drug resistant acute lymphoblastic leukemia

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Drug resistant acute lymphoblastic leukemia (ALL) patients do not respond to standard chemotherapy and there is an urgent need to develop new treatment strategies. Our study exploits the presence of B cell activating factor receptor (BAFF-R) on the surface of drug resistant B-ALL cells as a novel therapeutic target. We used anti-BAFF-R antibody (VAY736), optimized for natural killer (NK) cell mediated antibody dependent cellular cytotoxicity (ADCC), to kill drug resistant ALL cells. VAY736 antibody and NK cell treatments significantly decreased ALL disease burden and provided survival benefit *in vivo*. However, if the disease is advanced, the ADCC efficacy of NK cells is inhibited by micro-environmental transforming growth factor beta (TGF-beta). Inhibiting TGF-beta signaling in NK cells using TGF-beta R1 inhibitor (EW-7197) significantly enhanced VAY736 induced NK cell mediated ALL cell killing. Our results highlight the potential of using a combination of VAY736 antibody with EW-7197 to treat advance stage drug resistant B-ALL patients.

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DOI: 10.1002/eji.201970400

P2311

Human decidual-like NK cells induced and amplified in vitro as adjuvant therapy for recurrent spontaneous abortion

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Recurrent spontaneous abortion (RSA) with unknown reasons has become a common pregnancy disease that seriously affects reproductive health. Recent studies have shown that the RSA may mainly related to abnormal immune microenvironment at the maternal-fetal interface during pregnancy, especially abnormal decidual natural killer cells (dNKs). In order to establish an effective treatment for RSA with abnormal dNK cells, an amplification system of induced human decidual-like NK cells (idNKs) based on cytokine without feeder-cell in vitro was established. IdNKs have similar phenotypes and functions with dNKs, including high expression of CD49a, CD151 and low expression of CD16 and cytotoxic capability. Furthermore, the idNK cells also had high expression of growth-promoting factors (GPFs), which were very important for promoting fetal development during early pregnancy. The security of these induced idNK has been verified for low level of killing activity towards targets compared to peripheral blood NK cells. This research of inducing decidual-like GPF-secreting NK cells in a human system may improve the feasibility of applying these decidual-like NK cells to the RSA patients and give rise to achieve healthy reproduction.

P2312

Mesenchymal stem cells in sepsis: secondary pyroptotic cell death is mediated by dsDNA of neutrophil extracellular traps via cGAS-STING-AIM2 pathway

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It has been reported the effect of mesenchymal stem cells (MSCs) on sepsis is poor but the underlying causes and mechanisms remain unclear. Here we show that dsDNA of neutrophil extracellular traps (NETs) induces MSCs secondary pyroptosis that accounts for the poor efficacy of MSCs on sepsis. Patients with sepsis have increased dsDNA in plasma. In mice, dsDNA released through neutrophil NETosis activates cGAS and induces secondary pyroptosis of infused MSCs. Degradation of extracellular self-dsDNA by DNase I inhibits cGAS-STING signals and the downstream AIM2 inflammasome activation in MSCs. In vitro, both Calf thymus dsDNA and the dsDNA from the plasma of sepsis patients are recognized by cGAS, activate STING and the downstream AIM2-caspase-3-GSDME pathway in MSCs. These findings provide mechanistic insights into the poor effect of MSCs on sepsis and suggest that DNase I plus MSCs may have potential therapy value for sepsis.
Keywords: mesenchymal stem cell, dsDNA, cGAS, AIM2, GSDME

DOI: 10.1002/eji.201970400

P2313

Combined treatment with histone deacetylase inhibitor and checkpoint blockade rescues hypoxia induced immune dysfunction of gamma delta T cells in oral cancer

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Oral cancer is the most common cancer in India with a relatively poor prognosis. Hypoxia contributes to oral tumour progression, therapy resistance and poor clinical outcome. Understanding the complexity of tumour microenvironment is important for the development of immunotherapy. The present study was aimed at investigating the effector functions of gamma delta T cells (γδ T) in oral cancer exposed to hypoxia and understanding how combination of HDAC inhibitor and checkpoint blockade can augment the anti tumor cytotoxic potential of γδ T cells. γδ T cells showed increased oral tumour infiltration, decreased expression of activation markers, and effector molecules-perforin and IFNγ and enhanced expression of hypoxia inducible factor -1α. A marked decrease was observed in the anti-tumour cytotoxic ability of γδ T cells exposed to hypoxia correlating with decreased expression of Interferon γ, Lamp-1 and transcription factors Eomes and Tbet. Under hypoxia, γδ T cells showed increased expression of programmed death ligand-1 (PDL1), RORγt and secretion of cytokines favouring γδT17 differentiation. Antitumor cytotoxic potential of γδ T cells was attenuated after treatment with HDAC inhibitors which correlated with the enhanced expression of immune checkpoints programmed death-1 (PD1) and PDL1 in γδ T cells. Interestingly, PD-1 blockade improved the antitumor cytotoxic functions of HDAC inhibitor treated γδ T cells, which reflected in the increased expression of Granzyme B and Lamp-1. The study provides a rationale for designing γδ T cell immunotherapy for oral cancer with HDAC inhibitor and immune check point blockade as a combinatorial treatment modality.

P2314

Neoantigen reactive T cells combined chemotherapy and radiation improved the survival of advanced pancreatic cancer

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Background: Pancreatic cancer (PC) is one of the most aggressive and death-relating malignancy. The objective responsive rate (ORR) and medium progression free survival (PFS) of various therapeutic regimens are still unsatisfied. This study is to investigate the efficacy, safety and clinical beneficial of combination personalized immunotherapy with GEM and radiotherapy in locally advanced and metastatic PC patients.
Methods: Three locally advanced unresectable and seven metastatic PC patients received at least two cycles of GEM (1000mg/m^2 on day 1 and day 6), radiotherapy combing with neoantigen induced DC vaccination on day 7 and cytotoxic T lymphocyte transfer from day 12 to 15 per three weeks. The locally advanced unresectable PC patients received stereotactic body radiotherapy (SBRT) with a total amount of 50-66Gy during the first cycle. For metastatic patients, their partial lesions received a low dose radiation (0.5Gy bid*2days) on day 10 and 11 in each cycle.
Results: Two cases were observed with partial remission (PR), five with stable disease (SD), and three with progressive disease (PD). The disease control rate (DCR) was 70%. mPFS was 6.4 months. After the first treatment cycle, the total effectiveness for pain easement and increasing appetite are 100%（8/8）and 66.7%, respectively. Haematotoxicities were the most common adverse

DOI: 10.1002/eji.201970400

drug reactions. Two patients had grade 1 to 2 neutropenia, two with grade 3 to 4 thrombocytopenia. Three patients suffered grade 1 to 2 gemcitabine-induced skin rash. No treatment-related mortality occurred.
Conclusions: Neoantigen reactive T cells combined chemoradiotherapy demonstrated an acceptable response and safety in advanced pancreatic cancer patients.

P2315

The stimulatory effect of OK432 matured dendritic cells on T-cells from prostate cancer patients

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Cancer is one the leading causes of death in developed nations with WHO estimating nearly 10 million deaths worldwide in 2018. Prostate cancer is by far the most prevalent cancer for men and while the overall 5 years survival is high, it drops significantly to around 30% survival if the cancer metastasizes. Current treatment for metastatic prostate cancer is limited in both curative and stabilizing prospects and is in need for better treatment alternatives. With the success of checkpoint inhibitors in treating other types of metastatic cancers and in many cases leading to complete remission, it is evident that activating the patient's own immune system against metastatic prostate cancer is a promising treatment method.
While dendritic cell-based cancer vaccines have demonstrated repeatedly to be safe and feasible in clinical use, the therapeutic benefits still leave much to be desired. A prominent explanation is the inadequate T cell stimulatory effect of the dendritic cells used. How immature antigen-collecting dendritic cells are matured into immunogenic antigen-presenting dendritic cells is crucial to their immunostimulatory capabilities. Previously we have shown that dendritic cells matured with a cocktail containing OK432, a potent immunostimulatory agent, in combination with TLR7 ligand and PGE_2, have many desirable traits for cancer vaccination and showed great promise when applied to cells from healthy controls.
We will investigate the T cell stimulatory capacity of dendritic cells from prostate cancer patients stimulated with our OK432 cocktail to validate its potential as a new immunotherapeutic approach for dendritic cell-based cancer vaccines.

P2316

Adoptive natural killer cell therapy in murine cancer model

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Natural killer (NK) cells exert anti-tumor function through direct killing of tumor cells and indirect modulation of the function of other immune cells, such as T cells, dendritic cells and macrophages.However, the efficacy of adoptive NK cell transfer in cancer treatment has been limited, and only found in certain myeloid leukemia under allogeneic setting. The functional heterogeneity of NK cells and the immunosuppressive tumor microenvironment likely contribute to the limited efficacy. We establish a method to condition ex vivo expanded murine NK cells with anti-tumor activity. The conditioned NK cells are $EOMES^+CD127^-DX5^-CD49a^+$ and $CD11b^+CD27^+$. Encountering with solid

DOI: 10.1002/eji.201970400

tumor cell lines in vitro enhances the production of IFN-g and triggers killing of the tumor cells by conditioned NK cells.In a syngeneic orthotopic EO771 breast cancer model, adoptive transfer of conditioned NK cells into tumor-bearing mice slows tumor progression and alters the composition and function of intra-tumor immune cells. To better imitate clinical condition, we transferconditioned NK cells into tumor-resected mice, and found the therapy improved survival rate.

P2317

The immunomodulatory effects of mesenchymal stem cells on long term pulmonary complications in an animal model of exposed to a sulfur mustard analog (CEES)

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Sulfur Mustard (SM) is one of the most toxic chemical weapons. Exposure to SM induces short- and long-term toxicities, however pulmonary injury is major cause of mortality in the victims. 2-Chloroethyl Ethyl Sulfide (CEES), an analog of SM, shares similar properties with SM and is widely used to investigate the pathological mechanisms behind SM toxicity. To date, the immunopathogenesis of SM in the lung has remained unclear. Prolonged activation and the imbalance between two macrophage phenotypes (M1 vs M2) are known to be the main culprits of SM-induced lung injury. We tried to investigate the effectiveness of adipose mesenchymal stem cells (AD-MSC) on long-term CEES lesions in an animal model based on the modulation of pulmonary immune cells and alveolar macrophage phenotype alteration.
Treatment of mice with MSC after intraperitoneal administration of CEES (10 mg/kg) reduced progressive histopathologic changes in the lung. Flow cytometric analysis of isolated alveolar macrophages in the BAL showed that the accumulation of both M1 and M2 macrophages in response to CEES was reduced by MSC administration($p< 0.01$). The modulating effect in the M1-subset was much more significant compared to M2 ($p< 0.01$). AD-MSCs caused a marked reduction in the CD86 and CD206-expressing macrophages compared to the un-treated groups (98 vs 11% and 96 to 48% respectively).
These findings suggest that MSCs understand their environment and restore conditions to the balance in disorders associated with Th1 or Th2 imbalance. Our data suggests that MSCs may represent an efficacious approach to restore lung injury induced by mustards.

P3163

MICA-TM polymorphism (A5.1) as a potential predictive marker in response to NK immunotherapy for triple-negative breast cancer

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Objective: The major histocompatibility complex class I chain-related gene A (MICA) protein is upregulated under stressed conditions including oncogenic transformation. The membrane MICA (mMICA) protein is a target of immune attack on tumors by NK cells or γδ T cells. MICA has a triplet repeat microsatellite polymorphism (GCT)n in the transmembrane (TM) region. MICA-A5.1 polymorphism contribute to the development of cancer by promoting the release of soluble MICA（sMICA）to

DOI: 10.1002/eji.201970400

evade tumor immunosurveillance. In this article, we will determine the relationship between the status of MICA-TM polymorphism in Triple-Negative Breast cancer (TNBC) and the clinical outcome of adjuvant therapy combined with NK cells infusion.
Methods: MICA-TM polymorphism in 267 patients with TNBC was evaluated by PCR). Of which,120 patients received adjuvant chemotherapy after surgery combined with NK cell therapy. The survival outcomes were analyzed by using the log-rank test, Cox proportional models and Kaplan-Meier methods.
Result: Of the 267 tumor samples, 83 (31.1%) were found as MICA-TM polymorphism A5.1 (MICA-A5.1) and 184 (68.9%) were not found MICA polymorphism A5.1 (no MICA-A5.1). Univariate and Multivariate analysis showed that the TNM, level of soluble MICA and NK treatment were independent prognostic factors of TNBC. In subgroup analysis, in the group of no MICA-A5.1, the disease-free survival (DFS) and over**all survival**(OS) of patients who received NK therapy was longer than in those without NK therapy.
Conclusion: Our findings showed that adjuvant chemotherapy followed by NK therapy is a promising therapeutic strategy for TNBC cancer without MICA-A5.1.

P3164

Experimental study of adoptive immunotherapy with iNKT cells in RA mice induced by glucose-6-phosphoisomerase (GPI)

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INKT cells are a group of special bridging cells linking innate and adaptive immunity, and they are also the central cells of immune regulation. Decreased and functional deficits of iNKT cells, excessive polarization of Th1/Th17 cells, and immune imbalances have been found in patients with active rheumatoid arthritis (RA) . In this study, we established a RA animal model using mixed peptide of hGPI325-339 and hGPI469-483. Specific functional iNKT cells were obtained by in vivo induction and in vitro purification, and adoptive infusion therapy was performed in RA mice. On the 12th day after cell therapy, the disease progression was significantly improved. iNKT1 subset frequencies in the thymus and spleen were decreased, as well as splenic Th1 and Th17 cell subset rates, and serum TNF-α, IFN-γ and IL-6 levels. The rates of iNKT2 and Th2 subsets as well as IL-4 and IL-10 levels were increased. Thymus GATA-3 and splenic PLZF protein levels were increased . Adoptive infusion of iNKT cells exerts therapeutic effects in RA mice by increasing iNKT frequency, altering the proportions of iNKT cell subsets, correcting Th cell subset imbalance and reducing the amounts of inflammatory cytokines.
Keywords: rheumatoid arthritis, adoptive immunotherapy , iNKT cell, cytokines, glucose-6-phosphate isomerase(GPI)

P3165

Ex vivo expanded human circulating Vδ1 γδT cells exhibit favorable therapeutic potential for colon cancer

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DOI: 10.1002/eji.201970400

Background: Vγ9Vδ2 γδT cells-based adoptive immunotherapy for solid tumors has limited success. This study aims to determine whether Vδ1 γδT cells can be used as an effective adoptive cellular immunotherapy for colon cancer.
Methods: The characteristics and cytotoxicity against human colon cancer between fresh Vδ1 and Vδ2 cells were evaluated. To efficiently expand Vδ1 cells, we stimulated γδT cells with PHA and IL-7 for 14 days. The anti-cancer activity of expanded Vδ1 and Vδ2 cells was assessed in vitro and in vivo. To investigate the underlying killing mechanism, we performed cytotoxicity assay in a transwell co-culture system and antibody blocking experiment.
Results: Freshly isolated Vδ1 cells from human peripheral blood (PB) exhibit more potent cytotoxicity against colon cancer than Vδ2 cells *in vitro*. Upon stimulation with PHA and IL-7, Vδ1 cells from PB of both healthy donors and colon cancer patients were preferentially expanded and highly expressed cytotoxicity-related molecules, chemokine receptors and cytokines with enhanced cytotoxicity against adherent and sphere-forming colon cancer cells in a cell-contact dependent manner. In addition, *ex vivo* expanded Vδ1 cells by PHA and IL-7 showed proliferation and survival advantage partly through an IL-2 signaling pathway. Furthermore, PHA and IL-7 expanded Vδ1 T cells restrained the tumor growth and prolonged the tumor-burdened survival of human colon carcinoma xenografted mice. Moreover, we found TLR agonists and IL-4 could enhance Vδ1 cell expansion in vitro.
Conclusion: Our findings suggest that expanded human PB Vδ1 cells are a promising candidate for anticancer adoptive immunotherapy for colon cancer.

P3166

Epigenetic drug decitabine promotes the effect of immunotherapy for colorectal cancer with microsatellite stability

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Transforming 'cold tumor' (low immunogenicity) into 'hot tumor' (high immunogenicity) is the key role to improve the clinical outcome of tumor immunotherapy. Epigenetic modification has evidenced effects on tumor cells and immune cells. Our studies have shown that the epigenetic drug decitabine (DAC) could change the immunological characteristics of microsatellite stable (MSS) colorectal cancer (CRC) cells through demethylation of the gene promoter regions. DAC also increased the expression of antigen processing and presentation, cytokines and other genes, which induced the tumor cells to be more immunogenic. In mice or patient-derived xenograft model with MSS CRC established by us, DAC could also recruit more $CD4^+$ and $CD8^+$ T lymphocytes to infiltrate the tumor site and promote the secretion level of IFN-γ that construct suitable conditions for immunotherapy. We found that the combination usage of DAC and PD-1 blockade could play a synergistic role and significantly improve the anti-tumor effect. We also found that DAC could increase the expression of tumor associated antigens, such as NY-ESO-1. We then prepared NY-ESO-1 specific TCR engineering T (TCR-T) cells and observed that DAC remarkably improved the antitumor effect of the TCR-T cells on MSS CRC, which suggested that the pre-treatment of DAC induced tumor antigens overexpression on tumor cells. Therefore, DAC might be used as a unique synergistically therapeutic drug for tumor immunotherapy. The combination use of DAC and immunotherapy may be a new effective strategy for the treatment of MSS CRC.

DOI: 10.1002/eji.201970400

P3167

Clinical investigations of allogenic Vγ9Vδ2 T cell therapeutics for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. Vγ9Vδ2 T cells have been developing into a promising candidate for cancer immunotherapy. In our work, we had established a large-scale in vitro expansion protocol for Vγ9Vδ2 T cells from healthy donors. Our cultured Vγ9Vδ2 T cells possess optimal immune response function, including enhanced anti-apoptosis ability, higher expression killing receptors and cytokines, as well as elevated in vitro cytotoxicity against cancer cells. Notably, we conducted a phase I clinical study and for the first time verified that allogenic Vγ9Vδ2 T cells in the application of malignant tumor treatment are highly safe. We recruited nine liver cancer patients who had received routine surgery and chemotherapy. These patients were enrolled to receive allogenic Vγ9Vδ2 T cell treatment, and strikingly found that treatment with allogenic γδ T cells (minimal 5 times of infusion) could greatly prolong the survival of patients with a median surial of 16 months, and enhance the immunological functions of hosts. The overall responses were two patients had stable disease (SD), one patient had partial remission (PR), whereas other four patients experienced progressive disease (PD), and two patients had passed way after seven months from the last cell infusion. In conclusion, allogeneic γδ T cells are not only clinical safe, but also will become new alternative for liver cancer immunotherapy.

P3168

A nanobody against CTLA-4 increases the anti-tumor effects of specific $CD8^+$ T cells

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Cytotoxic lymphocytes (CTLs) are usually applied for adoptive cellular immunotherapy. The primary requirement for CTL therapy is to expand T cells ex vivo efficiently and economically. Traditional approach for T cells expansion uses autologous APCs to interact with T cells. However, incidental inhibitory program such as CTLA-4 pathway can inhibit T cell activation proliferation. We developed a nanobody against CTLA-4 (CTLA-4 Nb 16) and investigated the elimination of immunosuppression by blocking the negative costimulatory molecule CTLA-4 in the T cells. With CTLA-4 Nb16 stimulation, dendritic cell/hepatocellular carcinoma fusion cells (DC/HepG2-FCs) increased the proliferation activity of *in vitro* induced autologous $CD8^+$ T cells with IFN-γ secretion thus promoting tumor cell killing. Furthermore, this induced $CD8^+$ T cells adoptive immunotherapy significantly suppressed the proliferation and growth of tumor cells in NOD/SCID mice harboring hepatocarcinoma or breast cancer xenograft. Also, the tumor cell apoptosis was promoted resulting in prolonged survival times of the mice. Taken together, these results indicated that when stimulated by the nanobody, DC/tumor cells-FC-induced specific CTLs displayed better anti-tumor effects and may offer a new strategy of adoptive cellular immunotherapy for cancer.

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DOI: 10.1002/eji.201970400

P3169

Epigenetic modulation combined with PD-1/PD-L1 blockade enhance immunotherapy based on MAGE-A11 antigen specific CD8$^+$T cells

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In cancer cells, cancer-testis antigens (CTA) are promising targets for tumor immunotherapy, and are epigenetically depressed through DNA demethylation. Melanoma-associated antigen-A11 (MAGE-A11) as a cancer-testis antigen is frequently expressed in esophageal cancer and is correlated with poor prognosis of esophageal cancer. Consequently, MAGE-A11 may act as potential target for immunotherapy. In this study, we evaluated the expression of MAGE-A11 in esophageal cancer cells, and identified that MAGE-A11 expression was down-regulated in several tumor cell lines restricting the effect of immunotherapy. Also, the specific recognition and lytic potential of cytotoxic T lymphocytes (CTL) derived from cancer-testis antigen MAGE-A11 was determined. Specific CTLs can kill esophageal cancer cells expressing MAGE-A11, but hardly lyse the MAGE-A11-negtive tumor cells. Therefore, induction of MAGE-A11 expression is critical for cytotoxic T lymphocytes (CTL) recognizing and lysing esophageal cancer cells. Treatment with DNA methyltransferase inhibitor 5-Aza-2´-deoxycytidine increased MAGE-A11 expression in esophageal cancer cells and subsequently enhance the cytotoxicity of MAGE-A11 specific CD8$^+$T cells against cancer cell lines. Furthermore, we described PD-L1 expression in esophageal cancer cells which affects the antitumor function of CTLs. PD-1/PD-L1 blockade could increase the specific lyses of HLA-A2$^+$/MAGE-A11$^+$ tumor cell lines treated with 5-Aza-2´-deoxycytidine induced by CTLs. These findings indicate that treatment of tumor cells with DNA methyltransferase inhibitor 5-Aza-2´-deoxycytidine augmented MAGE-A11 expression in esophageal cancer cells. Combination of epigenetic modulation by 5-Aza-2´-deoxycytidine with PD-1/PD-L1 blockade could be useful against esophageal cancer on T cell-based immunotherapy.
Keywords: MAGE-A11; cytotoxic T-lymphocytes; peptide; 5-Aza-2´-deoxycytidine ; PD-1/PD-L1

Cytokine Antagonists in Therapy

O236

Discovery and development of highly potent small-molecule inhibitors of IL-17A

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IL-17A is the key cytokine driving multiple inflammatory and autoimmune diseases including Psoriasis, Psoriatic arthritis, and Ankylosing spondylitis as evidenced by extensive clinical data on IL17A-directed antibodies. The development of small molecule IL-17A inhibitors might offer a safer and more convenient oral and/or topical alternative to current injectables.
To search for novel small-molecule IL-17 inhibitors, we used our Chemetics platform to screen 40 trillion DNA-encoded small molecule compounds against the human IL-17A protein and discovered three distinct chemical series capable of inhibiting the IL-17A/IL-17RA interaction in the nM range. Co-crystal structures of 2 series complexed with IL-17A showed distinct mechanisms of IL-17A binding for the two chemotypes, allowing further lead optimization to yield IL-17A inhibitors with pM to low nM potency against human and mouse IL-17A, irrespective of IL17A glycosylation levels. The inhibitors also bind IL-17A/F but show selectivity against IL-17F. SPR revealed that the most potent inhibitors bind to IL-17A in a reversible manner, but with off-rates < 10^{-5}/s offering long duration of action. In primary human keratinocytes, the inhibitors suppress IL-17A-induced and IL-17A/TNFα-co-induced

DOI: 10.1002/eji.201970400

gene expression in the low nM range. Finally, in a mouse arthritis (CIA) model, when dosed starting 28 days after immunization a lead inhibitor completely blocked further progression of established arthritis and paralleled the efficacy attained with an IL-17A-neutralizing antibody.
In summary, we have developed highly potent first-in-class small molecule inhibitors of IL-17A with strong cell-based activity and convincing in vivo efficacy. We will provide a complete overview of the program including further recent data.

O237

NUE20798: an oral and selective clinical candidate targeting the first bromodomain of BRD2/3/4 with strong anti-inflammatory and anti-fibrotic activity in the absence of BET-associated toxicity

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Inflammatory and fibrotic diseases are major causes of morbidity and mortality with fibrosis estimated to cause ~40% of deaths in the western world. While clinical activity remains high, there is still a large unmet need for safe and effective treatments for inflammatory and fibrotic disease.
BET inhibitors (BETi) lower production of numerous cytokines/chemokines and show efficacy in autoimmune and fibrosis models. Humans express 4 BET proteins (BRD2/3/4/T), each containing 2 bromodomains (BD1 and BD2). Several panBETi are in clinical oncology trials, but these broadly inhibit all 8 BET bromodomains without selectivity and induce strong haematological and GI side effects hampering use outside oncology. Emerging data suggest that BD1 and BD2 have distinct functions.
Here we describe NUE20798, a clinical candidate with low nM affinity for BD1 of BRD2/3/4 and strong selectivity against BD2 and other bromodomains. In human primary cell assays, NUE20798 potently suppressed the production of pro-inflammatory and pro-fibrotic mediators in the mid-nM range, including TGFβ1-stimulated α-SMA, Gli1 and collagen in myofibroblasts and CCL2 in macrophages. In contrast, µM concentrations of NUE20798 are required to confer anti-proliferative activity suggesting this effect to require both BD1 and BD2 inhibition. In mouse disease models, 3 mg/kg of NUE20798 effectively inhibited inflammatory and fibrotic biomarkers including CCL2 in the absence of any toxicity such as thrombocytopenia. Our data provide a strong rationale for NUE20798 and BD1 inhibitors in general as safe and effective chronic oral treatments in inflammation and fibrosis.

P0532

Interleukin (IL)-33-NF-κB-CCL2 signaling pathway directs regulatory T cells to promote the metastasis of esophageal squamous cell carcinoma

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We aimed to clarify that whether IL-33/NF-kB/CCL2 is involved in the immune regulation of the tumor microenvironment in esophageal squamous cell carcinoma (ESCC). Our previous found that the

DOI: 10.1002/eji.201970400

expression of IL-33 in ESCC had a significant correlation with the degree of tumor differentiation, staging, invasion, and survival in 87 patients with ESCC. Kaplan-Meier curves analysis revealed that the high expression of IL-33 was negatively correlated with patients´ overall survival time, thus inferred that IL-33 can be used as an indicator to predict the prognosis of patients with ESCC. The role of IL-33 in ESCC was evaluated both in vitro and in vivo. Functional experiments in vitro were performed to elucidate the effect of IL-33 on the migration and invasion in KYSE450 and EC109 cell lines. Knockdown of IL-33 decreased the metastasis and invasion capacity of esophageal cancer cells, whereas IL-33 overexpression exhibited the opposite effect. Mechanistically, the study demonstrated that IL-33 played a vital role in regulating CCL2 expression of ESCC cells via NF-κB signaling pathway. Furthermore, IL-33 could promote tumor development and metastasis by recruiting the Treg cells via CCL2. Besides, knockdown of IL-33 also decreased the growth of human ESCC xenografts in BALB/c nude mice.
These results demonstrated that IL-33 could promote the secretion of CCL2 through the NF-κB signaling pathway, thus recruit Treg cells, promote the immune escape of tumors, and promote the development of ESCC.

P0533

Combined blockade of IL-25, IL-33 and TSLP further inhibits airways inflammation and remodelling in a murine surrogate of asthma

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Background and objective: The role of the epithelial cell-derived cytokines IL-25, IL-33 and TSLP in the pathogenesis of human asthma has been progressively revealed. While isolated blockade of any of these cytokines has been shown to inhibit airways inflammation and hyperresponsiveness in murine asthma surrogates, the hypothesis that combined blockade of all three cytokines can accomplish this more effectively has never been addressed.
Methods: We studied a murine surrogate of asthma employing sensitisation and challenge with ovalbumin (OVA) or saline control. To discern the effects of IL-33 blockade, we compared outcomes in strain identical, wild-type and IL-33 receptor (St2-/-) gene-deleted mice. We then examined, in the St2-/- animals, the effects of additional, single or combined blockade of IL-25 and TSLP with blocking antibodies. Outcomes included airways reactivity, inflammatory cellular infiltration, epithelial cell metaplasia, deposition of fibrosis-related proteins, local Th2-type cytokine expression and total and specific serum IgE concentrations measured by ELISA and quantitative immunohistochemistry.
Results: St2$^{-/-}$ gene deletion significantly reduced airways reactivity, inflammatory cellular infiltration, lung tissue expression of Th2 cytokines and fibrosis-related proteins and serum total IgE in response to OVA sensitisation and challenge. Additional, combined blockade of IL-25 and TSLP in the St2$^{-/-}$ mice further significantly reduced inflammation, Th2 cytokine expression, airways fibrosis and IgE production, while additional TSLP blockade alone reduced eosinophil infiltration and local IL-4 expression.
Conclusion: Our data show that combined blockade of these three cytokines can better ameliorate airways pathological changes in this murine asthma surrogate, with implications for human asthma.

DOI: 10.1002/eji.201970400

P0534

Regulation of IL-1α by calpains during tumor development

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IL-1α is a member of the IL-1 family and is known to be constitutively expressed in all cell types. The secreted form of IL-1α is rarely found in body fluid except in disease conditions. It may act as an alarmin that leads to induction of sterile inflammation, hence may result in immune-suppression and increased invasiveness in tumor development. IL-1α can be cleaved by calpain, a calcium-dependent cysteine protease expressed in all mammalian cells. The cleaved C-terminal portion of IL-1α is found to be more active than the full-length protein. However, it is not know which calpain in the 15 member calpain family is responsible for cleaving IL-1α and what is its role in tumor development. We investigated the effects of calpain 1, calpain 2 and calpain 5 knockout in IL-1α secretion and tumor growth in murine hepatocellular carcinoma models. We found that calpain 1 knockout had reduced IL-1α secretion and tumor growth compared with the controls. On the other hand, calpain 2 and calpain 5 knockouts had increased calpain activity and IL-1α secretion. The regulatory mechanisms among the different calpains are investigated through RNA sequencing and biochemical assays. In conclusion, calpain 1 is responsible for IL-1α cleavage and plays a critical role in tumor development, while other calpains may regulate calpain 1 expression through various mechanisms.

P1128

Blockade of the NLRP3/caspase-1 axis ameliorates airway neutrophilic inflammation in a toluene diisocyanate induced murine asthma model

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Objective: Multiple studies have addressed the vital role of NLRP3/caspase-1/IL-1β signaling in asthma. Yet, the role of NLRP3/caspase-1 in toluene diisocyanate (TDI)-induced asthma is still obscure. The aim of this study is to investigate the role of the NLRP3/caspase-1 axis in TDI-induced asthma.
Methods: Using an established murine model of TDI-induced asthma as previously described, we gave the asthmatic mice a highly-selective NLRP3 inhibitor, MCC950, as well as the specific caspase-1 inhibitors VX-765 and Ac-YVAD-CHO for therapeutic purposes. Airway resistance was measured and bronchoalveolar lavage fluid was analyzed. Lungs were examined by histology, immunohistochemistry, Western blotting and flow cytometry.
Results: TDI exposure elevated the expression of NLRP3 and caspase-1 that was coupled with increased airway hyperresponsiveness (AHR), neutrophil-dominated cell infiltration, pronounced goblet cell metaplasia, extensive collagen deposition and increased T_H2/T_H17 responses. Both VX-765 and Ac-YVAD-CHO effectively inhibited the activation of caspase-1 in TDI-asthmatic mice that was accompanied by dramatic attenuation of AHR, airway inflammation and airway remodeling, in addition to a decreased T_H2 response and lower levels of IL-18 and IL-1β. MCC950 blocked the activation of NLRP3 and down-regulated protein expression of caspase-1, IL-1β, IL-18 in TDI-exposed mice. Furthermore, MCC950 remarkably alleviated AHR, airway inflammation, airway remodeling, and significantly suppressed T_H2/T_H17 responses.
Conclusion : These findings suggested that blockade of the NLRP3/caspase-1 axis effectively

DOI: 10.1002/eji.201970400

prevents the progression of TDI-induced asthma and could be used as therapeutic targets for asthmatics.

P1129

Novel IL4/IL4RA double humanized mouse models for *in vivo* efficacy evaluation of human IL4 or IL4RA antibodies in asthma models

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Recently, the key role played by interleukin (IL)-4/13 and IL-4 receptor in the pathophysiology of asthma, atopic dermatitis (AD), and other auto-immune diseases has been widely accepted, and the pleiotropic cytokines are now considered to be suitable therapeutic targets. The monoclonal antibody Dupilumab is a very promising therapeutic agent because of its ability to inhibit the biological effects of both IL-4 and IL-13, and prevent the interaction of IL4/IL13 with the α-subunit of the IL-4 receptor complex (IL4RA). Because the monoclonal antibodies specific to human IL4RA can't cross-react with mouse endogenous IL4RA and mouse IL4 can't recognize human IL4RA, we generated a IL4/IL4RA double humanized mouse model for in vivo efficacy evaluation of IL4 or IL4RA antibodies. The double humanized mice are comparable to wildtype mice for basal leukocyte subpopulations, including T/B cells, NK cells, DC, granulocytes and monocytes/macrophages. The asthma model established in IL4/IL4R humanized mice showed increased level of eosinophils, neutrophils, IgE production and lung pathological phenotypes. After the treatment of Dupixent analog, the number of inflammatory cells in the lung and the serum IgE levels were much lower than the PBS control group in homozygous B-hIL4/hIL4RA mice.

Taken together, asthma model established in IL4/IL4R humanized mice is a useful tool for *in vivo* efficacy evaluation of human IL4 or IL4RA antibodies.

P1130

ART regimen and other sociodemographics does not affect cytokine expression in HIV patients in Ghana

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Background: Changes in the levels of cytokine in HIV infected individuals impact the function of the immune system with the potential to affect the course of HIV disease by enhancing or suppressing HIV replication.
Aim: The study sought to investigate the effect of sociodemographic indices, clinical laboratory parameters and ART regimen on Th1, Th2 and Th 17 cytokines in HIV patients.
Materials and method: A total of two hundred (200) HIV patients on either first or second line of ART were recruitedinto the study. Serum concentrations of two major immune promoting cytokines, IL 12 and IFN-γ as well as immune suppressive cytokines, IL 10 and IL 17 were measured using enzyme-linked immunosorbent assay (ELISA).
Results: AlthoughINF-γ and IL-17 levels were relatively high in males compared to females, it was vice versa for IL-10 and 12. However, none of these reached a statistical significance. Age significantly correlated inversely with IFN-γ [r=-0.27, p=0.00], IL 10 [r=-0.24, p=0.00], and IL 12 [r=-0.18, p=0.01] whilst duration on ART significantly correlated inversely with IFN-γ [r=-0.16, p=0.02]. CD4 counts at 6 months and 12 months on ART correlated inversely with IL-17 [r=-0.17, p=0.02] and plasma viral load

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DOI: 10.1002/eji.201970400

at 1yr [r=-0.22, p=0.00] respectively. A positive correlation was observed between IFN-γ and IL-12 [r=-0.84, p=0.00] and IL-17 [r=-0.50, p=0.00].
Conclusion: The levels ofIFN-γ, IL-12, IL-17 and 10 are not significantly affected by sociodemographicsandART regimen. This means both regimens are effective in the suppression of disease progression.

P1722

Pseudo-neutrophils as cytokine nano-sponges disrupt myeloid expansion and tumor trafficking to improve antitumor immunotherapy

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The immunosuppressive tumor microenvironment (TME) is critical for cancer progression, and remains a significant barrier to effective anti-tumor therapies. Myeloid derived suppressor cells (MDSCs) as one of the main drivers of immunosuppressive TME. The multiplicity of cytokines and their complex interactions limits the regulation of MDSCs expansion and trafficking. Herein, we prepared cytokines nano-sponge (NCSs) by fusing neutrophil membrane onto polymeric cores, which inherited the associated membrane receptors of neutrophil and made them ideal decoys of MDSCs expanded and chemotactic biological molecules. The blocking of MDSCs recruitment to peripheral lymphoid organs and tumors of breast and melanoma by NCSs treatment led to significantly increase the proportion of T lymphocytes, which efficiently inhibited the proliferation of tumor cells. Intriguingly, NCSs combined with PD-1 blockade showed synergistic efficacy in the treatment of melanoma. Overall, such a pseudo cell platform offers an effective means to disrupt MDSCs expansion and tumor trafficking and provides a novel nano-immunotherapy strategy to synergistically inhibit tumor growth.

P1723

Low dose IL-2 decreases P-glycoprotein activity and exerts synergistic anti-inflammatory effects with immunosuppressants in the treatment of lupus nephritis with steroid resistance

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Objective: P- glycoprotein (P-gp) is capable of transporting glucocorticoids and immunosuppressants out of lymphocytes, resulting in drug resistance in lupus nephritis (LN). IL-2 decreases P-gp expression in cultured cells from human colon carcinoma. Here, we investigated the effects of low-dose IL-2 on the functionality of P-gp in PBMCs of steroid resistant LN patients and in kidneys in lupus prone MRL/lpr mice.
Methods: 30 steroid resistant LN patients were treated with glucocorticoids and immunosuppressants with or without low-dose IL-2 for 12 weeks. Serum and urine collected before and after treatment were analyzed for anti-dsDNA, C3, C4, renal and liver function. P-gp expression and activity were detected in PBMCs by flow cytometry and rhodamine 123. MRL/lpr mice were treated with methylprednisolone with or without IL-2 for 4 weeks. Pathological changes as well as protein and mRNA expression of P-gp were assessed in kidneys.
Results: Compared to patients without low-dose IL-2 treatment, the patients treated with IL-2 had higher serum C3 (p=0.01) and C4 levels (p=0.04), while lower proteinuria(p=0.04), serum creatinine (p=0.03), anti-dsDNA levels (p< 0.001), and accordingly lower SLEDAI (p=0.03). These modifications were associated with reduced P-gp expression (p< 0.01) and activity (p=0.02) in PBMCs. Moreover,

DOI: 10.1002/eji.201970400

compared with controls, MRL/lpr mice treated with IL-2 and methylprednisolone showed reduced proteinuria (p=0.002) and fewer acute and chronic renal pathological lesions. IL-2 attenuated renal deposition of IgG/C3, decreased renal expression of P-gp.
Conclusion: Low-dose IL-2 decreases P-gp activity and exerts synergistic anti-inflammatory effects with immunosuppressants in the treatment of LN with steroid and immunosuppressants resistance.

P2318

TNF-α inhibitor combined with low-dose corticosteroids therapy for Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) syndrome

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Background: DRESS is a life-threatening durg eruption, while a long-term high-dose corticosteroids therapy has been widely used is often contraindicated.
Type of study: This is a small, uncontrolled case series study.
Methods: A total of five adult Han Chinese consecutive cases caused by allopurinol were enrolled using RegiSCAR diagnostic criteria. All patients received 25mg of tumor necrosis factor (TNF)-α inhibitor (Recombinant Human TNF Receptor-IgG Fusion Protein) subcutaneous injection once every two days for one to three weeks. According to systemic inflammatory response, initiating dose of 0.4mg/kg of corticosteroids daily was commenced. The serum cytokine level of TNF-α, IL-8, IL-6, IL-10 and IL-1β was measured at the first day of hospitalization and 4 days after treatment. Appropriate paired *t*-tests was used, and a time to healing curve was analyzed by the Kaplan-Meier method.
Results: All patients promptly respond to treatment and were cured without complications or side effect. The median time to healing was 14.5 days. Following-up 6 months, there was no reccurence. Four patients who had been tested drug genetics were postive for HLA-B*5801. The serum TNF-α concentration from all patients was above the normal range, which was significantly higher at day 4 after TNF-α inhibitor administered (p=0.0427). Meanwhile, the similar trend of serum IL-8 level was observed, although the difference was not significant (p=0.4997).
Conclusions: Etanercept combined with low-dose corticosteroids may be an effective therapeutic option for DRESS. Interestingly, in spite of the concentration of serum TNF-α was raised after introducing its receptor antagonist, disease was ameliorated.

P3170

Molybdenum disulfide nanoflowers mediated anti-inflammation macrophage modulation for spinal cord injury treatment

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Spinal cord injury (SCI) can cause locomotor dysfunctions and sensory deficits. Evidence shows that functional nanodrugs can regulate macrophage polarization and promote anti-inflammatory cytokine expression, which is feasible in SCI immunotherapeutic treatments. Molybdenum disulfide (MoS2) nanomaterials have garnered great attention as potential carriers for therapeutic payload. Herein, we synthesize MoS2@PEG(MoS2=molybdenum disulfide, PEG=poly (ethylene glycol)) nanoflowers as an effective carrier for loading etanercept (ET) to treat SCI. We characterize drug loading and release

DOI: 10.1002/eji.201970400

properties of MoS2@PEG in vitro and demonstrate that ET-loading MoS2@PEG obviously inhibits the expression of M1-related pro-inflammatory markers (TNF-α, CD86 and iNOS), while promoting M2-related anti-inflammatory markers (Agr1, CD206 and IL-10) levels. In vivo, the mouse model of SCI shows that long-circulating ET-MoS2@PEG nanodrugs can effectively extravasate into the injured spinal cord up to 96h after SCI, and promote macrophages towards M2 type polarization. As a result, the ET-loading MoS2@PEG administration in mice can protect survival motor neurons, thus, reducing injured areas at central lesion sites, and significantly improving locomotor recovery. This study demonstrates the anti-inflammatory and neuroprotective activities of ET-MoS2@PEG and promising utility of MoS2 nanomaterial-mediated drug delivery.

P3171

Inhibiting tumor necrosis factor-alpha diminishes desmoplasia and inflammation to overcome chemoresistance in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most common cancer death reasons. Anti-tumor necrosis factor-alpha (TNF-α) antibodies have shown promising effects in PDAC pre-clinical models. However, the prognostic values of TNF-α, underlying mechanisms by which anti-TNF-α treatments inhibit PDAC, and potential synergistic effects of anti-TNF-α treatments with chemotherapy are still unclear. To identify the targeting values of TNF-α in PDAC, we measured TNF-α expression in different stages of PDAC initiation and evaluated its prognostic significance in a pancreatic cancer cohort. We found that TNF-α expression elevated in PDAC initiation process, and high expression of TNF-α was an independent prognostic marker of poor survival. We further evaluated anti-tumor effects of anti-TNF-α treatments in PDAC. Anti-TNF-α treatments resulted in decreased cell viability in both PDAC tumor cells and pancreatic satellite cells in similar dose in vitro. In vivo, anti-TNF-α treatments showed effects in reducing desmoplasia and the tumor promoting inflammatory microenvironment in PDAC. Combination of anti-TNF-α treatments with chemotherapy partly overcame chemoresistance of PDAC tumor cells and prolonged the survival of PDAC mouse model. In conclusion, our findings indicated that TNF-α in PDAC can be a prognostic and therapeutic target. Inhibition of TNF-α synergized with chemotherapy in PDAC resulted in better pre-clinical responses via killing tumor.

P3172

Apilimod as an immunomodulator confers radioprotection of hematopoietic stem and progenitor cells through induction of granulocyte colony-stimulating factor

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Apilimod has been identified as an inhibitor of IL-12 and IL-23 cytokine production, and evaluated in clinical trials as an immunomodulatory agent for treatment of Th1- and Th17-mediated inflammatory diseases. Apilimod specifically targets PIKfyve, which has been reported to be a player in toll-like receptor (TLR) signaling and control TLR-mediated cytokine expression. As TLR signaling is critical for the pathogenesis of radiation injury and has been implicated in radiation protection, we therefore investigated whether systemic administration of apilimod could ameliorate hematopoietic injury after total body irradiation (TBI). Here we demonstrate that single administration of apilimod 3 hr before

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DOI: 10.1002/eji.201970400

irradiation conferred 100% survival in mice, against otherwise 100% death, at a 9.0-Gy lethal dose of TBI. Apilimod ameliorated radiation-induced pancytopenia, and augmented BM cellular recovery and colony-forming ability, promoted hematopoietic stem and progenitor cell recovery, and expanded the pool of functionally superior hematopoietic stem cells (HSCs) in the BM of sublethally irradiated mice. Apilimod injection resulted in strong transient induction of multiple cytokines with known roles in hematopoiesis, including granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6). The effects of apilimod on HSCs and survival in mice were abrogated by neutralization of G-CSF with antibody, indicating that radioprotective efficacy of apilimod is mediated through G-CSF. These findings thus suggest that induction of G-CSF with apilimod represents an attractive radiation countermeasure without known toxicity.

P3173

Triphenylethylene-coumarin hybrids inhibits tumorigenic progression and angiogenesis in breast cancer mouse model

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Coumarins are a wide group of naturally occurring compounds which exhibit a wide range of biological properties such as anti-cancer activities. Here, we characterized the biological functions of three triphenylethylene-coumarin hybrids (TCHs) both in cell culture and nude mouse model. We found that compound TCH-5c has inhibitory effects on both vascular endothelial cells and breast cancer cell lines. Compound TCH-5c inhibited proliferation, resulted in cell death, increased p21 protein expression to induce G0/G1 arrest and changed endothelial cell cytoskeleton organization and migration in EA.hy926 endothelial cells. Compound TCH-5c also inhibited breast cancer cell line derived VEGF secretion, decreased breast cancer cell induced endothelial cell tube formation *in vitro* and suppressed SK-BR-3 breast cancer cell-initiated tumor formation *in vivo*. Our study demonstrates that the coumarin derivative TCH-5c exerts its anti-cancer effects by 1. inhibiting endothelial cell proliferation, migration. 2. suppressing tube formation and angiogenesis induced by breast cancer cells *in vitro* and *in vivo*. Our results have potential implications in developing new approaches against breast cancer.

P3174

Chemokine receptor antagonist block inflammation and therapy Japanese encephalitis virus infection in mouse model

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Japanese encephalitis (JE) is viral encephalitis caused by Japanese encephalitis virus (JEV) which is characterized by cytokine imbalance and encephalitis. JEV can infect a broad host cells contain central nervous system cells. Once across the blood-brain barrier (BBB), JEV enters into central nervous system (CNS) and induces excessive inflammation, which leads to long-term sequelae or

DOI: 10.1002/eji.201970400

even host death in infected humans or animals. In the present study, we first investigated the distribution of JEV infection in brain and further analyzed the dynamic change in inflammation related genes, chemokines, as well as pathological characteristics. Results demonstrated that CCR5 (CCL3, CCL4, and CCL5) and CCR2 (CCL7 and CCL2) are highly correlated with viral encephalitis. CCR5 and CCR2 antagonist could significantly inhibit the inflammation. The mice treated with CCR5 and CCR2 antagonists had a higher survival rate between 70% to 60%, respectively. In summary, our study thoroughly illustrated the characteristics of the dynamic change in inflammation related genes and chemokines induced by JEV infection. We further indicated that CCR5 and CCR2 are potential targets for treatment of JE. In conclusion, this study illustrated CCR5 and CCR2 are crucial mediator for JEV-induced encephalitis and a potential target for the study of anti-inflammatory and antiviral drugs.

Adjuvant and Delivery System

O386

Nanoparticle-mediated delivery of MHC I-restricted autoantigen peptide to liver sinusoidal endothelial cells protects from experimental autoimmune cholangitis

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We have previously demonstrated that delivery of myelin autoantigens to liver sinusoidal endothelial cells (LSECs) using LSEC-targeting nanoparticles (NPs) provides effective protection from CD4 T cell-driven experimental autoimmune encephalomyelitis (Carambia et al. J Hepatol 2015). Here, we investigated whether this methodology might also serve antigen-specific treatment of CD8 T cell-driven autoimmune disease.

To that end, we used the model of OT-I T cell-driven cholangitis in K14-OVAp mice expressing the cognate SIINFEKL peptide in cholangiocytes (Schwinge et al. J Immunol. 2015). LSEC-targeting SIINFEKL peptide-conjugated NPs (SIINFEKL-NPs) were delivered intravenously one day before transfer of pathogenic OT-I T cells. Six days later, liver pathology and hepatic infiltrates were analysed.

Transfer of OT-I CD8 T cells to vehicle-treated K14-OVAp mice induced liver inflammation and injury, associated with significant weight loss and poor body condition. In contrast, K14-OVAp mice receiving SIINFEKL-NP manifested significantly reduced liver damage (serum ALT 33.2 vs. 140.1 U/ml; P=0.0317), and significantly improved body condition (weight change +0.86% vs. -8.95%; P=0.0149). Mechanistically, treatment with SIINFEKL-NP significantly reduced the number of liver-infiltrating OT-I T cells (42.2% vs. 77.3% of CD8; P=0.0159), which, moreover, manifested significant up-regulation of PD-1 (MFI 8708 vs. 5600, P=0.0079) and down-regulation of IFN gamma (76.8% vs. 49.8%, P=0.0012) and granzyme B (71.9% vs. 28.4%, P=0.0079).

In conclusion, NP-based delivery of cholangiocyte antigen to LSEC can provide effective treatment of CD8 T cell-driven autoimmune disease in a murine cholangitis model. Thus, LSEC-targeting NPs might function as treatment platform for antigen-specific treatment of CD8 T cell-driven autoimmune diseases.

DOI: 10.1002/eji.201970400

O387

Antibody repertoire in response to TLR7/8 and TLR9 agonists are different in India rhesus macaques and African green monkeys

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TLR7/8 and 9 agonists are common vaccine adjuvants. They facilitate the development of adaptive immunity, but their effects on antibody repertoire is still poorly understood. Here, we employed India rhesus macaques (IRM) and African green monkeys (AGM) that are currently used as vaccine research models and respond differentially to TLR7/8 and 9 agonists, to unveil dynamic changes of antibody repertoire at 0, 8, 24, 48, 72, 168 hours after dosing TLR7/8-agonist or TLR9-agonist. All samples were amplified for both heavy and light chain of antibody using rapid amplification of 5′ complementary DNA ends and then sequenced by high throughput sequencer. We found the change of antibody repertoire diversity and ratio of immunoglobulin kappa(IgK) and lambda (IgL) for TLR7/8-agonist were similar in both IRMs and AGMs, with diversity increased after 24 hours and ratio decreased, but the change of these indices were different for TLR9-agnoist in two groups. Furthermore, based on the analysis of mutation and isotype switch, we found the activated antibodies were increased by TLR7/8-agonist and TLR9-agonist before 24-hour, but only IRMs had a continued increase after 24-hour induced by TLR7/8-agonist. However, although such antibody variations had been observed, we found these variations were unbiased and we didn't find common TLR-specific antibodies expansion in all individuals as the repertoire similarity between individuals declined after TLR-agonist injection. The systematic assessment of antibody repertoire reaction by TLR 7/8 and 9 agonists in both IRMs and AGMs could provide cues for the development of vaccine adjuvants.

O392

Lactococcus lactis producing human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) used with metformin demonstrates antitumor activity on colon cancer cells in vitro and in vivo

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Introduction: TRAIL induces apoptosis in a variety of tumor cell lines *in vitro*, however in cancer patients was shown to be ineffective because of its short biological half-life after systemic administration. Moreover, many cancer cells have shown to be resistant for TRAIL-induced apoptosis. Here, we propose a TRAIL-based model therapy of colon cancer with the use of non-pathogenic *Lactococcus lactis* bacteria and metformin (MetF). The use of lactic acid bacteria as delivery system for TRAIL may enable production of TRAIL locally in the tumor site for a longer period of time, while MetF might restore sensitivity to TRAIL-induced apoptosis.
Objective: To assess the antitumor activity of the hsTRAIL-producing *L.lactis* bacteria used alone and in combination with MetF on colon cancer cells *in vitro* and *in vivo*.
Methods: Using codon-optimization and Nisin-Controlled Gene Expression System (NICE) we prepared *L.lactis* bacteria secreting human soluble TRAIL. By MTT-assay and subcutaneous NOD-SCID mice model we examined antitumor activity of *L.lactis(hsTRAIL+)* against HCT116 human colon cancer cells *in vitro* and *in vivo*, used in combination with MetF.
Results: The supernatant of *L.lactis(hsTRAIL+)* showed significant cytotoxicity against HCT116 cells

DOI: 10.1002/eji.201970400

in vitro while intratumor injections of *L.lactis(hsTRAIL+)* markedly reduced the tumor growth in NOD-SCID mice. Moreover, combination of *L.lactis(hsTRAIL+)* with MetF showed its synergistic antitumor effect both *in vitro* and *in vivo*.
Conclusions: Recombinant *L.lactis(hsTRAIL+)* produce hsTRAIL with antitumor activity on colon cancer cells *in vitro* and *in vivo*, acting synergistically with MetF.
Acknowledgments: This study was supported by National Science Centre in Poland (Grant no.UMO2014/15/B/NZ5/03484).

LB034

Use of alpha-glucan microparticles and selected mycobacterial peptides as immunotherapy for tuberculosis infection

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Tuberculosis (Tb) an infectious disease caused by *Mycobacterium tuberculosis* (MTb), is one of the leading causes of morbidity worldwide. In 2017, 10 million of new cases and 1.3 millions of deaths were reported (OMS, 2018). The cellular immune response is the most characterized and important response against this intracellular microorganism. The treatment against Tb consists in the administration of antibiotics for long periods of time which has been related to the generation of multidrug resistant strains, mainly because the lack of compliance. The use of immunotherapy as complement of the conventional treatment can be advantageous for a better bacterial elimination. Alpha-glucan microparticles have been used as antigen vehicle by mucosal routes (Patent No. 347183; Guillén *et al.,* 2014). It has also been demostrated their immunostimulant potential, administered as boost and adjuvant of the BCG vaccine in an experimental model of progressive pulmonary tuberculosis (Moreno-Mendieta *et al.,* 2017; 2019). We have isolated mycobacterial peptides that highly stimulate the immune protection activity producing significant therapeutic activity. Thus, we tested the hypothesis that the use of both alpha-glucans and mycobacterial peptides could be synergic and improve the immune response against MTb and thus shorten antibiotics regimens. Our preliminary results show that this combinated therapy extents survival rates but there is not significant decrease of bacilary loads, we are now studying the efficiency of this combined treatment to eliminate persistent mycobacteria.

LB035

Liposomal CpG-ODN: an in vitro and in vivo study on macrophage subtypes responses, biodistribution and subsequent therapeutic efficacy in mice models of cancers

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DOI: 10.1002/eji.201970400

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CpG oligodeoxynucleotides (CpG-ODN), a common immune stimulator and vaccine adjuvant, was reported to switch Tumor Associated Macrophages (TAMs) from M2 to M1 phenotype inducing anti-tumor responses. Liposomes are of the successfully applied carriers for CpG-ODN. The aim of present study was design and preparation of a liposomal formulation containing phosphodiester CpG-ODN, evaluation of its effect on macrophages responses, and subsequent antitumor responses in mice. Liposomal formulations containing phosphodiester CpG-ODN or non-CpG-ODN were prepared and characterized. MTT reduction assay in four different cell lines, uptake, arginase and iNOS activity evaluation in macrophage cell lines, biodistribution study and therapeutic anti-tumor effects of formulations in mice bearing C26 colon carcinoma or B16F0 melanoma were carried out. The size of liposomes containing CpG-ODN was ~200 nm with the encapsulation efficiency of 33%. The iNOS activity assay showed high nitric oxide (NO) level in M2 phenotype of macrophage cell lines treated by liposomes containing CpG-ODN. In mice which received liposomes containing CpG-ODN as a monotherapy, maximum tumor growth delay with remarkable survival improvement was observed compared to control groups. Biodistribution study showed the accumulation of liposomal formulation in tumor micro-environment. In conclusion, considerable anti-tumor responses observed by liposomes containing CpG-ODN was due to enhanced
delivery of CpG-ODN to immune cells and subsequent initiation of anti-tumoral immune responses.

LB072

Morphological changes in the organs of mice under the action of influenza vaccine, enhanced HGC adjuvant

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The experiment was conducted on mice CBA (18-20 g), combined in 3 groups. 1st group - animals received intranasally wild type of influenza B/Victoria/2/87 virus in 5×10^6 infectious units (Research Institute of Vaccines and Serums, Russia, Moscow); 2nd group - influenza virus in combination with 1-hydroxygermatranyl citrate (HGC), particle size 1.2 nm, highly soluble, hygroscopic, has low toxicity (Russia). 3rd group was control (intact mice). The maintenance and removal of animals from the experiment was carried out according to the "Rules of work with the use of experimental animals." After removal from the experiment, after 7 days, the lungs, small intestine, liver, and kidneys were taken from the mice. Histological sections were stained with hematoxylin and eosin, alcian blue. Image analysis was performed using the Olympus morphometric setup. With the introduction of influenza virus in the lungs formed distelectasis. In the epithelium of the bronchi, alveoli of the lungs, villi and crypts of the small intestine, tubules of the kidney and hepatocytes, cytopathic changes were observed. The secretory activity of the goblet cells in the epithelium of the villi of the small intestine increased. The joint introduction of influenza virus and HGC led to the development of lymphoplasmacytic infiltrates in the organs, an increase in the number of macrophages in them, but did not remove the cytopathic effect of the virus on epithelial tissue.

DOI: 10.1002/eji.201970400

LB108

LacVax®: OmpA- an affordable food grade vaccine platform against *Shigella*

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The problem of antimicrobial resistance (AMR) has been increasing with alarming rate for many enteric pathogens including *Shigella*. Vaccine is one of the promising approach that not only to provide immunity against the pathogens but also prevent the spread of antimicrobial resistance. Despite of extensive research during the last three decades, vaccine against *Shigella* is not available in the market. The major limitation is to provide the cross protection against multiple *Shigella spp*.
To ameliorate this, we have selected outer membrane proteins (Omps); OmpA and OmpC and their epitopes as conserved immunodominant antigens, which are present in most of the *Shigella spp*. The immunogenicity and protective efficacy of r-OmpA, r-OmpC and their selected epitopes were confimed in a robustly validated intraperitoneal murine shigellosis model. The delivery of these antigens is achieved using a safe, effective and oral delivery platform LacVax, developed using food grade bacteria, *Lactococcus lactis*. Oral immunisation withr-*L. lactis* expressing OmpA (LacVax®: OmpA) successfully elcited OmpA specific humoral (Serum IgG andfecal sIgA), and cellular(INF-γ and IL-10secretion) responses in BalB/C mice.The elevated markers significantly correlated with protection against *S. flexneri* 2a ATCC 12022 . The protective efficacy of r-*L. lactis* expressing OmpA against other *Shigella* strains, and the development of r-*L. lactis* expessing OmpC and epitopes as potential vaccine candidates is in progress.

LB109

Cytotoxicity, stimulatory and antitumor effects of liposomal delivery vehicles in cancer immunotherapies

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Liposome is one of the promising technologies for antigen delivery in cancer immunotherapies. It seems that the phospholipid content of liposomes can act as immunostimulatory molecules in cancer immunotherapy. In the present study, the immunological properties of different phospholipid content of liposomal antigen delivery platforms were investigated. To this aim, F1 to F4 naïve liposomes (without tumor-specific loaded antigens) of positively charged DOTAP/Cholesterol/DOPE (4/4/4 mol ratio), negatively charged DMPC/DMPG/Cholesterol/ DOPE (15/2/3/5), negatively charged DSPC/DSPG/Cholesterol/DOPE (15/2/3/5) and PEGylated HSPC/ mPEG2000-DSPE/Cholesterol (13/110) liposomal compositions were administered in mice bearing C26 colon carcinoma to assess tumor therapy. Moreover, In-vitro studies were conducted, including cytotoxicity assay, serum cytokines measurements, IFN-γ and IL-4 ELISpot assay, T cells subpopulation frequencies assay. The liposomes containing DOTAP and DOPE (F1 liposomes) were able to stimulate cytotoxic T lymphocytes signals such as IFN-γ secretions. In parallel, the aforementioned phospholipids stimulated secretion of IL-4 and IL-17 cytokines from T helper cells. Nonetheless, the survival analysis study showed neither cancer immunotherapy nor significant side effect resulting in death event. As conclusion, the liposomal formulations containing DOTAP as a positively charged phospholipid and

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DOI: 10.1002/eji.201970400

DOPE were able to stimulate cellular immunity and CTLs responses such as IFN-γ secretion as well as stimulation of IL-4 and IL-17. Taken together, fluid DOTAP and DOPE-containing liposomes can stimulate a mixture of Th1 and Th2-immune responses in an antigen-free manner.

P0535

Fingolimod can act as a facilitator to establish the primary T-cell response with reduced need of adjuvants

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The CD8+ T-cell response is an essential part of the adaptive immunity. Adjuvants are routinely required for priming of T cells against antigens encountered in lymph nodes (LNs) to generate antigen-specific immunity but may concomitantly trigger unexpected inflammatory responses. Sphingosine-1-phosphate (S1P) induces transient desensitization of S1P receptors on LN T cells and temporarily blocks their egress, leading to prolonged intranodal retention that allows effective immunosurveillance and increases the chance of priming. In light of the regulatory role of S1P in T-cell migration, we here develop a strategic approach to the T-cell priming with protein vaccine containing low-dose TLR-based adjuvants (LDAV) to induce antigen-specific CD8+ T cell responses as efficiently as using regular dose adjuvants in vaccine (RDAV). We found that when combined with one low dose of the S1P analog fingolimod administered into the same vaccination site posteriorly at a specific time, LDAV can elicit a primary response that reaches the level of that induced by RDAV with respect to the response magnitude and functionality. Time-course studies indicate that LDAV and fingolimod in combination act to mimic the expansion kinetics of RDAV-primed antigen-specific CD8+ T cells. Further, intranodal accumulation of cDC1 is markedly enhanced in mice receiving the combination vaccination despite the decrease in adjuvant use. Of particular note is the marginal cutaneous inflammation at the injection site, indicating an added benefit of using fingolimod. Therefore, fingolimod as a nonadjuvant agent essentially facilitates antigen-specific T-cell priming with reduced need of adjuvants and minimized adverse reactions.

P0536

Immune cells inspired granzyme B delivery for solid tumor therapy

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Immunotherapy for hematologic malignancies treatment such as leukemia and lymphoma has made a breakthrough and played anincreasingly important role in clinical studies. Nevertheless, the effect of immunotherapy in solid tumor still need to be improved due to the limitations of tumor microenvironment and tumor cell characteristics. To solve this problem,based on the mechanism that be involved in immune-targeted cell death, in this study, we prepared a new Granzyme B tumor targeting delivery system based on single protein nanocapsule technology and this system significantly inhibited the growth of tumor in nude mice. In order to further improve the transmembrane efficiency of granzyme B and the ability of inducing cell apoptosis, granzyme B was linked one kind of peptide named TAT that simulated cytotoxic T cells(CTLs)/NK cell mediate cancer immunotherapy process. The endocytosis method was discussed compared with perforin, showing similar cell uptake

DOI: 10.1002/eji.201970400

mechanism.The *in vitro* antitumor effects of granzyme B-TAT were defined. Also the granzyme B -TAT delivery system in tumor distribution and effective inhibition of solid tumors were established. Finally achieving the biomimetics function of immune cells and providing new ideas for the tumor immune therapy.

P0537

Vigorous rectum CD8+ T cells raised by an intranasal prime-rectal boost strategy protected from rectal challenges

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Successfully induction of mucosal immune responses may hold the key to curb the mucosal transmitted pathogens, like human immunodeficiency virus-1 (HIV-1). Accumulating evidence indicates that resident memory T cells (T_{RM}) is critical for controlling mucosal pathogen infection in lung, vagina and intestine areas. Therefore, novel vaccination strategies to effectively induce mucosal T_{RM} are demanded. Here we reported a novel mucosal vaccination strategy which led to vigorous induction of rectum T_{RM} responses and protection against intracellular bacterial infection in a murine model. The optimal strategy always involved a nasal prime and rectal boost regimen, but not by other prime and boost combinations via the same route. We demonstrated that early established circulating and not lung resident T cells are the main contributors to rectum T_{RM} formation during the boost. We confirmed that the efficacy of this mucosal vaccination strategies was independent of antibody responses but depends on regional T cell responses. Thus, we suggest that successful mucosal vaccination strategies likely require sequential systemic prime and local boost steps. Our data also highlights the vaccine-induced prophylactic potential of regional T_{RM} against infections by mucosal transmitted pathogens.

P0538

Nanovaccine against scorpion envenomation: zinc-hydroxyapatite enhance antigen delivery and improves adaptive immunity while inducing low inflammation

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Envenomation resulting after scorpion stings is an important public health problem in rural tropical area of Africa, Asia and America. Children and elderly are the most affected persons. The only specific treatment available is immunotherapy which remains limited due to its efficiency and the delay of its administration. The use of nanoparticle-based technology for the development of risk-free immunoprotective vaccine has received attention to improve vaccine efficacy and targeted delivery to achieve specific immune responses at the cellular level. The aim of this study is to develop inorganic biocompatible Zn^{2+}-substituted hydroxyapatite nanoparticles (Zn-HAP Nps) as efficient vaccine delivery system of native *Androctonus australis hector* (*Aah*) venom to enhance specific immune response. Results confirmed the adsorption of venom on crystallized nanoparticles with a median size

DOI: 10.1002/eji.201970400

of 230 nm. A non-toxic quality of free Zn-HAP Nps with low stimulation of circulating neutrophils cells and a non-significant myeloperoxidases secretion were observed after two injections in rabbits and mice. Zn-HAP-*Aah* was highly immunogenic and elicited antigen-specific adaptive immune responses, with high titer of IgG. IgG1/IgG2a balance revealed moreover an efficient activation of both Th-2 and Th-1 immune responses. Visualization of splenic CD3 co-receptor by immunohistochemestry reveals likewise important T-cell activation after two injections of Zn-HAP-*Aah*. Furthermore, an important immunoprotection up to 6 LD50 of native *Aah* venom without mortality was recorded in all immunized animals. The developed Zn^{2+}-substituted hydroxyapatite nanoparticles seems to be able to enhance activation of specific immune response. Their safety makes these preparations a promising candidate for long lasting anti-scorpionic vaccine.

P1131

Study on the safety and feasibility of HUCMSCs carrying reovirus

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Although reovirus has demonstrated potent oncolytic activities towards human cancers, it has not yet been widely adopted into clinical practice. The major hurdle is that the neutralizing antibody in the blood weakens the antitumor effect of the virus, especially the blood disseminated tumor. Appropriate systemically delivered can not only help oncolytic viruses escape immune neutralization, but also ensure the biological activity of viruses and systemically delivered, as well as the ability to target tumors and tumor matrix. Mesenchymal stromal cells (MSCs) appear to chemotactic migration to tumor focus location due to cytokine secretion. Among the MSCs, human umbilical cord mesenchymal stromal cells (HUMSCs) can be easily obtained from noninvasive collection methods and cultured with minimal manipulation. In the previous study, we first detected the effect of different multiplicity of infection (MOI) reovirus on the viability of HUCMSCs cells, and demonstrated that reovirus (MOI=1) infection had no effect on the viability of HUCMSCs cells. Then, we verified that biological activities such as multipotency and chemotactic migration of HUCMSCs are not affected after wild-type reovirus infected by osteogenic and adipogenic differentiation potential and transwell analysis. Collectively, our study shows the safety and feasibility of HUCMSCs during wild-type reoviral anti-cancer therapeutics in experimental research. In addition, the results support the possible clinical use of HUCMSCs as an effective cell carrier of oncolytic reovirus to maximize their anti-tumor activity.

P1132

PiggyBac transposon mediates IL-12 gene expression in human T cells

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Objection: To evaluate the T cells that were genetically modified using *piggyBac*(PB) transposon system to express human IL-12 gene *ex vivo*.
Methods: T cells were genetically modified using DNA plasmids from the *piggyBac* transposon system to constitutively expression single-chain IL-12 gene and propagated *ex vivo* with feeder cells

DOI: 10.1002/eji.201970400

and cytokines. Secreted IL-12 of the cultured supernatants, *ex vivo* expansion rate, immunophenotypes and T cells activation function assay of the final manufactured T cells were determined.
Results: *PiggyBac* transposon system could efficient introduction of IL-12 genes into T cells and secreted endogenous IL-12 (IL-12OE-T versus NT: 122.55 ± 13.8pg/ml versus 0.3 ± 0.23 pg/ml). Stimulated by constitutively secreted IL-12, IL-12OE-T cells showed even higher expansion rate, higher percentage of $CD4^+$ and $CD3^+CD45RO^+CD62L^+$ phenotype compared to non-transduced (NT) T cells (IL-12OE-T versus NT, $CD4^+$ subset: 20.8% versus 5.25% ; $CD3^+CD45RO^+CD62L^+$ subset: 54.8% versus 24.9%). In addition, IL-12OE-T cells exhibit enhanced cytokine secretion and cytotoxicity once encountered with tumor antigen stimulation. Most important of all, IL-12 secreted level was regulated by genetically modified T cells and increased almost 2-fold triggered by tumor antigen stimulation.
Conclusion: T cells may function as potential ideal carrier for foreign IL-12 gene delivered by *piggyBac*-based gene transfer. T-cell genetically modified to secret IL-12 not only improved its proliferation and antitumor effect, but also increased much higher percentage of $CD4^+$ T-cell and $CD3^+CD45RO^+CD62L^+$ T cell phenotype.
Keywords: Human IL-12 gene; genetically modified T cells; *PiggyBac* transposon system;

P1133

Cyclophilin A promotes intranasal immunization as adjuvant

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Lack of a potent and safe mucosal adjuvant has hampered the development of mucosal vaccination because of immune tolerance at mucosal sites. Efforts have focused on the exploitation of pathogen-associated molecular pattern (PAMP) molecules (e.g., TLR ligands and bacterial toxins). However, a similarly potent class of immune stimulators, which is the damage-associated molecular pattern (DAMP) molecules, have been largely neglected. DAMPs are a group of endogenous substances that can mobilize the immune system upon release from mammalian cells, e.g., during tissue injury. A major advantage of employing DAMP molecules lies in their better safety profile in clinical application because of their human origin. In this study, we explored the mucosal adjuvant activity of cyclophilin A, one of the DAMP molecules, using relevant mouse models and cellular analysis. After intranasal administration of mice with ovalbumin (OVA) adjuvanted with cyclophilin A, levels of serum OVA-specific antibodies including total IgG, IgG1, IgG2a and IgA were augmented. Cyclophilin A was also confirmed to be able to activate dendritic cells, the major antigen presenting cells. Dendritic cells upregulated mRNA transcript expression of IL-1b, IL-6, and CD40, factors critically important for inflammatory and adaptive immune responses upon treatment with cyclophilin A. Cyclophilin A augmented dendritic cell surface expression of CD80 and CD86, two co-stimulatory molecules. In conclusion, our data demonstrate that cyclophilin A exhibited mucosal adjuvanticity in nasal immunization by activating dendritic cells and this DAMP molecule warrants further consideration as a promising mucosal adjuvant.

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DOI: 10.1002/eji.201970400

P1134

Dendritic cell-based vaccine treated with polysorbitol transporter enhanced antigen-specific CD8⁺ T cell immune responses through cross-presentation

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Cross-presentation for the enhancement of CD8⁺ T cell activity is one of the most critical components in host protective immunity against viral infection and cancer. We previously reported that polysorbitol transporter (PST), prepared from sorbitol diacrylate and low-molecular-weight polyethylenimine, induces a long-term antigen specific B cell response and phagolysosomal escape of antigens via mechanism called proton-sponge effect in macrophage.
In the present study, we examined whether PST could induce cross-presentation of Ovalbumin (OVA) in dendritic cell (DC). PST formed a nano-sized complex with OVA (OVA-PST) induced increase of SIINFEKL-H-2Kb suggesting potential cross-presentation followed by enhanced OVA-specific CD8⁺ T cell proliferation. Monophospholipid A (MPLA) upregulated co-stimulatory molecules and expression of pro-inflammatory cytokines in DCs, necessary to induce adequate protective immune response. Together with the MPLA pretreatment, OVA-PST complex showed an effective upregulation of cross-presentation. In addition, applying OVA-PST-DC as DC-based cancer therapeutic vaccine showed remarkable protective effect in OVA-expressing metastatic tumor model.
In mechanism study for the enhancement of cross-presentation, we showed PST induces phagolysosomal escape of antigen to cytosol of DCs that allows the antigen to be processed by proteasome for MHC class I loading through the cytosolic pathway of cross-presentation. As the proof-of-concept, the inhibition of proteasome totally abrogated the enhanced cross-presentation. Collectively, these results suggested that the PST enhances cross-presentation in DCs together with antigen-specific CD8⁺ T cell immune responses that could be used as novel DC-based cancer therapeutic vaccine agent.

P1724

Peptide-based adjuvant optimizes the efficacy of HIV DNA vaccine

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HIV DNA vaccine suffers a modest antibody and cellular immune responses in vivo. We herein reported that a peptide-based adjuvant can optimize the efficacy of HIV DNA vaccine in vivo, via multiple vaccination routes including intramuscular, intradermal and subcutaneous injection. Our peptide-based adjuvant contains a four amino acids backbone (glycine-phenylalanine-phenylalanine-tyrosine) of which the N-terminal and C-terminal are modified by naphthalene acetic acid and methylamino group respectively. This peptide-based adjuvant can improve both the titer of IgG (8-fold) and the expression of IFN-γ (3-fold) induced by HIV DNA vaccines. Moreover, such a peptide-based adjuvant facilitates HIV DNA vaccine to produce polyfunctional T cell response, broad IgG subclasses response and V1/V2 loop-specific IgG response, all of which fail to be triggered by HIV DNA vaccine alone. This peptide-based adjuvant also shows a satisfactory bio-safety in vivo. Overall, our peptide-

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DOI: 10.1002/eji.201970400

based adjuvant shows a promising potential for improving the efficacy of HIV DNA vaccine.
Keywords: Adjuvant, HIV DNA vaccine, Peptide

P1725

Silver nanorods as a safe adjuvant for HIV vaccine

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It is meaningful for designing a safe adjuvant to improving the immune responses induced by HIV vaccine. We herein developed a series of surface-functionalized silver nanorods as adjuvant to improve the efficacy of HIV vaccine in mice. By reasonably designing the shape (rod) and size (< 100 nm) of silver nanomaterials, we realized a significantly decreased cellular uptake of such silver nanorods in vitro and in vivo, which directly results in a significantly reduced bio-toxicity. Moreover, we modified a series of immunoregulatory molecules (such as polyvinylpyrrolidone/PVP, polyethyleneglycol/PEG, amantadine/Ada) on the surface of silver nanorods. Such modifications on the surface of silver nanorods can improve HIV DNA vaccine to produce stronger antibody and cellular responses. Importantly, enhanced HIV-specific immune responses more effectively induced the death of HIV-infected cells and reduced HIV production. Our studies thus provide a new strategy for designing a safe and effective adjuvant for HIV vaccine.
Keywords: Adjuvant, HIV vaccine, Surface modification, Silver nanorods

P1726

Intradermal delivery of a fractional dose of influenza H7N9 split vaccine elicits protective immunity in mice and rats

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Vaccination is the most effective method of preventing the spread of the in fluenza virus. However, the traditional intramuscular (IM) immunization causes fear, pain, and cross infection. In contrast, needle-free (NF) immunization is quick and easy for medical personnel and painless and safe for patients. In this study, we assessed the safety and protective efficacy of NF intradermal (ID) immunization with the influenza H7N9 split vaccine (Anhui H7N9/PR8). A preliminary safety evaluation showed that ID immunization with 15mg of the H7N9 influenza vaccine was not toxic in rats. Moreover, the antigen was metabolized more rapidly after ID than after IM immunization, as determined by in vivo imaging, and ID immunization accelerated the generation of a specific immune response. Additionally, ID immunization with a 20% dose of the H7N9 split vaccine Anhui H7N9/PR8 offered complete protection against lethal challenge by the live H7N9 virus. Taken together, our findings suggest that NF ID immunization with the H7N9 influenza vaccine induces effective protection, has a good safety profi le, requires little antigen, and elicits an immune response more rapidly than does IM immunization. This approach may be used to improve the control of infl uenza H7N9 outbreaks.

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DOI: 10.1002/eji.201970400

P1727

Contribution to the improvement of antivenom immunotherapy: aluminium hydroxide nanoparticles as immune adjuvant

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Immunotherapy is the only specific treatemnt available for snake envenoming today. Aluminium hydroxide adjuvant is weidly used in the production of immune sera sdue to its high profile of security and effectiveness. However, applying this adjuvant to immunize large animals, like horses, requires using large quantities of venom antigens to produce sufficiently neutralizing antibodies. In this study synthesised aluminium hydroxide nanoparticles (Nps) are characterized and tested for their capacity to induce effective specific immune response and lower inflammation compared to classic Alum in order to improve the current immunotherapy approach.Aluminium hydroxide nanoparticles were synthetized andinvestigated for their size, shape and chemical composition. Two immunizing formulations were prepared using attenuated cerastes cerastes venom conjugated to either Alum or alum-nanoparticles, and used to immunize rabbits according to previously established schedule. After one month from the last immunizing boost, sera collected from rabbits were challenged with ascending lethal doses of native snake venom to evaluate their effectiveness in a passive immunoprotection procedure. Results showed that alum-Nps have 50-500 nm spherical shapes with the same chemical composition of Alum. They also showed greater effectiveness in activating lymphocytes, resulting in higher specific anti-venom antibody titers compared to Alum which could be atributed to the direct activation of B-cells in lymp nodes. Imunizing with nanoparticles also resulted in more potent passive immunoprotection against native venom. All together, these results showed that alum nanoparticles use in antivenom immunotherapy could improve the effectiveness of anti-venom immunotherapy in a more ethical enveronment of preoduction.

P1728

Immunomodulatory properties of a commercial product based on bacillus thuringiensis cry 1A-Cry2A against mycobacterial infections

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Immunomodulatory properties of a commercial product based on Bacillus thuringiensis Cry 1A-Cry2A against mycobacterial infections
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Bacillus thruingiensis Cry toxins are strong immunogen adjuvants and potential carriers of clinically important antigens, However, there are still safety and biological concerns for clinical use. In the present work, strong immunogenicity were measured through antibody production (IgM, IgA, IgG subclass)(in serum and broncheo-alveolar washes) and pro-inflammatory/anti-inflammatory cytokine induction after intranasal/oral immunization. First, we found that Th1-Th2 cytokines were induced (TNF-α, IFN-g, IL-6, IL-4) as well as IL-17 and IL-10. Second, a remarkable higher systemic IFN-g and TNF- α induction after oral immunization. These data were strengthened because no toxic effect in mammalian cells (pneumocytes classs II-A549 human cell line) at 10 µg/ml was observed. Conversely; Cry1A toxins acted as an adjuvant of BCG vaccine immunity after A549 co-stimulation with BCG, a boost in the Th1-type response (IFN-g and IL-12) was induced. In summary, all the data reported here, show that a commercial product based on *B. thuringiensis* Cry1A-Cry2A toxins is an

DOI: 10.1002/eji.201970400

strong immunogen and adjuvant, both properties could account for the development of non toxic therapeutic agents.

P1729

Comparative evaluation of MF59-chitosan, MF59 and alum adjuvanted influenza vaccine formulation in mice model

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Several adjuvants have been evaluated to enhance the immune response to influenza vaccines, among these, adjuants made from oil-in-water emulsions (O/W) have many advantages in protection against influenza viruses, and therefore, their adjuvant and immune stimulation efficacy make it a choice for influenza vaccines in high-risk groups. However, none has been currently reported to compare chitosan, MF59 and alum as potential adjuants in formulation of influenza vaccines. In the present study, we developed MF59 adjuvant containing squalene, tween and span with/without chitosan with good stability and biocompatibility and physicochemical characteristics and compared them with allum as conventional adjuant, using a prime-boost vaccination regimen in mice. We showed that vaccines containing MF59 and chitosan (MF59- chit) elicited higher humoral response, splenic lymphocyte proliferation as well as IFN-ɣ and IL-4 level than MF59, alum and non-adjuvant vaccines. In conclusion, we found that immunostimulatory potential, safety and effectiveness of MF59-chit can introduce it as a novel adjuvant for influenza vaccination so that it can successfully stimulate both humoral and cellular immunity better than MF59 alone.

P2319

TLR1/2 signaling enhances anti-tumor immunity by promoting differentiation of monocytic myeloid-derived suppressor cell into M1-type macrophage

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Myeloid derived suppressor cells (MDSCs) play a key role in tumor immunosuppressive microenvironment, which helps tumors avoid immune destruction. Blocking the suppressive activities of MDSCs could be an effective strategy to enhance the effect of anti-tumor therapies. In this study, we found that TLR1/2 expression predicted favorable prognosis of lung cancer patients. In the related mice tumor model, we demonstrated that activation of TLR2 by synthetic bacterial lipoprotein (BLP), a TLR1/2 agonist, inhibited tumor growth and decreased monocytic MDSCs (M-MDSCs). TLR2 deficiency abrogated the therapeutic effects of BLP. Furthermore, BLP treatment blocked the suppressive activity of M-MDSCs and redirected M-MDSC differentiation towards M1 macrophage through JNK pathway. This study demonstrated that TLR2 could be a biomarker and a potential therapeutic target of lung cancer patients.

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DOI: 10.1002/eji.201970400

P2320

Transcutaneous immunization of recombinant Staphylococcal enterotoxin B protein using a dissolving microneedle provides potent protection against lethal enterotoxin challenge

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Staphylococcal enterotoxin B (SEB) produced by the *Staphylococcus aureus* bacteriumis most commonly associated with food poisoning and is known to also cause toxic shock syndrome. Currently, no approved vaccine or specific drug is available to treat SEB intoxication. In this study, we fabricated dissolving microneedles (MNs) loaded with recombinant SEB (rSEB) protein, and evaluated its characteristics, including dissolution profile, protein particle size, insertion depth, antigen retention time in vivo, and skin irritation. Our results showed that rSEB protein-loaded dissolving MNs made of chondroitin sulfate (2%) and trehalose (0.8%) could easily penetrate into the mouse skin within 5 minutes. The rSEB particle size was unchanged before and after MN fabrication. The skin penetration depth of the MNs was 260µm. Moreover, the MNs also significantly extended the antigen retention time in vivo. rSEB protein-loaded dissolving MNs also triggered slight erythema at the beginning of administration, but this erythema disappeared within a few hours. More importantly, we investigated the immunogenicity and protective efficacy of rSEB protein-loaded dissolving MNs. Challenge studies in mice revealed that mice in full-dose MN group had a high level of SEB specific antibody response thatprovided100% protection against a lethal SEB toxin challenge. However, there was only 60% protection observed in mice that were in the half-dose MN (dose sparing) group. We also determined the pathological alterations in the tissues of the immunized mice. Taken together, these dissolving MNs may present a promising transcutaneous immunization strategy for treating SEB intoxication.

P2321

Targeting CNS extracellular vesicles enhanced bryostatin-1 therapeutic effect on experimental autoimmune encephalomyelitis

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Extracellular vesicles (EVs) play an important role in cell-to-cell communication in physiological and pathological conditions, increasingly application for disease diagnostics and therapeutic carriers. Lacking the ability of the target desired cell-derived EVs blocked their therapeutic potential to the clinic except modified EVs surface to enhance the tendency activity. Microarray acquired several target genes from the OPCs under inflammatory condition and immuneflurorescent staining confirmed PDGFRα is a potential target both in the CNS of EAE mice and active MS patient brain tissue. We provided a strategy to implant a ligand (PDGF-A) enables targeting through binding to PDGFRα-an oligodendrocyte lineage surface marker. Systemic injection of EVs modified with PDGF-A (EVPs) significantly enhanced the number of EVPs in the oligodendrocyte progenitor cells and oligodendrocytes. Loading Bryo-1 EVPs further ameliorate clinical disease development, decrease inflammation cells infiltration, blocked myelin loss and altered microglia phenotype. Lower dosage of Bryo-1 used acquired better therapeutic effect compared with free Bryo-1 administration. Our works

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DOI: 10.1002/eji.201970400

showed that EVs modified ligand PDGFA significantly enhanced target efficiency and loading Bryo-1 strengthened therapeutic effect, thus offer a strategy for EVs engineering, and targeted therapeutic drug delivery manner. Additionally, EVPs are valuable candidates for drug carrier, and capable of efficient administration of therapeutic agents with avoided nonspecific major distribution in vital organs.

P2322

Anti-inflammatory therapy using glucocorticoid hybrid nanoparticles

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Inflammatory diseases are a group of highly variable pathogenic conditions, often caused by a loss of self-tolerance or an immune response against foreign antigens. Notwithstanding the development of many new therapeutic regimens, glucocorticoids remain the measure of choice in the treatment of inflammatory diseases. Since a considerable number of patients suffer from adverse effects of glucocorticoids, efforts are needed to increase their specificity. Recently, we reported on hybrid nanoparticles as an alternative delivery vehicle for glucocorticoids. They are composed of an inorganic cation and functional organic molecules with a phosphate group, have a diameter of 30-40 nm, and are preferentially taken up by macrophages. Imaging flow cytometry was used to characterize the endocytosis pathway and the intracellular fate of these nanoparticles *in vitro*, and magnetic resonance imaging was used to determine their tissue distribution *in vivo*. We could further show that nanoparticles containing the glucocorticoid betamethasone efficiently ameliorate clinical symptoms in mouse models of multiple sclerosis and graft-versus-host disease, and that this effect is achieved by targeting macrophages. Current efforts aim to determine whether the use of nanoparticles might allow to avoid adverse effects of glucocorticoids such as muscle atrophy and osteoporosis. These findings should pave the way for a more tolerable anti-inflammatory glucocorticoid therapy.

P3175

β-Glucan induces autophagy in dendritic cells to influence T cell differentiation

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β-Glucan has been reported to activate dendritic cells (DCs), and activated DCs subsequently promote Th1 and cytotoxic T-lymphocyte priming and differentiation in vitro. However, the mechanism that regulates the immune response of β-glucan-induced DCs has not been thoroughly elucidated to date. Recent studies have drawn attention to a strong relationship between pathogen-associated molecular patterns (PAMP) recognition and autophagy for activation of DC function. In this study, we observed that β-glucan induced the expression of a number of autophagy-related genes and the formation of autophagosomes in DCs. To further investigate whether β-glucan-induced DC activation and innate cytokine production are associated with autophagy, we utilized 3-MA to block autophagosome formation and accessed the maturation and function of DCs induced by β-glucan. We found that autophagy-deficient DCs showed downregulated expression of MHC-II and CD80, decreased TNF-α secretion, and reduced production of iNOS upon β-glucan stimulation. Further examination demonstrated that blockade of autophagy in β-glucan-induced DCs significantly attenuated IFN-γ production by co-cultured CD4+ T cells and inhibited the proliferation and differentiation of CD4+ T cells. Thus, these data indicate that autophagy in β-glucan-induced DCs is a

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DOI: 10.1002/eji.201970400

crucial mechanism for maturation of DCs, and it drives innate cytokine production, thereby facilitating adaptive immune responses.

P3176

The enhanced effect of MPLA adjuvant on humoral response induced by ALVAC vectored HIV vaccine

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RV144 clinical trial testing the combination of ALVAC vectored HIV vaccine with alum adjuvanted gp120 protein boosts reported a modest efficacy (~31.2%). Immune-correlates analysis has demonstrated that the efficacy is associated with anti-HIV IgG antibody response. Our previous study demonstrated that ALVAC can induce inflammasome activation, and one following study suggested that ALVAC-induced inflammasome activation was associated with vaccine efficacy. Therefore, enhancing ALVAC-induced inflammasome activation may improve humoral response and then HIV vaccine efficacy. Based on this consideration, we created a strategy in this study to improve ALVAC vectored HIV vaccine-induced humoral response through enhancing inflammasome activation. We first determined that ALVAC-induced inflammasome activation contributed to vaccine-induced B-cell response. Based on the capability of LPS to activate inflammasomes, we demonstrated that Monophosphoryl Lipid A (MPLA) adjuvant, an approved LPS analogue, was able to enhance inflammasome activation and B-cell response induced by ALVAC vectored vaccine. At last, mice vaccination model further confirmed the enhanced effect of MPLA on the humoral response: MPLA can enhance HIV gp120-specific IgG antibody production, plasma cell generation and total B-cell response, which all are elicited by ALVAC vectored HIV vaccine. In conclusion, we demonstrate that MPLA adjuvant is able to increase humoral response induced by ALVAC vectored vaccines, and MPLA-triggered inflammasome activation may contribute to this effect.

P3177

Tumor-penetrating peptide fused EGFR single-domain antibody enhances cancer drug penetration into 3D multicellular spheroids and facilitates effective gastric cancer therapy

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Human tumors, including gastric cancer, frequently express high levels of epidermal growth factor receptors (EGFRs), which are associated with a poor prognosis. Targeted delivery of anticancer drugs to cancerous tissues shows potential in sparing unaffected tissues. However, it has been a major challenge for drug penetration in solid tumor tissues due to the complicated tumor microenvironment. We have constructed a recombinant protein named anti-EGFR-iRGD consisting of an anti-EGFR VHH (the variable domain from the heavy chain of the antibody) fused to iRGD, a tumor-specific binding peptide with high permeability. Anti-EGFR-iRGD, which targets EGFR and αvβ3, spreads extensively throughout both the multicellular spheroids and the tumor mass.The recombinant protein anti-EGFR-iRGD also exhibited antitumor activity in tumor cell lines, multicellular spheroids, and mice. Moreover, anti-EGFR-iRGD could improve anticancer drugs, such as doxorubicin (DOX), bevacizumab,

DOI: 10.1002/eji.201970400

nanoparticle permeability and efficacy in multicellular spheroids. This study draws attention to the importance of iRGD peptide in the therapeutic approach of anti-EGFR-iRGD. As a consequence, anti-EGFRiRGD could be a drug candidate for cancer treatment and a useful adjunct of other anticancer drugs.

P3178

Calmette-Guerin in vaccine development and tumor immunotherapy

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Bacillus Calmette-Guerin (BCG) vaccines are attenuated live strains of Mycobacterium bovis and are among the most widely used vaccines in the world. BCG is proven to be effective in preventing severe infant meningitis and miliary tuberculosis. Intravesical instillation of BCG is also a standard treatment for non-muscle invasive bladder cancer. In the past few decades, recombinant BCG (rBCG) technology had been extensively applied to develop vaccine candidates for a variety of infectious diseases, including bacterial, viral, and parasite infections, and to improve the efficacy of BCG in bladder cancer therapy. This review is intended to show the vast applications of BCG and recombinant BCG (rBCG) in the prevention of infectious diseases and cancer immunotherapy, with a special emphasis on recent approaches and trends on both pre-clinical and clinical levels.

P3179

Safety and improvement of aluminum adjuvant

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Aluminum adjuvant has a long history of application in human vaccines such as human papillomavirus vaccine, hepatitis B vaccine, pertussis-diphtheria-tetanus triple vaccine, etc. It has been used in vaccine production for more than 90 years. Currently recognized mechanisms of aluminum adjuvant include reservoir effects, phagocytosis, and inflammatory responses, but the exact mechanism remains unclear.
Although the aluminum adjuvant vaccine has a long-term safety record, but the relationship between aluminum adjuvant and the side effects of vaccines is unclear, there are still some potential problems in terms of safety. After vaccination the aluminum adjuvant may cause inflammation at the injection site and stimulate local redness, fever, subcutaneous nodules and allergic reactions, but it is usually mild and self-limiting.The risk of serious and rapid adverse reactions during the use of aluminum adjuvant vaccine is very low, and in terms of safety, it is safer and more reliable than other new adjuvants. In recent years, with the development of immunology, molecular biology, and genetics technology, many new vaccines have emerged, and the requirements for vaccine adjuvants have become higher and higher. Many new human vaccine adjuvants have appeared at home and abroad.At present, non-aluminum adjuvants cannot completely replace aluminum adjuvants.We still need to work harder to find better and safer adjuvants. Improvements in aluminum adjuvants have received much attention. Phosphorylation and nanocrystallization of aluminum adjuvants and the addition of other ingredients to aluminum adjuvants will expand the range of applications of aluminum adjuvants in vaccines and become a hot topic in future research.

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DOI: 10.1002/eji.201970400

Immunopharmacology

O369

CCL4 enhances blinatumomab therapy in a human B-ALL xenograft mouse model

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Blinatumomab, a recombinant single chain anti-CD19/anti-CD3 bispecific antibody, has been approved for the treatment of various B-cell precursor acute lymphoblastic leukemia (B-ALL), with an overall response rate of 42%. Much effort has been made to improve the efficacy of Blinatumomab, with limited success. Employing *in vitro* Blinatumomab cytotoxicity assay, multiple cytokine Luminex assay, and high-dimentional mass cytometry-based deep profiling of patient PBMCs samples, we found that T cell production of IL-2, IFN-g, perforin, granzyme B, and CCL4 strongly correlated with sensitivity to Blinatumomab-mediated cytotoxicity. We further demonstrated in a human B-ALL xenograft mouse model that a Blinatumomab and CCL4 combination therapy is more effective than Blinatumomab alone at clearing leukemia cells from the bone marrow, a primary hiding niche for leukemic cells. Our results suggest that the combination therapy of Blinatumomab and CCL4 is a better treat regimen for patients with B-ALL

O370

Suppression of a broad spectrum of liver autoimmune pathologies by single peptide-MHC-based nanomedicines

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Peptide-major histocompatibility complex class II (pMHCII)-based nanomedicines displaying tissue-specific autoantigenic epitopes can blunt specific autoimmune conditions by reprogramming cognate antigen-experienced CD4+T-cells into disease-suppressing Treg-regulatory type 1 (TR1) cells. Here, we show that single pMHCII-based nanomedicines displaying epitopes from mitochondrial, endoplasmic reticulum or cytoplasmic antigens associated with primary biliary cholangitis (PBC) or autoimmune hepatitis (AIH) can broadly blunt PBC, AIH and Primary Sclerosing Cholangitis in various murine models in an organ- rather than disease-specific manner, without suppressing general or local immunity against infection or metastatic tumors. Therapeutic activity is associated with cognate TR1 cell formation and expansion, TR1 cell recruitment to the liver and draining lymph nodes, local B regulatory cell formation and profound suppression of the pro-inflammatory capacity of liver and liver-proximal myeloid dendritic cells and Kupffer cells. Thus, autoreactivity against liver-enriched

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DOI: 10.1002/eji.201970400

autoantigens in liver autoimmunity is not disease-specific and can be harnessed to treat various liver autoimmune diseases broadly.

O371

Low dose interleukin-2 prevents infection in patients of rheumatic disease treated with corticosteroids and/or DMARDs

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Objective: Low dose interleukin-2 (IL-2) can selectively regulates the function of NK and CD8+ T cells. This study aimed to assess the infection incidence of low-dose IL-2 combined with corticosteroids and/or DMARDs for treatment of Rheumatic diseases.
Methods: We conducted a retrospective analysis and estimated incidence ratio of infection up to 3 months. The cohort included 365 patients who came from Peking University People's Hospital, followed from December 2016 to August 2018. We identified 164 low dose IL-2 and 201 corticosteroids and/or DMARDs users. The type and site of infection were recorded for each case. Chi-square test and multivariate analysis were performed to compare incidence ratio and factors associated with infection.
Results: Male-female ratio was 25/139 and 15/186, mean age was 43.5 and 43.3 years, major diseases, including SLE, RA and SS, with a total percent of 96.4% and 90.0% in low dose IL-2 group and traditional group respectively. Concomitant baseline treatments contain corticosteroid and DMARDs and there was no significant difference between two groups. Three months later, there were 10/164 (6.1%) patients in low dose IL-2 group and 39/201 (19.4%) patients in traditional treatment group, with a significant difference ($P < 0.001$). In multivariate analysis, low dose IL-2 was a significant positive factor to prevent infection, with an odd ratio of 0.218, and 95% confidence incidence was 0.098 to 0.486.
Conclusions: Low-dose IL-2 can prevent infection in patients of rheumatic disease treated with glucocorticoid and/or immunosuppressive agents.

O372

Radiotherapy combined with the immunocytokine L19-IL2 alters the systematic and tumor-infiltrating immune compartments

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Lung cancer is the leading cause of cancer-related death worldwide. Despite advances in cancer therapies, survival is poor and most patients die in the next years following diagnosis. New therapeutic strategies are therefore urgently needed. It has been shown that the administration of the tumor-targeted antibody-based immunocytokine L19-IL2 after radiotherapy (RT) resulted in synergistic anti-tumor effect. The combination of RT and L19-IL2 immunocytokine administration is thought to induce immunogenicity of cancer cells and local T cell activation in neovascular tissue and was found to induce long-lasting complete remission of C51 tumor in mice.
In this project, mice were subcutaneously injected with C51 colon carcinoma cells. Spleen and tumors were collected after single dose local tumor irradiation and systemic administration of L19-IL2 or equimolar controls.
We found the frequency of NK cells and pro-inflammatory macrophages increased after the

DOI: 10.1002/eji.201970400

combination therapy. We also showed that cytotoxic T cells expressed higher level of activation markers following the RT+L19-IL2 treatment. However, this phenomenon was associated with higher frequency of regulatory T cells in both spleen and tumor of mice treated with RT+L19-IL2. In the helper T cells compartment, we found the proportion of central memory and effector T cells increased after the combination therapy.
These data provide the first evidence for the anti-tumor immune response induced by a novel cancer-treatment strategy with increased therapeutic potential. We are investigating this combined therapy further in patients with non-small cell lung cancer treated with the combination of RT and L19-IL2 in a phase II clinical trial.

O373

Topical treatment of all-trans retinoic acid inhibits murine melanoma by promoting $CD8^+$ T and NK cell immunity

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All-trans retinoic acid (atRA), the main biologically active metabolite of vitamin A, has been implicated in immunoregulation and anti-cancer. A recent finding that vitamin A supplementation could decrease the risk of melanoma in human indicates the beneficial role of atRA in melanoma. However, it remains unknown whether topical application of atRA could inhibit melanoma growth by influencing tumor immunity. We here demonstrated that topical application of tretinoin ointment (atRA as the active ingredient) effectively inhibited B16F10 melanoma growth, which was accompanied by markedly enhanced $CD8^+$ T and NK cell responses. First, we found that topical atRA treatment promoted the differentiation of effector $CD8^+$ T cells in tumor-draining lymph nodes and enhanced cytotoxic function of effector CD8+ T cells by up-regulating MHCI expression in B16F10 cells. Importantly, $CD8^+$ T cell depletion partly restored tumor volumes in atRA-treated mice. Second, we found that chemerin, a known NK cell chemoattractant, was constitutively expressed in the skin, and down-regulated over the murine melanoma growth. Notably, topical treatment of atRA up-regulated skin expression of chemerin and promoted early recruitment of NK cells into melanoma. In contrast, atRA treatment failed to promote NK cell infiltration in tumors derived from mice which were deficient in chemerin or its receptor CMKLR1, suggesting a dependency of NK cell recruitment on the Chemerin-CMKLR1 axis in melanoma. Importantly, CMKLR1 deficiency partially abrogated the ability of atRA to inhibit melanoma growth in mice. Collectively, our study reveals new immunological mechanisms by which atRA inhibits murine melanoma growth via enhancement of anti-tumor immunity.

O374

$5\text{-}HT_2$ and $5\text{-}HT_{2B}$ receptor antagonism abrogate fibrotic potential of peritoneal fibroblasts by targeting STAT3 pathway in patients receiving continuous ambulatory peritoneal dialysis

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Peritoneal fibrosis results in ultrafiltration failure in continuous ambulatory peritoneal dialysis (CAPD) patients. 5-hydroxytryptamine (5-HT; Serotonin) strongly induces extracellular matrix synthesis in peritoneal fibroblasts in a Transforming growth factor beta 1 (TGF-β1) dependent manner. We aimed to evaluate anti-fibrotic role of inhibitors of $5\text{-}HT_2$ and $5\text{-}HT_{2B}$(Terguride and SB204741), respectively in

DOI: 10.1002/eji.201970400

human peritoneal fibroblasts (HPFB) isolated from peritoneum of CAPD patients. Peritoneal fibroblasts isolated from CAPD patients (n=6) and controls (n=8),were incubated with 5-HT (1µM)/TGF-β1 (10ng/ml) for 1 hour and later with 5-HT (1µM)/TGF-β1 (10ng/ml) and terguride or SB204741 (1µM, each) for 24 hours (Post-treatment strategy). In pre-treatment strategy, cells were pre-treated with terguride or SB204741 (1µM, each) for 1 hour and later with only 5-HT (1µM)/TGF-β1 (10ng/ml) for 24 hours.Real time PCR for pro-fibrotic (*TGF*B1, COL1A1, COL1A2, ACTA2, CTGFand *FN1*) and anti-fibrotic genes (*MMP2/TIMP1*) expression was performed. Type I collagen and α-SMA, phosphorylation status of Smad-3, ERK1/2, Src and STAT-3 was examined by western blotting. In 5-HT/TGF-β1stimulated HPFB, upregulated pro-fibrotic gene expressionwas observed, which significantly reduced on co-culture with 5-HT_2/5-HT_{2B}inhibitors, with no effect on anti-fibrotic genes mRNA expression. In 5-HT stimulated HPFB, treatment with both 5-HT inhibitors decreased type 1 collagen and α-SMA with reduced ERK1/2 phosphorylation, however, Smad-3 phosphorylation remain unaltered. In 5-HT/TGF-β1 simulated HPFB, 5-HT inhibitors decreased STAT3 phosphorylation, without affecting Src phosphorylation. TGF-β1 mediated non-canonical pathways, ERK1/2 and STAT3 have been implicated in in the development of fibrosis. 5-HT receptor antagonists might reduce fibrotic potential of HPFB via suppression of TGF-β1 mediated non-canonical pathways.

O375

Effectiveness of IgY in preventing risk of dental caries in animal and humans: systematic review

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Introduction: Immunoglobulin Y (IgY) is an egg yolk derived antibody which targets specific infectious entities within the alimentary tract. There are apparently no systemic immune response however allergic reactions may occur upon ingestion. Many researchers have reported the effectiveness of passive immunization against caries, in which specific antibodies against mutans streptococci are administered orally. IgY too has been found to reduce Streptococcus mutans count; a surrogate outcome for reduction of dental caries. However, there is a lack of evidence to enable recommendation of IgY as a caries preventive agent. Hence, a systematic review was planned to evaluate the effectiveness of IgY in preventing risk of dental caries in animal and humans.
Methodology: It was performed from January to April 2019 using PRISMA guidelines and specified design. A reproducible literature search strategy was employed in all the standard databases (PubMed-LILACS-Web of Science-Cochrane-CINHAL-Scopus databases, along-with Public-Health-Electronic-Library,TRoPHI & DoPHER). Observational, case-control, cohort and randomized clinical trial conducted in animals and humans with moderate or low risk of bias were assessed for performing systematic review.
Results: 12 studies were included in the systematic review. The majority of studies were conducted in animals and lacked standardization in terms of design and outcome variables. Human studies were mostly observational with no randomized clinical trial done till date, though they found efficacy of IgY in reducing dental caries in humans.
Conclusion: There is a lack of well designed studies and randomized clinical trial for establishing efficacy of IgY for prevention of dental caries.

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DOI: 10.1002/eji.201970400

O376

A mechanism for the induction of antitumor immunity by metformin against brain cancer

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Glioblastoma Multiforme (GBM) is the most malignant and aggressive brain tumor. Despite multiple immunotherapy and conventional therapies including chemotherapy and radiation, the average survival rate is less than 2 years. Recently, γδ T cells are thought to be an alternative target for immunotherapy. γδ T cells recognize stress induced surface molecules such as NKG2D ligands and phospho-antigens which are highly expressed on glioma cells. Likewise, γδ T cells in a xenograft model showed therapeutic effects. However, the role of γδ T cell in GBM is still ambiguous. Here, we suggested that metformin, the most common drug for type 2 diabetes, can inhibit progression of GBM via γδ T cells in vivo using a gl261 syngeneic mouse model. Unlike previous studies regarding extracranial tumors, the anti-tumor effect of metformin was not dependent on CD8 T cells. Moreover, the activation, exhaustion and cytokine production of CD8 T cells were not affected by metformin. However, metformin treatment enhanced infiltration of IFN-γ producing γδ T cells in GBM region. Metformin treated γδ T cells highly expressed NKG2D and multiple cytokines. Furthermore, metformin treatment reduced immunosuppressive microenvironment. In conclusion, this study provides information about the importance of γδ T cell on GBM and novel mechanism of metformin.

LB073

Anti-arthritis effect of berberine associated with regulating energy metabolism of macrophage through AMPK/ HIF-1α pathway

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Previously, we had been proved that berberine (BBR) alleviated the inflammatory response in adjuvant-induced arthritis (AA) rats by regulating polarization of macrophages. However, the intracellular molecular/pathways that BBR regulates macrophage polarization remains unclear. In this study, we showed that BBR treatment ameliorated inflammatory response and joint damage in AA rats by decreased the proportion of M1 macrophages and increased the proportion of M2 macrophages. Meanwhile, we found BBR treatment up- regulated the expression of AMP-activated protein kinase phosphorylation (p-AMPK) and the expression of Hypoxia inducible factor 1α (HIF-1α) was decreased in joint tissue. Further mechanistic studies were carried out in LPS-stimulated peritoneal macrophages from normal rats. Interestingly, we observed Compound C (AMPK inhibitor) abrogated the inhibition of BBR on migration of macrophages. Moreover, we found that pretreatment with BBR promoted M1 to M2 polarization transition by up-regulating the expression of p-AMPK and suppressing the expression of HIF-1α. We therefore further verified BBR suppressed LPS-induced glycolysis by decreasing lactate export and increasing ATP content. Remarkably, the effects of BBR on macrophage polarization and metabolic manners were reversed by Compound C. Hence, these observations taken together indicate that anti-arthritis effect of BBR associated with regulating energy metabolism of macrophage through AMPK/ HIF-1α pathway.
Keywords: Berberine, adjuvant arthritis, macrophage polarization, AMP-activated protein kinase, Hypoxia inducible factor 1α, energy metabolism

DOI: 10.1002/eji.201970400

LB110

A potent water-soluble ferrocenylquinoline clears Leishmania donovani infection by targeting the parasite survival enzymes inside the host cells effectively by both oral and intramuscular routes

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Since inception, every magic bullet developed against leishmaniasis traveled certain path and then dropped down either due to toxicity or emergence of resistance. Route of administration is also a concern as oral delivery is the most effective route due to flexibility of administration and cost-effectiveness. With this in mind, series of water-soluble ferrocenylquinoline molecules have been synthesized for target-specific therapy against *Leishmania donovani* infection based on our previously reported hydroimmiscible antileishmanial lead [7-Chloro-N-[2-(1H-5-ferrocenyl-1,2,3-triazol-1-yl) ethyl] quinolin-4-amine]. The best active water soluble ferrocenylquinoline molecule [7-Chloro-quinolin-4-yl-methyl-[2-(4-ferrocenyl-[1,2,3]triazol-1-yl)-ethyl]-amminium iodide] showed higher efficacy in comparison to other conventional antileishmanial drugs both, *in vitro* (IC_{50}: 0.5 µM), and *in vivo,* either in oral (IC_{50}: 0.8 mg/ kg body weight) or intramuscular (IC_{50}: 0.75 mg/ kg body weight) route. No toxicity was found on splenocytes and bone marrow hematopoiesis in treated animals; moreover, it induced pro-inflammation by skewing the Th2 response towards Th1. It acted through a putative target on parasite survival enzymes, precisely through the inhibition of trypanothione reductase (TryR) which is essential to overcome the drug-induced danger. More interestingly, the treatment also induced PARP1 expression followed by a reduction of Sir2 and associated NAD^+/NADH ratio to promote apoptosis of intracellular amastigotes. Neither the enteral or parenteral administration hampered the Phase I and Phase II drug metabolizing enzymes in the host liver. As the final point to mention, this easily bio-available molecule with a short elimination half-life did not induce transcription of MDR1 and MRPA resistance-conferring genes in laboratory-induced resistant-*L. donovani* lines unlike the conventional antileishmanial drugs.

P0539

Hydroxychloroquine enhances anti-tumor effect of BC001 via inhibiting autophagy and promoting immune responses in gastric cancer

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Gastric cancer is an important cancer type worldwide, the anti- angiogenic agent BC001 can target the vascular endothelial growth factor receptor 2 (VEGFR2), and significantly suppressed tumor growth of gastric cancer BGC823 cells in vitro and in vivo. However, numerous results indicated that anti-angiogenic drugs did not significantly improve overall survival of patients with gastric cancer. In addition, it is reported that some anti-angiogenic drugs could induce autophagy, and the inhibition of autophagy enhanced anti-cancer effects of anti- angiogenic agents. In this study, we found that Hydroxychloroquine (HCQ, an inhibitor of autophagy) increased the proliferation inhibition and apoptosis of BC001 in vitro, and enhanced the anti-tumor effect of BC001 on BGC823 xenograft tumor

DOI: 10.1002/eji.201970400

in vivo. Additionally, BC001 neither induced nor inhibited the autophagy. RNA-seq results revealed that HCQ influenced numerous autophagy or lysosomal- associated genes, such as TP53INP1, IL1B, TNF, MEFV, USP36, IL6, NEU1, ABCA1, PCSK9, MBP, NEU3. Importantly, HCQ can affect multiple pathways, including negative regulation of endothelial cell proliferation, blood vessel remodeling, cell surface receptor signaling pathways and notch receptor processing in signal transduction, cancers and immune system, through regulating CXCL8, TNF, IL6, ICAM1 and FOS genes. In summary, HCQ enhances anti-cancer effects of BC001 in gastric cancer via autophagy-dependent mechanism, also partially via promoting immune responses.

P0540

Oncolytic reovirus based combination anti-tumor strategy

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The naturally occurring oncolytic virus, reovirus, exhibits cytotoxic effects on cancer cells. Reovirus is safe, and currently is in multiple clinical trials testing for the treatment of different cancers. In order to improve the oncolytic activity of reovirus and allow for systemic administration despite the prevalence of neutralizing antibodies, we've tried three reovirus-based combination therapy strategies. Firstly, we tried to use immune cells such as CIK cells and NK cells as cell carriers for reovirus delivery, and found that cell carriers could transfer reovirus to tumor cells in the presence of neutralizing antibodies. In addition, we found that reovirus can promote the cytotoxicity of CIK and NK cells, so it can further enhance the anti-tumor effect when used in combination. Secondly, we confirmed that reovirus could enhance the effect of antibody-dependent cell-mediated cytotoxicity (ADCC), an important mechanism in antibody-mediated tumor clearance. The combination of reovirus and cetuximab produced augmented antitumor immunity and inhibited tumor growth in EGRR-overexpressing xenograft model of colorectal cancer. Thirdly, we've used a tumor-targeted delivery system by packaging reovirus with liposome. It is expected that the delivery system can reduce the recognition and response of the immune system to the virus, and further enhance the infectivity of the virus on tumor cells and tumor lytic effect. Further studies are ongoing to investigate the underlying mechanisms.

P0541

Isoforskolin modulates the Th17/Treg balance in a cigarette smoke induced chronic obstructive pulmonary disease animal model

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Chronic obstructive pulmonary disease (COPD) is the third largest cause of human mortality worldwide, and the imbalance of Th17/Treg cells plays an important role in the pathogenesis of COPD. Isoforskolin (ISOF) is the principle active component from the plant *Coleus forskohlii* native to Yunnan in China. Recently we found ISOF could improve pulmonary function in COPD model, but the mechanism remains unclear. In this study, a rat model of COPD was established by long-term exposure to cigarette smoke (1 cigarette each rat, once a day for 14 weeks), then the rats were exposed to cigarette smoke and orally treated with ISOF for 14 consecutive weeks. The pulmonary function, lung morphology, lymphocyte and inflammatory cytokines in peripheral blood and

DOI: 10.1002/eji.201970400

bronchoalveolar lavage fluid (BALF), Th17 and Treg related transcription factors and cytokines in lung tissue were evaluated. The results showed that ISOF significantly ameliorated pathological damage of lung tissue and improved pulmonary function in COPD rats. ISOF treatment decreased the number of leukocytes and lymphocytes, and also the levels of pro-inflammatory cytokines IL-1β and TNF-α in serum and BALF. Furthermore, ISOF significantly reduced the mRNA and protein expression of RORγδ and increased the expression of Foxp3 in lung tissue, and the IL-17A mRNA expression was downregulated while IL-10 mRNA level was upregulated, indicating that ISOF could restore the Th17/Treg balance. Overall, these findings demonstrated that ISOF exerted its anti-COPD efficacy by modulating the balance of Th17/Treg in lung tissue and reducing the levels of lymphocyte and inflammatory cytokines in serum and BLAF.

P0542

Romidepsin (FK228) regulates the expression of the immune checkpoint ligand PD-L1 and exerts synergistic anti-tumor activity with an anti-PD-1 antibody in colon cancer

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Romidepsin (FK228), a histone deacetylase inhibitor (HDACi), has anti-tumor effects against several types of solid tumors. Studies have suggested that HDACi could upregulate PD-L1 expression in tumor cells, changing the state of anti-tumor immune responses *in vivo*. However, the influence of enhanced PD-L1 expression in tumor cells induced by romidepsin on anti-tumor immune responses are still under debate. So, the purpose of this study was to explore the anti-tumor effects and influence on immune responses of romidepsin in colon cancer. The results indicated that romidepsin inhibited proliferation, induced G0/G1 cell cycle arrest and increased apoptosis in CT26 and MC38 cells. Romidepsin treatment increased PD-L1 expression in CT26 and MC38 cells via increasing the acetylation levels of histones H3 and H4 and regulating the transcription factor BRD4. In subcutaneous transplant tumor mice and colitis-associated cancer (CAC) mice, romidepsin increased the percentage of FOXP3+ regulatory T cells (Tregs), decreased the ratio of Th1/Th2 cells and decreased the percentage of IFN-γ+CD8+ T cells in the peripheral blood and the tumor microenvironment. Upon combination with an anti-PD-1 antibody, the anti-tumor effects were enhanced, and the influence on CD4+ and CD8+ T cells was partially reversed. Although romidepsin had direct anti-tumor effects in murine colon cancer, it increased PD-L1 expression in tumor cells and the percentage of immune-suppressive cells. Combination with anti-PD-1 antibody augmented the anti-tumor effects by reversing the influence of romidepsin on immune cells.

P0543

Activation of sigma-1 receptor enhanced pericyte survival via the interplay between apoptosis and autophagy: implications for blood-brain barrier integrity in stroke

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Stroke is a cerebrovascular disorder that affects many people worldwide. In addition to the well-established functions of astrocytes and microglia in stroke pathogenesis, pericytes also play an important role in stroke progression and recovery. The sigma-1 receptor (σ-1R) signal pathway has

DOI: 10.1002/eji.201970400

been envisioned to have promising neuroprotective potential in treating stroke, while whether activation of σ-1R regulates the function of pericytes remains elusive. **The aim of this study was to elucidate the role of σ-1R as well as a novel σ-1R agonist in pericytes following ischemic stroke.** An *in vivo* study was undertaken in σ-1R knockout (KO) mouse models, showing greater pericyte loss and less pericyte coverage in σ-1R KO mice than in wild-type (WT) mice. We demonstrated that a novel σ-1R agonist decreased infarct volume and blood-brain barrier damage with a concomitant amelioration of pericyte loss. Further mechanistic studies indicated that autophagy was induced by photothrombotic middle cerebral artery occlusion (pMCAO) or oxygen glucose deprivation (OGD) and that autophagy inhibition significantly lessened the apoptosis of pericytes. **This study identified unique roles for σ-1R in mediating pericyte survival via the regulation of the interplay between apoptosis and autophagy, suggesting that a novel σ-1R agonist may be a promising therapeutic agent for the treatment of stroke patients.**

P0544

Inhibitory effects and mechanisms of Methionine enkephalin (MENK) on cell proliferation and apoptosis of gastric cancer through opioid receptor (OGFr)

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Objective: The aim of this work was to investigate whether MENK could exhibit the activity against human gastric cancer in vitro and in vivo.
Methods: In vitro, cell proliferation, clone formation numbers, cell cycle and apoptosis were observed after HGC27 and SGC7901 cells treated with MENK. In vivo, xenograft tumor in nude mice was used to evaluate the effect of MENK on proliferation and apoptosis of SGC7901 cells. Meanwhile, IHC was used to detect the expression levels of M1/M2 tumor-associated macrophages (TAMs). Further more, siRNA liposome transient method was used to silence the expression of OGFr to observe the mechanism of MENK.
Results: The results indicate that, both in vitro and in vivo, MENK could significantly inhibit the growth of SGC7901 and HGC27 cells in a concentration- and time-dependent manner, decrease the number of cell colonies and arrest cell cycle in the G0/G1 phase. Furthermore, MENK could induce tumor cell apoptosis associated with the BCL-2/Bax/caspase-3/PARP signaling pathway. In addition, MENK may inhibit GC by enhancing the anti-tumor activity of TAMs through inducing the transformation of TAMs from M2 to M1 phenotype. Moreover, MENK could up-regulate OGFr expression and triggers the anti-tumor mechanism by binding to OGFr. After the silencing of OGFr, the inhibitory effect of MENK on the proliferation and apoptosis were significantly weakened.
Conclusions: MENK has a significant inhibitory effect on gastric cancer at the appropriate dose. MENK may be a potential drug for the treatment of gastric cancer.
Keywords: MENK; gastric cancer; opioid receptor; proliferation; apoptosis

DOI: 10.1002/eji.201970400

P0545

Effects of aconitum leucostomum vorosch. on inflammatory cytokines in collagen-induced arthritis rats

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Objective: We evaluate the effect of Aconitum leucostomum Vorosch. on the expression of TNF-α and IL-1β in synovium of CIA rats to explore the Aconitum leucostomum Vorosch. possible mechanism for the treatment of RA.
Methods: The 45 Wistar rats in clear level were randomly divided into normal control groups (8 rats),CIA model group(37 rats).The establishment of CIA model was used bovine collagen type II emulsion in rats at the end of the tail and paws multi-point injection. After the success of modeling, the 24 successful model rats were randomly divided into CIA model control group(8 rats), Tripterygium wilfordii group(8 rats) and Aconitum leucostomum Vorosch group.(8 rats). We evaluate the arthritis index of the four groups weekly. After treated by intragastric administration for 4 weeks, the expression of TNF-α and IL-1β in the synovium were tested by Immunohistochemical staining method, the mRNA expression were tested by qRT-PCR, and in the blood serum were tested by ELISA.
Results: Compared with the model control group, the arthritis index of arthritis index Tripterygium wilfordii and Aconitum leucostomum Vorosch. group decrease significantly after treatment. The expression of TNF-α and IL-1β in synovium of CIA model group were significantly higher than the control group. The expressions of TNF- and IL-1βin synovium and serum were decreased markedly after treatment with Tripterygium wilfordii and Aconitum leucostomum Vorosch.
Conclusion: The mechanism that Aconitum leucostomum Vorosch. can treat RA is related to inhibition of TNF-α and IL-1β.
Keywords: Aconitum leucostomum Vorosch.;CIA; TNF-α; IL-1β;RA

P0546

Bovine dializable leukocyte extract synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells *in vitro*

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Breast cancer is the most common malignancy among women worldwide. Triple negative breast cancer (TNBC), an aggressive subtype of breast cancer, has no effective therapy at present, as cancer cells often become resistant to chemotherapy. It has been proposed that combinational therapy may offer some advantages. Immunepotent $_{CRP}$® (I-CRP), a bovine dializable leukocyte extract, has clinically shown myelo-protection and improvement in quality of life; and *in vitro* cytotoxicity in different cancer cell lines. The aim of the present study was to evaluate the synergistic effect of I-CRP plus first-line chemotherapeutic agents, cyclophosphamide and epirubicin, against breast cancer cell lines MCF-7 and triple negative MDA-MB-231, *in vitro*. Results showed that I-CRP induces cell-death and cell morphology alterations, loss of mitochondrial membrane potential, and ROS production. Cell damage occurs in a ROS-dependent manner in both cell lines. Afterwards, we evaluated cell inhibition, cell death and cell morphology of non-constant ratio combinations with cyclophosphamide or epirubicin, which have demonstrated to induce ROS production through mitochondrial damage. Results show that, in contrast to cyclophosphamide or epirubicine alone, its combination with I-CRP, significantly increased cell inhibition in both cell lines, dependent of cell membrane permeabilization and phosphatidylserine exposure in most of them, in a synergistic or additional fashion. These results

DOI: 10.1002/eji.201970400

provide new evidence of the benefic effects of the use of I-CRP in combination with chemotherapy, as it increases their cytotoxicity, opening a new therapeutic window for breast cancer treatment and a potential approach to the poorly behaving TNBC subtype.

P0547

Targeting of endothelin type A receptor (ETAR) by the selective antagonist Ambrisentan improves outcome in a preclinical model of metastatic triple-negative breast cancer

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The endothelin receptor A (ETAR) and its ligand endothelin-1 (ET-1) are well known for their role in vasoconstriction. The ETAR antagonist Ambrisentan is FDA-approved for the treatment of pulmonary arterial hypertension. Interestingly, ETAR and ET-1 are also overexpressed in cancer cells. Using the orthotopic model of triple-negative breast cancer (4T1), we investigated the effects of Ambrisentan on tumor growth and metastasis. Ambrisentan was administered to BALB/c mice by daily oral gavage for 2 weeks before, and another 2 weeks post, tumor implantation. Tumor growth and animal survival were followed for up to 6 weeks. In some studies, mice were sacrificed 3 weeks post implantation and their tumor and organs (spleen, lung and liver) were processed for flowcytometric and histological analysis to assess the extent of tumor metastasis. Treatment with Ambrisentan led to a significant 43% reduction in metastatic tumor foci in the liver. Moreover, infiltration of granulocytic myeloid cells into the lungs, a correlate of tumor spread in this model, was inhibited by 66%. The decreased tumor metastasis also correlated with a significant enhancement in overall animal (median survival of 35 days in control group vs, 45.5 days in Ambrisentan group; $p = 0.017$). The potential effect of this treatment on anti-tumor immunity is currently being investigated. These findings demonstrate a significant and beneficial effect of Ambrisentan in this highly metastatic breast cancer model and provide a rationale for targeting ETAR as a potential adjuvant therapy in cancer patients

P1135

1,10-phenanthroline derivatives inhibit lung cancer progression and metastasis by regulating M2 macrophage polarization through IL4/STAT6 signaling pathway

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Tumor-associated macrophages (TAMs) are mostly characterized as alternatively activated M2-like macrophages. Recent studies indicated that the progression and prognosis of lung cancer are related to TAMs. Therefore, drug development targeting TAMs is of great interest for lung cancer therapy. In our previous research, a series of novel 1,10-phenanthroline derivatives were synthesized, which showed significant anti-tumor effect on A549 lung cancer cells. In the present study, we investigated whether 1,10-phenanthroline derivatives could inhibit M2 macrophages polarization and suppress

DOI: 10.1002/eji.201970400

tumor progression in vitro and in vivo. Lewis lung carcinoma (LLC) model was established in C57BL/6 mice and treated with 1, 10-phenanthroline derivative (compound 12c). The results showed that the compound 12c could significant inhibit the growth and metastasis of lung cancer in mice. The expression of M2 macrophage-related genes was decreased in tumor and lung tissues. In addition, 12c could inhibit IL-4-induced M2-like polarization of RAW264.7 and THP-1. Moreover, the proliferation and migration of LLC and A549 cells promoted by the conditioned media from M2-like macrophages could be restrained by 12c. Further research showed that 12c might inhibit M2-like polarization of macrophages through IL4/STAT6 signaling pathway. Our findings demonstrated that 1,10-phenanthroline derivatives could suppress tumor cell growth and metastasis by inhibiting the polarization of M2 macrophages, suggesting a promising lead compound and potential macrophage-based tumor therapeutic modality for lung cancer.
Keywords: M2 macrophage polarization, 1,10-phenanthroline derivatives, lung cancer
Acknowledgement: This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant No. LY18B020020.

P1136

Capsaicin ameliorates concanavalin A-induced hepatic injury in mice

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Objective and design: We aimed to investigate the effect of capsaicin in a concanavalin A-induced hepatitis mouse model.
Treatment: Capsaicin (1 mg/kg body weight) was administered by intraperitoneal injection. After 30 minutes, the mice were challenged intravenously with concanavalin A (20 µg/g body weight).
Methods: After concanavalin A injection, serum was withdrawn for plasma transaminase analysis. Pro inflammatory cytokine levels and hepatocyte apoptosis were assayed by ELISA and TUNEL, respectively. Liver samples were collected for real-time PCR, hematoxylin and eosin staining, and measuring oxidative stress and myeloperoxidase levels. Activation of splenocytes and hepatic mononuclear cells was analyzed by flow cytometry.
Results: Compared with control, the capsaicin-treated group showed significantly decreased aminotransferase levels and markedly prolonged mouse survival. Capsaicin pretreatment also attenuated hepatocyte apoptosis and oxidative stress. Furthermore, tumor necrosis factor-α and interferon-γ levels in serum and liver were significantly suppressed, while the percentage of myeloid-derived suppressor cells increased after capsaicin pretreatment.
Conclusions: Our findings indicate that capsaicin pretreatment protects mice from concanavalin A-induced hepatic damage and is partially involved in inhibiting hepatocyte apoptosis, oxidative stress, and inflammatory mediators as well as regulating activation and recruitment of intrahepatic leukocytes.

P1137

Toxicological studies of daphnetin

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Background: Numerous studies have demonstrated that daphnetin (DAP) has anti-inflammatory, antioxidant, antibacterial, antitumor, anti-arthritic, and other extensive pharmacological effects. However, its genotoxicity has rarely been examined.

DOI: 10.1002/eji.201970400

Methods: The safety of DAP was evaluated by an acute toxicity test using a Salmonella typhimurium/ mammalian microsomal enzyme assay (Ames test), bone marrow micronucleus test, acute skin allergy test, and local mucosal irritation test.
Results: Mice received a dose of 100 mg/kg body weight through lavage, which is more than 175 times the whole-body effective dose and were continuously observed for 14 d; mice showed no signs of poisoning. In the Ames test, each dose of DAP resulted in numbers of revertant colonies that were < 2 two times the number of mutation colonies in the negative control group, indicating a negative result. The micronucleus rate of each DAP dose group was not significantly different from that of the negative control group ($P > 0.05$) but was significantly different from that of the cyclophosphamide (CTX)-positive control group ($P < 0.05$). Rabbit skin showed no stimulation reaction to DAP or 10% dimethyl sulfoxide (DMSO), including no erythema, edema, or sensitization phenomena. Rabbits were generally in good condition after dosing the oral mucosa with DAP, and no oral mucosal erythema, erosion, ulcers, or other irritation reactions were observed.
Conclusions: Within the range of tested doses, DAP did not cause any observed toxic effects, mutagenic effects, sensitization, or stimulation.
Keywords: Acute skin allergy test, acute toxicity test, Ames test, daphnetin, local mucosa stimulation test, micronucleus assay

P1138

Antagonism of the Interleukin 17A pathway ameliorates hepatic fibrosis by restoring the STAT3/TRIB3-suppressed autophagy in mice

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Recent studies have identified critical roles for cytokine IL-17A in the development of chronic fibroproliferative diseases including hepatic fibrosis. However, the precise role and mechanism of IL-17A is largely unknown. In this study, we found that human cirrhosis tissues expressed higher levels of IL-17A, IL-17R and its transcription factor RORγt than normal hepatic tissues. Using the bile duct ligation (BDL)-induced hepatic fibrosis of mouse model, we observed that neutralizing IL-17A attenuated cholestatic liver injury-induced hepatic fibrosis and increased animal survival. In addition, IL-17A antagonism inhibited the phosphorylation of STAT3, as well as restored autophagy activity in fibrotic tissues. Blocking STAT3 by a STAT3 inhibitor STATTIC or STAT3 siRNA, IL-17A failed to suppress the autophagy in hepatocytes. We further identified the promoter of pseudokinase Tribbles 3 (TRIB3), a well-known stress sensor, could be activated by STAT3. Interestingly, the expression of TRIB3 was much higher in human cirrhosis tissues compared with normal tissues, which correlated positively with the expression of STAT3. Moreover, immunostaining and co-IP assays revealed the interaction between TRIB3 and p62/SQSTM1 (p62), an autophagic receptor, could cause p62 accumulation and obstruct autophagic flux. Accordingly, autophagy suppressing effect of IL-17A on hepatocytes was reversed by silencing TRB3, which were consistent with the *in vivo* findings that TRIB3 depletion protected against BDL-induced hepatic fibrosis and restored hepatocyte autophagy activity. Our findings demonstrate that the IL-17A-STAT3-TRIB3 signaling pathway involves in the pathogenesis of hepatic fibrosis through regulating autophagy, which can be a potential therapeutic target for the treatment of fibroproliferative liver diseases.

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DOI: 10.1002/eji.201970400

P1139

The function and mechanism of TRIM22 targeting eIF4E in the process of leukemia cells differentiation

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The interferon-inducible protein TRIM22 is one of the target genes of tumor suppressor gene P53, and its overexpression can activate NF-κB. It has been shown that TRIM22 with anti-tumor effect is involved in the inhibition of viral replication and has exhibits RING domain-dependent E3-ligase activity, but its specific molecular function has not been elucidated. Eukaryotic translation initiation factor 4E (eIF4E) is a cap-binding protein that specifically recognizes the 5' end of the mRNA´s cap structure and initiates translation. The reverse change between TRIM22 and eIF4E in the myeloid differentiation of acute myeloid leukemia cells induced by ATRA were observed in the early stage of our study. In order to investigate the function of TRIM22 and the interaction with eIF4E in the differentiation of human acute myeloid leukemia cells for further illustration the mechanism of TRIM22 targeting to regulate eIF4E, and to provide an important basis for finding new targets for leukemia treatment. The model of HL-60 and NB4 cells differentiation induced by ATRA was established in vitro experiment. The mRNA and protein expression of TRIM22 were gradually increased during the differentiation of HL-60 and NB4 cells induced by ATRA, while the expression of eIF4E mRNA and protein decreased gradually. TRIM22 inhibits cell proliferation and promotes cell differentiation during the process of NB4 cells differentiation induced by ATRA and plays an important role by targeting eIF4E to inhibit its expression.

P1140

CMTM3 is upregulated in B-ALL and knockdown of it increases the sensitivity to Imatinib

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CMTM（CKLF-like MARVEL transmembrane domain-containing family）is a gene family that was first reported in 2003 by our lab. In humans, CMTM comprises nine genes, which are *CKLF and CMTM1-8.* CMTM3 is a member of CMTM family, which has four transmembrane regions. ImmuSort database analysis indicates that CMTM3 is increased in B-ALL compared to normal B cells. Our study showed that CMTM3 had a higher expression level in the bone marrow cells from primary B-ALL patients than that from normal donors. It is also highly expressed in B-ALL cell lines SUP-B15 and BV173. In order to investigate the function of CMTM3, we suppressed its expression by shRNA and found that stable knockdown of CMTM3 inhibited cell proliferation, and slightly increased the proportion of apoptotic cells but had no effect on cell cycle. Imatinib, a BCR-ABL inhibitor, is a first-line drug for the treatment of B-ALL. Knockdown of CMTM3 increased the sensitivity to imatinib, but had no effect on BCR-ABL expression, ERK1/2 and Akt signaling pathways. We will explore the mechanisms through mass spectrometry analysis.

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DOI: 10.1002/eji.201970400

P1141

Small-molecule polyamides target-downregulate PD-L1 gene expression and promote cancer cell apoptosis *via* immune and non-immune pathways

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The blockade of PD-1/PD-L1 signaling by monoclonal antibodies (mAbs) has revolutionized cancer treatment. Although there have been no small molecular inhibitors of the PD-1/PD-L1 immune checkpoint on clinical trials, it is imperative to develop low-cost and easily available alternatives to mAbs. It was recently demonstrated that phosphorylated STAT3 directly binds to the promoter of PD-L1 and regulates its expression. In the present study, we designed and synthesized pyrrole-imidazole (Py-Im) polyamides, small molecule DNA binders, that interact with the STAT3 binding site in the PD-L1 promoter region, and evaluated their bioactivity in downregulating the expression of PD-L1 and promoting the apoptosis of tumor cells. It was demonstrated that Py-Im polyamides competitively bound to the STAT3-binding site, downregulating/affecting the mRNA and protein expression of PD-L1. Further results show that the down-expression of PD-L1 in tumor cells by the compounds, by which T cells exert effector functions against tumor cells efficiently, resulting in the reversal of immune escape. More importantly, the interaction of Py-Im polyamides with STAT3-binding site led to tumor apoptosis separately through the Akt/caspase-3 downregulation. Thus, the newly synthesized polyamides could be developed as promising small molecular anti-tumor drugs targeting the PD-L1 gene.

P1142

Structural properties of unique antibodies with TCR-like specificity utilized for cancer and autoimmune disease therapy

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For over a decade, our lab and others have been producing and characterizing a new family of antibodies (Abs) against cancerous and autoimmune diseases. These Abs are uniquely capable of recognizing MHC-restricted targets with a T cell receptor-like (*TCRL*) specificity, within a binding range of micro to nano molar disassociation constant affinity. These can be exceptionally utilized in different modes of action for therapeutic modalities. In this study, our aim is to understand the structural properties of the cognate ligand binding of the TCRLs at the atom level with X-ray Crystallography. The study considers both classes of the MHC, i.e. class I and class II. In class I-MHC, a massive over-presentation of Tyrosinase peptide has been observed on Melanoma lines in the context of HLA-A2 complex. As a therapeutic candidate target, many TCR-Like Abs have been produced against HLA-A2-Tyosrinase by utilizing phage-display and hybridoma techniques, thus yielding Abs with different amino-acid sequences that are translated into different binding affinities and specificity qualities. Our purpose is to solve structures of TCRL Abs selected from a scale of high to low affinity levels, in their bound state, to analyze their modes of binding with diverged affinities and understand the structural basis for this .In class II-MHC, novel TCRL Abs have been isolated and characterized in our lab towards MHC-peptide complexes associated with Multiple sclerosis autoimmune disease. Structural

DOI: 10.1002/eji.201970400

analysis of class II-MHC TCRL Abs is considered novel and thus may provide pivotal insights into an undiscovered family of immune-modulatory therapeutic TCRL Abs.

P1143

Dulaglutide modulates the development of tissue-infiltrating TH1/TH17 cells and the pathogenicity of encephalitogenic TH1 cells in the central nervous system

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GLP-1 (glucagon-like peptide-1) has been reported to play a vital role in neuroprotection. Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model widely used to study human multiple sclerosis, a chronic demyelination disease in the central nervous system (CNS). Recently, important studies have designated that the signaling axis of GLP-1 and its receptor controls the clinical manifestations and pathogenesis of EAE. However, it is elusive whether GLP-1 receptor signaling regulates the phenotype of autoreactive T cells in the CNS. We administered dulaglutide, a well-established GLP-1 receptor agonist (GLP-1 RA), to treat EAE mice prophylactically or semi-therapeutically and subsequently analyzed the mononuclear cells of the CNS. In this study, dulaglutide treatment significantly alleviates the clinical manifestations and histopathological outcomes of EAE. Dulaglutide decreases incidences of encephalitogenic Th1/Th17 cells and Th1 granulocyte-macrophage-colony-stimulating factor (GM-CSF) expression in the CNS. Administration of dulaglutide failed to control the chemotactic abilities of encephalitogenic Th1 and Th17 cells; however, prophylactic treatment considerably decreased the populations of dendritic cells and macrophages in the CNS parenchyma. These results obtained indicate that dulaglutide modulates the differentiation of encephalitogenic Th1/Th17 and the pathogenicity of Th1 cells by influencing antigen presenting cells quantities, providing mechanism insight on T cells regulation in ameliorating EAE by GLP-1.

P1730

A small mycobacteriophage-derived peptide and its improved isomer restrict mycobacterial infection via dual mycobactericidal-immunoregulatory activities

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Mycobacteriophages express various peptides/proteins to infect *Mycobacterium tuberculosis* (*M. tb*). Particular attention has been paid to mycobacteriophage-derived endolysin proteins. We herein characterized a small mycobacteriophage-derived peptide designated AK15 with potent anti-*M. tb* activity. AK15 adopted cationic amphiphilic α-helical structure, and on the basis of this structure, we designed six isomers with increased hydrophobic moment by rearranging amino acid residues of the helix. We found that one of these isomers, AK15-6, exhibits enhanced anti-mycobacterial efficiency. Both AK15 and AK15-6 directly inhibited *M. tb* by trehalose 6, 6'-dimycolate (TDM)-binding and membrane-disruption. They both exhibited bactericidal activity, cell selectivity, and synergistic effects with rifampicin, and none induced drug resistance to *M. tb*. They efficiently attenuated mycobacterial load in the lung of *M. tb*-infected mice. We observed that lysine, arginine, tryptophan and an α-helix are key structural requirements for their direct anti-mycobacterial action. Of note, they also exhibited immunomodulatory effects, includinginhibition of proinflammatory response in TDM-stimulated or *M. tb*-infected murine bone marrow-derived macrophages (BMDMs) and *M. tb*-infected mice, and

DOI: 10.1002/eji.201970400

induction of only a modest level of cytokine (tumor necrosis factor α [TNF-α] and interleukin-6 [IL-6]) production in murine BMDMs and a T-cell cytokine (interferin-γ [IFN-γ] and TNF-α) response in murine lung and spleen. In summary, characterization of a small mycobacteriophage-derived peptide and its improved isomer revealed that both efficiently restrain *M. tb* infection via dual mycobactericidal-immunoregulatory activities. Our work provides clues for identifying small mycobacteriophage-derived anti-mycobacterial peptides and improving those that have cationic amphiphilic α-helices.

P1731

The design, synthesis of chirally-modified polyamides and their bioactivity of anti-tumors by downregulating PD-1 gene

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Programmed death receptor-1 (PD-1) is a 50-55 kDa type I transmembrane glycoprotein and is a member of the immunoglobulin superfamily. In humans and mice, it is expressed on activated T and B cells and myeloid cells. PD-1 has two ligands: the CD80/CD86 IgSF members PD-L1 (PD-L1, also known as B7-H1, CD274) and PD-L2 (B7-DC, CD273). PD-1:PD-L1 blockade by monoclonal antibodies has proven to be an effective modality to overcome immune evasion and mobilize specific antitumor responses in mouse models and clinically in patients.
However, monoclonal antibody therapy inherently carries a number of disadvantages such as their immunogenicity (following repeated administration) and high production costs which may limit their use and broad availability to patients. Thus, searching for small organic molecules targeting PD-1/PD-L1/PD-L2 pathway may be an alternative. Herein, we designed and synthesized several chirally-modified polyamides and their ability against cancers through target downregulating PD-1 gene has been examined by using EMSA, CD, SPR, fluorescence confocal microscopy, RT-PCR, Western Blot and Small Animal in vivo Imaging techniques. Results show that polyamides can strongly bind PD-1 DNA sequence with affinity and specificity features rivaling that of antibodies and induce apoptosis of cancer cells, implying they have a potential as PD-1-targeted anti-cancer drugs.

P1732

Efficacy and safety of low-dose interleukin-2 in combination with methotrexate in patients with active rheumatoid arthritis: clinical results from the randomized, double-blind, placebo-controlled trial

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Objective: Impaired immunological homeostasis has been implicated in the pathogenesis of rheumatoid arthritis (RA). The study aimed to assess the efficacy and safety of low-dose interleukin (IL)-2 for the treatment of RA.
Methods: We conducted a randomized, double-blind, placebo-controlled clinical trial. Patients were randomly assigned (1:1) to receive low-dose IL-2, defined as a dose of 1 million IU, or placebo, and followed up for 24 weeks. All patients received a stable dose of methotrexate (MTX). The primary outcomes were the proportion of patients achieving ACR20 response and DAS28-ESR remission, the change of CDAI and SDAI at week 24 (NCT 02467504).
Results: Forty-seven patients were randomly assigned to low-dose IL-2 (n=23) or placebo

DOI: 10.1002/eji.201970400

group(n=24). Forty of 47 patients completed the trial. The primary outcomes were achieved in per-protocol population. The improvement from baseline in CDAI and SDAI cross time points in IL-2 group was significantly superior to that in placebo group (p=0.018 and p=0.015, respectively). Significantly higher proportion of patients in low-dose IL-2 group achieved ACR20 (p=0.014). Treg cells and $CD56^{bri}$ NK cells were increased in association with low-dose IL-2 administration, while levels of IL-17A and IFN-γ were significantly decreased compared to MTX monotherapy. Improvement by IL-2 therapy was probably correlated with higher IL-21 in serum (p=0.010). Though week 24, the proportions of patients with adverse event were similar between the two group.
Conclusions: Treatment with low-dose IL-2 significantly reduced the disease activity of RA, and were associated with suppressive effects on autoimmunity.

P1733

Low-dose IL-2 treatment in patients with primary Sjögren's syndrome: a randomised, double-blind, placebo-controlled trial

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Objective: Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease associated with dysregulated immune cells and impaired interleukin-2 (IL-2) signaling. This study aimed to assess the efficacy and safety of low-dose interleukin (IL)-2 for the treatment of pSS.
Methods: We conducted a randomized, double-blind, placebo-controlled clinical trial. Patients were randomly assigned (1:1) to receive low-dose IL-2, defined as a dose of 1 million IU, or placebo, and followed up for 24 weeks. The primary endpoint was the achievement of more reduction for either ESSPRI or ESSDAI or MFI-20 by IL-2 treatment at week 12 and 24. The study was registered with ClinicalTrials.gov (NCT02464319).
Results: Between June, 2015, and August, 2017, 60 patients were enrolled and randomly assigned. Twenty-nine patients in low-dose IL-2 group and 28 in placebo group who completed the trial were included in the primary efficacy analysis. Compared with placebo group, ESSPRI, ESSDAI and MFI-20 were decreased significantly in IL-2 group at both week 12 and week 24. The resolution of systemic manifestations was observed after IL-2 treatment, including decreased pulmonary and renal tubular abnormalities, arthritis, thrombocytopenia, leukopenia and fatigue. Immunological analyses revealed expansion of regulatory T (Treg) and B10 cells, with reduction of serum IL-17A and IFN-a in IL-2 group. No severe adverse event was observed in either group.
Conclusions: Low-dose IL-2 was effective and well tolerated in pSS and restored immune balance through enhancing Treg and B10 cells.

P1734

Tyrosine phosphatase SHP2 mediates mitochondrial homeostasis for the resolution of NLRP3 inflammasome activation and its promotion by small molecule compounds

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Aberrant activation of NLRP3 inflammasome is a key molecular event in the pathogenesis of various inflammatory diseases. Although the activation mechanism of NLRP3 inflammasome was relatively clear, there remains unknown for its resolution process. Here we demonstrate that NLRP3 inflammasome stimulators activate Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2), which translocates to mitochondria and dephosphorylates adenine nucleotide translocase 1

DOI: 10.1002/eji.201970400

(ANT1), the key molecule controlling mitochondrial permeability transition. Such mechanism prevents collapse of mitochondrial membrane potential and the subsequent release of mitochondrial DNA and reactive oxygen species (ROS), thus preventing hyper-activation of NLRP3 inflammasome. Ablation or inhibition of SHP2 in macrophage caused unresolved activation of NLRP3 inflammasome, excessive production of proinflammatory cytokines IL-1β/IL-18, and increased sensitivity to peritonitis and experimental autoimmune encephalomyelitis. By establishing an SHP2-specific phosphatase activity assay, we screened out several compounds that can stimulate phosphatase activity of SHP2. These SHP2 agonists were proved to be effective for the restoration of mitochondrial homeostasis and inflammation inhibition. Collectively, our data here highlighted a SHP2-mediated endogenous mechanism for NLRP3 inflammasome resolution, which could be served as a potential target for the treatment of inflammatory diseases.

P1735

14, 15-EET induces breast cancer cell EMT and cisplatin resistance by up-regulating integrin αvβ3 and activating FAK/PI3K/AKT signaling

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14,15-epoxyeicosatrienoic acid is an important lipid signaling molecule involved in the regulation of tumor metastasis, however, the role and molecular mechanisms of 14,15-EET activity in breast cancer cell epithelial-mesenchymal transition (EMT) and drug resistance remain enigmatic.In the present study, we show that serum level of 14, 15-EET increases in breast cancer patients and 14, 15-EET level of tumor tissue is higher than that of non-cancerous tissue. Moreover, 14, 15-EET increases integrin αvβ3 expression, leading to FAK activation. 14, 15-EET induces breast cancer cell EMT via integrin αvβ3 and FAK/PI3K/AKT cascade activation in vitro. Furthermore, we find that 14, 15-EET induces breast cancer cells EMT and cisplatin resistance in vivo, αvβ3 integrin and the resulting FAK/PI3K/AKT signaling pathway are responsible for 14, 15-EET induced-breast cancer cells cisplatin resistance. Our findings suggest that inhibition of 14, 15-EET or inactivation of integrin αvβ3/FAK/PI3K/AKT pathway could serve as a novel approach to reverse EMT and cisplatin resistance in breast cancer cells.

P1736

Plants: allergy versus therapy in West Africa

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Introduction: Pollinosis is sharply increasing in our countries. In addition ,the rich traditional pharmacopoeia remains popular with people because of their high levels of impoverishment,the low accessibilityof pharmaceutical drugs,and the mistrust of pharmaceuticals contraband.
Objective: To characterize plants with allergenic and /or therapeutic potential.
Material and methods: Three studies were conducted at Institut Pasteur of Côte d´Ivoire on plants.The first study identified local pollens plants.The two following studies measured in vitro activity of total and fraction extract of some plants.
Results: Implementation of procedure for conservation and characterization of tropical pollens was done.Reverse sickling activity in sickle cell disease and antimycosis effects on patients suffering of HIV,has been demonstrated.

DOI: 10.1002/eji.201970400

Conclusion: It would be interesting to perform an immunological,molecular and genetic characterization of plant extract compound useful for Iimmunodiagnosis and herbal medicine.
Keywords: Plants,Pollinosis,mycosis,sickle cell disease.

P1737

Glucocorticoid induced leucine zipper in the central nervous system and therapeutic strategies to target neuroinflammation

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Although traditionally accepted as anti-inflammatory agents, extensive studies provide compelling evidence that glucocorticoids (GC) exerts both neuroprotective and neurodegenerative responses in the central nervous system. Accruing oxidative stress with aging negatively regulates the glucocorticoid, preventing suppression of the transcriptional factor, nuclear factor-κB (NF-κB). Stress induced high GC also accelerate beta amyloid (Aβ) plaque formation and tau phosphorylation which in turn upregulate reactive oxygen species in vulnerable neurons. The ensuing inflammatory distress enhance further Aβ accumulation initiating a vicious cycle. The glucocorticoid induced leucine zipper (GILZ) is a gene strongly upregulated by GC and mediates many of the anti-inflammatory and anti-proliferative effects of GC. Mechanistically GILZ inhibits NF-κB by binding the p65 subunit of NF-κB in the cytoplasm and thereby prevent transactivation of inflammatory and apoptosis mediators. Constitutive GILZ expression has been shown to occur with varying dynamics suggesting that the local milieu modulates its expression with consequent effects on cellular responses. We investigated the expression profile of GILZ in the lipopolysaccharide mediated neuroinflammation model of Alzheimer's disease. Our data suggest that the GILZ expression is downregulated in neuroinflammation correlating inversely with the NF-kB p65 mediated innate immune responses and pro-inflammatory cytokines. Incorporating rational amino acid substitutions in the p65 binding motif, we designed low molecular weight GILZ analogs that exhibit increased propensity to form stable PPII helical conformation in the context of the p65-transactivation domain. Treatment with GILZ analogs suppressed neuroinflammation and prevented cognitive decline in 5xFAD transgenic mice, an aggressive model of Alzheimer's disease.

P1738

NK1R blockage using Aprepitant exerts apoptotic and anti-proliferative effects in REH cells

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Purpose: Following the first description of neurokinin-1 receptor (NK1R) network as a carcinogenic pathway, overwhelming number of studies have reported the inappropriate activity of this cascade in a wide assortment of human cancers. On the basis of the principal importance of the NK1R in the pathogenesis of acute lymphoblastic leukemia (ALL), it was of great interest to dissect the effect of Aprepitant, a competing non peptide NK1R antagonist, on REH pre-B-ALL cells.
Methods: To evaluate the anti-leukemic effect of the NK1R antagonist, REH cells was subjected to the increasing concentrations of Aprepitant and subsequent cell viability, growth kinetics, and metabolic activity were investigated. Moreover, to underscore whether the Aprepitant-induced

DOI: 10.1002/eji.201970400

cytotoxic effect could be attributed to either induction of apoptotsis or cell cycle arrest, Annexin-V/PI staining and DNA content analysis were performed. Finally, the transcriptional alteration of apoptosis-related target genes were studied using RT-PCR.
Results: We found that abrogation of the NK1R signaling pathway exerts cytotoxic and anti-proliferative effect against REH cells, as evidenced by the increased externalization of phosphatidyl serine (PS), elevated sub-G1 cell population, and decreased number of inhibitor-treated viable cells. In addition, our results also suggest that Aprepitant-induced apoptotic cell death in REH cells is mediated, at least partially, through shifting the ratio of death promoters to death repressors via alteration of Bax and Bcl2 expression.
Conclusions: This study highlighted the potent efficacy of Aprepitant in pre-B-ALL cells and shed light on pro-apoptotic property of this inhibitor.

P2323

SHP2 inhibition triggers anti-tumor immunity and synergizes with PD-1 blockade

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Tyrosine phosphatase SHP2 is a promising drug target in cancer immunotherapy due to its bidirectional role in both tumor growth promotion and T-cell inactivation. Its allosteric inhibitor SHP099 is known to inhibit cancer cell growth both *in vitro* and *in vivo*. However, whether SHP099-mediated SHP2 inhibition retards tumor growth *in vivo* via anti-tumor immunity remains elusive. To address this, a CT-26 colon cancer xenograft model was established in mice since this cell line is insensitive to SHP099. Consequently, SHP099 minimally affected CT-26 tumor growth in immuno-deficient nude mice, but significantly decreased the tumor burden in CT-26 tumor-bearing mice with intact immune system. SHP099 augmented anti-tumor immunity, as shown by the elevated proportion of $CD8^{+}IFN\text{-}\gamma^{+}$ T cells and the upregulation of cytotoxic T-cell related genes including *Granzyme B* and *Perforin*, which decreased the tumor load. In addition, tumor growth in mice with SHP2-deficient T-cells was markedly slowed down because of enhanced anti-tumor responses. Finally, the combination of SHP099 and anti-PD-1 antibody showed a higher therapeutic efficacy than either monotherapy in controlling tumor growth in two colon cancer xenograft models, indicating that these agents complement each other. Our study suggests that SHP2 inhibitor SHP099 is a promising candidate drug for cancer immunotherapy.

P2324

The anti-tumor effects of a novel MyD88 inhibitor TJ-M2010-5 on hepatocellular carcinoma growth

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Objective: Compared with normal liver, MyD88 is highly expressed in hepatocellular carcinoma (HCC), promoting tumor proliferation and metastasis. In this study, we synthesized a novel MyD88 inhibitor, which is a small molecule derivative of aminothiazole, named as TJ-M2010-5 to explore its direct therapeutic effect on HCC.
Result: In vitro**,** we revealed that TJ-M2010-5 inhibited the proliferation of H22 cells in a dose-

DOI: 10.1002/eji.201970400

dependent manner by inducing G0/G1 phase cell cycle arrest. In vivo, we observed a higher percentage of F4/80^{+}CD11c^{+} macrophages in TJ-M2010-5-treated group than the control group. The peritoneal macrophages treated with 10 mmol/L of TJ-M2010-5 showed no significant difference in the percentage of F4/80+CD11c+ macrophages, suggesting that TJ-M2010-5 has no direct inhibitory effects on macrophages. However, the percentage of F4/80+CD11c+ macrophages was increased after they cultured with the supernatant of H22 cells treated with TJ-M2010-5. Interestingly, no difference was found in F4/80+CD206+ macrophages between two groups in this setting. We demonstrated that TJ-M2010-5 led to an increase in the proportion of M1 macrophages indirectly, while has no effect on M2 macrophages. These data confirmed the indirectly upregulation of M1 macrophages by TJ-M2010-5.
Conclusion: In our study, we demonstrated the mechanisms by which TJ-M2010-5 inhibited the proliferation of HCC, including induction of G0/G1 phase cell cycle arrest and recruitment of M1 macrophage into tumor microenvironment. Taken together, these results indicate that MyD88 is a feasible target for anti-cancer treatment and TJ-M2010-5 may be a promising therapeutic drug for HCCs.

P2325

A novel irreversible and selective BTK inhibitor, ameliorates rheumatoid arthritis by dual inhibition of BCR and osteoclastogenic signaling

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Background: Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint destruction and autoantibody production. The Bruton's tyrosine kinase (BTK) signaling contributes to disease through mechanisms including both antibody/cytokines production and bone homeostasis. SIMM-016 is a highly potent and selective BTK inhibitor, this study was designed to investigate the potential effect and mechanism of SIMM-016 on RA.
Methods: The mouse model of collagen-induced arthritis (CIA) was used to evaluate the therapeutic effects of SIMM-016. Activated markers of splenic B cells from mice treated with BTK inhibitors upon anti-IgM or type II collagen (CII) stimulation were examined by flow cytometry. To define direct effects of BTK inhibitors on osteoclastogenesis, osteoclasts (OCs) were enumerated by tartrate-resistant acid phosphatase (TRAP) staining, RANK-BTK signaling was assessed by Western Blot.
Results: *In vitro,* SIMM-016 has far superior selectivity to Ibrutinib and better activity than Acalabrutinib (ACP-196) on BTK inhibition in B cell. Notably, SIMM-016 inhibited osteoclast differentiation and abolished transduction of RANK-BTK-PLCγ2-NFATc1 signaling in RANKL/M-CSF stimulated RAW264.7 cell. *In vivo*, SIMM-016 dose-dependently reduced serum CII specific antibodies and ameliorated bone destruction in CIA mice, exhibited more potent efficacy than Ibrutinib and ACP-196.Moreover, SIMM-016 treatment decreased expression of inflammatory cytokines and osteoclastogenic genes in joints of CIA mice.
Conclusion: SIMM-016 is a highly potent and selective BTK inhibitor, which presents distinguished therapeutic effects in the CIA model. Our results demonstrated a dual inhibition of B cell function and osteogenesis by SIMM-016, and characterized it as a promising drug candidate for RA treatment.

DOI: 10.1002/eji.201970400

P2326

Reversible SAHH inhibitor ameliorates psoriasis-like skin inflammation in mice by modulating keratinocyte differentiation and reducing inflammatory infiltration via regulating DNA methylation of GATA3 and Lcn2

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Background: Psoriasis is the most prevalent inflammatory skin disorders, affecting 1%-3% of the worldwide population. Genetic, immunologic, and environmental factors contribute to its pathogenesis. In psoriasis, immune cell activation and abnormal epidermal differentiation are key pathogenic events. DZ2002 is a reversible S-adenosyl-l-homocysteine hydrolase (SAHH) inhibitor, although our previous study reported its therapeutic effects on psoriatic skin inflammation, the molecular mechanisms of DZ2002 remain unclear.
Methods: We performed whole-genome bisulfite sequencing (WGBS) to determine altered CpG methylation in skin of imiquimod (IMQ)-induced psoriasis mouse model topically treated with DZ2002. Immunohistochemical analysis and flow cytometry examination were used to detect skin infiltration of immune cells. *In vitro* validation of correlations between methylation and gene expression was conducted in TNF-α/IFN-γ-stimulated HaCaT human keratinocytes by real-time quantitative PCR and Western blot.
Results: *In vivo*, topical use of DZ2002 on IMQ-induced psoriasis mouse model downregulated GATA binding protein 3 (GATA3) promoter methylation in skin, which inhibited keratinocyte proliferation and regulated Epidermal Differentiation Complex (EDC) gene expression; DZ2002 concurrently upregulated lipocalin 2 (Lcn2) promoter methylation, resulted in reduction of chemotaxis and skin infiltration of monocytes. *In vitro*, DZ2002 directly rectified the altered expression of Loricrin, filaggrin, S100 gene families as well as antimicrobial peptide and chemokines in TNF-α/IFN-γ-stimulated HaCaT human keratinocytes by modulating DNA methylation of GATA3 and Lcn2 promoters.
Conclusion: Topical administration of DZ2002 effectively modulated keratinocyte differentiation and reducing inflammatory infiltration in psoriatic mouse model via epigenetic mechanism of regulating DNA methylation of GATA3 and Lcn2 promoters.

P2327

Identification of anti-leishmanial leads from open access pathogen box

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Leishmaniasis is a collective term used to describe various pathological conditions caused by an obligate intracellular protozoan of the genus Leishmania. It is one of the neglected diseases and has been given low attention in drug discovery researches. Aiming to look for potential anti-leishmanialhits and leads, we screened MMV Pathogen Box for two strains of *Leishmania* parasites. Compounds were screened against promastigote, and then against amastigote stages of *L. donovani* and *L. aethiopica* isolates. Of the 400, 35 compounds showed >50% inhibition on *L. donovani* promastigotes, while 28 showed similar inhibition on *L. aethiopica* promastigotes in the initial screen (1 µM). Out of these compounds, sixteen (11 against *L. aethiopica* and 9 against *L. donovani*) showed >70% inhibition with IC_{50} ranges from 0.01 to 0.555 µM on anti-promastigote assay and 0.05 to 0.7 µM on

DOI: 10.1002/eji.201970400

intracellular amastigote assay. Four compounds showed >70% inhibition on both species of the parasite. Identified compounds demonstrated good safety on THP-1 cell lines and sheep RBCs, and possessed good physico-chemical property suitable for further drug development. Two compounds (MMV690102 and MMV688262)were identified as lead compounds. From which the anti-TB agent MMV688262(delamanid) showed synergistic effect with Amphoterecin B, indicating the prospect of this compound for combination therapy. These results indicate the presence of additional hits which may hold promise as starting points for anti-leishmanial drug discovery and in-depth structure activity relation studies. Future works should investigate antiamastigotes activity of remaining 'hits', which were not covered in the present study.

P2329

Inonotus obliquus polysaccharide regulates the polarization of CD4$^+$ T cells in vitro

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Inonotus obliquus polysaccharide (IOP), an edible fungal polysaccharide, has immuno-regulatory and anti-inflammatory activity in variety inflammatory diseases, which mechanism may associate with regulation the differentiation of CD4$^+$ T cell subsets. CD4$^+$ T cells regulate humoral and cellular immunity by polarization into specific subsets and secrete of multiple specific cytokines, and participate in the development and regression of inflammation. In present study, we explored whether IOP (10 µg/ml, 20 µg/ml, 40 µg/ml) directly regulates the polarization process of specific CD4$^+$ T cells in vitro. Flow cytometry analysis suggested that IOP can inhibit the activation of CD4$^+$ T cells with detect surface marker CD25 and CD69, and inhibit proliferation of CD4$^+$ T cells with CFSE stain. ELISA and RT-PCR analysis showed that IOP down-regulation the expression of Th1 related transcription factor T-bet and inflammatory cytokine IFN-γ, and up-regulation the expression of Th2 related transcription factor GATA3 and anti-inflammatory factor IL-4. The expression levels of transcription factor ROR-γt and pro-inflammatory factor IL-17 were decreased, and the expression levels of Treg-associated transcription factor Foxp3 and anti-inflammatory factor IL-10 were increased. Flow cytometry was used to detect the differentiation of CD4$^+$ T cell subsets. Results suggested that IOP significantly inhibited the differentiation of CD4$^+$IFN-γ$^+$ T cells and CD4$^+$IL-17$^+$ T cells and promoted the differentiation of CD4$^+$CD25$^+$Foxp3$^+$ T cells. The collective results indicated that IOP regulates CD4$^+$ T cells polarization directly, and aid in further clarifying the mechanism underlying the anti-inflammatory and immuno-regulatory effect of IOP.

P2330

Immunogenicity assessment for 3 anti-TNFα biotherapies (infliximab, adalimumab and etanercept) in 42 chronic inflammatory disease patients

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Introduction: Chronic inflammatory diseases therapy has been revolutionized since the introduction of Anti-TNFα drugs (Infliximab, Adalimumab and Etanercept). However, as most genetically engineered proteins, they are immunogenic, inducing the production of anti-drug antibodies (ADA) that lead to drug failure.
The aim of our study is to assess the immunogenicity of these 3 anti-TNFα therapies.
Materials and methods: Our study involved 42 patients (19 females & 23 males, age 38±13 yrs,

DOI: 10.1002/eji.201970400

disease duration 10±6 yrs) with established Rheumatoid arthritis, Crohn disease or ankylosing spondylitis and presenting a primary/secondary therapeutic failure to anti-TNF-α. Both drug and ADA levels were measured in patients' sera using ELISA test [Promonitor® Progenika Biopharma SA, Spain].
Results: The percentage of patients with ADA is 29% (ETN 0%, ADL 32% and IFX 36%). The presence of ADA is associated with drug levels below therapeutic range (>80%). females (58%) and older patients (60%) are most likely to produce ADAs. Moreover, ADAs develops after a fewer number of injections in IFX patients vs ADL and in females vs males. However, the association of Methotrexate (MTX) to the anti-TNFα decreases significantly its immunogenicity. The ADA frequency varies with the underlying disease but no significant influence of a previous biotherapy switch has been demonstrated in our study.
Conclusion: Drug failure to anti-TNF-α therapies is due to ADA production in 29%. This immunogenicity is influenced by numerous factors that are either drug related (structure; chimeric (IFX) or humanized (ADL), association to an immunosuppressant agent) or patient related (gender, age and the underlying disease).

P2331

Defective localization with impaired tumor cytotoxicity contributes to the immune escape of NK cells in pancreatic cancer patients

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Tumor infiltrating lymphocytes (TILs), found in patients with advanced pancreatic ductal adenocarcinoma (PDAC), are shown to correlate with overall survival (OS) rate. Although majority of TILs consist of CD8+/CD4+ T cells, the presence of NK cells and their role in the pathogenesis of PDAC remains elusive. We performed comprehensive analyses of TIL, PBMC, and autologous tumor cells from 80 enrolled resectable PDAC patients to comprehend the NK cell defects within PDAC. Extremely low frequencies of NK cells (< 0.5 %) were found within PDAC tumors, which was attributable not to the low expression of tumor chemokines, but to the lack of chemokine receptor, CXCR2. Forced expression of CXCR2 in patients' NK cells rendered them capable of trafficking into PDAC. Furthermore, NK cells exhibited impaired cell-mediated killing of autologous PDAC cells, primarily due to insufficient ligation of NKG2D and DNAM-1, and failed to proliferate within the hypoxic tumor microenvironment. Importantly, these defects could be overcome by ex vivo stimulation of NK cells from such patients. Importantly, when the proliferative capacity of NK cells in vitro was used to stratify patients on the basis of cell expansion, patients whose NK cells proliferated < 250-fold experienced significantly lower DFS and OS than those with ≥ 250-fold. Ex vivo activation of NK cells restored tumor trafficking and reactivity, hence provided a therapeutic modality while their fold expansion could be a potentially significant prognostic indicator of OS and DFS in such patients.

DOI: 10.1002/eji.201970400

P2332

Optimizing frog-derived antimicrobial peptides for use as antivirals

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Influenza infections are responsible for much of the annual disease and economic burden worldwide, occurring both as seasonal epidemics and sporadic pandemics. Each year, illnesses worldwide number in the millions with 250-500,000 deaths, so reliable anti-influenza methods are important. The first defense against influenza is vaccination, however in pandemics or when the predicted vaccine strains are mismatched, antiviral drugs become the next option. As resistance to conventional antiviral agents can occur, the development of novel anti-viral compounds is necessary to continue to treat influenza infections. Antimicrobial peptides (AMPs) are one large potential pool of therapeutics, as they are produced innately by most forms of life and often have broad activities. We have synthesized a frog-AMP-derived peptide, Yodha, which is an effective inhibitor of various H1N1 and H3N2 influenza viruses in *in vitro* focus-forming assays. The peptide appears to form large conglomerates of fibrils in solution that can be imaged with electron microscopy, however, which is a hindrance to in vivo delivery. We have designed and screened several panels of modified peptides based on Yodha's primary structure to examine the structure-function relationship of the antiviral activity. From these screens, we have determined that polymerization is not required for anti-viral activity and several modified peptides have been found to out-perform wildtype Yodha and are primary candidates for the development of a deliverable drug for seasonal and pandemic subtypes of influenza.

LB118

Inhibition of AIM2 inflammasome-mediated pyroptosis by Andrographolide contributes to amelioration of radiation-induced lung inflammation and fibrosis

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Radiation-induced lung injury (RILI) is one of the most common and fatal complications of thoracic radiotherapy, whereas no effective interventions are available. Andrographolide, an active component extracted from *Andrographis paniculate*, is prescribed as a treatment for upper respiratory tract infection. Here, we report the potential radioprotective effect and mechanism of Andrographolide on RILI. C57BL/6 mice were exposed to 18 Gy of whole thorax irradiation, followed by intraperitoneal injection of Andrographolide every other day for 4 weeks. Andrographolide significantly ameliorated radiation-induced lung tissue damage, inflammatory cell infiltration, and pro-inflammatory cytokine release in the early phase and progressive fibrosis in the late phase. Moreover, Andrographolide markedly hampered radiation-induced activation of the AIM2 inflammasome and pyroptosis *in vivo*. Furthermore, bone marrow-derived macrophages (BMDMs) were exposed to 8 Gy of X-ray radiation *in vitro* and Andrographolide significantly inhibited AIM2 inflammasome mediated-pyroptosis in BMDMs. Mechanistically, Andrographolide effectively prevented AIM2 from translocating into the nucleus to sense DNA damage induced by radiation or chemotherapeutic agents in BMDMs. Taken together, Andrographolide ameliorates RILI by suppressing AIM2 inflammasome mediated-pyroptosis in macrophage, identifying Andrographolide as a novel potential protective agent for RILI.

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DOI: 10.1002/eji.201970400

LB125

Lymphoid interstitial pneumonia in common variable immune deficiency - case report with disease monitoring in various therapeutic options. Pleiotropic effects of rituximab regimens

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LIP in common variable immune deficiency (CVID) was observed in a patient during immunomodulatory therapy after progression of the disease (i.e. glucocorticoids, immunoglobulin dose escalation and finally rituximab). Due to humoral immunodeficiency and serious serum sickness rituximab was used initially at a low dose (150 mg/m2 weekly). It resulted in temporary remission-increase leading parameters: FVC, paraproteinemia and splenomegaly normalization was observed. Owing to the relapse after six-month remission in the second cycle a standard dose of rituximab was used (375mg/m2). Therapeutic regimen with 375 mg/m2 of Rtx in optimal schedule (every 3 weeks) resulted in no longer remission but higher incidence of opportunistic infections. In laboratory and immunological progress the increase of NK and NKT cells was observed after the initial dose but the standard one caused NK cell increase only. Unfortunately, the decrease of CD19+Bcells was comparable between both doses, as was the decline of FoxP3+ regulatory T cell. Rtx (especially in low dose) brought further increase of persistent T cell activation (CD38+ T cells made up 79%). Innate immune response and the decrease of Treg are a compensatory pathways for the decrease of B and T cells. Significant microbiome pressure under influence of infectious process, the immunedysregulation as a hallmark immunodeficiency indicate the risk of complications after newly introduced cancer immunotherapies. It makes us stay vigilant and act in non-standard manner beyond clinical trials and large cohort in clinical oncology.
Thus CVID as well as other immunodeficiency requires a different clinical and investigative approach to a cancer immunotherapy.

P3180

Construction of a novel staphylokinase (SAK) mutant with low immunogenicity and evaluation of the dynamic changes of its antibody in rhesus monkey

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The heterologous nature of SAK, which is a throsmbolytic drug, elicits high titers of neutralizing antibodies, which limits its use. Here, we aimed to establish a SAK mutant with equivalent activity to the wild type but reduced antibody reactivity, which would allow for multiple injections. Biosun software was used to predict SAK antigenic epitopes, and several main epitopes were modified by gene deletion and mutation. Ten SAK mutants were constructed, and their thrombolytic activity and immunogenicity were analyzed in vitro. Then the dynamic changes of antibodies after repeated injections of SAK mutant and SAK in the rhesus monkey were examined. SAK6, with a high expression level (45%), similar thrombolysis activity, and lower antibody reaction, was chosen for in vivo analysis. In the 9-month experimental period, the antibody level of the SAK6 group was significantly lower than that of the SAK group. Moreover, only 5% of SAK activity was retained, whereas 75.6% of SAK6 activity was retained after incubating with the respective antiserum. Overall, these results demonstrate that SAK6, as a novel SAK mutant, had identical thrombolysis activity to

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DOI: 10.1002/eji.201970400

SAK, low immunogenicity, and good safety, demonstrating its great clinical potential for thrombus disease.

P3181

Fusion SAK-HV protein attenuates atherosclerosis in *Apoe*$^{-/-}$ mice via an anti-inflammatory mechanism mediated by PAR1 and GPIIb/IIIa

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Objective: Atherosclerosis (AS) is the primary cause of acute coronary syndrome (ACS) caused by atherosclerotic lesion rupture, erosion, or calcified nodules. Inflammation has been reported to be involved in the development of AS. We examined anti-AS effects and the anti-inflammatory effect of the fusion SAK-HV protein that harbors three structural parts associated with anticoagulation, antiplatelet, and thrombolysis activities, namely mutant staphylokinase, dodecapeptide of hirudin, and an RGD peptide.
Approach and Results: The effects of SAK-HV on AS were investigated in *Apoe*$^{-/-}$ mice and in quails fed with high-fat diet. SAK-HV decreased hypercoagulability and inhibited platelet activation. Furthermore, it reduced plaque size, intima-media thickness, macrophage infiltration, and smooth muscle cell migration without increasing plaque destabilization. SAK-HV down-regulated expression levels of inflammatory markers in *Apoe*$^{-/-}$ mouse aorta by inhibiting PI3K/Akt and NF-κB activation mediated by protease-activated receptor 1 (PAR1). Furthermore, serum levels of oxidative stress factors and lipids were lower in mice treated with SAK-HV. SAK-HV extended the activated partial thromboplastin time and decreased the fibrinogen value in mice, which indicated that SAK-HV had an anticoagulatory effect. In addition, SAK-HV inhibited platelet activation in mice and platelet aggregation induced by thrombin and ADP *in vitro*. Moreover, SAK-HV down-regulated thrombin-induced signaling pathways and their downstream inflammatory genes, and impaired monocyte adhesion on thrombin-activated vascular endothelial cells.
Conclusions: SAK-HV attenuated AS development by constraining the expression of inflammatory markers mediated by PAR1 and GPIIb/IIIa receptors, which may be the novel mechanism of action for drugs against ACS.

P3182

MEK inhibitor-selumetinib as an immunosuppressor in Th17-cell-mediated immunopathy

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Selumetinib, a highly potent MEK inhibitor, is under investigation for the treatment of carcinoma, thymic cancer , non-Small cell lung cancer, melanoma, breast cancer, small cell lung cancer, among others. However, because of the complexity of MEK signaling pathway, inhibition of MEK will cause many anti-tumor irrelevant effects. Later clinical trials found its poor efficacy in tumor therapy, so the drug was announced failure at phase III clinical trial for anti-tumor therapy. The side effects experienced by patients taking selumetinib include rash, edema, and vision changes. So we focused on how to avoid drug side effects and discovery new therapeutic indications, new use of old drugs. Herein, our research found its anti-inflammatory effect, inhibiting Th17 producing IL-17. In our study, IL-17-GFP reporter mice were used for drug efficacy test by evaluating the production of IL-17. Our results showed selumetinib inhibited IL-17 producing in a dose-dependent manner with an IC_{50} of 2μM. Then we stimulated the human PBMC with this compound, also showed a good inhibitory effect

DOI: 10.1002/eji.201970400

on IL-17. Further study found the selumetinib executed inflammation suppression roles through Erk/Stat3/RORγt pathway. We are trying to investigate the therapeutic effect of selumetinib in a mouse model of IMQ-induced psoriasis and experimental autoimmune encephalomyelitis (EAE) mouse model, Th17-cell-mediated immunopathy. Our research defined a novel therapeutic effect that MEK/ERK inhibitors as therapeutic agent for inflammatory diseases.

P3183

Ligustrazine ameliorates hypoxia-induced NOD2-inflammatory response in acute kidney injury

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Ligustrazine has been used to alleviate clinical acute kidney injury; however, the underlying molecular mechanisms are poorly understood. To address this, we focused on the activation of nucleotide-binding oligomerization domain containing 2 (NOD2) in this study. We showed that ligustrazine exerts its protective effect on acute kidney injury in vivo by inhibiting the upregulation of NOD2 expression after ischemia-reperfusion injury in rat models. Ligustrazine was also found to significantly inhibit NOD2 upregulation induced by CoCl2 and OGD followed by reoxygenation and to markedly induce autophagy in vitro. Further, the effect of ligustrazine on NOD2 downregulation was partially blocked by inhibiting autophagy. For the first time, these results provide evidence that ligustrazine can inhibit NOD2-mediated inflammation to protect against renal injury, in part through the induction of autophagy. This could help to design and develop new approaches and therapeutic strategies to prevent the deterioration of renal function and for the treatment of acute kidney injury.

P3184

Ursolic acid inhibits LPS-induced NLRP3 Inflammasome activation via suppressing NF-κB pathway and ROS production

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Ursolic acid (UA), a pentacyclic triterpenoid, is used as an anti-inflammatory and anti-cancer agent. NLRP3 inflammasome is a cytosolic multiprotein complex detecting pathogen infections, tissue damage, or metabolic imbalances, leading to maturation and release of several proinflammatory cytokines, including IL-1β. In the present study, we aimed to examine whether UA could activate NLRP3 inflammasome. Cell culture study showed that UA reduced pro-interleukin (IL)-1β protein levels and mature IL-1β secretion in macrophages in response to lipopolysaccharide (LPS) and suppresses LPS-induced NLRP3 inflammasome activation via repressing the NF-κB signal pathway. Moreover UA inhibits the caspase-1 activation and IL-1β production by decreasing intracellular ROS production through suppressing NADPH oxidase activation. UA treatment has good effects on mice liver injury, which lays a foundation for future treatment and prevention of liver injury. In conclusion, the data suggest that UA has anti-inflammatory effects in vitro and in vivo, which will be further developed as novel pharmacological strategies in order to defeat inflammatory diseases.

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DOI: 10.1002/eji.201970400

P3185

IgD and IgDR signals pathway in the regulation of T cell activity

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Immunoglobulin D (IgD), which remains a rather enigmatic player in immune system, can be expressed as membrane IgD (mIgD) representing one of B cell receptors with an attached signaling machinery or can be secreted as an antibody (sIgD). sIgD has been found to be over-expressed in several diseases, such as IgD myeloma, leukemia, tuberculosis, hyper IgD syndromes and autoimmune diseases. Blocking the binding between IgD and IgDR may become a new strategy in the treatment of autoimmune diseases and hematological diseases. Targeting IgD and IgDR, our group firstly synthesized a fusion protein called IgD-Fc-Ig through connecting IgD-Fc and IgG-Fc domains, and this fusion protein aims to competitively inhibit the binding of IgD and IgDR. *In vitro* study, IgD-Fc-Ig per se did not show a significant effect on the proliferation of $CD4^+$T cells, Jurkat and MOLT-4 cell lines but specifically inhibited the IgD-induced pro-proliferation and anti-apoptosis effect via IgD/IgDR/mTORC2/Akt/EGR1 pathway. In the peripheral blood from RA patients, IgD-Fc-Ig could clearly down-regulate IgD-induced proliferation and activation of T cells, and restore IgD-induced imbalance of Th17/Treg cell subsets. In collagen-induced arthritis mouse, IgD-Fc-Ig treatment significantly reduced the severity of established arthritis and histopathological changes. IgD-Fc-Ig could also attenuate the related clinical manifestation due to its inhibitory effect on T cell activation and restore Th17/Treg imbalance. With a particular emphasis on the novel T cell-targeted therapeutic strategies, our work highlights the immunologic features of IgD/IgDR and a novel direction of discovery and development of new drugs for the treatment of T cell related diseases.

P3186

Immunotherapy strategy targeting Nogo-66 and Amino-Nogo-A for spinal cord injury in rats

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Nogo-A, as an important myelin-associated inhibitors (MAIs) in the microenvironment of the injured site inhibits axon regeneration after spinal cord injury (SCI). Therefore, intervening inhibition signal mediated by Nogo-A may be one of the effective methods for promoting neural axon regeneration in the injured spinal cord. Nogo-66 and Amino-Nogo-A, two mainly functional domains of Nogo-A, have proven to play a critical role in inhibiting axon regeneration in the injured spinal cord. Based on the concept of protective autoimmunity, vaccine approaches could induce the production of antibodies against inhibitors in myelin to block the inhibitory effects and promote functional recovery in SCI models. Therefore, the choice and modification of self-antigens, and co-regulating Nogo-66 and Amino-Nogo-A targets may be more effective in repairing the injured spinal cord and improving functional recovery. To test this hypothesis, Nogo-66 and Amino-Nogo-A were selected to construct a granulocyte-macrophage colony stimulating factor-Nogo (GMCSF-Nogo) nucleic acid vaccine, and the efficacy of this immunotherapy was investigated in spinal cord-injured rats. The results showed that this vaccination could stimulate the production of antibodies against Nogo-A, promotes nerve regeneration and functional recovery after SCI. These findings suggest that nucleic acid vaccination against Nogo-A may be a promising therapeutic strategy for SCI and other central nervous system (CNS) injuries, which may pave a way to the further research of CNS regeneration on the target of Nogo-A.

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DOI: 10.1002/eji.201970400

P3187

Sodium butyrate alleviates LPS-induced acute lung injury in mice via inhibiting HMGB1 release

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Sodium butyrate (SB) is a short chain 4-carbon fatty acid salt naturally exists in animal fats. Previous studies have proven that sodium butyrate has many beneficial functions such as anti-tumor and anti-inflammatory actions. In the current study we investigated the effect and possible mechanism of sodium butyrate in LPS-induced acute lung injury (ALI). ALI was induced by intratracheal administration of LPS (10 mg/kg) in male BALB/c mice. Sodium butyrate (500 mg/kg) was administered intraperitoneally 30 min prior to LPS exposure. We found that sodium butyrate significantly protected animals from LPS-induced ALI as evidenced by decreased the lung wet to dry weight ratio, total cells, neutrophils, macrophages, myeloperoxidase (MPO) activity, and lung histological damage compared to vehicle control. Sodium butyrate pretreatment markedly inhibited the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). Furthermore, sodium butyrate pretreatment dramatically suppressed HMGB1 release and NF-kappa B activation. Together, these results suggest that sodium butyrate pretreatment protects mice from LPS-induced acute lung injury, possibly through the modulation of HMGB1 and inflammatory responses.

P3188

Characterization of HLA-A2-MART1$_{27-35}$ peptide complex-specific chimeric scFv-Ig antibody

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Recognition of tumor cells by HLA class I antigen restricted, tumor antigen (TA)-specific cytotoxic T lymphocytes (CTL) is mediated by the HLA class I antigen-TA derived peptide complex,which has emphasized the need to develop antibody probes which monitor HLA class I antigen-TA derived peptide complex expression on tumor cells. MART1 (melanoma antigen recognized by T cells 1) is an intracellular transmembrane protein expressed in melanocyte and over expressed in most malignant melanomas. The MART1$_{27-35}$ peptide is recognized by CD8+ tumor infiltrating T lymphocytes from HLA-A*0201 melanoma patients. We have stably transfected a novel scFv 8.3.21-pXIg construct into myeloma cells P3X63 Ag8 and purified the chimeric antibody scFv 8.3.21-Ig (also called P2F9), which was single chain Fv fragment (scFv 8.3) specific to HLA-A2-MART1$_{27-35}$ peptide complex linked to murine Ig γ1 heavy chain with enhanced avidity and stability. In the present study, we have characterized the reactivity and specificity of the antibody P2F9 to target antigen on cells. The antibody displayed specific reactivity with T2 cells pulsed with MART1$_{27-35}$ peptide and also stained melanoma cell lines (Mel 624,1520) which express both HLA-A2 and MART1 molecules, but did not stain melanoma cell lines which do not express HLA-A2 (Mel 1011, Colo38) or MART1 molecules (MV3, Colo38). However the antibody stained HLA-A2+/MART1- tumor cells (MV3) which had been pulsed with MART1$_{27-35}$ peptide, following elution of endogenous peptide with low pH. These results suggest that the scFv 8.3.21-Ig could monitor the level of expression of HLA-A*0201-MART1$_{27-35}$ peptide complex on melanoma cells.

DOI: 10.1002/eji.201970400

P3189

JSI-124 may induces apoptosis of leukaemia cells independent of JAK2/STAT3 pathway and compromise immune response

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Objectives: Evaluate the effects of JSI-124 on leukemia cells and explore whether the expression of STAT3 and phospho-STAT3 (pSTAT3) in tumor cells is related to their sensitivity to JSI-124, and further assess the effect of JSI-124 on the immune system.
Methods: We examined the effects of JSI-124 on five leukemia cell lines. Dose and time-dependent cytotoxicity were assessed by flow cytometry using Annexin-V/7AAD staining. Target proteins were detected by western blot and flow cytometry. The effects of JSI-124 on the immune system were assessed *in vitro* by CFSE labelling for cell proliferation, ELISA for cytokine production and Annexin-V/7AAD staining for apoptosis of splenocytes and *in vivo* by skin transplantation and BrdU assay in mice model.
Results: JSI-124 inhibited growth and was cytotoxic to both leukemia and immune cells in a time and dose dependent manner. Cytotoxicity was higher in TPH-1 and HL-60 than in KG-1a, HEL and K562 cells. HEL cells expressed the most prominent levels of JAK2 and STAT3 proteins. HL-60 showed the lowest constitutive pSTAT3 expression, implying that the sensitivity did not relate to pSTAT3 expression. JSI-124 alleviated allo-skin graft rejection and angiogenesis within the graft, inhibited BrdU incorporation and reduced double positive cells and thymus size.
Conclusion: JSI-124 inhibits proliferation and induces apoptosis in leukemia cells with variable sensitivities and such effects may be independent of pSTAT3 expression levels; at an effective dosage, JSI-124 may compromise immune function. Moreover, JSI-124 may not be suitable for an individual who is undergoing surgery or having a severe wound.

P3190

WP1066 supresses the growth of pancreatic cancer cells and modulates the tumor microenviroment

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Pancreatic cancer is the high fatal cancer with poor prognosis. It has been reported that JAK2-STAT3 which is an important signaling pathway mediating immunosuppression in tumor microenvironment is abnormally activated in animal models of pancreatic cancer. WP1066 as a novel inhibitor of JAK2 and STAT3 shows a positive effect on some solid tumors. To explore the effect of WP1066 on pancreatic cancer, the pancreatic cancer cell lines PANC-1 and PAN02 and C57BL/6 mouse isograft pancreatic tumor were hired. Our study demonstrated that WP1066 inhibited cell survival and proliferation in a dose-dependent manner. It prevented the phosphorylation of JAK2/STAT3 to regulate downstream signaling molecules, and induced apoptosis of PANC-1 and PAN02 cells. Meanwhile, WP1066 blocked the cell cycle of PANC-1 PAN02 cells in G2/G1. Interestingly, we also found the expression of MHC on the PANC-1 PAN02 cells treated by WP1066 was increased both in vitro and in vivo. Further study in vivo suggested that WP1066 could potently increase the ratio of $CD8^+$ T, NK, NKT cells, and reduce frequency of Tregs in the isograft pancreatic tumor. As a result, the tumor growth was suppressed significantly owning to the enhanced tumor immunity. Improtantly our experiments data

DOI: 10.1002/eji.201970400

showed that WP1066 obviously increased the cell counts of TECs and ETPs in thymus with increasing of naïve T cells migrated to spleen recently. Taken together, our findings indicate that WP1066 effectively supresses the growth and further modulating the tumor microenviroment. WP1066 can be a potential candidate of small molecule therapeutics for pancreatic cancer.

P3191

Epigenetic drugs enhance therapeutic efficacy of immunogenic chemotherapy through modulating T cell chemokines expression

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Certain chemotherapy drugs such as oxaliplatin can reinstate anticancer immunosurveillance through inducing tumor immunogenic cell death (ICD), which is critical to achieve long-term therapeutic efficiency. Here, using syngeneic ovarian and colorectal cancer mouse model, we show that low-does DNA methyltransferase inhibitor 5-azacitidine (AZA) and/or H3K27me3 epigenetic modifier EZH2 inhibitor GSK126 can enhance the therapeutic efficiency of oxaliplatin in immune-competent mice, but not in immune-deficient mice. The combination of chemotherapy with epigenetic drugs has led to enhanced accumulation of CD8 cytotoxic T lymphocyte cells and NK cells in ascites and tumor beds. Mechanismly, low-does epigenetic drugs treatment can directly enhance oxaliplatin induced T helper 1 (Th1)-type chemokines CXCL9 and CXCL10 production, and indirectly through increasing the expression of type-1 interferons of tumor cells. Finally, *in vivo* blockade type-1 interferons receptor or CXCL9/CXCL10 receptor CXCR3, abolished the improved effect of epigenetic drugs to chemotherapy. These findings indicate that selective low-does epigenetic drugs combination may enhance the clinical efficacy of chemotherapy by altering tumor immune cells infiltration.

P3192

Ginsenoside metabolite compound-K inhibits macrophage activation through downregulating β-arrestin2 in mice with collagen-induced arthritis

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Studies have shown that ginsenoside metabolite compound-K (C-K), which is an active metabolite of ginsenoside *in vivo*, inhibits the abnormal activation of immune cells including macrophage in immunological diseases, however, the potential mechanism is currently unclear. In present work, we treated collagen-induced arthritis (CIA) mice with C-K (112 mg/kg/day) and detected a reduced proportion of type I phenotypic macrophages (M1) and an inhibited phagocytosis of macrophage comparing with model mice as expected. Of note, C-K restrained the overexpression of β-arrestin2, Gαi, TLR4 and overactive NF-κB signaling in macrophages of CIA mice, while increased the expression of Gαs. Furthermore, we revealed that C-K promoted TLR4-Gαs coupling and prohibited TLR4-Gαi coupling through downregulating β-arrestin2 expression in macrophages, and finally decreased the proportion of M1 to M2 macrophages and improved clinical outcomes in CIA mice. These data reveals a correlation between TLR4 and G protein in the activation process of macrophage, moreover, β-arrestin2 induces biased coupling of TLR4 to Gαi contributes to the pathological function of macrophage in CIA model. C-K exerts its anti-inflammatory effects partly through inhibiting β-arrestin2 expression and promoting TLR4-Gαs coupling in macrophages.

DOI: 10.1002/eji.201970400

Receptor triggered biased signaling may become a promising therapeutic target for autoimmune diseases.

Keywords: collagen-induced arthritis; ginsenoside metabolite compound-K; macrophage; β-arrestin2; toll-like receptor 4.

Fundings: This work was supported by the National Natural Science Foundation of China (81202541, 81330081), and the Anhui Provincial Natural Science Foundation for Distinguished Young Scholars (1808085J28).

P3193

Construction and identification of a truncated tissue factor-coagulation based composite system for selective thrombosis in tumor blood vessels*

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Selectively inducing tumor vascular thrombosis with truncated tissue factor (tTF) delivered by a target ligand is a novel and promising antitumor strategy. In this study, we established the anti-NRP-1 mAb-SA:tTF-B composite system, wherein anti-neuropilin-1 monoclonal antibody (mAb) could locate tTF to the surface of tumor vascular endothelial cells and trigger the subsequent vascular embolization. Streptavidin (SA) and biotin (B) were employed to enhance the thrombogenic activity by virtue of their high binding affinity. MAb was conjugated with SA by a coupling method with water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccimide (sulfo-NHS). Biotinylated tTF (tTF-B) was prepared using a Biotin Labeling Kit after the fusion protein tTF being generated and purified. Confocal microscopy and flow cytometry indicated the anti-NRP-1 mAb-SA (mAb-SA) conjugate retained the targeting activity of mAb. The preservation of biotin-binding capacity of conjugate was confirmed by a competitive ELASA. Factor X-activation analysis revealed the tTF-B retained the procoagulant activity of tTF. Live imaging was applied to assess the biodistribution and tumor-targeting capability of mAb-SA, which yielded promising results. Results of *in vivo* studies demonstrated that this composite system could significantly induce tumor vascular thrombosis and inhibit tumor growth in mice bearing subcutaneous xenografts, and histological changes were scarcely observed in normal organs.

*This work was supported by the National Natural Science Foundation of China (81773770, 81472458), Science and Technology Foundation of Fujian Province, China (2017R1036-3, 2017R1036-5, 2018R1036-1, 2018R1036-3).

P3194

NK cell-mediated anti-leukemia cytotoxicity is enhanced using a NKG2D ligand MICA and anti-CD20 scfv chimeric protein

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Natural killer (NK) cells are important innate cytotoxic lymphocytes that have potential in treatment of leukemia. Engagement of NKG2D receptor on NK cells enhances the target cytotoxicity. To test the binding and signaling regulation of human NKG2D receptor, we constructed a NKG2D-DAP12-GFP reporter cell system. Results showed that the immobilized recombinant MICA is able to engage a

DOI: 10.1002/eji.201970400

clusters the NKG2D receptors, which signals through the adaptor DAP12 to activate NFAT promoter-driven GFP expression in the stable NKG2D-2B4 cell model. Here we produced a fusion protein consisting of the extracellular domain of the NKG2D ligand MICA and the anti-CD20 single-chain variable fragment (scfv). This recombinant protein is capable of binding both NK cells and $CD20^+$ tumor cells. Using a human NKG2D reporter cell system we developed, we showed that this fusion protein could decorate $CD20^+$ tumor cells with MICA extracellular domain and activate NK through NKG2D pathway. We further demonstrated that this protein could specifically induce the ability of a NK cell line (NKL) and primary NK cells to lyse $CD20^+$ leukemia cells. Moreover, we found that in the presence of fusion protein MICA-scfv the target cells with down-regulation of surface HLA class I expression improved NKL-mediated killing . Our results demonstrated that this recombinant protein with ScFv specifically mediate NK cells to lyse leukemia cells but not to HLA-I normal expressing cells may lead to development of a novel strategy for treating leukemia and other tumors.

P3195

The Brd4/c-myc/hTert signal pathway was involved in the differentiation induction of HL-60 cells induced by low concentration As_2O_3 plus VPA

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Differentiation inducers have different mechanisms of action, and effective combined induction of differentiation not only reduces the single dose, reduces toxic side effects, but also increases the degree of cell differentiation. Therefore, the study of the combined effects of two or more differentiation inducers can provide a powerful theoretical and experimental basis for reducing side effects, improving remission rates, and shortening the course of treatment in clinical applications. Therefore, this study intends to use VPA in combination with low-dose As_2O_3 to induce PML/RARα-negative HL60 cell differentiation, and further reveal the molecular mechanism of this synergistic differentiation from the perspective of Brd4/c-myc, and c-myc/hTert. Wright-Gimesa staining, CD11b differentiation antigen expression, and NBT reduction experiments confirmed that the differentiation model was established successfully, and the ability of HL-60 cells to induce differentiation by the two drugs was stronger. The results of ChIP assay showed that Brd4 can play a targeted regulatory role in combination with the c-myc promoter, and c-myc can bind to the promoter of hTert to target and regulate target cell differentiation. In vivo experiments, As_2O_3+VPA significantly inhibited the growth of tumor-bearing cells in vivo and was associated with apoptosis and apoptosis after terminal differentiation. VPA promoted the differentiation of PML/RARα-negative HL60 cells induced by low-dose As_2O_3, and the Brd4/c-myc/hTert signaling pathway was involved in the differentiation of HL60 cells induced by drugs.

P3196

The effect of (DTX)2-VRT on inducing apoptosis of HL60 leukemia cells and approach to its mechanism

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Objective: To select a novel targeted anticancer drug, voramide peptide docetaxel (VRT-DTX), and to target leukemia HL60 cells, focusing on the anti-leukemia cell proliferation of the drug, and analyzing the molecular mechanism of its action.
Methods: The effect of VRT-DTX on the proliferation of HL60 cells was detected by CCK8, and the

DOI: 10.1002/eji.201970400

inhibition rate of cell proliferation was calculated. Cell cycle changes were analyzed by flow cytometry, or changes in apoptosis were measured by Annexin V-PI double staining. RT-PCR was used to detect the target gene changes. Western Blot assay was used to detect the expression of bcl-2 and bax protein in liver cancer cells.
Results: VRT-DTX inhibited the proliferation of HL60, and the inhibitory effect was enhanced with the increase of drug concentration and duration of action. The expression of G0/G1 phase is prolonged, S phase is shortened, cell cycle is arrested in G0/G1 phase, and with the increase of VRT-DTX concentration, cell cycle arrest is more obvious; VRT-DTX induces apoptosis of HL60, and apoptosis rate increases as the concentration of action increases. After VRT-DTX treatment, the expression of Bcl-2 protein was decreased, while the expression of Bax was increased, and the change was more significant with the prolongation of action time. The ratio of Bax/Bcl-2 was increased, and Cleaved Caspase-3 and Cleaved Caspase-9 liter were induced.
Conclusion: VRT-DTX inhibited the proliferation of HL60, arrested it in the G0 /G1 phase, and showed significant apoptosis. After apoptosis occurs, the Bax/Bcl-2 ratio increases.

P3197

PADI/P53 signaling pathway was involved in the proliferation inhibition of SMMC-7721 cells

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Objective: To investigate the molecular mechanism of the target gene regulated by extracting objects based on the changes of biological indexes before and after treatment with Chinese herbal medicine Xiangsha Liujunzi prescription.
Methods: Immunohistochemical detection of P53 in gastric cancer tissues; qRT-PCR was used to detect gene expression in gastric cancer cell lines; CCK-8 was used to detect cell proliferation; cell cycle and apoptosis were measured by flow cytometry; Western blot was used to detect the expression level of related proteins.
Results: Immunohistochemistry showed the expression level of P53 was increased in 3 patients with gastric cancer after typical treatment. In vitro, Xiangsha Liujunzi have a value-inhibiting effect on gastric cancer SMMC-7721 cells in a time- and concentration-dependent manner. The extract of Xiangsha Liujunzi prescription could induced apoptosis of SMMC-7721 cells, PADI4 gene expression was down-regulated and P53 gene expression was up-regulated, and PADI4 affect the expression of P53. The expression of Bcl-2 gene and protein was decreased in SMMC-7721 cells induced, while Bax gene and protein expression was increased, and the ratio of Bcl-2/Bax decreased Caspase-3 and Caspase-9 activation.
Conclusion: P53 can be used as a therapeutic index for the treatment of gastric cancer with Xiangsha Liujunzi prescription. The extract of Chinese herbal medicine Xiangsha Liujunzi can reduce the apoptosis of gastric cancer SMMC-7721 cells by regulating the expression of P53, and it is related to the decrease of Bcl-2/Bax ratio and the activation of Caspase-3 and Caspase-9.

P3198

miR-338-3p/EVI1/Smad3 was involved in the differentiation induction of K562 cells induced by PMA

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Objective: To use the TGFβ/SMAD signaling pathway as an entry point for research, focusing on the upstream and downstream genes involved in the expression of EVI1 after phorbol ester (PMA)-induced leukemia cells, and their interactions.

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DOI: 10.1002/eji.201970400

Methods: The CCK8 was used to determine the value of target cells, and the optimal concentration of phorbol ester (PMA) was selected. A PMA-induced K562 cell model was established, and the cells morphological changes were observed by Swiss-Gemsa staining. Flow cytometry was used to detect changes in cell cycle before and after induction. RT-PCR was used to detect the changes of EVI1 and SMAD3 genes expression. Western blotting was used to detect protein expression of EVI1 and SMAD3. Chip analysis and software predicted EVI1 upstream miRNA, dual fluorescein reporter gene to detect its targeted binding.
Results: Based on the previous CCK8 experiment to determine the IC50 of about 1 ng/ml, the PMA-induced K562 cell model was established with 1 ng/ml PMA. After 72 hours of induction, the tendency from leukemia cells to differentiation of mononuclear-macrophage was enhanced. The expression changes of the EVI1 and SMAD3 genes were decreased and increased, respectively. Western blotting revealed that the protein expression patterns of EVI1 and SMAD3 were consistent with the above gene expression results. miR-338-3p can target EVI1 and has promoter regulation.
Conclusion: miR-338-3p can target EVI1 and regulate the expression of downstream SMAD3. Reduced Evi1 restores the differentiation of Smad3, thereby regulating the differentiation of leukemia cells into monocytes/macrophages.

P3199

The enhancement of DTX-VRT by TSA on inducing apoptosis of hepatoma cells and approach to its mechanism

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Trichostatin A (TSA) is an oxindole HDAC inhibitor that inhibits the activity of histone deacetylases, restores acetylation to histones, activates gene expression, and inhibits cell proliferation. Vapreotide (VRT) is one of the chemically-engineered highly-targeted peptides, with stronger STTR affinity and stability. Therefore,the novel targeted anti-cancer drugs VRT-DTX and HDAC inhibitor TSA were selected as candidate drugs, and liver cancer cells were used as target cells. Based on the demonstration of their individual effects, the two drugs were mainly investigated. The synergistic effect between the anti- proliferation of hepatoma cells and its molecular mechanism, which provides new solutions for the treatment of liver cancer. The deacetylase transferase inhibitor TSA+VRT-DTX has the synergistic effect of inhibiting the proliferation of hepatocellular carcinoma cell line BEL-7402, and is an important drug combination system for the anti-hepatoma cell proliferation. TSA significantly inhibited the growth of hepatocellular carcinoma cell line BEL-7402, blocked hepatoma cell line BEL-7402 at G0/G1 phase, and showed significant cell apoptosis. After apoptosis occurred, the ratio of Bax/Bcl-2 increased. VRT-DTX can also induce apoptosis in hepatoma cells and is associated with apoptosis induction. Compared with the effects of the above two drugs alone, they can produce significant synergistic anti-hepatoma cell proliferation and are associated with activation of Cleaved Casapase-3 and -9 induced by an increase in the ratio of Bax/Bcl-2.

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DOI: 10.1002/eji.201970400

P3200

The novel dual effects of oridonin on relieving GVHD response and inhibiting growth of leukemia by inducing apoptosis of lymphocytes and HL60 leukemia cells respectively

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Acute GVHD remains the major complication and cause of death after allogeneic hematopoietic stem cell transplantation. Therefore, it is necessary to develop some new drugs to optimize efficacy and reduce this kind of side effects. Oridonin is one kind of natural organic compounds, which is extracted from Rabdosia rubescens. Demonstrated the role of oridonin in inducing apoptosis of leukemia cells and lymphocyte subsets in vitro experiments, we further detected its dual effects on both anti-GVHD and anti-leukemia cells in vivo. The present results indicated that oridonin not only could obviously inhibit the proliferation of peripheral blood mononuclear cells in vitro, but also could induce obvious apoptosis of all above-mentioned subsets of lymphocytes from peripheral blood, liver or spleen in vitro or in vivo, which contributed to the activation of mitochondrial apoptosis signaling pathway. While there was no obvious cytotoxicity against red blood cells, hemoglobin and platelets. Meanwhile, oridonin could reduce the severity and liver/lung damage of GVHD indicated by in vivo experiment. The apoptosis of HL60 cells was obviously induced by oridonin, VPA or their combination in vitro, and the growth of transplanted tumor of HL60 cells was inhibited by oridonin, VPA or oridonin plus VPA. Taken together, oridonin not only could induce apoptosis of different lymphocyte subsets, which may be taken as one kind of immune modulator to prevent GVHD, but also could do as treatment drugs for leukemia cells by inducing apoptosis of HL60 cells in vitro and in vivo, which was enhanced by HDAC inhibitor VPA.

P3201

Study on molecular regulation mechanism of VEGF in HL-60 cell after induced differentiation

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Vascular endothelial growth factor (VEGF) is known as the most important positive angiogenesis factor, which can stimulate vascular endothelial cell proliferation, strengthen blood vessel´s permeability, and promote the occurrence and metastasis of tumor cells. This project first established cell model of ATRA induced HL-60 cell differentiation, and at the same time VEGF level was decreased. And we proceeded on to screeded from several tumor related transcription factor, and find the upstream key factor that can regulate the expression VEGF. We meant to explore the key molecular mechanism of VEGF expression, which can break a path for leukemia treatment.1μM ATRA treated for 96 hours can obviously restrain HL-60 cell proliferation, and in addition, the cells appeared apparent differentiation tendency under the inverted microscope, which growed like mature granulocyte; The expression of VEGF and c-myc in cytoplasm of HL-60 cell was remarkable decreased .When c-myc gene expression was blocked, VEGF gene expression level decreased obviously; ChIP experiment furtherly verify c-myc is binding onto the promoter of VEGF gene.In the model of ATRA induced HL-60 cell differentiation, VEGF expression level was reduced with the procession of HL-60 cell differentiation, which may be largely correlated with the down regulation of c-myc and STAT3. And additional, c-myc is binding onto the promoter of VEGF gene. Maybe HIF-1α,

DOI: 10.1002/eji.201970400

P53, VHL, COX-2, SP-1 and AP-1 gene act as transcription repressors in the activity of VEGF regulation, and maybe they are largely relevant to cell differentiation.

P3202

Leukemia cell differentiation process of nm - 23 h1 expression changes and molecular regulation mechanism research

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Nm23-H1 was identified as a newtumor metastasis suppressor gene by subtractive hybridization. In order to discussed impacts of differentiation of leukemia cells caused by down-regulation of Nm23-H1 gene and whether there is an interaction between Nm23-H1 and C-myc, G-CSF, We choosed optimal inducing concentration of ATRA and PMA by CCK-8 method; built a ATRA-inducing HL-60 model and PMA-inducing THP-1 model, detected expression changes of CD11b and CD14 by flow Cytometry, verified the correctness of the models by Switzerland Giemsa staining. Gene expression changes of Nm23-H1 and C-myc, G-CSF were detected on the transcriptional and translational level. Changes of G-CSF level in supernatants of ATRA-inducing HL-60 in differentiation process were measured by ELISA. PMA inhibited THP-1 cells proliferation, and TUP-1 emerged the trend of differentation to mature mononuclear-macrophage in vitro experiments, thus illustrated stronger inducing effect making THP-1 differentiation. HL-60 had the trend of being differentiated into mature granulocyte after ATRA inducing. In the process of differentiations by these two differentiation-inducing agent, Nm23-H1 had decreased in varying degrees. It is illustrated that Nm23-H1 gene may had a crucial role in the differentiation of drug-inducing leukemia cells. Research findings of relationships between Nm23-H1 gene and malignant hematologic diseases were mainly based on leukemic cell lines. It has been identified that high expression of Nm23-H1 is one of the poor prognosis index of malignant hematological tumors, and this positive relationship prompt that Nm23-H1 gene may be the new therapeutic target of leukemia and lymphoma.

P3203

Identification of small molecule metabolites that promote immune cell survival from conditioned medium from non-immune cells

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The high incidence of cancer in China is a serious problem that jeopardizes the health of our nationals and is a top problem that plagues all human beings. After traditional chemoradiotherapy, the patient´s immunity is low, and the quality of life and overall survival rate are not optimistic. This project intends to find and identify new types of immune promoters, and provide a new theoretical basis for the development of drugs that enhance immunity. Compared to traditional cytokine preparations and synthetic drugs, small molecule drugs have some significant advantages, such as lower immunogenicity, more easily resolved structures, longer half-lives, and looser storage conditions. We have long been committed to finding new small factors that promote T cell survival from biological tissue sources. The results indicate that conditioned medium of various non-immune cell lines

DOI: 10.1002/eji.201970400

promotes survival of mouse T cells and B cells. In addition, the heat-treated conditioned medium and the ≤3KD fraction separated by the ultrafiltration concentrating tube still have the effect of promoting the survival of T and B cells, indicating that non-immune cells may secrete a new type of small molecules that promote the survival of immune cells.

P3204

Valproic acid reduces inflammation after spinal cord injury by inhibiting the activation of NF-κB signaling pathway in microglia cells

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Valproic acid (2-propylpentanoic acid, VPA), a drug demonstrated to be effective for the treatment of bipolar disorder and epilepsy, was recently reported to play important roles in improving inflammation. However, the time-dependent activities and the long-term treatment effects of the drug has not been fully elucidated. To examine the anti-inflammatory action of VPA in spinal cord injury (SCI), we performed spinal cord injury model in adult mice and hypoxia-treated primary spinal cord microglia cultures, and compare the time-dependent characteristics and of efficacy VPA. Our results demonstrated proinflammatory cytokine (TNF-a, IL-1b, and IL-6) expression levels significantly increased 3 days after spinal cord injury and decreased after 14 days, accompanied by the increased activation of NF-κB, This increase was reversed by the Valproic acid treatment. Double immunofluorescence labeling showed that the activation of NF-κB was particularly prominent in microglia cells. Immunohistochemistry confirmed that there was no significant change in the number of microglia. However, we confirmed the effect of VPA on TNF-a, IL-1b, and IL-6 expression and NF-κB activation in hypoxia-treated spinal cord microglia. Moreover, the results in hypoxia-treated primary spinal cord microglia confirmed the effect of FK-506 on TNF-a, IL-1b, and IL-6 expression and NF-κB activation. These results suggest that valproic acid may be used as a drug to regulate inflammation in the treatment of spinal cord injury.

P3205

Reprogramming tumor associated macrophage phenotype by a polysaccharide from ilex asprella for sarcoma immunotherapy

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We report here the discovery of an acidic polysaccharide, namely IAPS-2, from the root of Ilex asprella, with anti-tumor activity via a repolarizing tumor associated macrophages (TAMs) phenotype. We obtained IAPS-2 polysaccharide from this herb based on acidity and found that IAPS-2 expressed the activity of promoting the secretion of anti-tumor cytokines in macrophages. Furthermore, we evaluated its anti-tumor effect on TAM cells, through the activation of nuclear factor-κB (NF-κB) and signal transducer and activator of transcription (STAT) signaling. In particular, in the tumor murine model, IAPS-2 demonstrated that it could significantly inhibit the growth of tumors via modulating the function of TAMs and increase the animal survival rate. In summary, IAPS-2, with a clearly illustrated chemical composition, potent anti-tumor activity, and a solid mechanism of action, may be developed into a valuable therapeutic tool for cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P3206

Co-delivery of rifampicin and curcumin by targeted PLGA-PEG nanoparticles to promote anti-tuberculosis responses through activation of alveolar macrophage autophagy

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Tuberculosis (TB) is a major disease burden globally causing more than 1.5 million deaths per year. Systemic administration of anti-tubercular drugs can be complicated by off-target toxicity to cells and tissues that are not infected by Mycobacterium tuberculosis. Autophagy, a lysosomal degradation pathway for cellular constituents and organelles, is an adaptive and essential process required for cellular homeostasis. Autophagy is a newly recognized innate and adaptive immunity defense against intracellular pathogens. To address these issues, we fabricated the novel multifunctional PLGA nanoparticles that could co-deliver rifampicin, a first-line antibiotics for TB and curcumin, one drug for anti-tuberculosis via induction of cell autophagy, to macrophages (Cur-Rif-PLGA NPs). And, with further functional modification such as chitosan, mannose, the as-synthesized nanoparticles could directly target and enter into the Mtb infected macrophages by the process of phagocytosis and localize in the lysosomes. The released drugs from nanopartilces into lysosomes, rifampicin will kill Mtb in macrophages, and curcumin, will induce macrophage autophagy and subsequently kill Mtb in macrophages. This approach provides a new means for controlled and efficient release of Cur using the NPs delivery system and is promising for the treatment of TB.

P3207

Multiple factors affecting the pharmacokinetic and pharmacodynamic processes of tacrolimus in Chinese myasthenia gravis patients

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Myasthenia gravis (MG) is an autoimmune disease mediated by autoimmune antibodies that destabilize the neuromuscular junction. Tacrolimus was recently considered as a novel effective immunosuppressant for MG patients. However, the narrow therapeutic window, and high inter- and intrapatient variation in bioavailability largely limited the clinical application of tacrolimus in MG patients. Based on the finding in Human Genome Project, researchers began to realize that genetic and epigenetic factors may play important roles in affecting tacrolimus-related clinical outcomes. In this article, we found that single nucleotide polymorphisms(SNPs) rs15524 and rs4646440 were predicted to affect tacrolimus serum concentration, while SNPs rs8392 and rs6021183 were predicted to affect clinical outcome. CpG sites, including chr4:102267890, chr18:77153995, chr4:102267995, chr18:77153977 and chr4:103422333 were discovered to be critical in relation to tacrolimus serum concentration, and the clinical outcomes of patients treated with tacrolimus. Based on our findings, we further investigated the interactions between SNPs, and we found that the relationship between rs2235856 and rs4646437 may explain why a SNP variant of NFATC2 could influence the pharmacokinetic process. We hope our study can provide support for tacrolimus dose prediction and set the basis for further research.

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DOI: 10.1002/eji.201970400

P3208

Cathelicidin-related antimicrobial peptide limits gluten-sensitive enteropathy

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Background: Celiac disease (CeD) is a gluten-sensitive enteropathy that develops in genetically susceptible individuals and shares key features with autoimmune diseases. Cathelicidin-related antimicrobial peptide (CRAMP) expressed by epithelial and immune cells exhibits immune modulatory effects in a variety of diseases. In the gut, CRAMP has been largely described for antimicrobial effects against intestinal infections. However, the immunomodulatory role of CRAMP in CeD is still unknown.
Methods: A mouse model of CeD was induced by adoptive transfer of gliadin-presensitized $CD4^{+}CD45RB^{low}CD25^{-}$ T cells into lymphopenic *Rag1*$^{-/-}$ or *Rag1*$^{-/-}$ *CRAMP*$^{-/-}$ mice. Effects of prophylactic or therapeutic CRAMP treatment on CeD development were evaluated. Regulatory effects and mechanisms of CRAMP on gliadin-induced barrier dysfunction and immune dysregulation were evaluated *ex vivo*.
Results: Epithelial CRAMP expression was negatively correlated with CeD development. *Rag1*$^{-/-}$ *CRAMP*$^{-/-}$ mice exhibited aggravated CeD with decreased tight junction protein (TJP) expression and a higher zonulin production. CRAMP treatment markedly improved CeD, accompanied by increased TJP expression and downregulated zonulin expression. *Ex vivo* investigations revealed that CRAMP regulated zonulin production in macrophages and epithelial cells by regulating TLR4-MyD88 signaling and EGFR activation. Additionally, CRAMP downregulated epithelial IL-15 expession, thereby decreasing cytotoxic $NKG2D^{+}$ intraepithelial lymphocytes (IELs), and modulated dendritic cell and macrophage phenotypes exhibiting enhanced ALDH activities to induce regulatory T cells at lamina propria. Finally, gut dysbiosis contributed to decreased epithelial CRAMP production.
Conclusion: Taken together, our results suggest that CRAMP is a key modulatory mediator in CeD and modulation of CRAMP represents a promising therapeutic strategy for CeD.

P3209

Triptolide suppresses the non-small cell lung cancer epithelial mesenchymal transition via the ribosome-MDM2-P53-IL6 pathway

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Triptolide is extracted from Tripterygium wilfordii used in traditional Chinese medicine to treat autoimmune disorders. Triptolide has anticancer effects in vitro and is reported to impair lung cancer cell migration, while the underlying mechanism is elusive. The present study investigated the effects and underlying mechanisms of triptolide in A549 and H1299 lung cancer cells. It was revealed that triptolide led to the ribosome transcription factors UBF and RNA pol I which was mainly located in nucleolus disperse throughout nucleus. Triptolide significantly reduced UBF and RNA pol I binding to the promoter of rRNA gene, interrupted the synthesis of rRNA through inhibition of transcriptional activation of RNA Pol I and UBF. In the case of ribosome synthesis disorder by triptolide, the binding of ribosomal protein L23 to MDM2 was increased and the binding of p53 and to MDM2 was reduced, resulting in stabilization/activation of p53. Increased expression of P53 significantly inhibits IL-6-induced epithelial mesenchymal transition. In conclusion, the results demonstrated that triptolide suppressed lung cancer cells epithelial mesenchymal transition in a ribosome-MDM2-P53-IL6-dependent manner.

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DOI: 10.1002/eji.201970400

P3210

The ameliorative effects of Probiotic V_9 on oxidative stress and lipid metabolism in non-alcoholic fatty liver rats

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Aims: To study the effects and mechanisms of Probiotic Bifidobacterium V_9 on oxidative stress and lipid metabolism in NAFLD rats.
Methods: Wister rats were randomly divided into Control group（Control）、model group（HFD）、Berberine group(HFD+BR)、Probiotics V_9 treatment group(HFD+V_9)and Probiotics V_9 control group (V_9). Except for the Control group and the V_9 group, the other groups were given high-fat diet. After 6 weeks, the high-fat diet was changed into normal diet and probiotics V9（1×109 CFU/ml）was orally administered for 4 weeks, and the positive control Berberine(300 mg/kg)was also orally administered. Serum and liver were collected after 4 weeks.
Results: Compared with the Control group, the levels of AST, ALT, MDA and TC in the HFD group were increased($p< 0.05$), and the expression levels of SOD and SOD2 were decreased($p< 0.05$), the expression levels of PPAR-α and PPAR-γ are inhibited ($P< 0.05$). Compared with the HFD group, the expression levels of ALT、AST、MDA、TC were decreased in Berberine and HFD/V_9 groups ($P< 0.05$), and the expression levels of SOD and SOD2 were increased ($P< 0.05$), the expression levels of PPAR-α and PPAR-γ were significantly increased ($p< 0.05$).
Conclusion: Probiotics V_9 can improve NAFLD in rats by inhibiting oxidative stress and alleviating lipid metabolism disorders.

P3211

Apolipoprotein A-I mimetic peptide 4F suppresses PMN-myeloid-derived suppressor cells in mouse pancreatic cancer

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An Apolipoprotein A-I (ApoA-I) mimetic peptide, named L-4F, was engineered to mimic the anti-inflammatory and antioxidative functionalities of ApoA-I. In this paper, H7 cells were orthotopically implanted in mice, and the mice were treated with L-4F. Then, the development of pancreatic cancer and myeloid-derived suppressor cell (MDSC) infiltration were investigated *in vivo*. After L-4F treatment, the polarization, proliferation and apoptosis of MDSCs were detected *in vitro*. Moreover, we explored the effects of L-4F on the immunosuppressive function of MDSCs *ex vivo*. The results show that L-4F obviously reduced the tumorigenicity of the H7 cells. L-4F suppressed granulocytic myeloid-derived suppressor cell (PMN-MDSC) polarization and inhibited increases in the accumulation of PMN-MDSCs in the mouse spleen and tumor tissue. L-4F weakened the immunosuppressive function of MDSCs, resulting in decreased production of ROS and H_2O_2 by MDSCs, and increased T cell proliferation, IFN-γ secretion, and $CD4^+$ T and $CD8^+$ T cell infiltration into the mouse spleen and pancreatic cancer tissue. Furthermore, L-4F significantly down regulated the STAT3 signaling pathway in PMN-MDSCs. These results indicated that L-4F exerts an effective in anti-tumor and immunomodulatory effect in pancreatic cancer by inhibiting PMN-MDSCs.

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DOI: 10.1002/eji.201970400

P3212

Carboxyamidotriazole combined with IDO-KYN-AhR pathway inhibitors profoundly potentiate cancer immunotherapy

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Cancer immunotherapy has generated significant excitement, predominantly with immune checkpoint inhibitors. Blockade of PD-1 or its ligand with antibodies has brought impressive clinical activity. However, a subset of patients does not respond to biologic therapeutics or severe immune-related adverse events occur in certain cases. Modulation of the immune system with small molecules might bring surprising benefits. The present study highlights two combinations consisting of carboxyamidotriazole (CAI) and an inhibitor of IDO1-Kyn-AhR pathway as well as the complementary mechanisms of the combinations towards the tumor-associated immune modulation. CAI plays a critical role in promoting IFN-γ release from activated T cells that might further strengthen its anti-proliferative and anti-metastasis effect on cancer cells and reducing the transcriptional activity of NFAT2 leading to down-regulated expression of PD-1. However, CAI also increases kynurenine (Kyn) levels in tumor microenvironment facilitating tumor cell immune evasion. Two inhibitors of IDO1-Kyn-AhR pathway were severally combined with CAI, leading to enhanced PD-1 blockade in CD8+ T cells and synergistic anti-cancer capacity in terms of inhibiting tumor growth and prolonging life span of tumor-bearing mice.

P3213

Evaluation of the cytotoxicity of stearoyl gemcitabine-loaded PLGA nanoparticles to Lewis lung cancer cells

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Objective: To investigate the anti-proliferation effect of 4-(N)-stearoyl gemcitabine-loaded poly (lactic-co-glycolic) acid nanoparticles (GemC18-PLGA-NPs) on Lewis lung cancer cells (LLC) *in vitro*.
Methods: Lewis cells were incubated with GemC18-PLGA-NPs, free GemC18, gemcitabine HCl (GemHCl) or GemC18-free blank nanoparticles (PLGA-NPs) respectively and cell viability was determined using an MTT assay after 24h,48h or 72h of incubation. The apoptosis rate after 48 and 72 hours of incubation were measured by flow cytometry.
Results: GemC18-PLGA-NPs, GemC18, and GemHCl all significantly inhibited the growth of LLC cells, but it took a longer time for GemC18-PLGA-NPs than for GemHCl or free GemC18 to inhibit LLC growth to the same extent, likely because it took time for the GemC18 to be released from the GemC18-PLGA-NPs before or after they were taken up by tumor cells.
Conclusion: GemC18-PLGA-NPs have significant anti-proliferation effect on mouse Lewis lung cancer cells *in vitro*.

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DOI: 10.1002/eji.201970400

P3214

The progress of pH-sensitive nanomedicines in tumor chemotherapy

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Nanomedicines have many advantages compared with traditional drugs. Nanomedcines can prolong the action time of drugs and have good pharmacokinetic characteristics. Medicines are released in the microacidic environment, and the EPR effect is used to increase the distribution of nanomedicine in tumors and more easily spread to tumor cells and their vicinity. However, the absorption of nanomaterials in tumor tissues is worse than that of conventional small molecule drugs, and the PEG on the surface of nanomaterials can reduce the phagocytic effect of tumor cells, and the problems of above can be solved by using the micro-acidic tumor microenvironment. Because the acidity of many types of tumor tissue is slightly stronger than that of normal tissue, it is possible to release pH-sensitive nanomedicine directly around tumor tissue. Moreover, in acidic tumor microenvironment, a kind of nanomedicine can be designed target to tumor-associated macrophages (TAMs), thus reducing the uptake of drugs by normal macrophages in mononuclear phagocytic system. PEG-sheddable in tumor tissues can increase the absorption of TAMs to nanomedicine and improve the effect of nanomedicine. So that nanomedicine can be better absorbed and utilized in tumor tissues, thus enhancing the effectiveness of drugs and reducing systemic damage. In recent years, the research on acid-sensitive nanomedicine in the treatment of cancer has made rapid progress. This paper focuses on the role of pH-sensitive nanomedicine in the treatment of tumor chemotherapy in the micro-acidic environment.

P3215

Oral 4-(N)-stearoyl gemcitabine nanoparticles inhibit tumor growth in mouse models

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In spite of recent advances in targeted tumor therapy, systemic chemotherapy with cytotoxic agents remains a vital cancer treatment modality. Gemcitabine is a nucleoside analog commonly used in the treatment of various solid tumors, but an oral gemcitabine dosage form remain unavailable. Previously, we developed the 4-(N)-stearoyl gemcitabine solid lipid nanoparticles (GemC18-SLNs) by incorporating 4-(N)-stearoyl gemcitabine (GemC18), an amide prodrug of gemcitabine, into solid lipid nanoparticles. GemC18-SLNs, when administered intravenously, showed strong antitumor activity against various human and mouse tumors in mouse models. In the study, we defined the plasma pharmacokinetics of gemcitabine when GemC18-SLNs were given orally to healthy mice and evaluated the antitumor activity of GemC18-SLNs when given orally in mouse models of lung cancer. In mice orally gavaged with GemC18-SLNs, plasma gemcitabine concentration followed an absorption phase and then clearance phase, with a T max of ~2 h. The absolute oral bioavailability of gemcitabine in the GemC18-SLNs was ~70% (based on AUC 0-24 h values). In mice with pre-established tumors (i.e. mouse TC-1 or LLC lung cancer cells), oral GemC18-SLNs significantly inhibited the tumor growth and increased mouse survival time, as compared to the molar equivalent dose of gemcitabine hydrochloride or GemC18 in vegetable oil or in Tween 20. Immunohistostaining revealed that oral GemC18-SLNs also have significant antiproliferative, antiangiogenic, and proapoptotic activity in LLC tumors. Formulating a lipophilic amide prodrug of gemcitabine into solid

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DOI: 10.1002/eji.201970400

lipid nanoparticles may represent a viable approach toward developing a safe and efficacious gemcitabine oral dosage form.

P3216

Gemcitabine derivative improved antitumor activity and potential to circumvent gemcitabine resistance

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Gemcitabine (2',2'-difluorodeoxycytidine) is a deoxycytidine analog with antitumor activity against a wide variety of solid tumors such as pancreatic, non-small cell lung, breast, and ovarian cancers, alone or in combination with other chemotherapeutic agents. However, short half-life due to extensive deamination at 4-amino site by action of cytidine deaminase (CDA), side effects and drug resistance limit its application. To improve its antitumor efficacy and/or safety profiles, modification of gemcitabine was explored. The 4-amino groupand/or 5'position were usually modified. The main purpose of the synthesis of gemcitabine derivativemay involved (i) protect the 4-N group from deamination, (ii) deliver more therapeutics to tumorsand/orreduce toxicity, (iii) improve storage stability, (iv) possible apply in deoxycytidine kinase (dCK) deficient tumor cells and (v) overcome nucleoside transporters deficiency-based cancer cell resistance. Remarkably, **coupling of some compounds can significantly increase lipid solubility of gemcitabine, which is potential to circumvent gemcitabine resistance caused by decreased expression of nucleoside transporters**. Interestingly, Sobot and coworkers reported gemcitabine-squalene conjugate (SQ-Gem) encapsulated into nanoparticles, which were shown to be strongly interacted with the low-density lipoproteins (LDLs) in the human blood, and improved pharmacological activity. These prompted us to study the mechanism of gemcitabine derivative more deeply. This review is intended to show the research progress of gemcitabine derivative, with a special emphasis on the application of gemcitabine derivativein overcoming cancer cell resistance to gemcitabine and its increased antitumor activity.

P3217

Curcumin suppressed activation of dendritic cells via JAK/STAT/SOCS signal in mice with experimental colitis

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Dendritic cells (DCs) play a pivotal role as initiators in the pathogenesis of inflammatory bowel disease and are regulated by the JAK/STAT/SOCS signaling pathway. As a potent anti-inflammatory compound, curcumin represents a viable treatment alternative or adjunctive therapy in the management of chronic inflammatory bowel disease (IBD). The mechanism of curcumin treated IBD on DCs is not completely understood. In the present study, we explored the mechanism of curcumin treated experimental colitis by observing activation of DCs via JAK/STAT/SOCS signaling pathway in colitis mice. Experimental colitis was induced by 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). After 7 days treatment with curcumin, its therapeutic effect was verified by decreased colonic weight, histological scores and remitting pathological injury. Meanwhile, the levels of major histocompatibility complex class II (MHC-II) and DC costimulatory molecules (CD83, CD28, B7-DC, CD40, CD40 L and

DOI: 10.1002/eji.201970400

TLR2) were inhibited and followed the up-regulated levels of IL-4, IL-10 and IFN-γ, and down-regulated GM-CSF, IL-12p70, IL-15, IL-23 and TGF-β1. A key finding was that the phosphorylation of the 3 members (JAK2, STAT3 and STAT6) of the JAK/STAT/SOCS signaling pathway was inhibited, and the 3 downstream proteins (SOCS1, SOCS3 and PIAS3) from this pathway were highly expressed. In conclusion, curcumin suppressed the activation of DCs by modulating the JAK/STAT/SOCS signaling pathway to restore immunologic balance to effectively treat experimental colitis.

Traditional Medicine and Immunity

O297

Feiji Recipe(Jinfukang) inhibits the growth of lung cancer by modulating T-cell through IDO pathway in an orthotopic implantation tumor model

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Objective: Immune escape is a basic characteristic of cancer metastasis, and IDO plays a key role in mediating immune escape of non-small cell lung cancer. Feiji Recipe(FJR),a Jinfukang associated prescription, has the effect of stabilizing lesions and prolonging survival in patients with lung cancer. The purpose of this study was to investigate the immune mechanisms of FJR to anticancer.
Methods: In this article, we established an orthotopic transplant model of mouse Lewis lung cancer, with stable expression of IDO gene, in C57BL/6 mice; we use optical imaging to observe the effect of FJR in the treatment of lung cancer *in vivo*. We also investigated the effect of FJR on the proliferation of mouse Lewis lung cancer cell line 2LL, 2LL-EGFP and 2LL-EGFP-IDO, and the apoptosis of T-cell by MTT using flow cytometry.
Results: Compared to the control group, we found that the survival of animals treated with FJR was significantly prolonged ($P = 0.0074$), and the IDO protein level also decreased ($P = 0.0072$). Moreover, the percentages of $CD4^+CD25^+$ T-cells and $Foxp3^+$ T-cells were significantly decreased ($P < 0.05$). And the molecular mechanism of FJR against lung cancer may relate to the regulation of immune cell, such as T-cells and regulatory T-cells.
Conclusion: The molecular mechanism of FJR's treatment of lung cancer is to restore the function of T-cells in the tumor microenvironment through interfering the IDO.

O298

Helminthes associated derivative tuftsin-phosphorycholine, to treat autoimmunity

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Where there are helminthes , autoimmunity is rare. The aim of the helminthes is to protect themselves *via* immunomodulation of the host immune network. The immunoregulatory functions of helminthes were attributed to the phosphorylcholine (PC) moiety on the helminthes' secretory molecules. We have

DOI: 10.1002/eji.201970400

constructed a bi-functional molecule PC-tuftsin (TPC) and showed its immunomodulatory activity in 4 mouse models of autoimmune diseases and *ex-vivo* using human peripheral mononuclear cells (PBMCs) or biopsies of patients with giant cell arteritis (GCA) and lupus.
TPC attenuated the clinical score of murine autoimmune models|: lupus in NZBxW/F1 mice, collagen-induced-arthritis, DSS induced colitis and experimental-autoimmune-encephalomyelitis . In all the three autoimmune conditions, TPC decreased the secretion of inflammatory cytokines (TNFα, IL-1β, IL-6, IFNγ) and enhanced production of anti-inflammatory IL-10; expanded T and B regulatory cells. Likewise, TPC had an equal effect as methylprednisolone in a mouse model of lupus, inhibiting disease development in established lupus. Using *ex-vivo* lupus derived PBMCs, TPC could replace methylprednisolone. The immunomodulatory activity of TPC was proven also *ex-vivo* in lupus and GCA, reducing the inflammatory cytokines production by patients PBMCs and in biopsies from GCA. The bi-functional immunomodulatory activity of TPC was attributed to: a) The PC part which inhibited significantly TLR4 expression by HEK^{TM}-mTLR4 cells, through NFkB expression; b) TPC shifts macrophage cells from pro-inflammatory macrophages M1 to anti-inflammatory M2-secreting IL-10 and induce Tregs through the neuropilin-1.
Our data propose the potential for this small molecule as a novel therapy to treat autoimmune state (TPCera LTD).

O299

Berberine suppresses influenza virus triggered NLRP3 inflammasome activation in macrophages via inducing mitophagy

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Berberine is a natural extract from Rhizoma Coptidis, a herbal medicine wildly used in Chinese medicine. Our previous studies indicated that berberine can inhibit the lung inflammatory injury and mortality rate of mice with viral pneumonia. Recently, the argument of autophagy against inflammatory response arouses wide concern. This study based on ROS-NLRP3 pathway to discuss whether berberine inhibits NLRP3 inflammasome activation by inducing mitophagy. In the present study, J774A.1 macrophage infected by PR8 influenza virus was used as the model. Our results demonstrated that berberine and mito-TEMPO (a specific mitochondria ROS scavenger) significantly restricted NLRP3 inflammasome activation as reflected by increased NLRP3 expression as well as enhanced caspase-1 activity and secretion of mature interleukin-1β, while increased mitochondrial membrane potential(MMP) and decreased mitochondrial ROS(mtROS), suggesting that ROS was involved in NLRP3 inflammasome activation. We further demonstrated that the activation of NLRP3 inflammasome and generation of mtROS were elevated after treatment with mitophagy inhibitor 3-MA, indicating the involvement of mitophagy in ROS-NLRP3 pathway. Berberine treatment attenuated the effects of 3-AM. Furthermore, the mitophagy-related proteins including LC3II and Bnip3 were all significantly upregulated during berberine intervention, which suggested that mitophagy was stimulated in response to the berberine treatment. Berberine induced mitophagy was further confirmed by images under the confocal microscope and transmission electron microscopy.Collectively, these findings suggest that restricting NLRP3 inflammasome activation in macrophages by decreasing mitochondrial ROS generation via up-regulation of mitophagy may be a crucial mechanism for berberine to restrain influenza virus-induced inflammation.

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DOI: 10.1002/eji.201970400

O300

The Th17/Treg balance and defined micro-RNA-regulated genes mediate the anti-arthritic activity of traditional Chinese medicine Celastrus and its bioactive Celastrol

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovial joints, deformities, and disability. Owing to adverse reactions following the long term use of currently used anti-arthritic drugs, increasing numbers of RA patients are resorting to the use of natural herbal products that might offer effective, safe, and less expensive alternatives/adjuncts. Celastrol is a bioactive compound derived from traditional Chinese medicine Celastrus. However, the mechanisms underlying the anti-arthritic activity of celastrol /celastrus have not been fully defined. We determined the frequency of pathogenic T helper 17 (Th17) and protective T regulatory (Treg) cells in the joints of rats with adjuvant arthritis (AA), a model of human RA. We also assessed the impact of celastrol on Th17/Treg balance in arthritic joints. Celastrol treatment reduced Th17 cells but increased Treg in the joints, and it inhibited Th17 differentiation but promoted Treg differentiation in vitro by blocking the activation of phospho-STAT3. Furthermore, celastrol limited the production of Th17-differentiating cytokines (e.g., IL-6) as well as chemokines (CCL3, CCL5) that mediate cellular infiltration into the joints. Examination of the underlying molecular mechanisms revealed that celastrol treatment of arthritic rats reduced the expression of micro-RNA (miR)-22, miR-27a, miR-96, miR-142, and miR-223, but increased that of miR-296, coupled with changes in their defined target genes. Celastrol and Celastrus share many of the above functional attributes. These results have identified novel molecular targets for RA therapy and set the stage for clinical testing of celastrol/celastrus in RA.
(Supported by NIH R01AT004321, F31AT007278 and F31AT009421.)

O301

Wogonin induces neutrophil apoptosis and inhibits MAPK/Akt signaling to attenuate neutrophilic inflammation and tissue remodeling in allergic airways

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Severe neutrophilic asthma is characterized by persistent airway inflammation and irreversible airway remodeling, which are overstimulated by the key inflammatory mediator, high-mobility group box protein 1 (HMGB1). Whereas wogonin, an O-methylated flavone, was widely used to treat inflammatory and allergic diseases, its therapeutic effects and potential mechanisms on severe neutrophilic asthma have not been established. We examined the effect of wogonin on reducing neutrophilic airway inflammation in a mouse model of severe neutrophilic asthma. We further manifested the effect of wogonin on inducing human neutrophil apoptosis. Furthermore, we determined the effect of wogonin on inhibiting ASMC proliferation and migration mediated by HMGB1.

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DOI: 10.1002/eji.201970400

We demonstrated that wogonin reduced the frequency of neutrophils and inhibited the production of multiple inflammatory mediators in bronchoalveolar lavage fluid and lung tissues of the neutrophilic asthmatic mouse model. These data strongly support a significantly suppressed neutrophilic airway inflammation, functionally consistent to the relieved airway hyperresponsiveness by wogonin *in vivo*. To further understand cellular and molecular bases, we showed that wogonin induced human neutrophil apoptosis in a dose-dependent manner by activating caspase-8 and caspase-3 *in vitro*. As ASMC proliferation and migration contribute to airway tissue remodeling, wogonin pretreatment abolished HMGB1-induced ASMC proliferation and migration, which can be explained by the inhibition of phosphorylation in the MAPK/Akt singling pathways. Our findings support that wogonin augments caspase-dependent apoptosis in neutrophils to alleviate neutrophilic inflammatory responses and regulates intracellular signaling to inhibit HMGB1-mediated ASMC activation, providing a promising novel therapeutic agent for severe neutrophilic asthma.

O302

Naringenin decreases numbers of activated splenic T and B cells and ameliorates autoimmunity in lupus-prone mice

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Systemic lupus erythematosus-(SLE) is a disorder that results from several factors, including the abnormal behavior of T and B cell subsets. The main characteristics of SLE include high titers of anti-dsDNA, anti-nuclear antibodies and glomerulonephritis. Currently, immunosuppressive drugs are used to treat it, but unfortunately these agents produce side effects such as recurrent infections. Thus, it is necessary to find alternative therapies with higher effectiveness and security for the patients. Naringenin is a flavonoid found in grapefruit, which has been suggested to possesses anti-inflammatory effects. The main objective of this work was to prove the ability of Naringenin to reduce the splenic autoreactive T or B cell pool in a lupus-prone mice model. Twelve-week-old MRL/MpJ-Fas< lpr>/J mice were orally administered with Naringenin at 50 and 100 mg/kg every day for 6 weeks. Our results have shown that Naringenin treatment reduces the splenomegaly and absolute numbers of splenocytes. Furthermore, Naringenin treatment reduces the titers of anti-dsDNA and anti-nuclear antibodies and ameliorates glomerulonephritis. Naringenin decreased effector T cells and reduced their intracellular production of IFN-g. Regulatory T cells were increased in the animals that received Naringenin treatment. Besides, Naringenin reduced marginal zone B cells and downregulated the expression of CD86 and MHC-II. To evaluate the long-term effect, B6.MRL-Fas< lpr>/J were administered for 5-7 months. Similar effectiveness was observed in these mice without obvious side effects. Together, these results suggest that Naringenin ameliorates autoimmunity by the reduction of activated T and B cells and decreasing the signs of lupus-like disease in mice.

DOI: 10.1002/eji.201970400

O303

Lupeol in combination of AmphotericinB mediates synergistic anti-leishmanial immunomodulatory effects on Leishmania donovani AG83 infected BALB/c mice

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Visceral leishmaniasis, caused by protozoan parasite *Leishmania donovani*, is a neglected tropical disease affecting worldwide. The only orally bioavailable drug miltefosine is toxic whereas amphotericin B (AmpB) is expensive. Therefore, novel therapeutic strategies based on natural resources with less toxicity and low cost are urgently needed. Lupeol, a tri-terpenoid isolated from the bark of *Sterculia villosa* was reported to exhibit immunomodulatory and antileishmanial activity in experimental Visceral Leishmaniasis.
The present study was designed to understand the synergistic effect of sub-optimal dose of antileishmanial compound lupeol on anti-leishmanial and immunomodulatory activities of Amp B. It was observed that combination of sub-optimum dose of lupeol and AmpB significantly reduced the parasitic burden in *L. donovani* infected BALB/c mice. The antileishmanial effect was supported by the rise in nitric oxide production, robust induction in Th1 cytokines (IL-12 and IFN-γ) level and significant suppression in th2 cytokine (IL-10 and TGF- β) production. The treatment with the lupeol-AmpB combination enhances p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation but phosphorylation of extracellular signal related kinase (ERK 1/2) was reduced.
The present work indicates that combination of lupeol-AmpB mediated effective immunotherapeutic response for eliminating the parasite-induced immunosuppression. Therefore, lupeol-AmpB combination can be further explored as effective therapeutic regime against visceral leishmaniasis.

O304

Ursolic acid induced anti-tumor effect on adult T cell leukemia cells

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Ursolic acid (UA) is one of the components of *Oldenlandia diffusa*, and its antitumor effect has been reported. In this study, we investigated the anti-tumor effect of UA on adult T cell leukemia (ATL) cells. ATL cell lines MT-1, MT-2, MT-4, and acute lymphoblastic leukemia cell Jurkat were treated with UA at various concentrations (10 µM to 70 µM) and cell proliferation was measured by Cell Titer -Glo luminescent cell viability assay system. UA treatment inhibited cell proliferation of all cell lines in dose-dependent manner and MT-4 was most sensitive. Additionally, low concentration of UA (10 µM) was not toxic to human lymphocytes obtain from healthy donors while the proliferation of lymphocytes from ATL patient was predominantly inhibited by UA. In order to investigate the cellular events, Cyto-ID detection kit which detect monodansylcadaverine as a lysosome/autophagy vacuoles marker was used. MT-2, MT-4, and Jurkat cells were analyzed by flow cytometry at different time point after treatment with UA. All of the cell lines showed vacuoles positive, especially Jurkat cell showed much positive cells compare to other cell lines. Western blotting showed slight activation of mammalian target of rapamycin (mTOR) which plays a crucial role in regulating autophagy on MT-4 cells after UA treatment. Electron microscopy revealed the presence of large number of autophagic vacuoles and abnormal shaped mitochondria compare to control group, indicated that UA might induce mitochondrial disorders. Taken together, these results suggest that UA induced cell death might associate with mitochondria disorder otherwise apoptosis in ATL cells.

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DOI: 10.1002/eji.201970400

P0548

Emodin suppresses tumor growth through downregulation of CD155

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Emodin is a Chinese herb-derived compound and presents in a wide variety of plants. Although emodin has been shown to inhibit growth of cancer cells, its antineoplastic function is incompletely understood. CD155 is a member of poliovirus receptor-related (PRR) family of adhesion molecules; it is constitutively expressed on many tumor cell lines and tissues and has diverse functions. CD155 has been reported to mediate activation of T cells via CD226 or inhibition of T cells via T-cell immunoreceptor with Ig and ITIM domains (TIGIT). In addition, CD155 may play a critical role through non-immunological mechanisms in cancer. In this study, we tested the ability of emodin to modulate CD155 expression in cancer cells. We found that emodin significantly decreased the expression of CD155 in tumor cells and inhibited tumor cell proliferation and migration, and the antineoplastic properties of emodin are at least partially CD155 dependent. Therefore, we propose that emodin could inhibit tumor growth via downregulation of CD155. Our study provides new insights into the mechanisms by which emodin inhibits tumor growth.

P0549

Natural products promote susceptibility of tumor cells to natural killer cell-mediated immune surveillance

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Natural products are good sources for the discovery of novel anticancer drug candidates, therefore, much effort was devoted to screen these drug leads. NK cell-based immunotherapy is an attractive strategy to treat cancers. The engagement of natural-killer group 2 member D (NKG2D) receptor with its ligands on target cells play a critical role in stimulating NK cells cytotoxicity activity. In our screening model, we found the natural ent-kaurane diterpenoids exhibited great potential to enhance the cytotoxicity activity of NK cells via upregulating the expression of NKG2D ligands on tumor cells. As previously reported, ent-kaurane diterpenoids show great potency to kill cancer cells, but their immunoregulatory activities are rarely studied. Recently, **we enriched the anti-tumor role of some Star molecules from ent-kaurane diterpenoids in NK cell-based immunomodulatory activation.** The reactive oxygen species (ROS)/ERK axis was revealed to be involved in the regulation of NKG2D ligand expression. What's more, remarkable tumor growth inhibition by ent-kaurane diterpenoids was also observed in vivo in different types of cancer model, depending on the activation of NK cells. Our data not only confirmed the antitumor activation of traditional Chinese medicine, but also provide considerable evidence for the development of ent-kaurane diterpenoids as sensitizing agents in NK cell-mediated cancer immunotherapy.

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DOI: 10.1002/eji.201970400

P0550

Anti-inflammatory and anti-fibrosis effects of curcumin and curcumol

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E-Zhu (Curcuma zedoaria) is known as a classical traditional Chinese medicine and widely used in the treatment of cancers, cardiovascular disease, inflammatory disease. Its main components include curcumol and curcumin, which have anti-inflammatory and anti-fibrosis effects. Here we established an in vitro cigarette smoke extract (CSE) inflammatory injury model in Raw 246.7 cells to detect the effects of curcumin and curcumol. Our work demonstrated that curcumol and curcumin could reduce the release of intracellular ROS from macrophages, and inhibit the phosphorylation of p65 in NF-κB signaling pathway, also down-regulated the expression of pro-inflammatory factor IL-1β, IL-6, and TNF-α. After administration inhibitor of NF-κB, the effect of curcuminin in anti-infammatory was crippled in CSE-treated cells. Furthermore, the content of hydroxyproline and the expression of fibrosis related genes including α-SMA, Col-I, Col-III were down-regulated via TGF-β/Smad2/3 signaling pathway in pulmonary fibroblasts.

P0551

Yin and Yang in escaping from immune system of Brucella

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Chinese Medicine (CM) has been used for several thousand years, Yin and Yang theory is an important theoretical basis of Chinese medicine culture. The five elements theory of Yin and Yang are used to explain various phenomena of human physiology and pathology, and it is also used to guide the summary of medical knowledge and clinical experience. Brucellosis, one of the major zoonotic infections, is caused by the intracellular pathogens Brucella. Brucella is able to survive and replicate within host cells and using various strategies to avoid the host's immune response. The Yin and Yang theory is also suitable for the Brucella in the processes of escaping from the immune system. The macrophages which parasitic by Brucella are called Yin, the T cells which resist to Brucella´s offense are called Yang. The immune system in Yin and Yang are converted to each other, the macrophages are components of innate immune to phagocytosis of pathogens which called yang, but at the final stage of infection when it parasitic they are called Yin. In the paper, I will summarize the immune escaping process of Brucella including the immune cells, immune molecular, and the whole immune system in Yin and Yang theory.

P0552

Colorectal cancer cell-derived CCL20 recruits regulatory T cells to promote chemoresistance via FOXO1/CEBPB/NF-kB signaling

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DOI: 10.1002/eji.201970400

Background: Colorectal cancer (CRC) is one of the most common forms of cancer worldwide. The tumor microenvironment plays a key role in promoting the occurrence of chemoresistance in solid cancers. The effective targets to overcome resistance are necessary to improve the survival and prognosis of CRC patients. The aim of this study was to evaluate the underlying molecular mechanisms of the tumor microenvironment that might involve in the chemoresistance in patients with CRC.
Methods: We evaluated the effects of CCL20 on chemoresistance of CRC by recruitment of regulatory T cells (Tregs) *in vitro* and *in vivo*.
Results: We found that the level of CCL20 derived from tumor cells was significantly higher in Folfox-resistant patients that that in Folfox-sensitive patients. The high level of CCL20 was closely associated with chemoresistance and poor survival in CRC patients. Among the drugs of Folfox chemotherapy, we confirmed that 5-FU increased the expression of CCL20 in CRC. Moreover, CCL20 derived from 5-FU-resistant CRC cells promoted recruitment of Tregs. Tregs further enhanced the chemoresistance of CRC cells to 5-FU. FOXO1/CEBPB/NF-kB signaling was activated in CRC cells after 5-FU treatment, and was required for CCL20 upregulation mediated by 5-FU. Furthermore, blockade of CCL20 suppresses tumor progression and restored 5-FU sensitivity in CRC. Lastly, the expression of this signaling molecules mediating chemoresistance was closely correlated with poor survival of CRC patients.
Conclusions: CRC cell-secreted CCL20 can recruit Tregs to promote chemoresistance via FOXO1/CEBPB/NF-kB signaling, indicating that FOXO1/CEBPB/NF-kB/CCL20 axis might provide a promising target for treatment of CRC.

P0553

Hypofractionated stereotactic radiation therapy activates the peripheral immune response in operable stage I non-small-cell lung cancer

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It has been reported that in patients with operable stage I non-small cell lung cancer (NSCLC), overall survival (OS) is better in those who undergo hypofractionated stereotactic radiation therapy (HSRT) than in those who undergo surgery. However, the reason that HSRT has a better OS has not been fully explored. Here, we analyzed reconstitution kinetics in immune cells in the peripheral blood of NSCLC patients after HSRT. We found that HSRT increased the frequency of total T cells, especially the proportion of CD8+ T cells, but decreased the frequency of inhibitory Tregs. Intracellular staining showed that after HSRT, peripheral CD8+ T cells were transformed into activated T cells, which express high levels of TNF-α, IFN-γ, granzyme B and IL-2. HSRT also increased the production of IL-2, TNF-α, and IFN-γ but down-regulated the production of TGF-β in CD4+ T cells. The frequencies of naïve B cells and double-negative B cells were lower, while the proportions of MZ-like B cells, transitional B cells and plasmablast cells were higher after HSRT. Collectively, our results demonstrate that HSRT activates the peripheral immune response and indicate the dynamic variation in peripheral lymphocytes after HSRT, which is very important for optimizing combination treatments in clinical practice.

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DOI: 10.1002/eji.201970400

P0554

Xuanbai Chengqi decoction alleviates pulmonary inflammation in mouse model of chronic obstructive pulmonary disease through inhibiting the ratio of Th17/Treg via gut commensal microbiota

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Objective: We aimed at exploring whether Xuanbai Chengqi decoction (XBCQ) could modify the gut microbiota and inhibit the ratio of Th17/Treg so as to reduce lung inflammation in mouse model of COPD.
Methods: 48 female C57/BL6 mice were divided randomly into following groups: Wild type control, COPD model, XBCQ treatment (including low-, medium-, high-dose), Dexamethasone positive control. COPD model was established by intra-tracheal administration of cigarette smoking extract and lipopolysaccharide mixture. Pulmonary pathology by HE staining, inflammatory cytokines by real time PCR, Th17/Treg by flow cytometry and composition as well as richness of gut microbiota by 16S rRNA sequencing were detected.
Results: It clearly showed that there was enlargement of alveolar air spaces, destruction of lung parenchyma and inflammatory cell infiltration of bronchia in COPD mice. After XBCQ´s treatment, the destruction of alveolar walls and inflammatory exudation was obviously alleviated in COPD mice. What's more, obviously high expression of TNF-α, IFN-γ and IL-1β as well as a dominance of Th17 over Treg induction was showed from lung tissue of COPD mice while the treatment of XBCQ decoction significantly reduced the secretion of these inflammatory cytokines and corrected the imbalance of Th17/Treg ($p< 0.05$). Finally, 16SrRNA sequencing results showed lower diversity and richness of gut microbes from COPD mice while XBCQ decoction ameliorated flora disequilibrium in COPD mice.
Conclusion: XBCQ decoction has a remarkable effect on the maintenance intestinal immune homeostasis, which might be associated with adjusting gut commensal flora on COPD mice.
This project was supported by NSFC (No.81473656)

P0555

The effect of sanshui baihu decoction on bone destruction in hTNF transgenic mice with rheumatoid arthritis

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Objective:To study the curative effect of sanshui white tiger decoction (SSBH) on hTNF alpha transgenic mice (Tgtc) with rheumatoid arthritis (RA) , and discusse the mechanism of action of the inhibit bone destruction on Tgtc mice.
Methods: 24 Tgtc rats were randomly divided into normal saline (NS) group, SSBH group and leflunomide (LEF) group.The arthritis index of each group was recorded during the drug administration. Imaging evaluation and serum was taken to detect TNF-α was conducted after 60 days of continuous drug intervention. .
Results: After intervention, the serum TNF- level in the SSBH LEF group was lower than that in the NS group (all $P < 0.05$).Spleens index, ankle cortical bone density, ankle trabecular bone density of each group showed no significant difference ($P>0.05$).MR indicated that all the knee joints of mice in each group had bone marrow edema, synovial hyperplasia, but the SSBH group and NS group

DOI: 10.1002/eji.201970400

showed no synovial erosion of bone joint space stenosis and partial articular surface fusion.
Conclusion:SSBH had a good inhibitory effect on chronic arthritis of Tgtc rats.By inhibiting the release of inflammatory factor TNF-α, the progression of joint bone destruction in Tgtc rats was slowed down to some extent.
Keywords: sanshui baihu decoction, rheumatoid arthritis,Tgtc mice, bone destruction

P0556

The essential oil of Zanthoxylum myriacanthum var. pubescens attenuates diabetic nephropathy by reducing oxidative stress and inflammation

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Background: *Zanthoxylum myriacanthum* var. *pubescens* known as Ma Qian is an indigenous aromatic plant in xishuangbanna and is commonly used in Dai ethnic medicine to treat digestive disorders. We have previously shown that the essential oil extracted from Ma Qian (MQEO) exhibited strong anti-oxidative and anti-inflammatory effects in human THP-1 cells.
Aim of the study: To explore the therapeutic value of MQEO in diabetic nephropathy.
Methods: Diabetes in ICR mice was induced by a single dose of streptozotocin injection. Seven days later, the diabetic mice were treated by oral administration of MQEO. At the end of seven-day treatment, the urine levels of urea and creatinine were evaluated. Kidney tissues were analyzed for oxidative stress biomarkers by biochemical assays, pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-β (IL-1β) by ELISA, protein expressions of thioredoxin-interacting protein (TxNIP), iκB kinase β (IKKβ), and nuclear factor-erythroid-1-related factor 2 (Nrf2) by western blotting, and histological changes by H&E staining.
Results: Oral administration of MQEO significantly decreased urine urea and creatinine levels and normalized the kidney histology in the diabetic mice. MQEO also normalized several oxidative stress biomarkers including malondialdehyde, vitamin C, and antioxidant enzymes in the diabetic kidneys. In addition, renal levels of TNF-α and IL-1β were significantly reduced following MQEO treatment. Furthermore, MQEO normalized renal protein expressions of TxNIP, IKKβ, and Nrf2.
Conclusion: These findings demonstrated the renoprotective effect of MQEO through attenuating oxidative stress and inflammation and highlighted its potential as a unique therapy for treating diabetic nephropathy.

P0557

Myricetin inhibits NLRP3 inflammasome activation by reducing ROS-dependent ubiquitination of ASC and promoting NLRP3 ubiquitination

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Myricetin is a plant-derived flavonoid with diverse pharmacological properties. NLRP3 (NLR Family, Pyrin Domain-Containing 3 Protein) inflammasome is a cytosolic multi-protein complex that plays a critical role in an innate immune system and is involved in the pathogenesis of multiple inflammatory disorders. In this study, we found that myricetin could inhibit NLRP3 inflammasome assembly by promoting ROS-independent ubiquitination NLRP3 and reducing ROS-dependent ubiquitination of ASC (apoptosis-associated speck-like protein containing a CARD), disrupting the interaction between ASC and NLRP3 and thereby inhibiting ASC oligomerization. Mice models of LPS-induced sepsis and

DOI: 10.1002/eji.201970400

alum-induced peritonitis further confirmed this effect *in vivo*. Levels of IL-1β and IL-18 in serum were significantly reduced after short-term myricetin treatment, while TNF-α was not affected. These results suggest the potential therapeutic value of myricetin by targeting NLRP3-driven inflammatory diseases.

P0558

Tranilast directly targets NLRP3 to treat inflammasome-driven diseases

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The dysregulation of NLRP3 inflammasome can cause uncontrolled inflammation and drive the development of a wide variety of human diseases, but the medications targeting NLRP3 inflammasome are not available in clinic. Here, we show that tranilast (TR), an old anti-allergic clinical drug, is a direct NLRP3 inhibitor. TR inhibits NLRP3 inflammasome activation in macrophages, but has no effects on AIM2 or NLRC4 inflammasome activation. Mechanismly, TR directly binds to the NACHT domain of NLRP3 and suppresses the assembly of NLRP3 inflammasome by blocking NLRP3 oligomerization. In vivo experiments show that TR has remarkable preventive or therapeutic effects on the mouse models of NLRP3 inflammasome-related human diseases, including gouty arthritis, cryopyrin-associated autoinflammatory syndromes, and type 2 diabetes. Furthermore, TR is active ex vivo for synovial fluid mononuclear cells from patients with gout. Thus, our study identifies the old drug TR as a direct NLRP3 inhibitor and provides a potentially practical pharmacological approach for treating NLRP3- driven diseases.

P0559

The role and mechanism of shikonin in the regulation of Th cell differentiation by SOCS1 in the treatment of RA.

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Background: Rheumatoid arthritis (RA) is a refractory autoimmune disease, and T cells play an important role in its development. Traditional Chinese medicine for clearing away heat and promoting blood circulation lithospermum has a good curative effect on Arthromyodynia, but its target and mechanism are unclear. shikonin, which is an anti-inflammatory active ingredient of lithospermum, can effectively act on RA.
Methods: We predicted the possible mechanism of shikonin acting on Collagen-Induced Arthritis (CIA) by whole-gene spectroscopy and network pharmacology, and established a CIA model in DBA/1 mice to validate this prediction. Treatment with SKN (4 mg/kg, p.o., daily), or vehicle began from day 21 to day 40 of first immunization. The differentiation of Helper T (Th)1,Th2, Th17 and Regulatory T (Treg) cells was measured by Flow cytometry in lymphonodus. We used Western blot to study its mechanism in joints of CIA mice.
Results: When shikonin on the dose of 4 mg/kg,it can inhibit the differentiation of Th1 and Th17 cells and promote the differentiation of Th2 and Treg cells by up-regulating Cytokine signal transduction inhibitor protein 1(SOCS1), p-JAK1,p-STAT1and p-STAT6.
Conclusion: shikonin can inhibit the differentiation of Th1 and Th17 cells and promote the differentiation of Th2 and Treg cells by up-regulating SOCS1, and its mechanism is related to JAK/STAT pathway.

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DOI: 10.1002/eji.201970400

P0560

Low molecular weight extracts from Bovine spleen promotes CAR-T cell therapy against cancer through the modification of gut microbiota in human tumor transplanted mouse model

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The gut microbiota influences the result of immunotherapy in cancer patients through reciprocal interactions with the host immune system. In this study, the late stage of NPG mice injected with Raji cells, a human tumor cell line, through the tail vein, lead to a poor quality of life and shortened survival due to the gut microbiota imbalance. Injecting CAR-T cell therapy with the intragastric administration of the Low molecular weight extracts from Bovine spleen (LMWEBS) prolonged a longer lifetime （average value: 96 days） of the NPG mice with the injection of human cancer cells than those mice received CAR-T cell therapy without the treatment of LMWEBS (average value: 81 days). According to the results of the intestinal bacteria sequencing, the gut microbiota in mice treated by LMWEBS had a more similar pattern to the health mice rather than those of mice with tumor cell injection but without LMWEBS treatment. In mice with CAR-T cell treatment, the number of Clostridium in intestinal flora and their product, short-chain fatty acids, which may affect the efficacy of immunotherapy, decreased after the intragastric administration of LMWEBS. Furthermore, both the number and function of injected CAR-T cells in the mice were increased with LMWEBS treatment according to the results of cytometry and immunohistochemical analysis. In conclusion, our results suggest that LMWEBS treatment promotes the CAR-T cell therapy against tumor growth and prolongs the life of NPG mice transplanted with human tumor cells, though preventing the microbial community dysregulation and bacteria toxic metabolites releasing.

P0561

Immunogenic chemotherapy effectively constrains KRAS-mutant lung cancer

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Immunogenic chemotherapies have proven effective for the treatment of several cancers. Here we identified tra as an immunogenic cell death inducer and observed effective antitumor activity when used in combination with IL-12, in a *Kras*-mutant spontaneous lung cancer model. Tumor cells treated with tra showed the hallmarks of immunogenic cell death, including surface calreticulin expression, release of HMGB1 and DC activation. Mice treated with both tra and IL-12 showed increased T cell infiltration and proliferation within the tumor, and increased effector function of NK and T cells, indicating that this therapeutic combination can improve the quality of the immune response. Taken together, our results provided a potential new therapeutic regimen for the treatment of *Kras* mutant lung adenocarcinoma.

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DOI: 10.1002/eji.201970400

P0562

Tanshinone IIA attenuate atherosclerosis by mediating the crosstalk of autophagy and polarization in macrophages via miR-375

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Objective: Macrophages play a key role in destabilizing atherosclerotic plaque. We aim to identify the exact effect of Tanshinone IIA (TNA) that regulate the crosstalk between autophagy and polarization in macrophages to attenuate atherosclerosis.
Methods: C57BL/6J mice were divided into three groups. ApoE$^{-/-}$ mice in model group and TNA group were fed with high-fat diet for 20 weeks, and the mice in TNA group were treated with TNA by intraperitoneal injection. Wildtype mice in control group were fed with normal diet. Afterward, the levels of plasma cholesterol and triglycerides were measured. Intimal lesion areas and lipid accumulation were assessed by staining. The expression of RNAs/protein was evaluated by PCR or WB.
Results: TAN group had lower levels of TG, CHO, LDL and a higher level of HDL vs model group. Lipid accumulation was more severe in model group and TAN group vs. control group, while TAN group was milder than model group. Macrophages expressed much more LC3 puncta in TAN group vs. model group. A significant decrease of TNF-a, IL-6, iNOS, and IL-12 was found in TNA group vs. WT group. The level of miR-375 was strongly increased in ApoE-/- mice vs. WT mice, but it is no significant difference between TNA group and WT group. After treatment with miR-375 mimics, the expression of Beclin1, LC3 II and phosphorylation of STAT6 were attenuated, and phosphorylation of NF-κB was enhanced.
Conclusion: The results revealed TNA mediated the activation of autophagy and M2 polarization via miR-375 in anti-inflammation to attenuate atherosclerosis.

P0563

Quercetin protects against RSV infection by promoting alveolar macrophage production type I interferons

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Background and Purpose: RSV is a leading cause of upper and lower respiratory tract infections in young children. Alveolar macrophage (AMs)-derived type I interferons orchestrate innate immunity are the first and earliest line of defense against RSV. Here, we investigated how quercetin affects AMs produce type I IFNs to protect against RSV infection.
Experimental Approach: The mice were infected intranasally with RSV to establish RSV infected mice model, and model mice were intragastrically administered quercetin for evaluating therapeutic effect in vivo. Multiple molecular experiments including real-time PCR, Western blot, flow cytometry and immunofluorescence were used to elucidate the underlying mechanisms. For depletion of AMs, mice were inoculated intranasally with a single dose of Clodronate liposome. For depletion of gut microbiota and metabolites, mice were treated broad-spectrum ABX cocktail.
Key Results: Here, we find that quercetin reduces RSV-induced lung pathology and RSV replication through the modulation of the innate antiviral response. Furthermore, quercetin induces the production

DOI: 10.1002/eji.201970400

of type I IFNs in AMs through the activation of RIG-I and TLR3 pathways. We demonstrate the antiviral immunity responses of quercetin are dependent on desaminotyrosine (DAT), the degradation product of quercetin from the action of gut microbiota. DAT could promote type I IFN secretion from macrophages via RIG-I and TLR3 pathways following RSV infection. We show that DAT is responsible for the antiviral response of quercetin in vivo through the depletion of DAT levels.
Conclusions: Our study reveals that quercetin restricts severe RSV infection through the promotion of macrophage-mediated host anti-viral innate immunity.

P0565

MAPK can regulate anti-cancer drug, cucurbitacin D (CuD)-induced effects on adult T-cell leukemia (ATL) cells

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Adult T-cell leukemia (ATL) is an aggressive and malignant blood disease. We previously reported that the steroid-structured cucurbitacin D (CuD) induces apoptosis in ATL cells. Here, we investigate the effects of MAPK signaling inhibitors on CuD-induced cell death in PBL isolated from ATL patient and cell lines. The serum from of this ATL patient contained higher levels of several cytokines, such as IL-8, IL-10, IL-17A, IL-18, and IFN-γ than that of a healthy donor. As we have reported, CuD induced cell death, which was enhanced by a MEK1/2 inhibitor, U0126. However, a JNK inhibitor interrupted CuD-induced cell death. Immunoblot analysis revealed that CuD reduced phosphorylation of Erk, p38, and JNK, and co-treatment with CuD and U0126 did not affect the phosphorylation of Erk. MEK1/2 and p38 inhibitors enhanced CuD-induced cell death. U0126 enhanced CuD-induced de-phosphorylation of Erk in MT-1and MT-4 cells. Thus, CuD can reduce Erk activation resulting in enhanced anti-tumor effects in the ATL cells.

P1144

Periplocin sensitizes human oesophageal cancer cells to TRAIL-induced apoptosis via upregulating death receptors and inhibiting the Wnt/β-catenin pathway

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Oesophageal squamous cell carcinoma (ESCC) is a kind of malignant tumour that associated with poor prognosis frequently. Tumour necrosis factor- related apoptosis-inducing ligand (TRAIL) has become a promising anti-tumour drug because of its ability to induce apoptosis in a variety of tumour cells without affecting normal cells. However, drug resistance in some of tumours makes it less effective than expected in clinical applications. Therefore, it is urgent to find agents that can enhance sensitivity to TRAIL. In this experiment, most of ESCC cell lines were resistant to TRAIL, while its sensitivity to TRAIL were enhanced after treatment with periplocin extracted from cortex periplocae (CPP). We explored the mechanism of cells resistance to TRAIL is that the expression of death receptors (DRs) is relative low. Moreover, CPP could enhance the expression of DRs in ESCC cells by downregulating a transcriptional repressor of the DRs, FoxP3, and decrease the expression of survivin by inhibiting the Wnt/β-catenin pathway. Therefore, CPP could further enhance TRAIL-induced apoptosis of cells, thereby facility TRAIL to generate anti-tumour effects. In addition, after treatment with CPP and TRAIL in vivo, the content of IFN-γ in nude mice serum was increased while the content of IL-1β, IL-6 and CXCL1 were decreased. Through changing the expression of cytokines, the immune

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DOI: 10.1002/eji.201970400

microenvironment in vivo was improved, thereby rescuing the immunosuppressive in tumour microenvironment, further enhancing the efficacy of the drug.
Keywords: TRAIL; CPP; Oesophageal squamous cell carcinoma; DRs; Wnt/β-catenin pathway

P1145

A study of the efficacy and safety of excess iodine Chinese herbal decoction for the treatment of Graves disease

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Purpose: To explore the efficacy and safety of excess iodine Chinese Herbal Decoction for the treatment of Graves disease.
Method: 65 Graves disease patients were recruited and randomly divided into two groups: The treatment group was treated with excess iodine Chinese Herbal Decoction, plus 5mg Methimazole per day. The control group was given 10-20mg Methimazole per day. The scores of syndromes, FT3, FT4, TSH, TgAb, TPOAb,TRAb, adverse reactions and the Median urinary iodine index were observed.
Results: The therapeutic efficacy in the Treatment group and the Controls was 96.9% and 90.6% respectively,but without significant difference(P＞0.05).Compared with the controls, serum FT3,FT4 levels 2 and 4 weeks after treatment with excess iodine Chinese Herbal Decoction were reduced markedly,
FT310.64±2.74pmol/Lvs18.70±8.02pmol/L.FT421.45±9.20pmol/Lvs36.29±12.31pmol/L;FT36.04±2.56 pmol/Lvs8.67±4.25pmol/L, FT414.46±4.94pmol/Lvs20.60±13.30pmol/L(all P＜0.05).excess iodine Chinese Herbal Decoction can significantly reduce TRAb levels. No obvious adverse reaction was found in treatment group.
Conclusion: The treatment of Graves Disease with excess iodine Chinese Herbal Decoction is effective and safe..
Keywords: Graves disease; excess iodine Chinese Herbal Decoction; TRAb.

P1146

A Clinical Study on the therapeutic effect of Hashimoto´s Thyroiditis with Yiqi Huatan Huoxue Decoction

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Purpose：To observe the therapeutic effect of Hashimoto´s Thyroiditis(HT) with Yiqi Huatan Huoxue Decoction.
Method：64 patients with HT were randomly divided into two groups:the treatment group and control group. The controls were given a lifestyle intervention education,including low iodine diet, mental regulation,etc. The treatments were given a Chinese herbal decoction (Yiqi Huatan Huoxue Decoction)with the function of invigorating qi,resolving phlegm and promoting blood circulation. The treatment course last for 3 months.The syndrome scores, FT3,FT4,TSH, TPOAb, TGAb and thyroid size parameters were observed.
Results: Compared with the controls,Yiqi Huatan Huoxue Decoction could significantly improve the symptom scores,markedly reduce elevated serum TSH levels,

DOI: 10.1002/eji.201970400

could significantly reduce the serum TPOAb levels in HT patients.The recipe could improve serum TGAb levels but without significant difference.Furthermore,the recipe could significantly reduce the anteroposterior diameter, left and right diameter of the lobes and isthmus thickness.
Conclusion: Yiqi Huatan Huoxue Decoction can reduce the serum TPOAb levels and relieve goiter in HT cases.
Keyword： Yiqi Huatan Huoxue Decoction；Hashimoto´s thyroiditis；Chinese Medicine.

P1147

p-Hydroxylcinnamaldehyde reverses 4NQO-induced esophageal tumorigenesis via the RhoA-MAPK signaling pathway

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p-Hydroxylcinnamaldehyde isolated from the Cochinchina momordica seed (CMSP) has been identified as an inhibitor of the growth and metastasis of esophageal squamous cell carcinoma (ESCC) by inducing differentiation. The aim of the present study was to evaluate the effect of CMSP on 4-nitroquinoline-1-oxide (4NQO)-induced esophageal tumorigenesis and its underlying mechanism. In the present study, a mouse model of esophageal preneoplastic lesions was established by providing 4NQO-containing drinking water to C57BL/6 mice. The function of CMSP against tumorigenesis induced by the chemical mutagen and the effect of CMSP on immune function were investigated. The results showed that the incidence and pathological stage of atypical hyperplasia in esophageal tissues were significantly alleviated in CMSP-treated mice compared with those in untreated mice. Immunohistochemistry and pull-down results revealed that the expression levels of p-ERK1/2, p-SAPK/JNK and GTP-RhoA were significantly downregulated in the esophageal tissues of CMSP-treated mice. In addition, the proportions of $CD4^+$ T cells, $CD8^+$ T cells and NK cells were increased, while the proportion of $CD4^+$ $CD25^+$ regulatory T cells (Tregs) was decreased in the peripheral blood of CMSP-treated mice. These results indicated that CMSP could reverse 4NQO-induced esophageal tumorigenesis by regulating the RhoA-ERK/JNK signaling pathway and promoting immune function, providing a new potential strategy for treating preneoplastic lesions of the esophagus.
Key words: p-Hydroxylcinnamaldehyde (CMSP); Esophageal squamous cell carcinoma (ESCC); Preneoplastic lesion; Immune regulation

P1148

Qing-Re Chu-Shi Decoction improves 2,4-dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice via anti-inflammatory and immune regulation

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Atopic dermatitis (AD) is reported to be an allergic dermatitis characterized by eczematous lesions and pruritus. Qing-Re Chu-Shi Decoction (QRCSD) is the herbal formula long used as a complementary and alternative therapy for inflammatory skin diseases in China, with pooly understanding of role and mechanism of the action. The study was designed to explore the underlying effects of QRCSD and identify the components accounting for the therapeutic effects in 2,4-Dinitrochlorobenzene (DNCB)-

DOI: 10.1002/eji.201970400

induced AD-like model of NC/Nga mice. Network pharmacological analysis first predicted the pharmacological mechanisms of QRCSD, as well as UPLC-mass analysis identifying active components. QRCSD or prednisolone (positive control) was administered by gavage every other day over d14~d21. Dermatitis severity score and scratching behavior showed that oral administration of QRCSD could relieve the manifestations, and pathology observation of skin lesions including HE and Toluidine blue staining revealed that epidermal thickness and number of mast cell were decreased compared with the model. Immunohistochemistry illustrated that QRCSD could increase the tight junction protein (Claudin 1, Claudin 3) levels and downregulate IgE level in serum. Flowcytometry displayed that Th1/Th2 balance was regulated by QRCSD. Substantial levels of interleukin (IL)-4, IL-5, IL-6, IL-17A and Eotaxin were revealed by protein arrays in AD-like skin lesions, and western blotting confirmed that the JAK, IL-6, ERK proteins were down-regulated by treatment of QRCSD. The study contributes to an understanding of anti-inflammatory and immunomodulatory effects of QRCSD that plays a significantly protective role on AD. QRCSD treatment could be considered as a candidate complementary and alternative therapy for AD.

P1149

Expression and clinical significance of PD-1, PD-L1 and PARP-1 in hepatocellular carcinoma and paracancerous tissues

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Objective: To intestigate the expression of programmed death-1(PD-1) ,programmed death ligard-1 (PD-L1), poly ADP-ribose polymerase-1 (PARP-1) in hepatocellular carcinoma tissue and its adjacent liver tissues. And analyze the clinical significance in pathological features as well as prognosis.
Methods: Seventy-one HCC specimens and matched tumor-adjacent specimens were collected. Immunohistochemistry (IHC) was used to determine the expression of PD-1, PD-L1 and PARP-1 in the above tissues. The relationship of PD-1, PD-L1, PARP-1 expression with the clinics- pathological features and the prognosis was investigated.
Results: The positive expression rate of PD-1, PD-L1 and PARP-1 in HCC tissues were significantly higher than in its adjacent liver tissues (X^2 =75.59 .X^2 =187.20 .X^2 =47.03, P< 0.05). The rates of PARP-1 expression in the poorly differentiated HCC s (X^2 =21.72, P< 0.05), or with recurrence and metastasiss (X^2 =19.01 ,P< 0.05) were higher than others. The positive expression rates of PD-1, PD-L1 in HCC tissue with different pathological classification varied .Being the highest in moderately differentiated HCC tissuess (X^2 =201.94, P< 0.05) or which with Barcelona clinical liver cancer stage C (X^2 =36.37, P< 0.05). Kaplan-Meier analysis showed the HCC patients with high PARP-1 expression or with negative PD-1 PD-L1 jade shorter overall survival time than those with low PARP-1 expression s (X^2 =5.64, P< 0.05) or with positive PD-1, PD-L1 (X^2 =26.12, P< 0.05).
Conclusion: PD-1, PD-L1, PARP-1 have higher positive rates in HCC ,which might be associated with the invasion of HCC, and help evaluate the prognosis of HCC patients.

DOI: 10.1002/eji.201970400

P1150

Effect of diosgenin on expression of Treg cell and its specific transcription factor in collagen-induced arthritis mice in vitro

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This study aimed to explore the effects of diosgenin on expression of Treg cell and its specific transcription factors in vitro, then clarify the immune regulation mechanism of diosgenin on collagen-induced arthritis mouse model.Lymphocytes were isolated from DBA/1J mice, and CCK-8 assay was used to explore the toxicity and proliferation inhibition of diosgenin in lymphocytes. Chicken collaged II was used to construct CIA model in DBA/1J mice. Then, lymphocytes were isolated, cultured in vitro, and divided into five groups: control (IL-2 stimuation), CIA model (IL-2+collagen II),high-/medium-/low-dose-Diosgenin (IL-2+collagen II). Following this, the percentage of Treg cells was determined using flow cytometer. Expression of Foxp3 was determined using quantitative real-time PCR and western blot.Diosgenin had no obvious toxicity on lymphocytes with concentration ranged from 3.125 to 25 μmol/l, and could inhibit the proliferation of lymphocytes with a dose-dependent manner. Three doses of drug stimulation in vitro were selected at 6.25, 12.5 and 25 mol/L respectively.Diosgenin could obviously increase the elevation of Treg cells in the CIA model ($P < 0.05$). Compared with the control group, Foxp3 mRNA expression was increased in the model group ($P< 0.05$), and significantly increased in the high-dose group ($P< 0.05$).The protein expression of Foxp3 was decreased in the model group compared with the control group ($P< 0.05$).Compared with the model group, Foxp3 was increased in the high-dose group ($P< 0.01$).Diosgenin could serve as a potential agents in ameliorating the pathogenesis of RA via upregulating the generation of Treg cells and its specific transcription factor.

P1151

Fraction from Lycium barbarum polysaccharides synergizes with chemotherapy or adoptive immunotherapy in the inhibition of mouse H22 solid tumor growth

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It has been demonstrated that combination therapies have some beneficial effects for tumor treatment. In previous study, we found that a fraction isolated from Lycium barbarum polysaccharides, designated LBP3, could inhibit the solid tumor growth in H22 tumor-bearing mice and simultaneously induced systemic and local immune responses by alleviating immunosuppression and maintaining antitumor immune responses. In the present study, we investigated whether LBP3 could synergize with chemotherapy or adoptive immunotherapy in the inhibition of mouse H22 solid tumor growth. Our results showed that LBP3 enhanced antitumor activity of Doxorubicin, and improved peripheral blood lymphocyte counts and the cytotoxicity of splenocytes in the tumor-bearing mice. Furthermore, LBP3 improved peripheral blood lymphocyte counts, promoted cell cycle recovery in bone marrow cells, and restored the cytotoxicity of natural killer cells in Doxorubicin treated tumor-free mice. Thus, LBP3 enhanced antitumor activity of doxorubicin partly via reducing the immunotoxicity of doxorubicin in mice. In another study, we found that LBP3 plus adoptive tumor infiltration lymphocytes (TILs) therapy had better inhibition effects in mouse H22 solid tumor growth than adoptive TILs therapy alone.

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DOI: 10.1002/eji.201970400

Besides, $CD8^+$T cells of mice in LBP3 plus adoptive TILs therapy group had higher level of mitochondria than those in adoptive TILs therapy group. Taken together, our results demonstrated that LBP3 could synergize with chemotherapy or adoptive immunotherapy in the inhibition of mouse H22 solid tumor growth.

P1152

Preliminary study on anti-tumor effect and related mechanism of Nidus Vespae in vitro

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Objective: To study the anti-tumor effect of *Nidus Vespae* and the related anti-tumor mechanisms.
Methods: The inhibitory or promoting effects of different concentrations of *Nidus Vespae* decoction (NVD) on tumor cell and immune cell were detected through CCK8 Assay kit. We also used enzyme-linked immunoadsorption to detect the content of various cytokines in the cell culture solutions. The apoptotic effect and the cell cycle were evaluated by flow cytometry.
Results: High concentration of NVD could significantly inhibit tumor cell proliferation, and low concentration of NVD had no direct inhibitory effect, but could significantly promote the proliferation and differentiation of THP-1. When THP-1 was co-cultured with serum-containing NVD, its proliferative ability was significantly improved; while co-cultured with serum-free NVD, THP-1 could differentiate into macrophages with distinct characteristic stellate morphological, although its proliferative capacity decreased. The serum-free NVD also could induce THP-1-derived macrophages to produce cytokines IL-6, IL-12p40 and TNF-α. Furthermore, the supernatant of THP-1-drived macrophages could significantly inhibit tumor cells proliferation, by inducing apoptosis and arresting the cell cycle.
Conclusion: Different concentrations of NVD had different anti-tumor mechanisms. High concentration of NVD could directly kill tumor cells, while low concentration of NVD could exert indirect anti-tumor effects by enhancing immune function.

P1153

Neferine exerts its protective effect on acetaminophen-induced liver injury via Nrf2-mediated anti-oxidant signaling pathway

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Drug-induced liver injury (DILI) has been a thorny issue of safety medication due to the high occurrence rate and perniciousness, whose pathogenesis is closely correlated with reactive oxygen species (ROS) formation caused by toxicity metabolites of drugs. Neferine, an extraction of “lianzixin”, is validated to exert biological effect on disorder in cardiovascular system, neural system and fibrinolytic system via anti-oxidant capacity. The study was established to explore whether neferine could play protective role in DILI. APAP overdose was adopted to mimic DILI in mice and the grouping was set up as: control, APAP+ saline, APAP+ neferine (5/10/20 mg/kg) group. Our results showed that neferine alleviated APAP-induced liver histological impairment and lowered serum levels of ALT and AST. With inhibiting accumulation of ROS, neferine increased the levels of SOD, GSH and GSH-px while MDA decreased. Serum levels of TNF-α and IL-6 as well as expression levels of 3-NT and iNOS were reduced by neferine. Also, neferine improved APAP-caused apoptosis and promoted expression level of Bcl-2 while down-regulating that of Bax and caspase 3. Nrf2/HO-1/NQO1 signaling pathway was detected as possible molecular mechanism and was found of higher expression under neferine

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DOI: 10.1002/eji.201970400

treatment. The results suggested that neferine could protect from APAP-induced liver injury by suppressing oxidative stress via up-regulation of Nrf-2/HO-1/NQO1 signaling pathway.

P1154

Immunization of male mice with B-cell epitopes of uPA inhibits fertility

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Objective: To evaluate the contraceptive ability of three B-cell epitopes in uPA which we denominated as GF34, K121, SP231.
Methods: The three predicted B-cell epitopes of uPA were synthesized to immunize male mice. BALB/c male mice were randomly divided into three experimental groups (n=12) and two control groups (n=10) for immunization. Fertility, epididymal sperm function, and the presence and reactivity of antibodies in immunized males were investigated.
Results: Significant reduction of fertility was observed in GF34 and K121group in mating trial with no evident systemic illness or abnormal mating behavior. However, SP231 and control groups show similar normal results. Epididymal sperms of GF34 and K121 group males exhibited impaired progressive motility and ability to fertilize eggs in vitro. High titers of antibodies were induced in the sera in all three experimental groups.
Conclusions: Subcutaneous injection of uPA B-cell epitope members GF34 and K121 to male mice could effectively reduce their fertility, and uPA epitope vaccine could become a new strategy for male immunocontraception development.

P1155

Glycyrrhiza polysaccharides regulates the differentiation of intestinal epithelial γδt cells to inhibit tumor growth through modulating gut microbiota composition

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Objectives: Glycyrrhiza Polysaccharide (GCP), a macromolecular polysaccharide isolated from Licorice, has been proved to inhibit tumor growth in vitro and in vivo. However, the specific anti-tumor mechanism of GCP remains unclear. It has been recognized that polysaccharides could regulate gut microbiota and affect immune cell function. In this study, we investigated the effect of GCP on the differentiation of intestinal epithelial γδT (IELγδT) cells and the regulation of gut microbiota.
Methods: Colon carcinoma cell line (CT-26) cells were inoculated subcutaneously to set up a tumor-bearing mouse model. After 14 days of GCP treatment, the tumors were stripped to estimate the tumor inhibition rate. The differentiation of intestinal epithelial γδT cells was detected using Flow Cytometry. Feces were collected for 16SrRNA high-throughput sequencing to detect the composition change of gut microbiota after GCP treatment. The mechanism was further verified by depleting gut microbiota through a combination of antibiotic treatment and fecal transplantations.
Results: GCP could effectively inhibit tumor growth and promote the number of IFN-γ+γδT in intraepithelial lymphocytes. The 16SrRNA high-throughput sequencing result showed that the gut microbiota composition changed significantly after GCP treatment. After depleting the gut microbiota, the anti-tumor effect of GCP was abolished. Fecal transplantation experiments found that recepient tumor-bearing mice administrated the feces from GCP treated mice, to a certain extent, could inhibit tumor growth and promote the percentage of IFN-γ+ IELγδT.

DOI: 10.1002/eji.201970400

Conclusions: These results demonstrated that Glycyrrhiza Polysaccharides exert anti-tumor effects by changing gut microbiota composition and regulating the differentiation of IFN-γ+IEL γδT Cells.

P1156

The protective effects of acupuncture and drug combined anesthesia on immune system

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In the traditional Chinese medicine, acupucture has been used in clinical practice for over 2000 years, its curative effects have been recognized.Many evidence have shown that acupucture and drug combined anesthesia can reduce the stress of operation and regulate immune function compared to the traditional anesthesia. However, the mechanism still remains unclear. In this study, we stimulate points called zusanli with electroacupuncture in the process of mouse abdominal operation(EA group) which simulate the process of clinical application. Another two control groups were only accept operation(Non-EA group) and with no disposal(Control). 4 days after operation, mouse sacrificed and we analyse the effect of operation and electroacupuncture on innate and adaptive immune cells separately. In innate immunity, proinflammatory cells include monocytes in the peripheral blood and peritoneal macrophages, all increased after operation. The number of NK cells decreased after operation. However electroacupuncture inhibit these events and induce more MDSC. In adaptive immunity, markedly, the number of CD8+ cells decreased after operation, moreover, the expression of Fas and FasL increased on T and B cells after operation, which suggested that operation lead to AICD (activated inducing cell death) and impair Cellular and humoral immune responses. As expected, these phenomena attenuated in EA group, moreover, the survival of either T cells or B cells in EA group is siginificantly inceased than Non-EA group when stimulated with bacteria. All together, these results may imply that electroacupuncture can inhibit inflammatory and has protective effects on immune cells in operation. The mechanism is still being studied.

P1157

5-FU-induced up-regulation of exosomal PD-L1 reflects immunosuppression in gastric cancer patients and might predict treatment efficacy

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Although 5-Fluorouracil (5-FU) chemotherapy has been thought to directly kill cancer cells and even play an immunostimulatory role, accumulating evidence indicates 5-FU also damages function of T cells through up-regulating programmed death-1-ligand 1(PD-L1), which is a negative regulator of T cell immune ability. Previous studies have shown that PD-L1 has exosomal forms secreted in the microenvironment, except for membrane bound forms and extracellular soluble forms. In addition, exosomal PD-L1 retains stronger immunosuppressive activity. However, whether 5-FU can change the expression of exosomal PD-L1 and induce immunosuppression is unknown. In the present study, we found that compared with the level at baseline, exosomal PD-L1 in the peripheral blood of gastric cancer patients was up-regulated gradually after 2, 4, 6 repeated cycles of fluorouracil treatment, accompanied by decreased concentration of cytokines IFN-γ ($p< 0.05$), TNF-α ($p< 0.01$), IL-2 ($p< 0.01$), IL-6 ($p< 0.05$) and GM-CSF ($p< 0.05$). Importantly, there existed statistical differences in the change of exosomal PD-L1 between responders and non-responders groups ($p< 0.05$). Furthermore, exosomal PD-L1 derived from MKN74 induced apoptosis of Jurkat T cells and inhibited the activation

DOI: 10.1002/eji.201970400

of T cells in PBMCs, which could be attenuated by nivolumab. These results suggested that 5-FU-induced up-regulation of exosomal PD-L1 caused immunosuppression in gastric cancer patients after multiple cycles chemotherapy, especially after 2 cycles.

P1158

The experimental study on the anti-inflammatory effect of eye-acupuncture on the formation of focal corpuscle in brain tissue of CIRI rats

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Objective：To observe the effect of eye-acupuncture on the formation of focal corpuscle, IL-1, IL-18 content, P2X7R, NLRP3, pro-caspase-1, ASC and caspase-1 expression in CIRI rats, and to explore the anti-inflammatory mechanism of eye-acupuncture on CIRI rats. **Methods：**Eighty rats were randomly divided into the Sham-operated group (15 rats) and the model group (65 rats);the modified suture method was used to replicate the CIRI model in rats of the model group,and the Sham-operated group was performed same operation, but the middle cerebral artery embolization was not performed. The model quality of CIRI rats was evaluated by neurological defect score, brain TTC and HE staining. After successful modeling, the model rats were randomly divided into model control group, eye-acupuncture group and inhibitor group. The expression levels of P2X7R, NLRP3, pro-caspase-1, ASC and caspase-1 in brain tissues were detected by the methods of immuneohistochemistry and western-blot. ELISA was used to detect the contents of IL-1β and IL-18 in cerebral ischemic penumbra tissues. **Results:** 1.A large number of pyroptosis cells were observed in the brain tissue of CIRI rats.2.Compared with the model control group, the contents of IL-1β and IL-18 in cerebral ischemic penumbra tissue of rats in eye-acupuncture group and inhibitor group decreased significantly, while the expressions of P2X7R, NLRP3, pro-caspase-1, ASC and caspase-1 decreased significantly ($p < 0.01$).**Conclusion：**In CIRI rats, the happening of pyroptosis was positively correlated with the expression level of cytokines. Eye-acupuncture therapy can inhibit the occurrence of pyroptosis by reducing the expression of cytokines and and play an anti-inflammatory role.

P1159

Epoxide micheliolide protects mice from carbapenem-resistant *Klebsiella pneumoniae*-induced sepsis by inhibiting inflammatory response via targeting Akt

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Sepsis is life-threatening organ dysfunction caused by dysregulated host response to infectionofbacteria, etc. Itis especially difficult to deal with when the pathogens are resistant to antibiotics.Recently, host-directed therapies (HDTs)aimedto control exacerbated inflammation and to relieve tissue damage are becoming available adjuncts to standard antimicrobial treatment.Epoxide micheliolide (EMCL) is epoxidizedfrom micheliolide, an anti-inflammatory natural compound found in *Michelia compressa*(Magnoliaceae). Here we demonstrated that EMCL reduced the expression of inflammatory cytokines and chemokines induced by lipopolysaccharide (LPS) in macrophagesanddendritic cells. Italso inhibited heat-killed carbapenem-resistant *Klebsiella pneumoniae* (CR-KPN)-induced IL-6 and TNF-αin macrophages. The levels of IL-6, TNF-α, MCP-1,

DOI: 10.1002/eji.201970400

IFN-βandMIP-2 in sera were inhibited by EMCL in LPS-challenged acute peritonitis mice. Accordingly, EMCL increased the survival rate in lethal CR-KPN-induced sepsis mouse model. Liver and kidney damage were relieved and the secretion of IL-6 and MCP-1 was also down-regulated by EMCL though it has no effects on the growth of CR-KPN.The combination of EMCL and levofloxacindecreased bacterial load in spleen and kidney. Results showed that EMCL decreased Akt and its downstream signaling after LPS stimulation. Furthermore, analysis of crystal structure confirmed that EMCL interacts via a pair of H-bonds to a deep pocket of Akt1 at Leu156 and Ala230 and the matchingscore was -7.79, showing that EMCL may be a potential immune-regulatory agent via targeting Akt. EMCLisa potential agent of HDT, treating severe bacterial infection and even sepsis caused by antibiotic-resistant bacteria.

P1160

Bortezomib-based regimens for primary multiple myeloma patients in China: from 2006 to 2018

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Since the approval of Bortezomib for the treatment of Chinese MM patients in 2006, the understanding of the efficacy and adverse reactions of the drug has gradually become clear, therefore the clinical application form also have corresponding changes and thereby affect the characteristics of the patient population who receiving treatment and the treatment effect. A total of 530 MM patients who received bortezomib-based treatment in 4 hematological disease treatment centers in China from February 2006 to August 2018 were enrolled in this study ,the general characteristics, treatment regimen, treatment course, efficacy, survival and adverse reactions of the patients in this group who received treatment before and after August 2013 were analyzed and compared.The results indicated that optimized treatment regimen and administration enable patients to complete more courses of treatment after 2013, the ORR was similar in this two group (88.6% vs 90.5%), but there was a higher rate of CR (39.1% vs 28.6%) and a better PFS after 2013.Subgroup analysis suggested that non-high-risk patients with D-S stage, ISS stage and R-ISS stage had significant PFS benefits in elderly patients aged 65 and above.Therefore, in the clinical practice in China,by reducing the financial burden of patients´ treatment and optimizing the treatment regimen, more patients can adopt the better regimen and receive more treatment, thus achieving better efficacy and survival.

P1161

Resveratrol enhances the efficacy of effector T-cells (Teff) function in tumor microenvironment to suppress colitis associated colorectal cancer

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Colorectal cancer (CRC) is the one of the most prevalent cancers and cause of cancer-related death and disability in the USmaking preventive strategies imperative. Despite of significant advances in diagnosis and treatment, many patients look for alternative treatment options including non-toxic dietary supplements to abate symptoms of colitis-associated CRC. To this end, a naturally occurring non-toxic compound, resveratrol (RES)has received much attention as a potent anti-cancer agent.

DOI: 10.1002/eji.201970400

CXCR3 and its ligands (CXCL9 and CXCL10) exert exquisite control over T-cells activation, differentiation and play an important role in mediating CD4 T-cell function. In this study, we investigated how RES abates CRC symptoms in azoxymethane (AOM) and dextran sodium sulphate (DSS)-induced models of CRC. RES effectively suppresses mucosal, peripheral activated T-cells, systemic and mucosal inflammatory cytokines as compared to vehicle. RES induces $CD8^+CXCR3^+$ regulatory T cells (Tregs) number and frequency and CXCR3ligands levels (CXCL9 and CXCL10) in the CRC tumor microenvironment as compared to vehicle. Further, RES reduces the incidence and size of colon polyps in DSS and AOM induced CRC as compared to vehicle. We also noticed that CXCR3 activation further induces the differentiation of CD4 T-cells into $CXCR3^+$Th1 cells and enhances Teff function in the tumor microenvironment. Taken together, these results indicate that RES modulates CXCR3 ligands at tumor sites to inhibit immunosuppressive property of Tregs that induces IFN-γ and effector T-cells function to reduce tumor size and incidence. Our results suggest that RES, a phytoestrogen, differentially mediates the peripheral vs tumor site CXCR3 expression and lessens colitis associated CRC.

P1162

Oral administration of Manuka honey leads to enhanced anti-tumor immunity in a syngeneic preclinical model of colorectal cancer

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Due to the adverse side effects associated with conventional anti-cancer therapies, the focus on using alternative natural products for cancer prevention and treatment has been increasing over the past few years. Among these natural products, manuka honey (MH) has been extensively researched. We previously demonstrated that MH can inhibit the growth of several cancer cell types including melanoma, breast adenocarcinoma, and colorectal cancer. In the current study, we investigated the effect of oral administration of MH on tumor growth using a syngeneic model of colorectal cancer. MH was administered daily by oral gavage starting 2 weeks prior to tumor implantation and continued for one more week after implantation. Control mice were given a mixture of the three main sugars found in MH, at equivalent concentrations. The results showed that tumor growth was significantly inhibited (~65%; p=0.025) in MH-treated mice, in a dose-dependent manner. In order to understand the underlying cause of MH-induced inhibition, tumors were excised and tissue sections analyzed by immunohistochemistry to quantify the number of tumor infiltrating leukocytes (TILs). The data revealed a 2.5-, 2.4-, and 1.7-fold increase in CD8 T cells, CD4 T cells and neutrophils, respectively, which were statistically significant compared to control mice. In addition, analysis of gene expression in tumor tissue by qRT-PCR highlighted a significant enhancement in expression of proinflammatory mediators in MH-treated group and decreased expression of immunosuppressive cytokines. These findings suggest that in addition to its demonstrated tumoricidal potential, MH may adversely influence tumor growth by boosting anti-cancer immune responses.

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DOI: 10.1002/eji.201970400

P1739

Regulation of Guiqi Baizhu decoction on immune factor and intestinal microflora in spleen deficiency mice

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Objective: To investigate the regulatory effect of Guiqi Baizhu Decoction on immune factors and intestinal flora in mice with spleen deficiency.
Methods: Sixty SPF mice were randomly divided into blank group, model group, high, medium and low dose group of Chinese medicine. The spleen deficiency model was established by gastric administration of rhubarb. After successful modeling, Guiqi Baizhu decoction was given to treat the mice (blank group and model group were given distilled water). The contents of AQP3 and AQP4 in small intestine tissue were detected by immunohistochemistry. The levels of serum immune factors IL-10 and IFN-gamma were detected by ELISA. 16SrDNA amplifier sequencing was used to analyze the changes of intestinal flora structure in feces.
Results: The protein content of AQP3 and AQP4 in model group was lower than that in blank group ($P < 0.05$). AQP3 and AQP4 in high, middle and low dose group were higher than those in model group ($P < 0.05$), and AQP3 and AQP4 in middle dose group were the highest. The levels of IL-10 and IFN-gamma in blank group, high, middle and low dose group were higher than those in model group ($P < 0.01$). Compared with model group, the diversity index and similarity coefficient ratio of blank group, high, middle and low dose group were higher ($P < 0.01$).
Conclusion: Guiqi Baizhu Decoction can correct the intestinal flora disorder of mice with spleen deficiency and restore their immune function in a dose-dependent manner.

P1740

Therapeutic effect of Nr-CWS in the treatment of high-risk human papilloma vrius persistengt infecction or cervical lesions with high-risk human papilloma vrius

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Objective: To study the therapeutic effect of Nr-CWS in the treatment of high-risk human papilloma vrius (HPV) persistengt infecction and cervical lesions with high-risk human papilloma vrius infecction.
Methods: 24 patients with high-risk HPV infection who admitted to Shengjing hospital from March 2016 to October 2017 were divided into 4 groups. The experimental group was given Nr-CWS for one course,20 days. The positive control group were given Baofukang or Andafen suppository for 3 months. The blank control group did not receive any special treatment. All patients were detected HPV and thinprep cytologic test (TCT) at 1st month, 4th month, 8th month and 12th month after treatment, colposcopy and histopathology were performed if necessary.
Results: The cure rate of HPV infection in experimental group was higher than positive control groups at 8th month($P< 0.05$). At 12th month ,the cure rate of HPV inection was higher in the experimental group than blank control group ($P< 0.05$).The abnormal TCT of the experimental group were all changed to normal at 1st,4th ,8th month,and didn´t aggravated, while in the other groups, TCT was aggravated to varying degrees, but the difference was not statistically significant($P>0.05$).There was aslo no significant difference in colposcopy and histopathological among the groups who underwent colposcopy and histopathological examination ($P>0.05$).
Conclusion: Nr-CWS has a certain therapeutic effect on the clearance of HPV infection, but effect on TCT, colposcopy and histopathological is not clear, which may require further research.

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DOI: 10.1002/eji.201970400

P1741

Efficacy and advantages of modified traditional Chinese medicine treatments based on "kidney reinforcing" for chronic aplastic anemia: a randomized controlled clinical trial

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Objective: Compare the efficacy of modified treatments based on "kidney reinforcing" in the management of chronic aplastic anemia (CAA).
Methods: 111 patients with CAA were randomly divided into three groups: kidney reinforcing alone (KA), "kidney reinforcing and Qi tonifying" (KQ), and "kidney reinforcing and blood circulation invigorating" (KC). Normal and positive control groups were also formed. All patients were treated for 6 months. Hemograms, Traditional Chinese Medicine (TCM) syndrome scores, and therapeutic effects were assessed, and changes in T-lymphocyte subsets, regulatory T cells and cytokines were detected.
Results: The KQ and KC groups had lower TCM syndrome scores than the positive control after 6 months ($P < 0.05$). The KQ group had a higher overall efficacy than the positive control after 3 months ($P < 0.05$), while platelet increased in the KC group after 6 months ($P < 0.05$). CD3+ T-lymphocyte ratios decreased only in the KQ group, while CD3+CD4+CD8− Tlymphocytes increased only in the KC group after 6 months ($P < 0.05$). Levels of interferon-γ, tumor necrosis factor-α, interleukin (IL)-2 and IL-6 decreased and levels of IL-4 and IL-10 increased in all treated groups after 6 months. Levels of IL-6 in the KQ and KC groups were lower than those in the positive control ($P < 0.05$).
Conclusions: Treatments based on kidney reinforcing have a rebalancing effect on cytotoxic and T helper cells, and regulate expression of interferon-γ, IL-2, IL-6 and IL-4. KQ may be more effective in treating CAA, and KC may have an advantage in platelet recovery.

P1742

Identification of a molecular target of anti-arthritic compound isolated from traditional Chinese medicine

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SNM is a purified compound isolated from Chinese Medicinal plant to treat arthritis. In the current study, we found that SNM not only ameliorated the progression of collagen induced arthritis (CIA), but protected joints from destruction in mice, indicating that SNM probably restricted the local inflammation of arthritic joints. As synovium in the joints is the major target tissue of rheumatoid arthritis, the synovium fibroblasts derived from RA patients (RASFs) were employed to validate the anti-arthritic effect and explored underlying mechanism of SNM. The results demonstrated that SNM significantly inhibited IL-6 and IL-33 secretion, COX-2 expression and ROS production in RASFs. Mass spectrometry results demonstrated that cysteine 26 (C26) and lysine 873 (K873) of C15 are a direct target of SNM. Underlying mechanistic study showed that knockdown C15 resulted in the accumulation and phosphorylation of p62 and aggregation of Nrf2 and HO-1 in cytoplasma of RASFs. Consistently, SNM activated p62/Nrf2 signaling in RASFs *via* covalently binding at C26 and K873 of C15. Furthermore, the collagen antibody- induced arthritis (CAIA) model was established in Nrf2-/- mice to verify the role of Nrf2 in the anti-arthritic effect of SNM, and the results explored that Nrf2-/- mice are resistant to the anti-arthritic effect of SNM. Our findings explored that C15 is an actionable

DOI: 10.1002/eji.201970400

drug target for anti-arthritis *via* activation Nrf2 signaling.
Acknowledgments
This work is supported by Macau Science and Technology Development Fund (0017/2018/A1)

P1743

Yu-Ping-Feng-San ameliorates recurrent allergic inflammation of atopic dermatitis by repairing tight junction defects of the epithelial barrier

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Background: Atopic dermatitis (AD) is a common allergic inflammatory disease with high recurrence rate. *Yu-Ping-Feng-San* (YPFS) clinically reduces AD relapse rate and recurring severity incidence. However, the underlying mechanism is still unknown and further study is needed.
Methods: A FITC-induced AD relapsing mouse model was established to evaluate the effects of YPFS and three components, claycosin, formononetin, and cimifugin, on recurrent allergic inflammation *in vivo*. HaCaT cells were utilized for *ex vivo* cellular analyses. Mice ear tissue inflammation were evaluated by H&E staining. Production of IL-4, IL-5, IL-13, and IgE in ear tissue homogenate or serum, and TSLP in culture medium was assessed by ELISAs. Tight junction (TJ) expression was detected by IHC and WB. Epithelial barrier integrity was observed with electron microscopy, transepithelial electric resistance (TER), and paracellular flux measurements.
Results: In the recurrent phase of AD, YPFS and three components remarkably reduced ear tissue inflammation and decreased IL-4, IL-5, IL-13, and IgE production. Decreased TER and increased FITC-dextran flux in air-liquid interface cultures of HaCaT cells were significantly repaired by YPFS and three components. Notably, the upregulated TJ (CLDN-1 and occludin) expression of epithelium was observed in YPFS and three components-treated mice. Restored TJ expression by three components was even detectable in the remission phase. Moreover, impaired TJ expression influenced the effects of YPFS on epithelium-derived TSLP production.
Conclusions: YPFS ameliorated recurrent allergic inflammation of AD by repairing TJ defects of epithelial barriers. Intervening epithelial barrier might be a preventive and therapeutic target for allergic inflammation diseases.

P1744

The effect of Invigorating kidney and activating blood to the mice with chronic aplastic anemia

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Objective: To explore the effect of Invigorating kidney and activating blood to the mice with chronic aplastic anemia（CAA）.
Methods: The mice of Chinese medicine group are given orally herbal soup , negative control group and normal control group are given orally the same dose of normal saline. The mice are observed daily life, tested the number of white blood cells, hemoglobin and platelets by using blood cell analyzer, detected serum VEGF and SDF-1a levels by ELISA, counted bone marrow nucleated cells under electron microscope, observed bone marrow pathological features through HE staining.
Results: The mice of Chinese medicine group and negative control group gradually show signs of weight loss, fatigue, dry and sparse hair, pale limbs and so on. With the Chinese medicine intervention

DOI: 10.1002/eji.201970400

time prolonged, the mice Chinese medicine group are recovered varying degree from above symptoms.When benzene reagent enters twenty-fifth times, compared with negative control group, the decrease of white blood cells, hemoglobin and platelets in group Chinese medicine is smaller($p< 0.05$), and bone marrow nucleated cells have a smaller reduction in Chinese medicine group($P< 0.05$), and the levels of serum VEGF and SDF-1a are higher in Chinese medicine group($P< 0.05$).HE staining shows that the degree of granulocyte, erythrocyte and megakaryocyte series is decreased less in Chinese medicine group than that of in negative control group.
Conclusions: Invigorating kidney and activating blood can improve the symptoms and recover hematopoietic of the mice with CAA. The possible mechanism is that the levels of serum VEGF and SDF-1a is increased.

P1745

LK7 increased NK-cell mediated lysis of non-small cell lung cancer cells by down-regulating CD155 and CD112

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Immunotherapy has revolutionized cancer therapy, however response rate between cancers and patients are varied due to many potential innate resistance mechanisms. Therefore, other immune checkpoint receptors become more important. CD155 (poliovirus receptor [PVR] or NECL5) is the ligands of TIGIT, which is one of the NK cell inhibitory receptors. High levels of CD155 expression are associated with a poor prognosis in cancer patients and CD155 is highly expressed on tumor cells in both human and mouse. LK7, a natural product extracted from Traditional Chinese Medicine, was found to increase NK cell-mediated lysis of non-small cell lung cancer cells. We then determined the degranulation level of NK cells, the expression of CD107a was increased in LK7 treated A549 and H1299 after incubated with NK cells but not in LK7 treated NK cells, indicating that degranulation of NK cells induced by LK7 was independent of PKC activation. The production of interferon-gamma and granzyme B which are the main cytotoxic factors critical for NK cells were further investigated. Results showed that LK7 could significantly increase the production of interferon-gamma and granzyme B in a dose-dependent manner. We next determined the mechanism of action. The inhibitory ligands, CD112 and CD155 were found to down-regulate in LK7 treated A549 and H1299 cells. Consistently, LK7 treatment increased NK cell-target cell conjugates in a dose-dependent manner. Taken together, LK7 could enhance NK cell recognition of target cells thus increased NK cell-mediated killing, suggesting the potential application of targeting CD155 function in NK-based immunotherapy.

P1746

Puerarin reduces hyperglycemia-induced inter-endothelial junction by interfering endothelial NLRP3 inflammasome signaling

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Background: Inflammatory-mediated endothelial dysfunction is a crucial mechanism for diabetes mellitus. Therefore, it's imperative to explore an effective method for inhibiting endothelial dysfunction by inhibiting inflammatory responses. Puerarin has been widely and successfully used to treat cardiovascular diseases in China for many years. In this study, we investigate the underlying

DOI: 10.1002/eji.201970400

mechanism of puerarin.
Methods: We examined the co-localization of NLRP3 with TXNIP and the formation of Nlrp3 inflammasome in mMVECs pretreated with hyperglycemia and puerarin using confocal microscopy approach. The monolayer cell permeability by immunofluorescence and ELISA analysis in endothelial cells. The expression of TXNIP, HMGB1, NLRP3, ASC, Caspase1 was detected by western blot. ROS was measured through immunofluorescence microscopy analyses and flow cytometry. ROS scavenger (APO) and Nlrp3 gene silencing were used to determine the roles of the ROS-Nlrp3 pathway involved in the molecular mechanism of Pu.
Results: Puerarin inhibit hyperglycemia-induced inter-endothelial junction which mechanism relative to decrease ROS-dependent activation of endothelial Nlrp3 inflammasomes.
Conclusions: Puerarin may developed as effective drugs for hyperglycemia-related cardiovascular disease.
Acknowledgement: The National Key Research and Development Program of China (No. 2017YFC1700400); The project supported by National Natural Science Foundation of China (NO.81603587, 81603668); Guangdong Natural Science Funds for Distinguished Young Scholar (No. 2018B030306027)

P1747

Aspirin alleviates LPS-induced noxious heat with blood stasis syndrome endothelial dysfunction through inhibition of NLRP3 inflammasome activation

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Objective: LPS-induced endothelial connective integrity disruption can lead to increased vascular injury, which is related to the activation of endothelial inflammasomes. In China, vascular injury is closely related to noxious heat with blood stasis Syndrome. There are evidences that aspirin can effectively prevent cardiovascular diseases. We hypothesized that aspirin could ameliorate endothelial injury by inhibiting the activation of NLRP3 inflammasome and ultimately prevent noxious heat with blood stasis Syndrome.
Methods: Endothelial cells were stimulated by lipopolysaccharide (2μg/mL) and administrated by 0.1~3 mmol/L aspirin. Western Blot, Immunofluorescence, Real-time Cell Detection, and other test methods were used to detect the Nlrp3 inflammasome relevant indexes. The Wild Type mice were stimulated with LPS (100μg/kg/d) and, 1 hour later, treated with aspirin (12.5, 62.5, or 125 mg/kg/d) and dexamethasone (0.0182 mg/kg/d) for 7 days. Plasma and heart were harvested for ELISA and immunofluorescence analyses.
Results: We found aspirin could inhibit NLRP3 inflammasome formation and activation in vitro with dose-dependent, and it has correlation between the NLRP3 inflammasome and the ROS/TXNIP pathway.We found that low-concentration aspirin could inhibit the formation and activation of NLRP3 inflammasome and restore the expression of the endothelial tight junction protein ZO1/2.We assume that aspirin can ameliorate the endothelial layer dysfunction by suppressing the activation of NLRP3 inflammasome.
Conclusions: Aspirin could alleviate LPS-induced endothelial dysfunction through the inhibition of NLRP3 inflammasome in the noxious heat with blood stasis Syndrome.
Acknowledgement: The National Key Research and Development Program of China (No. 2017YFC1700400); Guangdong Natural Science Funds for Distinguished Young Scholar (No. 2018B030306027)

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DOI: 10.1002/eji.201970400

P1748

Effect of du-moxibustion on the related Th1 and Th2 cytokines of collagen-induced arthritis

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Objective: To study the effect of du-moxibustion on the related Th1 and Th2 cytokines of laboratory rats with collagen-induced arthritis, and discuss its mechanism of action in the treatment of rheumatoid arthritis.
Methods：Sixty healthy and clean grade female Wistar rats aged 6-8 weeks were randomly divided into blank group、model group and du-moxibustion group. After 28 days of treatment with du-moxibustion, observe the changes of the body weight and pathological conditions of synovial membrane of the laboratory rats with CIA, meanwhile test the content of IL-6、TNF-α、IFN-γ、IL-4、IL-10、TGF-β in the serum by using ELISA method and the expression of IL-6、TNF-α、IFN-γ、IL-4、IL-10、TGF-β in synovial membrane by using immunohistochemistry assay.
Results: After the treatment with du-moxibustion, the body weight of the laboratory rats in du-moxibustion group are markedly increased and their temperament are much more tender when compared with the rats in model group. There are 5-6 observed cell layers of the synovial membrane(normally 1-3 cell layers can be observed), all of them had not showed inflammatory cell infiltration. In the serum, the content of IL-6、TNF-α、IFN-γ decreased remarkably($P< 0.05$), and the content of IL-4、IL-10、TGF-β increased significantly($P< 0.05$). In synovial membrane, the expression of IL-6、TNF-α、IFN-γ reduced remarkably($P< 0.05$), and the espression of IL-4、IL-10、TGF-β increased significantly($P< 0.05$).
Conclusion: Du-moxibustion can lower the expression of IL-6、TNF-α、IFN-γ and enhance the expression of IL-4、IL-10、TGF-β, thereby play a role in the treatment of rheumatoid arthritis by regulating the imbalance of Th1/Th2 ratio.

P1749

Exploring the material basis and molecular mechanism of Angelica treatment of tumor based on network pharmacology

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Objective: To study the material basis and molecular mechanism of Angelica treatment of tumor based on network pharmacology.
Methods: The bioactive components of Angelica and their corresponding targets are predicted by the BATMAN-TCM database. "Tumor" is used as a screening term to obtain all tumor-related targets in the HPO database. Then input the targets into the String database to obtain the "component targets-disease targets" interaction map and analyze the key targets, David database is used to perform the KEGG Pathway and Metascape database to analysis GO (gene ontology) biological process enrichment.
Results: A total of 81 bioactive components are obtained from Angelica corresponding to 1159 targets. From the tumor-related pathological phenotypic analysis 111 phenotypes are obtained corresponding to 321 disease targets, and the interactions between component targets and disease targets are analyzed to obtain the key targets . There are 17 key targets in the disease targets, including AKT1, ATM, PIK3CA, PTEN, etc., corresponding to 22 chemical components, namely: Angelicin, Beta-Myrcene, Ten Tetradecane, Beta-Elemene, Dodecenoic Acid, etc., All targets involves in direct tumor correlation, direct inflammation and tumor, inflammation-related signaling pathways,

DOI: 10.1002/eji.201970400

enriches in following GO terms: tumor, inflammation, DNA methylation and so on.
Conclusion: This study predicts the molecular mechanism and material basis of Angelica treatment of tumor based on network pharmacology. 22 effective drug components corresponded to 17 key therapeutic targets are obtained which are enriched in tumor and inflammation-related pathways. It provides a scientific basis for Angelica treatment of tumor and ideas for subsequent experimental research.

P1750

Anti-inflammatory effects of MXSGD on pulmonary macrophages infected with influenza A virus via TLR2/TLR4-MyD88 signal pathways

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The “cytokine store” and excessive inflammation triggered by influenza A virus(IAV) are responsible for its high mortality. In this study, we investigated the anti-inflammatory effects of Maxingshigan Decoction (MXSGD), a classical Chinese herbal formula, on pulmonary macrophages infected with influenza A virus(IAV) through TLR2/4-MyD88 signaling pathway. MTT method was used to detect the cytotoxicity of MXSGD-containing serum on pulmonary macrophages. IAV-infected macrophages were cultured with MXSGD-containing serum for 24h and 48h, using the TLR2/4 activator and inhibitor groups as control. The secretion levels of IL-1, IL-8, TNF-α/β in cell culture supernatants were detected by ELISA, the expression levels of TLR2, TLR4 and MyD88 were detected by Western Blotting. Compared with the corresponding concentration of fetal bovine serum groups, 40%, 20%, 10%, 5% MXSGD-containing serum was not toxic on macrophages. Compared with the normal group, the levels of IL-1, IL-8 and TNF-α were increased significantly in activator groups, IAV infected group (P< 0.05). Meanwhile, the levels of these proteins were decreased significantly (P < 0.05) in MXSGD group compared to IAV infected group at 24h. Compared with the normal group, the levels of TLR2, TLR4 and MyD88 in activator groups, IAV infected group were increased significantly(P< 0.05).The levels of these proteins were decreased significantly (P< 0.05) in MXSGD group compared to the IAV infected group at 24h. No statistical significance was found in the above indicators at 48h. This study suggested that MXSGD can downregulate the over-activation of TLR2/4-MyD88 pathway to inhibit the over-expression of proinflammatory cytokines which induced by IAV.

P1751

Effect of MXSGD on the expression of chemotactic factors of CCL3 and CCL25 in mice infected by influenza virus

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Objective: To explore the effect of MaxingShigan Decoction（**MXSGD**）on the expression of chemokine CCL3,CCL25 in lung tissue of influenza virus infected mice**.**
Methods: The mouse model of influenza virus pulmonary infection was established by nasal inoculation. The body weight and lung tissue were measured and calculated after intervention of MXSGD 3 days and 7 days later.The levels of CCL3 and CCL25 in serum were detected by ELISA, meanwhile the expression of these proteins in lung tissue was analysed through Immunohistochemical and Western blotting .
Results: The lung index of model control group was significantly higher cmpared to normal control group both at 3days later or 7days later of administration(P < 0.05) . It was consistent with the results

DOI: 10.1002/eji.201970400

in MXSGD at 3days later($P < 0.05$) , while not in 7days later($P > 0.05$) . The levels of CCL3 and CCL25 in serum was increased dramatically in the model control group compared to the normal control group($P < 0.05$), while it was descreased obviously in MXSGD group compared to the model control group at 3days later of administration($P < 0.05$). But it wasn´t significant difference at 7days later of administration($P > 0.05$). The results detected by IHC and WB in model group was significantly higher compared to normal group and MXSGD group at 3days later of administration($P < 0.05$) , while not in 7days later.
Conclusion: The lung index and the expression of CCL3 and CCL25 could be significantly reduced by MXSGD in mice infected by influenza virus.

P1752

Efficacy and prognostic analysis of R-DA-EPOCH/R-CHOP regimen in double-hit/double-copy diffuse large B-cell lymphoma

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Purpose: To compare the efficacy of R-DA-EPOCH with R-CHOP regimens in double-hit and double-copy diffuse large B-cell lymphoma (DLBCL) and to contrast the prognosis of these two disease types.
Methods: We retrospectively examined 127 cases of newly diagnosed DLBCL, and used fluorescence in situ hybridization (FISH) to detect genetic abnormalities in *MYC, BCL2,* and *BCL6*.
Results: In two schemes, the 2-year progression-free survival (PFS) was higher for R-DA-EPOCH than R-CHOP (79.8% *vs*. 57.5%, p=0.002), this advantage was also reflected in 2-year overall survival (OS) (81.6% *vs*. 58.5%, p=0.002). Subgroup analysis showed that in 26 patients with DHL, the 2-years survival of R-DA-EPOCH was better than R-CHOP (PFS: 73.0% *vs*. 28.1%, p=0.001; OS: 68.4% *vs*. 38.2%, p=0.047). In 101 cases of double-copy DLBCL, there was also a statistically significant difference between two regimens (PFS: 82.8% *vs*. 63.2%, p=0.007; OS: 85.3% *vs*. 65.0%, p=0.010). For two disease types, the PFS for DHL was inferior to that for double-copy DLBCL (52.9% vs. 72.4%, p=0.008), while the OS was not significantly difference (52.9% *vs*. 75.2%, p=0.050). Multivariate analyses showed that in DLBCL with genetic abnormality detected by FISH, the treatment and disease types were independent prognostic factors. The adverse reaction were similar in R-DA-EPOCH and R-CHOP (p>0.05).
Conclusion: In DHL and double-copy DLBCL, DHL had a worse prognosis than double-copy DLBCL. R-DA-EPOCH was superior to R-CHOP and which was well tolerated. Treatment regimens and disease types may be independent prognosis factors.
Keywords: R-DA-EPOCH; R-CHOP; DHL; double-copy; diffuse large B-cell lymphoma, efficacy and prognosis

P1753

Berberine alleviates influenza viral pneumonia by inhibiting the NLRP3 inflammasome-induced pyroptosis

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Pyroptosis is a proinflammatory form of cell death that is associated with pathogenesis of many inflammatory diseases. Berberine has been reported to possess anti-inflammatory properties by inhibitng inflammasome activation. However, the effects of pyroptosis on inflammation and berberine on inflammasome-induced pyroptosis in influenza viral (IV) pneumonia remain unknown. Here, we

DOI: 10.1002/eji.201970400

demonstrated that pyroptosis induced by NLRP3 inflammasome was involved in the development of lung inflammation in IV-infected mice. MCC950, as a specific inhibitor of NLRP3 inflammasome, inhibits pyroptosis by preventing the formation of inflammasome. In this study, MCC950 reduced IV-induced mortality, ameliorated inflammatory cell infiltration and decreased IL-1β, IL-18 levels in lung tissue, but had no effect on virus reproduction. We also proved that berberine can alleviate lung inflammatory injury by inhibiting NLRP3 inflammasome-induced pyroptosis. Berberine caused the decrease of virus reproduction only at an early stage (day2) and had obvious therapeutic effect on IV-infected mice at all time points (day2/4/6). Furthermore, berberine markedly decreased the mRNA and protein expression of GSDMD, which is the symbolic protein of pyroptosis, and reduced the content of amino terminal of GSDMD (GSDMD-N). Free GSDMD-N is the main domain of GSDMD leading to pyroptosis and it is produced as a result of the cleavage of GSDMD by activated NLRP3 inflammasome, which content and activation were also inhibited by berberine. Our data reveal the function of berberine on pyroptosis in IV pneumonia, suggesting a new potential mechanism for berberine to treat excessive inflammation caused by IV infection.

P1754

Effect of Litsea cubeba oil on breast cancer and its relationship with EGFR/PI3K/Akt pathway

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Objective: To study the in-vitro inhibition and its mechanism of Litsea cubeba oil on MDA-MB-231 cells of human breast cancer.
Methods: MDA-MB-231 cells were treated with different concentrations of Litsea cubeba oil for 12、24and 36 hours. The cytostasis rate was measured by CCK-8 method. Flow cytometry (FCM) was used to analyze cell cycle and apoptosis rate. Tile expression levels of Bax, Bcl-2, caspase-3, EGFR and p-AKT proteins of MDA-MB-231 cells were detected after cultured for 12h with difierent concentrations.
Results: MDA-MB-231 cells were significantly inhibited by Litsea cubeba oil in dose and time dependent manner. Cell cycle analysis indicated that the percentage of MDA-MB-231 cells was blocked at S/G2 phase . The results also indicated that Litsea cubeba oil could induce apoptosis of MDA-MB-231 cells in dose and time dependent manner. Litsea cubeba oil also up regulated the expression of Bax、p-caspase-3 protein and down regulated the expression of Bcl-2、EGFR、p-AKT protein($P< 0.05$).
Conclusion: Litsea cubeba oil can obviously inhibit the proliferation of MDA-MB-231 cells in vitro and the inhibition of Litsea cubeba essential oil on the proliferation of MDA-MB-231 cells may be related to its induction of cell cycle arrest and apoptosis, and its mechanism may be related to the inhibition of EGFR/PI3K/Akt signaling pathway
Keywords: Litsea cubeba oil；Breast cancer；Signaling pathway

P1755

Berberine ameliorates atherosclerosis and inhibites inflammatory reaction by regulating TLR4/NF-κB signaling pathways in ApoE$^{-/-}$ mice

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DOI: 10.1002/eji.201970400

Atherosclerosis(AS) is a chronic inflammatory vasculopathy characterized by lipid accumulation and inflammatory cell infiltration. Coronary heart disease caused by AS remains the leading reason of death in industrialized countries. Berberine (BBR) is used to treat obesity, diabetes and atherosclerosis. The potential role of BBR in modulating inflammatory reaction is not fully clear because the oral bioavailability of BBR is poor. The aim of the present study was to evaluated the therapeutic effects of BBR and its anti-inflammatory mechanism in apolipoprotein E-deficient mice ($ApoE^{-/-}$). The results indicated that BBR significantly decreased the aortic plaque area of $ApoE^{-/-}$ mice with high fat diet compared with Untreated group. BBR treatment downregulated the serum concentrations of total cholesterol(TC), triglyceride(TG) and low-density lipoprotein-cholesterol(LDL-C), and the expression of inflammatory factors including interleukin (IL)-6, IL-1β and tumor necrosis factor-α(TNF-α) in $ApoE^{-/-}$ atherosclerotic model mice. Moreover, the expressions of TLR4 and MyD88 protein as well as the phosphorylation of IκBα and NF-κB p65 were significantly reduced in arterial tissues of mice treated with BBR. Taken together, these results suggested that BBR could ameliorates atherosclerotic inflammation by regulating and controlling the TLR4/MyD88/NF-κB pathway. The research will afford experimental basis for the application BBR in the treatment of atherosclerosis and other chronic inflammatory disease.
Keywords: atherosclerosis, Berberine, inflammatory, TLR4/NF-κB

P1756

Tian Immune Booster: traditional therapy with clinical benefits

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Tian Immune Booster (TIB) is a Chinese herbal medication used clinically in central Africa, mostly in Kenya, to manage HIV by reducing viral load. TIB is also used for anticancer therapy and a number of other ailments. Clinical evidence on the immune boosting properties of TIB exists. In vitro studies on the antioxidant potential and antimicrobial study have been conducted, and preliminary anti-HIV data is also available. An in vitro study on the anticancer potential of TIB is underway. The available scientific findings suggest that TIB extracts have immune boosting properties, supporting the traditional use of the product.
Keywords: Tian Immune Booster, antioxidant, anti-HIV, anti-cancer

P1757

Efficacy and underlying mechanisms of sinomenine in treating rheumatoid arthritis

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Sinomenine (SIN) is an alkaloid derived from the Chinese medicinal plant, *Sinomenium acutum* (Family Menispermaceae). In a randomized controlled trial, oral administration of sinomenine for 6 months demonstrated similar effect to MTX in rheumatoid arthritis patients in ameliorating morning stiffness, grip strength, joint swelling, tender and joint count index, ESR, and rheumatoid factor with

DOI: 10.1002/eji.201970400

significantly lower rate of ADR. In an open-label, 24-week, parallel randomized controlled trial, 65.3% of patients treated with MTX+SIN showed improved disease activity as determined by the ACR50 response at week 24 compared to 69.6% of patients treated with MTX+LEF. Notably, significant reductions in gastrointestinal adverse reactions and liver toxicity were found in patients treated with MTX+SIN compared to patients treated with MTX+LEF ($p< 0.05$). Our pharmacological studies showed that sinomenine significantly ameliorated the clinical signs, such as hind paw swelling, arthritic scores and ESR of adjuvant arthritis, antigen-induced arthritis and collagen-induced arthritis in rats. These actions are closely associated with the inhibition of proliferation and activation of lymphocytes and macrophages, reduction in the secretion of inflammatory mediators in macrophages and arthritic animal models, including LTC_4, PGE_2, NO, mPGES-1, TNF-α, IL-1, IL-6, and IL-8, attenuation of the productions and activities of MMP-2 and MMP-9, augmentation of the activities of TIMP-1 and TIMP-3. Interestingly, sinomenine decreased PGE_2 levels without affecting prostacyclin PGI_2 and thromboxane TXA_2 synthesis via selectively inhibiting mPGES-1 expression. This may explain the low risk of cardiovascular event of sinomenine compared with NSAIDs.

P1758

A traditional herbal medicine formula reprograms melanoma immune microenvironment

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A traditional Chinese medicine formula comprising Sophorae Flos and Lonicerae Japonicae Flos (SL for short) was used for treating melanoma. We have previously shown that an ethanolic extract of SL (SLE) possesses anti-melanoma effects and suppresses STAT3 signaling *in vivo* and *in vitro*. STAT3 has been linked to the development of melanoma immunosuppressive microenvironment. In this work, we investigated whether SLE inhibits melanoma growth by reprogramming the tumor microenvironment in mouse and co-culture cell models. In B16F10 melanoma bearing mice, we found that intragastric administration of SLE inhibited tumor growth and angiogenesis. These observations were associated with the downregulation of protein levels of p-STAT3 (Tyr 705) and STAT3-targeted genes. We also observed decreased immunosuppressive cytokines and increased Th, Tc and dendritic cells in B16F10 melanomas of the SLE-treated mice. In a co-culture system composed of B16F10 cells and mouse splenic lymphocytes, we found that SLE inhibited the proliferation of and STAT3 activation in B16F10 cells, and increased the percentages of Th, Tc and dendritic cells. In the co-culture setting, SLE downregulated mRNA levels of STAT3-targeted genes in the splenic lymphocytes. Over-activation of STAT3 diminished SLE's effects in remodeling immune cell subsets and in lowering immunosuppressive cytokine levels in a co-culture system. These findings indicate that reprograming immune microenvironment, partially mediated by inhibiting STAT3 signaling, contributes to the anti-melanoma mechanisms of SLE. This study provides pharmacological groundwork for developing SLE as a modern agent for melanoma prevention/treatment.

This work was supported by HMRF: 14150571; NSFC: 81673649; and GRF: 12125116.

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DOI: 10.1002/eji.201970400

P1759

Evaluation of the immunomodulatory activity of drugs in the form of beverages sold on the Côte d'Ivoire market

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Introduction: The plants using in therapy is very old and is currently experiencing renewed interest among the population. It is possible to use the entire plants or their products extracted (Marc, 2001). This recourse to traditional medicine is mainly based on the idea that plants are a natural means of treatment without any risk. Consumers often believed that natural is synonymous of harmless. But a plant can be both useful and toxic. For most of the past decade, WHO has been working on developing international guidelines and technical standards to help countries to formulate policies and regulations to control the traditional medicines. On the other hand, very few scientific tests are carried out to evaluate the products and practices of traditional medicine.
General objective: This study is to evaluate the immunomodulatory activity caused by some of traditional drugs sold on the markets in Côte d´Ivoire.
Methodology: To do the inventory of traditional medicines in the form of beverages sold on the markets,
To determine the different secondary metabolites in identified drugs,
To evaluate the immunomodulatory activity of the identified drugs.
Expected results :
Traditional medicines in the form of beverages sold in Abidjan markets are listed
The different secondary metabolites of beverages and their immunomodulatory activity are known
Conclusion: Traditional medicines sold on the Ivorian market could be used to boost the immune system
Key words: immunomodulatory, secondary metabolites

P2333

Brief talk about the relationship between Yetianshi' beneficial to Qipulse approach and immune reconstruction

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In modern times, under the promotion and influence of science and technology, medicine continues to elicit various problems under the micro-angle of view. Recent animal experiments have shown that intestinal microbiota can not only regulate mucosal immunity, but also promote systemic immunity and hematopoiesis. Depletion of intestinal biota can resulting in impaired recovery of lymphocytes and neutrophils,and reduction dietary energy intake and visceral fat storage. Many a clinical of Clostridium diffcil infection also focus on the relationship between normal intestinal biota and immunity, thus putting forward the treatment approach of fecal bacteria transplantation. A great febrile disease master YeTianshi not only had provide his experience of ancient fecal bacteria transplantation in his treatise. But also addressed to a new treatment of beneficial Qi-pulse for a long illness,inordinately fits with the exploration of immune reconstruction nowadays. This paper, by combing the intestinal flora, immune reconstruction and YeTianshi practice,aiming for establish an efficacy and safety spectrum effect relationship and soughing new breakthrough for clinical dilemma.

DOI: 10.1002/eji.201970400

P2334

Protective effect of isoflavones extracted from *semen sojae praeparatum* on endothelial cell model induced by oxidative damage

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Soybean isoflavones are common phytoestrogens, which can delay the process of oxidative stress injury of vascular endothelium by binding estrogen receptors on vascular endothelial cells and regulating the antioxidant system in vivo.*Semen Sojae praeparatum* is a kind of traditional Chinese medicine which is fermented from soybean. The content of isoflavones in *Semen Sojae praeparatum* was significantly higher than that in soybean. Isoflavones were extracted from *Semen Sojae praeparatum* made from fermented soybeans by Bacillus cereus. The oxidative damage model of EA. hy926 cells induced by ox-LDL to evaluate the effect and mechanism of isoflavones. **The results showed that isoflavones extracted from *Semen Sojae praeparatum* could regulate oxidative damage of EA. hy926 cells induced by ox-LDL, such as reducing the levels of LDH, MDA and ET-1 in cells, and increasing the activity of SOD. Moreover, these changes are closely correlated with the expression levels of some protein of Nrf2/ARE signaling pathway.** It is significant for further understanding of the pharmacological effects and utilization of *Semen Sojae praeparatum.*

P2335

Compounds from *Cohosh* ameliorate autoimmune hepatitis by synergistically suppressing Th1 immune response

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Autoimmune hepatitis (AIH) is a severe necrotizing inflammatory liver disease associated with significant mortality. The progression of disease relies heavily on the exorbitant Th1 immune response. The mutual regulation of IFN-γ from T cells and interleukin (IL)-12 from myeloid cells creates a positive feedback loop to promote and maintain Th1 immunity. Here, we tried to investigate how Cohosh, a natural medicine, attenuates Th1 immunity to improve AIH. We challenged C57BL/6 mice with Concanavalin (Con) A followed by oral treatment with or without Cohosh or its compounds, including one phenol compound A, two triterpenoid saponin compounds X and Y. Cohosh dramatically increased the survival rate of Con A-challenged mice with decreasing the serum levels of ALT and AST, reducing the serum levels of IL-12 and IFN-γ within 10 hours after Con A injection. In T lymphocytes, phenol compound A or combination of triterpenoid saponin compounds X and Y repressed IFN-γ production by inhibiting phosphorylation of STAT4. In macrophages, triterpenoid saponin compounds X or Y controlled lipopolysaccharide-induced IL-12 secretion through NF-κB and MAPK pathway, while phenol compound A inhibited IFN-γ-induced amplification of IL-12 secretion by blocking STAT1 signals. Consistent with above results, the three compounds showed synergistically therapeutic effect on Con A-induced AIH. Consistently, combination of three compounds from Cohosh significantly decrease the serum levels of ALT and AST in Con A-challenged mice. Thus, Cohosh and the active compounds alleviated Con A-induced AIH by block the feedback loop of Th1 immunity.

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DOI: 10.1002/eji.201970400

P2336

Astragalus polysaccharides ameliorates DSS induced colitis in mice by regulating gut microbiota

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Inflammatory bowel disease (IBD) is a chronic inflammation of the gut mucosa. The considerable role of the gut flora in IBD pathology presumes a new therapeutic approach to restore it and treat IBD. Astragalus polysaccharide (APS) is a bioactive extract of Astragalus membranaceus (AM) and used in traditional Chinese medicine as a tonic remedy. The beneficial effects of APS on immune-based pathologies such as inflammatory bowel disease (IBD) have been well reported. However, their exact mechanisms have not been fully elucidated. We evaluated the therapeutic effect of APS and its potential mechanisms in a mouse model of dextran sulfate sodium (DSS)-induced colitis. The APS treatment have significantly improved the disease activity index (DAI) of colitis and histological scores, as well as a significant increase in weight and colon length in colitis mice as compared to the control group. Finally, the gut microbial profiling based on the Illumina MiSeq platform, 16S rRNA sequencing of the mice gut contents demonstrated that the gut microbiota structure remarkably changed in the model group, was restored to a certain degree by APS treatment in treated groups compared to model and control. Astragalus polysaccharide selectively improved the growth of important bacteria *Lachnospiraceae, Cellulosimicrobium* and *Microbacterium*, while reduced the abundance of negative bacteria *Helicobacter, Erysipelotrichaceae* and *Enterococcus i*n colitis mice*.* These findings suggest that APS could be a promising therapeutic approach in inflammatory diseases such as IBD.
Keywords: Gut microbiota; Astragalus polysaccharide; Inflammatory bowel diseases (IBD); High-throughput sequencing

P2337

Suppression of tumor immune evasion is induced by natural herbal medicine mediated through the PD-1/PD-Ls signaling pathway

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Programmed death receptor 1 (PD-1) is induced on peripheral T and B cells upon activation. Interaction of PD-1 with its ligands PD-L1 (B7-H1) or PD-L2 (B7-DC) negatively regulates immune responses. Many human cancers have been reported to aberrantly express PD-Ls. It has been shown that tumor eradication can be accelerated by PD-1/PD-Ls blockade. Therefore, regulation of the PD-1/PD-Ls signaling might improve proliferation of T cells and thus allow for improved lysis of target cells. It would be a very attractive possibility for tumor immunotherapy. The present study investigated the mechanisms of the natural herbal medicine (NHM) in suppressing tumor immune evasion and in treating cancer diseases mediated by the PD-1/PD-Ls signaling pathway. Through both clinical trial and experimental research, the present study demonstrated the therapeutic effects of the NHM on human lung cancer. Meanwhile, using both the animal models and the cell culture system, the regulatory effects on expression of the PD-1/PD-Ls by the NHM were observed in melanoma. It showed that the NHM had the significant effects on human cancer, and it could inhibit the growth of tumor, decrease the symptoms, and improve the living quality of the host with cancer. It was reported that the NHM could inhibit the PD-1/PD-Ls pathway both in vivo and in vitro, and could enhance T lymphocyte proliferation and cytokine production. It indicated that the NHM could play a crucial role in

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DOI: 10.1002/eji.201970400

regulating the PD-1/PD-Ls signaling pathway, which might mediate its therapeutic effects in suppressing tumor immune evasion and in treating cancer diseases.

P2338

Design of polyhydroxyalkanoate-mycophenolic acid nanoparticles for systemic lupus erythematosus therapy with enhanced anti-inflammatory efficacy

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Autoimmune disorder is a kind of common chronic disease, which is difficult to cure throughout life. Small chemical drugs such as glucocorticoids, immunosuppressors and non-steroidal anti-inflammatory drugs have been widely used for the treatment of autoimmune disorders. However, these small chemical drugs suffer from poor solubility, short circulating half-life and adverse side effects, which lead to poor compliance to patients and limit the widely clinical use. One of the most effective strategies to extend the circulating time is loading drugs into nanocarriers to form nanomedicines, which is of particular interest for cancer and viral diseases therapy but seldom applied in autoimmune disorder treatment. Furthermore, current carriers have many drawbacks such as poor biocompatibility, low stability and over-complicated design. In this study, we developed an easy but general drug delivery platform based on the new polyhydroxyalkanoate terpolymer-poly(3- hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) (PHBVHHx). We reported the first example of PHBVHHx nanoparticle loaded immunosuppressant, ultimately being applied in systemic lupus erythematosus therapy. These nanoparticles are biodegradable, stable, and show improved pharmacokinetics, optimized biodistribution, low systemic toxicity and excellent in vivo therapeutic efficacy in the MLR/lpr murine model of systemic lupus erythematosus. This delivery system may provide a new and general platform for the development of nanomedicines with enhanced therapeutic efficacy and reduced side effects.

P2339

Astragaloside Ⅳ regulates differentiation and induces apoptosis of activated $CD4^+$ T cells in the pathogenesis of experimental autoimmune encephalomyelitis

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$CD4^+$ T cells, especially T-helper (Th) cells (Th1, Th2 and Th17) and regulatory T cells (Treg), play pivotal role in the pathogenesis of multiple sclerosis (MS), a demyelinating autoimmune disease occurring in central nervous system (CNS). Astragaloside IV (ASI, CAS: 84687-43-4) is one of the saponins isolated from *Astragalus membranceus*, a traditional Chinese medicine with immunomodulatory effect. So far, whether ASI has curative effect on experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and how it affects the subsets of $CD4^+$ T cells, as well as the underlying mechanism have not been clearly elucidated. In the present study, ASI was found to ameliorate the progression and hamper the recurrence of EAE effectively in the treatment regimens. It significantly reduced the demyelination and inflammatory infiltration of CNS in EAE mice by suppressing the percentage of Th1 and Th17 cells, which was closely associated with the inhibition of JAK/STAT and NF-κB signaling pathways. ASI also increased the percentage of Treg cells in spleen and CNS, which was accompanied by elevated Foxp3. However, *in vitro* experiments disclosed

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DOI: 10.1002/eji.201970400

that ASI could regulate the differentiation of Th17 and Treg cells but not Th1 cells. In addition, it induced the apoptosis of MOG-stimulated $CD4^+$ T cells probably through modulating STAT3/Bcl-2/Bax signaling pathways. Together, our findings suggested that ASI can modulate the differentiation of autoreactive $CD4^+$ T cells and is a potential prodrug or drug for the treatment of MS and other similar autoimmune diseases.

P2340

Paeonol inhibits pancreatic cancer cell migration and invasion through the inhibition of TGF-β/Smad signaling and Epithelial-mesenchymal-transition

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignant tumours with growing incidence worldwide. The treatment outcome has not improved over the past decade. Thus, an urgent need remains to identify a novel therapeutic approach for patients with metastatic disease. Paeonol, a natural product derived from the root of *Cynanchum paniculatum* (Bunge) K. Schum, has attracted extensive attention for its anti-proliferation effect in the recent years. However, the anti-metastatic effect of Paeonol, especially in PDAC and the underlying mechanisms of action lack conclusive evidence.
Methods: Cell viability and apoptosis were evaluated by MTT and Annexin V-PE/7AAD double staining. Migration and invasion capabilities were examined by cell scratch-wound healing assay and Chamber assay. Western Blot and qRT-PCR were used to measure the protein and RNA levels of Vimentin, E-Cadherin, N-Cadherin, and TGF-β/Smad signaling.
Results: In the present study, the results showed that at non-cytotoxic doses, Paeonol showed significant migration and invasion inhibition on PDAC cells ($p< 0.01$). Paeonol inhibits Epithelial-mesenchymal-transition of PDAC cells through TGF-β/Smad signalling pathway and inhibits the TGF-β/Smad signalling pathway.
Conclusion: These findings revealed that Paeonol could suppress proliferation and inhibit migration and invasion in PDAC cells, at least partially due to its anti-inflammatory effect. The underlying mechanism of its anti-cancer effect might involve the inhibition of TGF-β/Smad signaling and EMT. Our study provided scientific evidence for the potential therapeutic value of Paeonol in the treatment of pancreatic cancer, yet, further in vivo evaluation is warranted to further elicited the interaction between tumour inflammatory environment and metastasis.

P2341

Scoring system for tumor-infiltrating lymphocytes and its prognostic value for gastric cancer

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The tumor microenvironment (TME) is the internal environment of malignant tumor progression, and the host antitumor immune response and normal tissue destruction occur in the TME. Tumor-infiltrating lymphocytes (TIL) is a crucial component of the TME and reflect the host antitumor immune

DOI: 10.1002/eji.201970400

response. The purpose of this study was to discuss the methodology for TIL evaluation and assess the prognostic value of TIL in gastric cancer. In total, 1033 gastrectomy cases were reviewed between 2002 and 2008 at the Third Affiliated Hospital of Soochow University. TIL were assessed by optical microscopy and verified by immunohistochemistry. There is no current consensus on TIL scoring in GC. In this study, we discussed a TIL evaluation system that includes an analysis of the amount and percentage of TIL in a tumor. Ultimately, there was a statistically significant relationship among TIL, tumor size, histological grade, LN metastasis, nerve invasion, pTN stage. The high level of TIL was a positive significant predictor of overall survival (OS) in Kaplan-Meier survival analysis and multivariate Cox regression analysis. After surgery, patients with malignant tumors underwent chemoradiotherapy according to standard therapeutic guidelines based on TNM stage. The TNM scoring system cannot reflect the full information of TME; therefore, TIL can be used as a diagnostic supplement. We constructed a nomogram model that showed more predictive accuracy for OS than pTN stage. In summary, this study proves that high levels of TIL are associated with a positive prognosis and that TIL reflect the protective host antitumor immune response.

P2342

GRd could suppress LPS-stimulated proinflammatory factors production of macrophages through NF-kB and p38 MAPK pathway in DSS-mice

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Introduction: Inflammatory bowel disease has been dramatically increased in recent years. Over-reactive immune system may be one of the etiologies. Ginseng has been widely used in various clinical diseases, especially inflammation. Ginsenosides Rd (GRd), one of the main active steroid-like-saponin components of ginseng, has been reported to suppress inflammatory responses.
Objective: to investigate whether GRd could play an anti-inflammatory role in inflammatory regulation effect of macrophages in DSS-mice.
Methods:
1. DSS and DSS-GRd treated mice were assessed inflammation by their weight, colon length, DAI/Cooper scores and bowel inflammation by immunohistochemistry.
2. Intestinal macrophages and LPS stimulated peritoneal macrophages were obtained from mice, TNF-a, IL-6, IL-12/23p40, IFN-γ and critical factors of NF-kB, p38 MAPK, JNK and other pathways were tested by ELISA, Western-blotting and qRT-PCR.
3. After intestinal and LPS stimulated peritoneal macrophages were administrated with DSS and DSS-GRd in vitro, we measured inflammatory factors' expression, and critical factors of NF-kB, p38 MAPK by ELISA, Western-blotting and qRT-PCR.
Results:
1. GRd could relieve colonic inflammation of DSS-mice.
2. Intestinal macrophages and LPS stimulated peritoneal macrophages in GRd-DSS mice showed suppressed activation of NF-kB and P38MAPK pathways and reduced level of proinflammatory factors.
3. In vitro experiments showed, GRd also decreased proinflammatory factors' expression through NF-kB and P38MAPK pathways in intestinal macrophages and LPS stimulated peritoneal macrophages.
Conclusions: GRd could suppress LPS-stimulated proinflammatory factors production of macrophages through NF-kB and p38 MAPK pathway in DSS-mice. It may provide a new effective treatment for IBD in the future.

DOI: 10.1002/eji.201970400

P2343

Dahuang mudan decoction regulated the metabolism of rats with Crohn´s disease induced by TNBS/ethanol

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Background: Crohn's disease (CD) is a chronic and refractory inflammatory disease of the intestinal tract. Previous studies had showed the effectiveness of Dahuang Mudan Decoction (DMD) on patients or animals. which is a classical prescription deriving from *Synopsis of Golden Chamber* written by Zhongjing Zhang. However, its functional mechanism remains unclear.
Objective: This study is to investigate the effect of Dahuang Mudan Decoction on metabolism in CD rat, accounting for its functional mechanism.
Methods: Sprague-dawley rats were randomly divided into control, model, mesalazine and DMD group (7.56g/kg). Rats were given a mixture of TNBS and ethanol (90mg/kg) to induce CD except the control group. The changes of body weight, disease activity index (DAI), pathological changes of colon analyzed with H&E staining, and the level of IL-6 and TNF-α in serum were measured by ELISA to evaluate the therapeutic effect of DMD on IBD rats. Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS) was applied to analyzing the serum metabolic profile changes.
Results: DMD showed its well therapeutic effects on the CD rats. And 8 potential serum metabolic markers were found to be closely related with the treatment mechanism of DMD on CD.
Conclusions: The study proved that DMD might relieve CD by regulating the serum metabolomics.
Keywords: Dahuang Mudan decoction(DMD); Crohn's disease; UPLC-QTOF-MS; metabolomics.

P2344

Huangqin Decoction restored intestinal barrier function to relieve inflammatory bowel disease

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Therapeutic effect of Huangqin Decoction (HQD) on inflammatory bowel disease was well known. However, its molecular mechanism has not yet been determined. To investigate the mechanism of HQD, C57BL/6 mice was given 3% DSS to induce inflammatory bowel disease model. Abundance and diversity of intestinal flora were analyzed by high-throughput 16S rRNA gene amplicon sequencing. In addition, bacteria invasion in lamina propria of colon was measured with fluorescence in situ hybridization (FISH). FITC-Dextran was administrated by gavage, and fluorescence intensity in serum of mice was measured with flow cytometry. Transmission electron microscopy was used to observe microstructure of colonic epithelial tight junction. Protein and gene expression level of MLCK, pMLC2, Occludin, and ZO-1 were respectively detected by western blot and RT-PCR. Furthermore, Caco-2 monolayer cells were stimulated by TNF-α to imitate gut barrier dysfunction in vitro. We found that HQD regulated the abundance and diversity of gut microbiota, inhibited bacteria invasion in lamina propria of colon, restored intestinal epithelium permeability, protected integrity of colonic epithelial tight junction. Besides, the active ingredient of HQD inhibited the MLCK/pMLC2 signaling pathway, protected Caco-2 monolayer cells from gut barrier dysfunction induced by TNF-α. In conclusion, these findings suggested that Huangqin Decoction relieved inflammatory bowel disease, which is related with restoring the intestinal barrier function.
Keywords: Huangqin Decoction (HQD), inflammatory bowel disease, intestinal barrier function

DOI: 10.1002/eji.201970400

P2345

Anti-tumor effect and mechanism study of Astragalus Polysaccharide promotes dendritic cell maturation through TLR4 signaling pathwayy

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Background: Astragalus polysaccharides(APS) is widely used as an anti-tumor immunomodulator in clinic. Accumulated studies demonstrated that APS could induce dendritic cell (DC) maturation to exert anti-tumor effect. However, the mechanism remains unclear. Here, we investigated the anti-tumor mechanisms of APS inducing DC maturation through TLR4 signaling pathway.
Methods: In vitro, DC was treated with APS and the maturation of DCs, T cells activation and the killing ratio of tumor cells were detected. In vivo, DCs was injected into tumor-bearing mice and tumor-related assays were carried out.
Results: Flow cytometry and ELISA assays revealed that APS could increase $CD80^+CD86^+$, IL-6, IL-23, IL-12P-70 expression in DCs, which can promote T cell proliferation and IL-2 secretion. These effects were diminished in the presence of TLR4 inhibitor. Furthermore, Q-PCR results revealed that APS could stimulate TLR4, MyD88 and IKK2 expression, which are key molecules of TLR4-MyD88 dependent signaling pathway. Celigo® Image Cytometer assays indicated that APS induced DCs could increase the tumor killing ratio of CTL on tumor cells, and this effect was decreased by TLR4 inhibitor. In BALB/c tumor-bearing mice, APS induced DCs transplantation significantly decreased tumor growth and increased immune organ indexes, but the addition of TLR4 inhibitor inhibit the anti-tumor effect of DCs transplantation.
Conclusions: APS could induce DC maturation and enhance killing effect of T cells on tumor through TLR4 signaling pathway, which provide the theoretical basis for clinical application of APS and a new perspective for the development of new drugs.

P2346

Research on the molecular mechanism of promoting the apoptosis of human colorectal carcinoma cell line sw480 in vitro induced by stewed dried toad

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Colorectal carcinoma is a common digestive tract cancer with high morbidity and mortality. The traditional Chinese medicine of Toad has plenty of advantages in the field of the treatment of tumor, for example, multiple targets and less adverse reactions. The aim of our study was to investigate the mechanism of Stewed Dried Toad (SDT) promoting apoptosis of human colorectal carcinoma cell line SW480 cells; to explore the mechanism of action of SDT in inhibiting the epithelial-mesenchymal transition (EMT) of SW480 cells. Analyzed with flow cytometry, SDT (0.05, 0.25, 1.25, 6.25mg/ml) could efficiently induce apoptosis and necrosis in SW480 cells with a concentration-dependent and time-dependent manner. SDT could inhibit SW480 cells growth and arrest cells in S phase of cell cycle. SDT may stimulate the apoptosis and inhibit migration of SW480, its mechanism may be related to the decrease of expression of apoptosis-related proteins PD-L1, cytokine IL-6 and gene PD-1 and increase gene expression of Bax, Fas and Caspase 3. SDT could up-regulate the expression of E-cadherin mRNA and down-regulate the expression of β-catenin and Vimentin mRNA. These data suggest that SDT has an anti-tumor effect by promoting the apoptosis and inhibit the migration of SW480 partly by triggering apoptosis pathway and inhibiting the epithelial-mesenchymal transition.

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DOI: 10.1002/eji.201970400

SDT (10g/kg/d) does not have acute toxicity to mice. Our study provides a theoretical basis for the molecular mechanism of the treatment of colorectal carcinoma in SDT.

P2347

Development of an anti malarial- immunomodulator based combination therapy for Cerebral Malaria in mice

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There is an urgent need to develop combination therapy for Cerebral malaria as quinine and artemesinin based therapy are becomig ineffective. Using the C57Bl6-Plasmodium berghei ANKA model we are testing a combination of Artemesinin and other anti malarials together with the immunomodulator curcumin for effectiveness. Earlier work had shown that injection of Artether into infected mice followed by feeding of curcumin- PLGA nano particles was effective. We have developed artemisinin nano particles and synthesized (2E)-1-(4-Chlorophenyl)-3-[3-methoxy-4-(prop-2-en-1-yloxy)phenyl]prop-2-en-1-one (chalcone-9) a molecule capable of potentiating artemesinin activity and used another antimalarial Andrographolide. When all the three molecules were fed to infected mice on 2nd,3rd and 4th day after infection using nano artemisinin at 1mg/mice , chalcone 9 at 2mg/mice and Andrographolide at 1mg/mice the parasitemia was controlled and feeding nano curcumin at 3mg/mice on 5th and 6th day post infection restored the blood brain barrier breakage. All the treated mice survived for 30 days post infection without any cerebral malaria pathology but all infected untreated mice died after 8 days post infection. Separate experiment showed that nano curcumin can enter into brain as indicated by detection of fluorescence and can modulated the expression of several proteins like Rab11 family interacting protein and Retinoic acid induced protein 1 needed for survival of neurons. Analysis of brain tissue profile of infected mice without treatment and after treatment showed them to be very different. Therefore our combination therapy is effective in keeping the brain free of inflammation by restoring the blood brain barrier breakage.

P2348

Effect of Wuzi Yanzong pill on inflammatory response in Parkinson's disease mouse

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Background: Wuzi Yanzong Pill (WYP, a classical Chinese herbal formula) has a potentia a potential therapeutic effect on Parkinson´s Disease(PD) Mice, but the mechanism is not clear.
Objective: To explore the neuroprotective effect and inflammatory response mechanism of WYP on PD mice.

DOI: 10.1002/eji.201970400

Methods: C57BL/6 mice were randomly divided into model and WYP groups. PD models were established by MPTP. WYP group mice received WYP for 14 days.The behavior and autonomous activity were detected by average speed and rest time. The positive expression of TH was detected by Immunohistochemical method . The levels of cytokine IL-6, IL-10, IL-1β, and TNF-α in brain were detected by ELISA.
Results: Compared with the model group, rest time, expression of IL-1β, secretion of inflammatory factors IL-6 and TNF-α were decreased in WYP group. TH positive cells in substantia nigra, the total distance, the average speed of movement, the amount of autonomous activity and the expression of IL-10 were increased.
Conclusion: WYP can improve the activity of PD mice and protect dopaminergic neurons. Its mechanism may be related to reduce the secretion of inflammatory factors and promote the expression of anti-inflammatory factors.
(NNSF of China 81102552 and 81703978, Shanxi Scholarship Council project 2017-129 and 2017-19, Shanxi International Science and Technology cooperation project 201703D421016, Scientific and technological innovation team of integrated Chinese and western medicine for the prevention and treatment on nervous system diseases of Shanxi university of Chinese medicine 2018TD-012, Shanxi Key Laboratory project SXIDL-2018-04, Shanxi Key project 201803D31209.).

P2349

Circulating Th1 and Th17 frequency normalized by LiangXueJieDu decoction through stratified gene expression regulation in psoriasis patients

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Psoriasis is a T cell mediated chronic inflammatory skin disease. It can be differentiated into distinct patterns in TCM. Blood-heat is one of the psoriatic patterns and conventionally treated with LiangXueJieDu (LXJD) decoction, an effective formula in clinic. To decipher the molecular basis of psoriatic “blood-heat” and the genes responding to LXJD treatment, fifteen patients diagnosed as psoriasis vulgaris with blood-heat pattern were enrolled and assessed every two weeks on LXJD treatment for 8 weeks. Psoriatic patients were sub-grouped into the Th17-high and the Th17- low group, based on baseline comparison of circulating T cell subsets to that of the healthy controls. LXJD treatment either lowered or increased Th17 and Th1 frequency and reached normal at the week 8th, accompanied with improved psoriasis area and severity index (PASI) score. Network analysis of differentially expressed genes (DEGs) from RNA-sequencing of peripheral blood cells indicated that LXJD regulated gene expression of various originated immune cells in circulation, and that the preferential pathways regulated by LXJD were different between the two subgroups. *In vitro* assay indicated that LXJD play an inhibitory role on T cell differentiation. These findings demonstrate that psoriatic blood-heat could be a kind of inflammatory status, and treatment of psoriasis with LXJD may balance the inflammatory circumstance either through inhibiting the differentiation of effective T helper cells directly or through modulating gene expression of myeloid or granule originated cells.

(The work is supported by NSFC 81774046, BNSF 7325032)
Keywords: psoriasis, T cell differentiation, RNA sequencing, Chinese medicine, PASI

DOI: 10.1002/eji.201970400

P2350

Daikenchuto contributes to metabolic activity in a mouse colitis model

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Daikenchuto (DKT) is composed of ginger dried rhizome, pepper and ginseng, and has been used for the treatment of inflammatory bowel disease (IBD). IBD is characterized by chronic inflammation in the intestine, which is triggered by disrupted balance of gut microbes and host immune responses. Clinical evidence has shown that DKT is beneficial to the patients with Crohn's disease. However, the underlying mechanisms has not been well documented. We therefore examined the anti-inflammatory effects of DKT by focusing on the changes of gut metabolites using mice. Mice were pre-treated with DKT and colitis was induced with 1.5% dextran sulfate sodium (DSS) in drinking water. These mice were also kept under the DKT treatment during and after DSS process. Interestingly, DKT treatment significantly promoted the recovery of experimental colitis, whereas DKT untreated group showed a remarkably high histological score. There were no significant differences in the concentration of short chain fatty acids (SCFAs) in their feces. However, DKT treatment led to the decrease of lactate in the post-DSS phase to the initial level. At the same time, the colon lengths of DKT-treated mice were longer than those of non-DKT treated mice, suggesting that DKT affects the microbial community to keep gut homeostasis, and suppression of excessive microbial lactate by DKT may prevent the intestinal inflammation. Our results indicate that the anti-colitis actions of DKT, likely through altered microbial metabolite production, might provide an effective therapy for IBD.

P2351

Artemisia absinthium extract loaded nanoparticles induce apoptosis in breast cancer cells via inhibiting vesicular trafficking related proteins

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The limitations of the currently available anti-breast cancer agents like they exert side effects can be overcome by combining two approaches together i.e. loading of herbal drugs into the nanoparticles. Keeping in mind the tumor micro environment, double triggered (temperature and pH responsive) polymeric nanoparticles (NPs) were prepared and characterized by TEM, DLS and IR. The cytotoxicity of the different part extracts of *A. absinthium* was evaluated on the breast cancer cell lines (MCF-7 & MDA MB-231) by MTT and LDH assay. The extract with least IC_{50} value (whole plant extract) was loaded into the NPs and various cytotoxic parameters like MTT assay, CFSE cell proliferation assay, apoptosis assay, cell cycle study and DAPI nuclear staining was performed to evaluate cytotoxicity against breast cancer cell lines by. To further delineate the mechanism of action of extract loaded NPs, nano-LC-MS/MS analysis was performed. The cytotoxic studies together with proteome analysis suggest that extract loaded NPs effectively inhibits cell proliferation and induce apoptosis and cell cycle arrest in G0/G1 phase in both the breast cancer cell lines via significantly modulating the expression of the key proteins related to the proliferation, vesicular trafficking, apoptosis, and tumor suppression. In conclusion, encapsulation of the whole extract of *A. absinthium* causes apoptosis at very low concentration in breast cancer cell lines and the identification of differentially expressed proteins as potential therapeutic targets may paved the way for future research.

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DOI: 10.1002/eji.201970400

P2352

Vitamin D - containing natural extracts (pig liver and Shiitake mushroom) as modulators of the immune system

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Vitamin D is a well-known regulator of bone mineralisation and muscular function; however, the expression of the vitamin D receptor in multiple cell types suggests its extra-skeletal role. Vitamin D deficiency is associated with an increased risk for autoimmune diseases, as it can influence the activity of both innate and adaptive immune cells, finally favouring the development of tolerogenic responses. Vitamin D supplementation is currently prescribed for the treatment of hypovitaminosis D; however, the pharmacokinetic profile of vitamin D from natural sources underlines a longer therapeutic effectiveness and a better pharmacologic tolerance compared to synthetic supplements. The aim of this work was to characterize the immuno-modulatory properties of two natural extracts (pig liver, containing vitamin D3, and Shiitake mushroom, containing vitamin D2), with a focus on innate immune cells. A commercially available vitamin D supplement was also used as a control. Flow cytometric analysis and Elisa on THP1-derived macrophages cultured under M1/M2 polarizing conditions in the presence of the extracts showed a strong modulation of the expression of surface markers (CD80, CD197, CD36, CD206) and pro- and anti-inflammatory cytokines secretion (IL1β, IL6, TNFα, IL12, IL10). Moreover, the evaluation of the expression of surface markers (CD80, CD86, CD40 and MHCII) on monocyte-derived dendritic cells (DCs) suggests that the extracts can influence their maturation. Our results show that pig liver and Shiitake extracts retain the ability to modulate the immune system even better than the control and they could be therefore used for the prevention and treatment of vitamin D deficiency.

P2353

Synergistic antitumor effect of *Ganoderma formosanum* polysaccharides and 5-fluorouracil against colorectal cancer in mice

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Ganoderma is a renowned medicinal fungus that has been used for treating various diseases for centuries in Asia, and *Ganoderma formosanum* is a native species of *Ganoderma* isolated in Taiwan. Our previous studies showed that a polysaccharide fraction, PS-F2, purified from the submerged culture fluid of *G. formosanum* exhibited immunostimulatory and adjuvant activities. In this study, we investigated the antitumor effect of PS-F2 combined with 5-fluorouracil **(**5-FU) on the treatment of C26 colorectal tumor in mice. Our data showed that oral administration of PS-F2 alone could suppress the growth of established tumor, and combined treatment with PS-F2 and 5-FU further suppressed tumor growth. In the spleen, PS-F2 and 5-FU monotherapies resulted in a significant increase in cytotoxic T lymphocytes (CTL), which was further augmented by the combined treatment. Conversely, the accumulation of immunosuppressive polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) and regulatory T (T_{reg}) cells were significantly reduced by mono- and combined therapy of PS-F2 and 5-FU. In the draining lymph nodes, the combined therapy also resulted in an increases in interferon (IFN)-γ-producing $CD4^+$ and $CD8^+$ T cells. In the tumor microenvironment, the combined therapy significantly increased the CTL population and reduced the accumulation of PMN-MDSCs,

DOI: 10.1002/eji.201970400

Treg cells and tumor-associated macrophages (TAMs). The combined therapy also promoted the maturation of monocytic myeloid-derived suppressor cells (M-MDSCs) in the spleen and the tumor. Overall, our data indicate that PS-F2 has the potential to be used in adjuvant immunotherapy alone or in combination with chemotherapy for the treatment of cancer.

P3218

Statins inhibit progress and reduce risk of hepatocellular carcinoma by enhancing activity of CD8+ T cell

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Hepatocellular carcinoma (HCC) has a dismal 5-year survival rate, and to date there is no effective chemotherapy treatment. Metabolic syndrome has been proved associated with the incidence in many cancer, and it has been reported that statins would reduce HCC risk and prolong overall survival,while we notice the serum cholesterol and triglyceride of HCC patients are increasing. It suggests that statins may suppress tumor in some unknown way. So we plant HCC cell in mice to form xenografts and then give mice rosuvastatin, atorvastatin, simvastatin, pravastatin, fluvastatin, cerivastatin, and lovastatin,some clinically available drugs inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a key enzyme in the rate-limiting step in cholesterol synthesis. We obtain that all drugs reduce tumor happening and inhibit it growing in different degrees, while make mice lower serum cholesterol, in especially, atorvastatin perform the best. Furthermore, we examined the activity and number of tumor-infiltrating lymphocytes by flow cytomertry, discover the CD8+ T cell is increasing and more active than control, as well in IHC. In brief, recipient of statins not only prevents cardiovascular and cerebrovascular disease, but also protects user from HCC.
Highlight: cholesterol metabolism is important for tumor immunity

P3219

AZT sensitizes hepatocellular carcinoma cells to As2O3 by upregulating the arsenic transporter aquaglyceroporin9

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Background: To investigate the role which aquaglyceroporin 9 (AQP9), plays in the process of As2O3 combined with AZT in inhibiting growth, migration and the invasion of hepatocellular carcinoma cells.
Methods: HepG2 and MHCC97H cells were treated using As2O3 (2 µM) combined with AZT (0, 10, 20 µM), and the proliferation inhibition rates were detected. AQP9 mRNA/protein expression was determined by RT-PCR and WB, respectively. HepG2 cells were electro-transfected with AQP9 siRNA. The effect of the 2 µM As2O3 combined with 20 µM AZT on the migration and invasion of HepG2 cells was then measured before and after an AQP9-siRNA transfection.
Results: The proliferation inhibition rate from the combination of As2O3 and AZT on the hepatocellular carcinoma cells was significantly higher than the rate from As2O3 alone ($P< 0.05$), and it had a greater dose-dependent effect when it was compared to AZT. As2O3 (2 µM) combined with AZT (20 µM) also significantly inhibited the migration and invasion of liver cell carcinoma. The expression levels of AQP9 in the combination of As2O3 (2 µM) and AZT (20 µM) group were significantly higher than those from the As2O3 alone group and the blank control group ($P< 0.05$).

DOI: 10.1002/eji.201970400

After silencing AQP9, the effect of the combination between As2O3 and AZT was significantly reduced (P< 0.05).
Conclusions: AZT improves the efficiency of As2O3 on inhibiting the proliferation, migration and invasion of hepatocellular carcinoma cells by up-regulating the expression of AQP9.

P3220

Research progress on two-way Immunoregulation of crude drugs in past ten years

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Immunoregulation is a process in which body regulates occurrence, development and outcome of immune response through coordination of multiple systems, cells and molecules, so that immune response can be maintained at a moderate level, thus maintaining stability of the internal environment of body. Once obstacles occur to body´s immunoregulation mechanism, it may lead to body´s immune dysfunction, and cause related immune diseases. Drug therapy and cellular immunotherapy for immune diseases have been widely reported. But drug therapy is still the best choice for the treatment of immune diseases. Chemical drugs can only be used to relieve symptoms, maintain function and delay tissue damage. It is hoped that the stronger immunoactive ingredients can fundamentally make disease recovery by regulating the patient´s self-immunity. It has shown that some crude drugs containing polysaccharides, glycosides, alkaloids and flavonoids have two-way immunoregulation effects on body´s immune system in the past ten years. they can not only regulate two-way immunoregulation at different levels of organ, tissue, cell and molecule. Specifically, they can enhance body´s immune function by promoting functions of phagocytes, lymphocytes and mononuclear macrophages. At the same time, they can also inhibit the body´s immune function by inhibiting lymphocyte proliferation and reducing the release of cytokines. Through which mechanisms do these different crude drug components achieve immune regulation to the body? This paper will summarize the previous relevant research.

P3221

Andrographolide exerted its antimycobacterial effects and anti-inflammatory effects by upregulation of autophagy in Mtb-infected macrophages by inhibiting PI3K/Akt/mTOR pathway

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Mycobacterium tuberculosis (Mtb) remains a significant menace to global health as it induces granulomatous and systemic inflammatory responses during active tuberculosis (TB). TB control faces a shortage of new drugs. Adjunct host-directed therapy (HDT) based on both antimycobacterial and anti-inflammatory interventions could be exploited to improve treatment efficacy and outcome. Andrographis paniculata is a Chinese traditional herbal medicine used in the treatment of upper respiratory tract infections etl. Andrographolide (Andro) is the major active components. Autophagy is increasingly recognized as a major host immune defense mechanism against Mtb. In the present study, we showed that Andro could induce autophagy in THP-1 and cause increased killing of intracellular Mtb. Results also showed that Andro decreased the secretion of Mtb-induced inflammatory cytokines (IL-1β, IL-6 and TNF-α) in a dose-dependent manner. We investigated the key signaling pathways involved in Andro-induced autophagy and Andro-induced anti-inflammation. Western blot assays indicated that Andro decreased the levels of p-Akt and p-mTOR in THP-1 and mouse primary peritoneal macrophages. In addition, Andro exerted an obvious inhibitory effect on NLRP3 inflammasome activation and NF-κB activity. Moreover, immunofluorescence studies

demonstrated that Andro promoted the co-localization of NLRP3 inflammasome with autophagosome which may serve as the underlying mechanism of autophagic degradative effect on reducing NLRP3 inflammasome. Together, Andro induced the activation of autophagy in Mtb-infected macrophages through inhibiting PI3K/Akt/mTOR pathway. Andro induced autophagy and NF-κB inhibition contribute to decrease cytokine secretion and NLRP3 inflammasome activation. Therefore, Andro may be a potential drug candidate in the adjuvant HDT of TB.

P3222

Regulatory effect of Zhui feng tou gu capsule on TLR2/4-NF- κ β signaling pathway in rheumatoid rrthritis rats

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Objective: To observe the effect of Zhui feng tou gu capsule on the syndrome of cold-dampness obstruction in rats with rheumatoid arthritis and to explore its mechanism from the TLR2/4-NF- κ β signal pathway.
Methods: Type II collagen and artificial climate box were used to construct collagen-induced arthritis rat model of (CIA) cold-dampness obstruction. The model rats were randomly divided into six groups: blank group, model group, high, middle, low dose group and methotrexate control group. After 28 days of treatment, the temperature, pain threshold and swelling degree of rat joints were measured. The expression of CRP, ESR, RF, TLR2/4, NF-κβ in rat blood was detected by ELISA, and the expression of TLR2/4, NF-κβ in synovial tissue was detected by immunohistochemistry. RT-PC R was used to detect the expression of TLR2, TLR4, NF-κβ mRNA in synovial tissue.
Results: The capsule can reduce arthritis rat´s joint swelling degree, blood viscosity degree, arthritis index, raise the pain threshold, reduce the pain times, and at the same time, reduce the related inflammation index of rheumatoid arthritis in the blood. The expression of ESR,RF,IL-1 β, IL-6,TNF- α and IL-17 decreased the expression level of TLR2,TLR4,NF- κ β protein and mRNA in synovial tissue of the diseased joints, and there was no significant difference compared with that of methotrexate in western medicine control group.
Conclusion: Zhui feng tou gu capsule can improve the joint symptoms of rheumatoid arthritis with cold-damp arthralgia syndrome, and Its mechanism may be to regulate the TLR2/4-NF- κ β signaling pathway.

P3223

Effects of Xie-Xin-decotion containing serum on expressions of TLR9/MyD88/NF-κB p65 signal pathway in foam cell

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Xie-Xin-Decotion is one of the traditional antipyretic detoxification formula, which has purging fire and drying dampness effect. Modern pharmacological studies have confirmed that it has the resistance function to atherosclerosis.To investigate the effect of Xie-Xin-Decotion containing serum on ox-LDL-induced TLR9 signal transduction pathway in RAW264.7 macrophage -derived foam cells, and to explore its anti-atherosclerosis mechanism. RAW264.7 macrophages were induced by ox-LDL to construct a foam cell model. Xie-Xin-Decotion containing serum at different doses was prepared and

DOI: 10.1002/eji.201970400

applied to foam cells. The degree of foaming was observed by oil red O staining. The contents of IL-1β and INF-γ in cell supernatant were detected by ELISA. The mRNA and protein expression of TLR9,MyD88 and NF-κB p65 were detected by real-time PCR and Western Blot. The results indicated that Oil red O staining under the microscope showed that the red lipid droplets in macrophages induced by ox-LDL were obvious, showing typical foam cell morphology. Compared with the normal serum control group, the model group induced TLR9, MyD88, NF-κB p65 ,IL-1β,INF-γ expression after intervention with ox-LDL; compared with the model group, Xie-Xin-Decotion containing serum reduced TLR9, MyD88, NF-κB p65, IL-1β, INF-γ expression. Xie-Xin-Decotion containing serum can inhibit ox-LDL-induced RAW264.7 macrophage foaming, and its mechanism may involve regulation of TLR9/MyD88/NF-κB p65 signaling pathway and inhibition of IL-1β and INF-γ overexpression. This may be one of its mechanisms of against atherosclerosis.
Keywords: Xie-Xin-Decotion; Foam cells; TLR9 signaling pathway; Atherosclerosis; Oxidized low density lipoprotein

P3224

Astragalus polysaccharides reduced biological stability change of MSCs induced by inflammatory microenvironment

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Aim: To study the effects of Astragalus Polysaccharides (APS) on biological stability of MSCs in inflammatory microenvironment and associated inflammatory pathways mechanism.
Methods: The MSCs were grouped as follow: Control group (cultured regularly), Model group (treated with IL-6 and TNF-α) and APS group (treated with APS, IL-6 and TNF-α). After 6weeks, all of cells were assessed for systematical evaluating the biological stability.
Results: Compared with control, morphology results showed that the pathological mitosis and apoptosis appeared, and the percentage of cells expressing $CD24^+$, $CD133^+$, and $CD73^+$ changed in model group. Also, the mRNA expression levels of stemness genes changed, and the ability to differentiate into lipid and the GJIC function were descended in model group. There were more than 2 000 difference genes in model group. The expression level of key genes regulating cell proliferation, apoptosis and differentiation changed obviously compared to control. After injecting interfered MSCs 12 weeks respectively, there was no visible tumor growth in the injection site of nude mice, similar to control. However, APS reduced biological characteristic changes induced by IL-6 and TNF-α such as immunophenotyping, the ability to differentiate into lipid, the expression level of some genes and so on.
Conclusion: APS can decrease biological instability of MSCs induced by inflammatory factors. The mechanism may be related to regulating the expression of key genes mediating inflammatory signaling pathways. It coincides with the theory of Traditional Chinese Medicine “Qi can produce Essence and strengthen Essence” and “Qi can control Sprinkle”.

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DOI: 10.1002/eji.201970400

P3225

Primary intestinal lymphoma: an analysis of 62 cases with localized lymphoma

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Purpose: To investigate the clinical characteristics and prognostic factors of the localized primary intestinal lymphoma (PIL).
Patients and methods: We reviewed 62 localized cases to explore the clinical characteristics, survival and some prognostic factors.
Results: The most common presentation was abdominal pain that often involved the ileocecum. The overall survival (OS) and progression-free survival (PFS) of patients treated with surgery followed by chemotherapy were significantly better than those of patients who received surgery alone. In CD20-positive B-cell lymphoma patients, rituximab combined with surgery followed by chemotherapy produced a similar result as in patients treated with surgery followed by chemotherapy. In univariate analysis, T-cell lymphoma, lactate dehydrogenase (LDH), β2-microglobulin level, hypoalbuminemia, perforation were the poor factors in the prognosis. In multivariate analysis, T-cell lymphoma (Relative Risk (RR)=25.405; 95% Confidence Interval (CI) 3.382-190.839; P=0.002) and LDH increased (RR=0.143; 95%CI 0.033-0.620; P=0.009) were independent poor prognostic factors.
Conclusions: In conclusion, patients with localized PIL can be successfully treated with chemotherapy alone or with resection and chemotherapy. Rituximab-based chemotherapy may provide some therapeutic benefits. T-cell derived and LDH level above normal were independent prognostic factors in localized PIL.

P3226

Gemcitabine, cisplatin, prednisone, and thalidomide for relapse and refractory peripheral T cell lymphoma: a retrospective study from China

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Peripheral T-cell lymphoma（PTCL）is often prone to relapse and progression even after formal first-line treatment, and there is no standard regimen for second-line treatment. What's more, the activity of thalidomide against this type of lymphoma is unknown. In this retrospective study, gemcitabine, cisplatin, prednisone, and thalidomide (GDPT) combination regimen was used as salvage protocol for PTCL that failed in first-line treatment for 29 patients, who were scheduled to receive up to four-six courses of GDPT therapy unless there was evidence of disease progression, unacceptable toxicities, or refusal by the patient. After a total of 106 cycles of GDPT regimen were administrated, the result showed that the disease control rate (DCR) achieved 86.2% and overall response rate (ORR) reached 69.0 % with 34.9% complete remission (CR) rate and 34.9% partial remission (PR). The median PFS was 8.0 months (95 % CI 3.9-12.1), and median OS was 26.0 months (95 % CI 20.5-31.5). And the 1-year PFS rate and 1-year OS were 43.7% and 64.4 %, respectively. Both hematologic and non-hematologic toxicities were moderate and well tolerated. There was no treatment-related death. Thalidomide in combination with gemcitabine, cisplatin, prednisone is a new promising approach to treating patients with relapse and refractory PTCL.

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DOI: 10.1002/eji.201970400

P3227

Advances in the study of Chinese medicinal herbs extracts on diseases by affecting macrophage polarization

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Radix Ranunculi Ternati is the root tuber of *Ranunculus ternatus Thunb*, which belongs to the *Ranunculaceae* family. It is wildly used to treat tuberculosis, pharyngitis and malaria. Our study investigated the effect of *Radix Ranunculi Ternati* on macrophage polarization and which composition contributed to this effect. Our study found that macrophages were fusiform and slender after treated with 100 μg/mL *Radix Ranunculi Ternat*i for 24h, the morphology of them were similar to classically activated macrophages (M1) phenotype macrophages. The results showed that Radix Ranunculi Ternati could markedly increase the expression of M1 related cytokines, such as iNOS, IL-6 and TNF-α. Meanwhile, we discovered that Nos2 and F4/80 double positive cells were increased to 86.2% after treated with *Radix Ranunculi Ternati* for 36h. Furthermore, we explored that *Palmitic Acid* and *Ternatolide* the main ingredient of *Radix Ranunculi Ternati* could promote macrophage to M1 polarization synergistically. Taken together, we demonstrated that *Radix Ranunculi Ternati* and its main ingredient could induce mouse bone marrow-derived macrophage to M1 polarization, which is dependent on MAPK and NF-KB signaling pathways. The results expanded our knowledge about the role of *Radix Ranunculi Ternati* in macrophage polarization and its underlying mechanisms

P3228

Correlation between chemical composition and antifungal activity of clausena lansium essential oil against candida spp

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Essential oils (EOs) have been shown to have a diversity of beneficial human health effects. *Clausena* is a large and highly diverse genus of plants with medicinal and cosmetic significance. The aim of this study was to analyze the composition of *Clausena lansium* EOs and to investigate their potential antifungal effects. The chemical compositions of *C. lansium* EOs obtained by hydrodistillation were analyzed by gas chromatography-mass spectrometry. A total of 101 compounds were identified among the diverse extracts of *C. lansium*. EOs of leaves and pericarps from different cultivars collected in Hainan (China) were classified into four clusters based on their compositions. These clusters showed different antifungal activities against five *Candida* species (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*) using the disc diffusion method. *Clausena lansium* EOs of pericarps displayed noteworthy antifungal activitives against all the tested *Candida* strains with inhibition zone diameters in the range of 11.1-23.1 mm. EOs of leaves showed relatively low antifungal activities with 6.5-22.2 mm. The rank order of antifungal activities among the four EO clusters was as follows: Cluster IV> Cluster III > Cluster I ≥ Cluster II. These results represent the first report about the correlation between chemical composition of *C. lansium* EOs and antifungal activity. Higher contents of β-phellandrene, β-sesquiphellandrene and β-bisabolene in EOs of pericarps were likely responsible for the high antifungal activity of Cluster IV EOs. Furthermore, the results showing a wide spectrum of antifungal activities provide scientific evidence for the traditional use of these plants.

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DOI: 10.1002/eji.201970400

P3229

GH2-34, a novel compound, inhibits Th17 cells and protects against Th17-mediated autoimmune diseases

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T helper 17 cells (Th17) are a subset of pro-inflammatory T helper cells defined by their production of interleukin 17 (IL-17), which are associated with autoimmune disease such as multiple sclerosis, rheumatoid arthritis, and colitis. In preliminary studies, we extracted a novel compound (GH2-34) from the *Myrothecium fungus*; however, there are no reports about its structure and any biological activity. Here, we showed that GH2-34 inhibited Th17 differentiation of mouse and human naïve CD4 T cells in vitro. Meanwhile, GH2-34 treatment had a very marked therapeutic effect on Th17-mediated autoimmune diseases in mouse models of colitis and EAE, and suppressed the proportion of IL-17$^+$ (Th17) in mice. Interestingly, GH2-34 was found to protect against sepsis and liver injury. Thus, our data demonstrates a novel compound GH2-34 has potential utility in the treatment of TH17-mediated autoimmune diseases.

P3230

Effects of pachyman in combination with vinorelbine and cisplatin on tumor growth and the expression of EGFR and K-ras in mouse model of lung cancer

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Objectives: To examine the effects of pachyman in combination with vinorelbine and cisplatin on tumor growth and the expression of epidermal growth factor receptor (EGFR) and K-ras in a mouse model of lung cancer induced, and to investigate the molecular mechanisms underlying the antitumor effects of pachyman.
Methods: The size of the tumor xenografts in mice was recorded after treatment with pachyman monotherapy or with pachyman in combination with vinorelbine and cisplatin. We performed immunohistochemical analysis to determine the levels of expression and distribution of EGFR and K-ras in lung cancer tissues, and RT-PCR was used to determine the relative mRNA expression levels.
Results: Vinorelbine and cisplatin significantly decreased the rate of growth of A549 xenografts, and pachyman increased the efficacy of vinorelbine and cisplatin. Vinorelbine and cisplatin can significantly decrease the expression, distribution, and mRNA expression levels of EGFR and K-ras in tumor tissues. Pachyman monotherapy significantly decreased the distribution and the mRNA expression levels of EGFR in lung cancer tissues. In addition, pachyman in combination with vinorelbine and cisplatin markedly decreased the distribution and expression levels of EGFR in lung cancer tissues. However, pachyman monotherapy or combination therapy did not significantly decrease the mRNA expression levels of K-ras.
Conclusions: Pachyman in combination with vinorelbine can significantly inhibit the growth of A549 xenografts, and pachyman can regulate the expression of the *EGFR* gene to increase the efficacy of vinorelbine and cisplatin in lung cancer and decrease the side effects associated with chemotherapy.

DOI: 10.1002/eji.201970400

P3231

Guiqi Baizhu Granule on blood routine and serum circular RNA (circRNA) in patients with gastric cancer before and after chemotherapy

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In recent years, traditional Chinese medicine(TCM) has played a significant role in enhancing efficacy and reducing toxicity in adjuvant chemotherapy of clinical malignant tumors. From the perspective of TCM and circRNA, we study the therapeutic effect and possible molecular mechanism of Guiqi Baizhu Granule assisted gastric cancer chemotherapy. It was found that the first-line chemotherapy regimen combining Capecitabine and Oxaliplatin can reduce the levels of WBC, Neu, Lym, RBC and PLT in peripheral blood of patients with gastric cancer chemotherapy through its hematological toxicity. It also has some influence on the immune response mechanism of the patients themselves. Chinese herbal compound Guiqi Baizhu Granule can effectively improve the WBC, Neu, Lym, RBC and PLT levels in peripheral blood after chemotherapy, and restore the immune response mechanism of the patients themselves.At the same time, we also found 120427 circRNAs in the serum of gastric cancer patients were different form that of healthy people, among which 137 circRNAs with different expression levels, 67 circRNAs with up-regulated expression and 70 circRNAs with down-regulated expression(Difference multiple ≥ 2.0, p value ≤ 0.05). Clinical chemotherapy can reduce the expression of circRNA. Guiqi Baizhu Granule can effectively improve the low expression of circRNA after chemotherapy. In summary, we conclude that Chinese herbal compound Guiqi Baizhu Granule can effectively reduce the side effects of chemotherapy drugs, restore the body´s tumor immune response mechanism, and possibly play its corresponding biological role by regulating the expression of circRNA in the body.

P3232

Improvement of cinnamon on impaired IFN-γ-producing T and NK cells decreased tumor metastasis after single-low dose irradiation

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Background: Irradiation-induced impaired gamma interferon (IFN-γ) production promotes tumor relapse and metastasis. Here we identified the characteristics of IFN-γ-producing T and natural killer (NK) cells following single low-dose total-body irradiation (SLTBI) and evaluated their sensitivity to Cinnamon treatment to mitigate lymphopenia in response to irradiation.
Materials and methods: Th1, Tc1 and NK cells, antigen presenting cells (APCs) following SLTBI were examined by flow cytometry in irradiated mice with or without cinnamon treatment. Homeostatic cytokines (including IL-2, IL-7, IL-12, IL-15 and IL-18) that support T and NK cell proliferation and related signaling (STAT1, STAT4, RUNX3, and Eomes) required for IFN-γ production were detected by real time-PCR. The risk of impaired IFN-γ-producing T and NK cells on tumor development after SLTBI and the reversal effects of cinnamon treatment were evaluated in a lung metastasis model of melanoma in SLTBI mice.
Results: The impaired IFN-γ-producing cells after SLTBI were characterized by diminished Th1 cell capacity due to defects in integrating signals from APCs and impaired sensitivity to elevated homeostatic cytokines; IFN-γ-producing CD8+ T and NK cells with high sensitivity to cytokines and

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DOI: 10.1002/eji.201970400

cinnamon treatment could rapidly recover after irradiation and strengthen anti-tumor immunity. The remarkable elevated Tc1 and NK1 cells following cinnamon-treatment post SLTBI was also confirmed in a lung metastasis tumor model using irradiated mice treated with cinnamon. These results provide new strategies for improving the reconstitution of IFN-γ-producing cells after irradiation, and this will be beneficial for rational design of radiotherapy and immune-checkpoint therapies.

P3233

Study on the proliferation and apoptosis of lung adenocarcinoma cells induced by a new anticancer prescription of Chan-Shan-Hu-Jin

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Objective: To investigate the effect of the serum containing drug of a new anticancer Prescription of Chan-Sha-Hu-Jin on the proliferation and apoptosis of lung adenocarcinoma A549 cells.
Methods: 30 SD rats were divided into three groups randomly, they were serum containing drug group, Xiao-Ai-Ping group and normal saline group, with 10 rats in each group. Each group was intragastric administrated successively with the Decoction of Chan-Shan-Hu-Jin Prescription、Xiao-Ai-Ping water solvent and normal saline, to prepare the serum containing drug. A549 cell proliferation inhibition test (MTT test), apoptosis assay and detection the expression of "death protease" Caspase-3 was carried out with serum containing drug.
Results: Through the MTT test, it was found that the group of Chan-Shan-Hu-Jin Prescription could inhibit the proliferation of A549 cells, and positive correlation with dose, compared with the negative group($P< 0.05$), and compared with the Xiao-Ai-Ping group ($P< 0.05$). The apoptotic detection was carried out by flow cytometry, it was found that the early apoptosis after the medium concentration treatment group and high concentration treatment group, compared with the negative group ($P< 0.05$, $P< 0.05$). Western-Blot test showed that the activation of caspase-3 was up-regulated.
Conclusion: the serum containing drug of Chan-Shan-Hu-Jin Prescription could inhibit the proliferation and induce the apoptosis of lung adenocarcinoma A549 cell, and positive correlation with dose.
Keywords: Prescription of Chan-Shan-Hu-Ji, Proliferation, Inhibition, Cell apoptosis

P3234

Effect of Celastrus orbiculatus extract and Genistein on regulation of inflammation and thrombus prone status in lymphoma-bearing mice

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To observe the regulation of Chinese herbal medicine *Celastrus orbiculatus extract* (COE) and *Genistein* (GEN) on regulation of inflammation and thrombus prone status in 38B9 lymphoma-bearing mice. 50 Balb/c mice were randomly divided into CON group, untreated group, COE intervention group, GEN intervention group and CTX intervention group. On the 14th day of the tumor-bearing, COE (3 mg/(kg·D)) and GEN (30 mg/(kg·D)) were intragastrically administered, and CTX was intraperitoneally injected (40 mg/(kg·d)) for one week. The untreated group was given the same amount of normal saline. Blood samples were collected respectively on the time of before injection (1d), tumor formation (14d) and after intervention (21d) to detect the changes of inflammation indexes, thrombosis indexes, platelet activation markers and Treg cells level. After the intervention of COE,

DOI: 10.1002/eji.201970400

GEN and CTX, the tumor size of the lymphoma-bearing mice decreased and the survival was significantly prolonged. The plasma level of TNF-α, IL-6, NETs, TF, D-D and vWF in three intervention groups was significantly decreased, the level of IL-10 increased ($P < 0.05$). The platelet activation index CLEC-2 was significantly decreased ($P< 0.05$), there was no significant difference with GPIIbIIIa and P-selectin. The number of Treg T cells was significantly decreased ($P< 0.05$). Among the three intervention groups, the CTX group was particularly effective, COE group and the GEN group showed on difference.COE and GEN may interfere with the tumor progression by improving the hypercoagulable and inflammable state of lymphoma-bearing mice.
Key words: Lymphoma bearing mice; *Celastrus orbiculatus* extract; Genistein; inflammation; hypercoagulable state

P3235

Effect of Tonifying Spleen-qi on miR-155/SOCS1/STAT3 signaling pathway in NOD.H-2h4 mice

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Objective: To observe the effect of Yiqi Huatan Huoxue Decotion on miR-155/SOCS1/STAT3 axis in autoimmune thyroiditis(AIT) mice.
Methods: Sixty 8-week-old NOD.H-2h4 mice were randomly divided into six groups: normal(NG), model(MG), Selenious yeast(SeG), Yiqi Huatan Huoxue Decotion(YG-2), 2 times dose(YG-1) and ½ times dose(YG-3). Mice in NG were fed with distilled water, the others were supplied with 0.05 % NaI for 8 weeks to induce AIT. 8 weeks later, thyrocyte morphology was observed under optical microscopy; IL-17 and miR-155 mRNA expressions in thyroid were detected by RT-PCR. SOCS1 and p-STAT3 protein expressions were determined by Western Blot.
Results: ①Compared with NG, intrathyroidal lymphocyte infiltration of MG was more obvious, and that of all the medicated groups were alleviated markedly.②IL-17 and miR-155 mRNA expressions and p-STAT3 protein expression were increasing markedly in MG ($P<0.01$);While,IL-17 mRNA expressions in SeG,YG-1 and YG-2 decreased remarkably($P<0.01$);miR-155 and p-STAT3 expressions were reduced markedly in all the medicated groups($P<0.01$).③ SOCS1 expression in SeG,YG-2 and YG-3 all increased remarkably($P<0.01$)when compared with MG group.
Conclusion: The mechanism of the treatment of AIT with the method of Tonifying Spleen-qi may include the regulation of miR-155/SOCS1/STAT3 signaling pathway.
Keyword: IL-17;miR-155;SOCS1;p-STAT3;NOD.H-2h4 mice;Tonifying Spleen-qi;Chinese Medicine.

P3236

Dihydroartemisinin alleviates imiquimod-induced psoriasis in mice via IL-23/Th17 axis

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Psoriasis is a chronic inflammatory autoimmune skin disease. IL-23/Th17 axis plays a crucial role during the development of psoriasis. Recent studies shown that dihydroartemisinin (DHA) can significantly improve the symptoms of autoimmune diseases. Our previous studies found DHA can inhibit the proliferation of HaCaT cells, and altered the expression of cytokines. We hypothesized DHA can improve the symptoms of psoriasis, and further explored the possible mechanism. BALB/c mice

DOI: 10.1002/eji.201970400

were treated with imiquimod for 5 days on back skin to establish psoriasis mice model, and DHA was given at concentrations of 25 mg/kg/d and 50 mg/kg/d for 8 days by intragastric administration. Compared with IMQ group, psoriasis symptoms were significantly improved, the PASI score decreased, the number of inflammatory cells infiltration reduced, thinning of the epidermis, the hyperkeratosis was alleviated, the spikes were shortenedand in skin by H&E staining. Flow Cytometry showed that the percentage of $CD4^+IL\text{-}17^+T$ cells, $CD4^+IFN\text{-}\gamma^+T$ cells and $CD8^+IFN\text{-}\gamma^+T$ cells decreased after DHA treatment in spleen. In addition, the mRNA levels of IFN-γ, IL-17, IL-23 declined, and IL-4, IL-10 significantly increased in skin. The expression of IFN-γ decreased and IL-6, IL-10 raised in DHA treatment groups in spleen by western blotting. Futhermore, DHA declined STAT3 phosphorylation levels. These results showed that DHA can alleviate psoriasis skin lesion and restore balance of inflammatory cytokines by IL-23/Th17 axis, and is promising to be an effective drug for psoriasis.
Key words: Psoriasis; DHA; IL-23/Th17 axis

P3237

Genistein suppresses psoriasis-related inflammation through a STAT3-NF-κB-dependent mechanism in keratinocytes

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Psoriasis is a chronic recurrent skin inflammatory disease, and inhibition of inflammation may be an effective means of treating psoriasis. The flavonoid genistein has a clear anti-inflammatory effect. However, the anti-psoriatic effects of genistein and their underlying mechanisms remain unclear. In this study, we investigated the effects of genistein on imiquimod (IMQ)-induced psoriasis-like skin lesions in vivo and explored the mechanisms underlying those effects in vitro. It was found that genistein can significantly improve IMQ-induced pathological scores of cutaneous skin lesions in mice, reduce epidermal thickness, and inhibit the expression of inflammatory factors, including interleukin (IL)-1β, IL-6, tumour necrosis factor-alpha (TNF-α), chemokine ligand 2 (CCL2), IL-17 and IL-23. In vitro studies, genistein inhibited the proliferation of human keratinocyte HaCaT cells and inhibited the expression of inflammatory factors in a dose-dependent manner which induced by TNFα. Further researches showed that genistein could also significantly inhibit phosphorylated STAT3 (pSAT3) expression in IMQ mice dorsal skin and in TNF-α-induced HaCaT cells. The inhibitory effect of genistein on the expression of IL-6, IL-23 and TNF-α was weakened after Stat3 siRNA in HaCaT cells. Genistein could also significantly inhibit TNF-α induced the nuclear translocation of NF-κB, and inhibit the phosphorylation of I-kBα (pI-kBα). After combining with NF-κB blocker BAY 11-7082, the effect of genistein down-regulate the expression of TNF-α and VEGFA was attenuated in HaCaT cells. The results suggest that genistein may be developed for the treatment of psoriasis lesions.

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DOI: 10.1002/eji.201970400

P3238

Lycopene protects against acetaminophen-Induced liver injury by attenuating oxidative stress in mice

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Background: Acetaminophen (APAP) overdose can cause severe liver failure and even lead to death. Oxidative stress has been validated as a critical event involved in APAP-induced liver failure. Lycopene has been widely studied for its antioxidant properties. This study seeks to investigate the protective effect and mechanisms of lycopene against APAP-induced hepatotoxicity.
Methods: Male C57BL/6 mice were randomly divided into six groups (n=8 per group):Control group(Ctrl),APAP-induced-liver injury group (APAP,400mg/kg),NAC pretreated group (NAC,150 mg/kg),10 mg/kg and 30 mg/kg Lycopene pretreated group (APAP/LP10 and APAP/LP30, respectively). Mice were treated with Lycopene for 14 consecutive days. NAC was administrated 1h before APAP challenge. Samples of blood and liver were collected 16h after APAP treatment. The levels of Nrf2 signaling pathway were analyzed by Real-time PCR and Western blotting.
Result: Pretreatment with lycopene leads to decreased ALT and AST levels, and increased GSH and GSH-PX levels in APAP-injected mice. Lycopene attenuates APAP-induced oxidative stress by promoting Nrf2 nuclear translocation, up-regulating Nrf2 target genes including GCLC,HO-1,NQO-1. In addition, Lycopene suppressed APAP-induced increases in the ratio of Bcl-2/Bax.
Conclusion: Lycopene shows a potent protective effect against APAP-induced hepatotoxicity by activation of the Nrf2 signaling pathway.

P3239

Tanshinone IIA induces autophagy associated cell cycle arrest to against lung cancer cells

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To figure out the mechanisms of the effects of Tanshinone IIA (T2A) against lung cancer. Cell proliferation of T2A treated lung cancer cell A549 was detected with CCK8 assay. Beclin-1, p62 and LC3B, which are associated with autophagy, were measured with Western blot on T2A treated A549 cells. Cell cycle distribution of T2A treated A549 cells was detected by flow cytometry. A549 cells, pretreated with autophagy agonist Rapamycin (RAPA) or autophagy antagonist 3-methyladenine (3-MA) for 1 h, were collected for cell proliferation, cell cycle distribution, and autophagy analysis after T2A treating for 24h. it suggested that cell proliferation was significantly inhibited in T2A treated A549 cells in a dose-dependent manner, which may be due to T2A induced cell cycle G0/G1 arrest in A549 cells. Autophagy was also found in T2A treated A549 cells. The ratio of LC3-II/LC3-I and the Beclin-1 increased, meanwhile p62 decreased. Compared to the A549 cells treated by T2A alone, RAPA, which is autophagy agonist, pretreated A549 cells showed more distinct cell cycle arrest and autophagy after T2A treatment. On the opposite, 3-MA, which is autophagy agonist, pretreated A549 cells showed less distinct cell cycle arrest and autophagy after T2A treatment. Above all, the data suggested an intense relation between T2A induced autophagy and cell cycle arrest in lung cancer cell A549, which means autophagy might play an important role in the pharmacology of Tanshinone IIA.
Keywords: Tanshinone IIA, lung cancer cells, autophagy, cell cycle arrest

DOI: 10.1002/eji.201970400

P3240

Isoorientin attenuates neuroinflammation and cognitive impairment in APPswe / PS1dE9 mice

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Background: Isoorientin, a 6-C-glycosylflavone, isolated from corn silks (*Zea mays* L.), is traditionally used as a medicinal herb and recently noted for their potentially cognitive benefits. Alzheimer´s disease (AD) is the most common neurodegenerative disorder in the aged population, with key pathological features: beta-amyloid (Aβ) aggregation, phosphorylated tau accumulation, and neuroinflammatory activity. Our previous *in vitro* data indicated that isoorientin inhibited glycogen synthase kinase 3β (GSK-3β), a key kinase responsible for tau hyperphosphorylation, and attenuated Aβ-induced NF-κB activation and neuronal damage. The neuroprotective effect of isoorientin *in vivo* remains unclear.
Methods: The effects of isoorientin on cognitive impairment, Aβ deposition, tau phosphorylation, inflammatory cytokines in amyloid precursor protein /presenilin 1 (APP/PS1) transgenic mice were assessed using water maze test, immunofluorescence, ELISA, RT-PCR and western blotting.
Results: Compared with Tg mice, isoorientin improved cognition potential. Isoorientin reduced Aβ deposition, tau phosphorylation and the quantity of activated microglia (marked by ionized calcium binding adapter molecule 1, IBA1) surrounding the Aβ plaque in brain. Isoorientin also reduced the expression of cyclooxygenase-2 and alpha 7 nicotinic acetylcholine receptor, TNF-α, IL-6 and IL-1β in brain. Isoorientin decreased the concentrations of TNF-α and IL-2, but increased IL-4 and IL-10 in serum. Isoorientin also reduced the concentrations of IgG anti-Aβ42 in serum.
Conclusion: Isoorientin can improve the cognitive function in AD model mice through decreasing Aβ production and tau phosphorylation, especially inhibiting neuroinflammation and regulating peripheral inflammation. Isoorientin is a promising lead compound to be developed as disease-modifying therapeutics for the prevention and treatment of AD.

P3241

Seabuckthorn polysaccharide inhibits NLRP3 signaling pathway in macrophages and attenuate ulcerative colitis in mice

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Purpose: To study the effect of Seabuckthorn Polysaccharide（SP）on the NLRP3 sginaling pathways in macrophages and to explore the alleviating mechanisms of SP on DSS-induced ulcerative colitis（UC）in mice.
Methods: RAW264.7 cells were pretreated with different doses of SP for 2h, and then stimulated with LPS (500ng/ml) for 6h plus stimulation with ATP (2.5μM) for 30min. The expression of the NLRP3 signaling pathway components was measured both at mRNA and protein level. Mice was orally administrared with SP (200mg/kg) or vehicle for three weeks. 3.5% DSS was given in dring water in the SP or vehicle treat group for 7 days. Mesalazine (60mg/kg) was used as a positive control in this experiment.
Result: SP dose-dependently inhibited LPS and ATP-induced expression of IL-6 and TNF-α,while

DOI: 10.1002/eji.201970400

increasing the expression of IL-10. The expression of NLRP3, ASC, cleaved caspase-1 and cleaved IL-1β was also suppressed by SP. SP ameliorated DSS-induced acute colitis mice by decreasing weight loss and improve the colon length and colon histology. SP inhibited DSS-induced colonic expression of NLRP3 , ASC cleaved caspase-1 and IL-1β. SP inhibited the expression of IL-6and TNF-α, while increasing the IL-10 expression in serum. Furthermore, SP increased the level of ZO-1 protein in colon.
Conclusion: SP attenuate DSS-induced UC through inhibiting the NLRP3 signaling pathway.

P3242

p-Hydroxylcinnamaldehyde induces the differentiation of oesophageal carcinoma cells via the cAMP-RhoA-MAPK signalling pathway

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p-Hydroxylcinnamaldehyde (CMSP) has been identified as an inhibitor of the growth of various cancer cells. However, its function in oesophageal squamous cell carcinoma (ESCC) and the underlying mechanism remain unclear. The aim of the present study was to characterize the differentiation effects of CMSP, as well as its mechanism in the differentiation of ESCC Kyse30 and TE-13 cells. The function of CMSP in the viability, colony formation, migration and invasion of Kyse30 and TE-13 cells was determined by MTS, colony-formation, wound healing and transwell assays. Western blotting and pull-down assays were used to investigate the effect of CMSP on the expression level of malignant markers of ESCC, as well as the activity of MAPKs, RhoA and GTP-RhoA in Kyse30 and TE-13 cells. We found that CMSP could inhibit proliferation and migration and induce Kyse30 and TE-13 cell differentiation, characterized by dendrite-like outgrowth, decreased expression of tumour-associated antigens, as well as the decreased expression of malignant markers. Furthermore, increased cAMP, p-P38 and decreased activities of ERK, JNK and GTP-RhoA, were detected after treatment with CMSP. These results indicated that CMSP induced the differentiation of Kyse30 and TE-13 cells through mediating the cAMP-RhoA-MAPK axis, which might provide new potential strategies for ESCC treatment.

P3243

Total flavone from Seabuckthorn leaves attenuates DSS-induced inflammatory responses by suppressing NF-kB and NLRP3 signaling pathways

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Total flavone, composed of flavones, flavonol, flavonones, flavanonol, etc., have been reported to have a variety of biological properties,such as anti-inflammatory, antioxidant and other important therapeutic effects.The purpose of this study was to investigate the anti-inflammatory effect and mechanism of total flavone from Seabuckthorn leave on dextran sulfate sodium (DSS)-induced colitis in mice. In vivo, DSS-induced mice colitis model was established by giving mice drinking water containing 3.5% (w/v) DSS for 7 days.Total flavone was administered through intragastrically daily at 100mg/kg prior to DSS for 7 days. In vitro,RAW264.7 macrophages were treated with total flavone and stimulated by lipopolysaccharide (LPS) and adenosine triphosphate(ATP). The results showed that total flavone reduced DSS-induced clinical-(weight loss, diarrhea) and histological scores (mucosal

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damage) . Total flavone increased colonic Zo-1 tight junction protein expression and attenuated expression of proinflammatory cytokines IL-1β, IL-6 and TNF-α as well as nuclear transcription factor-kappaB (NF-κB) and NLRP3 inflammasome. Some of the protective effects of total flavone were likely be mediated directly through local macrophages because total flavone dose-dependently inhibited IL-1β,IL-6 and down-regulate the expression of proteins related to NF-KB and NLRP3 signaling pathways after LPS in mouse macrophage cell line RAW264.7. In conclusion, this study demonstrated that total flavone had protective effects on DSS-induced colitis and may be a promising therapeutic reagent for colitis treatment.

P3244

Sinomenine inhibits fibroblast-like synoviocyte inflammatory response by regulating A2A and inhibiting NF-κB via targeting α7nAChR

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Backgroud and purpose: Alpha 7 nicotinic acetylcholine receptor (α7nAChR) is an important target of the anti-inflammatory effect of sinomenine (SIN), an alkaloid derived from the plant *Sinomenium acutum*. But the mechanism of SIN effect on rheumatoid arthritis (RA) via targeting α7nAChR remains unclear. RA is a chronic inflammatory disease characterized by hyperplasia of the synovial lining cells, especially fibroblast-like synoviocytes (FLS), resulting in progressive joint destruction. Adenosine receptor A2A always has anti-inflammatory and immunosuppressive function. NF-κB plays a central role in inflammatory response.
Methods and results: The adjuvant-induced arthritis (AA) model were induced in rats. FLS was isolated from AA rat synovial tissues and LPS was used to induce inflammatory response *in vitro*. SIN inhibited the release of TNF-α, IL-6, MCP-1 and VEGF induced by LPS. The expression of A2A was significantly decreased in FLS induced by LPS, but increased by SIN. SIN decreased the expression of TLR4 in FLS induced by LPS. SIN decreased p65 protein expression in nucleoprotein and increased IκBα protein expression in cytoplasm. Meanwhile, α-BTX, a specific antagonist of α7nAChR, or α7nAChR siRNA significantly attenuated the effect of SIN in FLS above, but not Indo.
Conclusion: Our study suggests that α7nAChR is the key target of SIN anti-arthritis effect in FLS. The expression of A2A is negatively correlated with inflammation of FLS. SIN can up regulate the expression of A2A and inhibit the activation of TLR4/NF-κB signal pathway via α7nAChR to play anti-arthritis effect.
Keywords: sinomenine; α7nAChR; fibroblast-like synoviocyte; adenosine receptor A2A; NF-κB

P3245

Astragaloside IV, the major bioactive component of Astragalus membranaceus (Fisch.) Radix, attenuates airway inflammation by modulating Th2 cytokines and STAT6 in an asthmatic mouse model

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DOI: 10.1002/eji.201970400

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Objective: Astragaloside IV (AST Ⅳ), the major bioactive component of *Astragalus membranaceus*（Fisch.）Radix, possesses anti-inflammatory, regular immunological balance and other pharmacological properties. Although *Astragalus* is associated with immunoregulatory functions of allergic diseases, the pathophysiological mechanisms of AST Ⅳ action in allergic inflammatory lung disease have not been examined. This study aims to explore the mechanisms of how AST Ⅳ suppresses airway inflammation in bronchial asthma by inhibiting the inflammatory cytokines and transcription factors pathway.
Methods: A total of 40 BALB/c male mice were selected and divided into control, asthma, AST Ⅳ and dexamethasone groups. Ovalbumin-induced mouse asthma model was established. Hematoxylin and eosin (HE) staining were conducted to observe the pulmonary pathological changes. Optical microscope was used to detect asthmatic lung pathology change. Enzyme-linked immunosorbent assay (ELISA) was adopted for the levels of IFN-γ, IL-5, IL-13, IL-17A and TGF-β, qRT-PCR for the transcription factors mRNA expressions of GATA-3, STAT6, T-bet, RORγt, and Foxp3.
Results: Compared with the control group, the asthma group had irregular tissue structure and severe inflammation. AST Ⅳ strongly suppressed injured airway epithelial cells, accumulation of inflammatory cells, and levels of T helper type 2 (Th2) cytokines IL-5 and IL-13 in lung homogenate ($p<0.05$). AST Ⅳ exerted immunomodulatory effects by suppressing Th2 cytokines (IL-5, IL-13) production through downregulation of signal transducer and activator of transcription (STAT-6) (2.65 time).
Conclusion: These results suggest that the anti-asthmatic activity of AST Ⅳ may occur by the suppression of IL-5, and IL-13 production through inhibition of the STAT6.

P3246

The extracts of Morinda offcinalis and its hairy roots attenuate dextran sodium sulfate-induced chronic ulcerative colitis in mice by regulating lymphocyte apoptosis

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Evidences showned that exaggerated activation of T lymphocytes is closely related to the pathogenesis of chronic ulcerative colitis (UC). Thus, induction of increased T lymphocytes apoptosis is an important strategy for the treatment of UC. Previous study illustrated that *Morinda offcinalis* can induce the apoptosis of T lymphocytes to effectively treat UC. The hairy root with higher genetic and biochemical stability cultured from *M. offcinalis* that might have similar effects on UC treatment. Here, we found that the hairy root extract of *M. offcinalis* (MOHRE) possesses many specifc peaks unobserved in the chromatogram of native plant. The content of iridoids in the root extracts of *M. offcinalis* (MORE) (3.10%) and MOHRE (3.01%) is somewhat similar but quite different for their anthraquinones's content (0.14 and 0.66%, respectively). Despite all this, treatment with MOHRE signifcantly attenuated the symptoms of colitis, including diarrhea, colon shortening, histological damage and decreased inflammatory cytokine levels. The therapeutic effect of MOHRE on colitis is similar to MORE. In addition, further investigation showed that MOHRE can also dose-dependently increase the apoptosis of T lymphocyte (such as helper T cells ($CD3^+CD4^+$) and cytotoxic T cells ($CD3^+CD8^+$)) *in vivo* and *in vitro*. The results demonstrated that though there are a few difference on their chemical composition, MORE and MOHRE have similar effect on UC treatment, which are largely dependent the increase of T lymphocyte apoptosis, indicating that the hairy root cultured from *M. offcinalis* might be able to replace its native plant on treatment of UC.

DOI: 10.1002/eji.201970400

P3247

Influences of the repeated male blood donation on hematological indices of CD4, CD8, CD34 and NK cells

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Objective: Previous studies have explored the influences of the repeated blood donation on hematological indices such as red blood cell count, white blood cell count platelet count and so on. The aim of this study was to evaluate the influences of the repeated male blood donation on hematological indices including CD4, CD8, CD34 and NK cells.
Methods: The hematological indices including CD4, CD8, CD34 and NK cells were determined by flow cytometry in 46 male blood donators with blood donation frequency ≥2 times/per year and 44 healthy controls (males without blood donation experiences). SPSS12.0 statistical software is used for experimental data analysis; a separate sample t test is used in testing and comparing the differences between the various observation indices of the observation group and the healthy controls.
Results: There are not differences between the two groups on the percentage of CD4/CD8 and CD34+ ($P>0.05$). However, the NK cell count in the repeated donors is significantly higher than that in the healthy controls ($P< 0.05$).
Conclusion：This study demonstrates that the repeated blood donation may promote the generation of NK in males.

P3248

The effects of Liuweidihuang Pills on the histological changes of liver, kidney and pancreas in KK-Ay mice with spontaneous type 2 diabetes mellitus

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Diabetes mellitus is a growing public health problem, in which more than 90% of patients are diagnosed type 2 diabetes mellitus. To investigate the effects of Liuweidihuang Pills on the histological changes of liver, kidney and pancreas from human spontaneous type 2 diabetes mellitus model mice (KK-Ay mice) administrated with different doses of Liuweidihuang Pills.
Methods: The 6-8 week-old KK-Ay mice were randomly divided into three groups including no drug control group, low-dose group and high-dose group, in addition C57BL/6J mice were used as a genetic control group. All the animals were given with different dose Liuweidihuang Pills solutions or sterile distilled water by intragastrical administration for fifteen weeks. The histological changes of liver, kidney and pancreas were assessed by H&E staining. All the results from different groups were analyzed.
Results: In the two treated groups, reduced pathological changes of liver, kidney and pancreas were observed, compared with no drug control group. The low-dose regimen exhibited preferable protection for liver tissue. The high-dose treatment achieved better curative effect than low-dose regimen to renal lesions. Furthermore, the low-dose regimen performed obviously protective effect to islet cells in pancreas.
Conclusions: The dose-dependent therapeutic effects of Liuweidihuang pills to the tissues including liver, kidney and pancreas from human spontaneous type 2 diabetes model mice were definite.

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DOI: 10.1002/eji.201970400

P3249

Clinical significance of detection of minimal residual diseases in 36 cases of multiple myeloma

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Objective: To investigate the correlation between bone marrow plasma cell count and M protein level in patients with multiple myeloma by multiparameter flow cytometry and immunofixation electrophoresis.
Methods: Flow cytometry and immunofixation electrophoresis were used to detect the number of plasma cells in bone marrow and the level of serum M protein in different stages of the patients.
Results:
1. Before treatment, the level of M protein in stage III was significantly different from that in stage I,P = 0.012 (P < 0.05); there was no significant difference between stage III and stage II, P = 0.18 (P > 0.05).
2. The correlation coefficient between bone marrow plasma cell count and serum M protein level before treatment was 0.371, P=0.00 (P< 0.05).
3. With the increase of course of treatment, the effective rate of treatment gradually increased, the overall level of MRD gradually decreased, and the low level of MRD increased, P = 0.00 (P < 0.05), the difference was statistically significant.
Conclusions:
1. To evaluate the prognosis, we must simultaneously detect the number of residual plasma cells, the level of M protein and free light chain, which is indispensable.
2. MRD monitoring can predict recurrence early, and early intervention can improve prognosis.
3. The number of bone marrow plasma cells before treatment was correlated with the level of M protein.
Keywords: Multiple myeloma, Minimal residual disease, Immunofixation electrophoresis, Flow cytometry

P3250

KIF23/MKLP1 promotes gastric cancer cell proliferation by direct interaction with Amer1 and activation of the Wnt/β-catenin signaling pathway

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The pivotal factors underlying gastric cancer (GC) development and outcomes are not well characterized, resulting in a paucity of validated therapeutic targets. We identified KIF23, encoding MKLP1, as a candidate oncogene target. KIF23/MKLP1 was upregulated in GC, and increased expression of KIF23/MKLP1 correlated with poor prognosis. KIF23/MKLP1 inhibition suppressed GC cell proliferation, tumorigenesis, migration and invasion, and arresting the cell cycle in the G2/M phase. Mechanistic investigations confirmed that miR-195 directly regulated KIF23/MKLP1 expression posttranscriptionally by binding with the 3'-UTR of KIF23/MKLP1, which activated the Wnt/β-catenin signaling pathway by directly interacting with Amer1. Furthermore, KIF23/MKLP1 exhibited competitive binding with Amer1 to block the association of Amer1 with APC, thus attenuating the ability of Amer1 to negatively regulate Wnt/β-catenin signaling, resulting in activation of this signaling pathway. Our findings demonstrated that KIF23/MKLP1, regulated by miR-195, promoted GC cell proliferation by directly interacting with Amer1 and activating the Wnt/β-catenin signaling pathway.

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DOI: 10.1002/eji.201970400

P3251

The immunoregulatory effects of traditional Chinese medicine on psoriasis *via* its action on interleukin: advances and considerations

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Psoriasis is a chronic inflammatory cutaneous disease, the main clinical manifestations of which are erythema and scaling on a background of polygenic inheritance, based on internal immune T-cell disorders and affected by infection, the environment, and emotion. As the pathogenesis of psoriasis is not yet clear, Western medicine treats it with combinations of hormones and physiotherapy. However, this may have side effects, and patients often relapse after withdrawal of the drugs. Traditional Chinese medicine is an efficient way of treating psoriasis clinically. Practitioners of integrated Chinese and Western medicine take full advantage of the features of Chinese medicine to provide a comprehensive treatment for psoriasis. They take into consideration both individual and regional differences, and prescribe medications flexibly according to dynamic changes in the disease. Chinese medicine has gradually become one of the most efficient treatment methods for psoriasis, being adopted by complementary and alternative medicine practitioners both in Asia and worldwide. More and more research indicates that Chinese medicine regulates the unbalanced immune system in several ways, which is one of the complicated mechanism of a clinically effective formula. However, interleukin is an important immune factor and treatment target during therapy for psoriasis. In this paper we review recent advances in research into how Chinese medicine immunoregulates psoriasis via interleukin, and systematically summarize the mechanism by which Chinese medicine affects interleukin. It is important for scholars to achieve a better understanding of the clinical value of Chinese medicine.

P3252

Bryostatin-1 ameliorated experimental colitis in Il-10-/- mice by protecting the intestinal barrier and limiting immune dysfunction

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Objectives: Bryostatin-1 (Bry-1) has been proven to be effective and safe in clinical trials of a variety of immune-related diseases. However, little is known about its effect on Crohn´s disease (CD). We aimed to investigate the impact of Bry-1 on CD-like colitis and determine the mechanism underlying this effect.
Methods: Fifteen-week-old male Il-10-/- mice with spontaneous colitis were divided into positive control and Bry-1-treated (Bry-1, 30 µg/kg /every other day, injected intraperitoneally for 4 weeks) groups. Age-matched, male wild-type (WT) mice were used as a negative control. The effects of Bry-1 on colitis, intestinal barrier function and T cell responses as well as the potential regulatory mechanisms were evaluated.
Results: The systemic delivery of Bry-1 significantly ameliorated colitis in Il-10-/- mice, as demonstrated by decreases in the disease activity index (DAI), inflammatory score and proinflammatory mediator levels. The protective effects of Bry-1 on CD-like colitis included the maintenance of intestinal barrier integrity and the helper T cell (Th)/regulatory T cell (Treg) balance. These effects of Bry-1 may act in part through nuclear factor erythroid 2-related factor 2 (Nrf2) signaling activation and STAT3/4 signaling inhibition.
Conclusions: The protective effect of Bry-1 on CD-like colitis suggests Bry-1 has therapeutic potential in human CD, particularly given the established clinical safety of Bry-1.

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DOI: 10.1002/eji.201970400

Emerging Techniques and Targets in Immune Diagnosis

O337

Serum antibody profiling reveals a stratification of healthy toddlers into low, intermediate and high igG responders towards autoantigens, infectious agents, and vaccine antigens

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The adaptive immune responses are relatively weak in newborns, with protection against infections largely due to maternal antibodies. As their immune response develops during the first two years of life, an infant/toddler begins making antibodies in response to infections, environmental exposures, and vaccinations. Yet, surprisingly little is known about the specificity of these antibodies. We have an IRB approved study for 1000 healthy 1- and 2-year old toddlers to characterize their antibody specificities. A new antigen array was developed comprising autoantigens, infectious agents, vaccine antigens, and allergens. Serum profiling of 160 samples to date reveals a stratification of these toddlers into low, intermediate, and high IgG response groups. The separation is based on the normalized aggregate response, with high responders selected at 1 SD beyond the mean. Sixteen % are high responders, with their IgGs recognizing many self-antigens and infectious agents. Interestingly, 26% and 9% of the cohort had moderate and high anti-nuclear antibody (ANA) titers, respectively. Comparing clinical data reveals a significant correlation with the high IgG responders and a family history of asthma and maternal gestational diabetes. Targeted DNA sequencing in the high responder group revealed a strong genetic association signal at the HLA locus, with genetic polymorphisms at this locus associated with high ANA and IgG titers to many antigens. Findings from our study may support implementation of a new wellness screen to identify toddlers at risk for immune system abnormalities later in life.

O338

A novel immunohistological profiling method for cancer tissue, Immunohistogram, that would contribute to personalized precision medicine

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Immunotherapy has become the fourth therapeutic modality for advanced cancer. However, only 20-30% of the patients responded to the immune checkpoint blockade monotherapy even in immune-sensitive lung cancer. Therefore, predictive biomarkers are required to select the most effective cancer therapy. We developed a novel immunohistological profiling method for cancer tissues, Immunohistogram, which could visualize the disorders of cancer-immunity cycle in each cancer tissue. Surgical specimens of cancers were immunostained with a panel of immunopathological markers, including MMR-related molecules (PMS2, MSH6), tumor infiltrating lymphocytes (CD8, TIA1), antigen presenting molecule (HLA class I), immune exhaustion marker (PD-1), and immune resistance marker (PD-L1). The immunohistogram of each cancer tissue was created from the expression level of these molecules, and evaluated for the correlation with prognosis. Comparison of the Immunohistogram

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DOI: 10.1002/eji.201970400

between DNA mismatch repair (MMR)-deficient cancer and MMR-proficient cancer revealed the distinctive characteristics in each cancer tissue. Cancer immunoprofiling using Immunohistogram would become a potent diagnostic tool for selection of therapeutic options and contribute to personalized precision medicine.

O339

Application of CD19$^+$ EV as an indicator in tumor diagnosis and prognosis

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Systemic immunosuppression greatly affects the chemotherapeutic antitumor effect. We have already verified that CD19$^+$ extracellular vesicles (EVs) from B cells through CD39 and CD73 vesicle-incorporated proteins hydrolyzed ATP from chemotherapy-treated tumor cells into adenosine, thus impairing CD8$^+$ T cell responses. The role of CD19$^+$ EV in tumor development has been clarified very clearly, especially in tumor chemotherapy. Here, we showed that cancer patients, including gastric, colon, lung, breast cancer patients, had higher serum CD19$^+$ EVs compared to healthy people. It showed better sensitivity and specificity compared with traditional tumor biomarker, such as CEA. Stratification analysis indicated that the patients with lower serum CD19$^+$ EVs had significantly longer PFSPs in gastric and colon cancer patients. Moreover, surgical removal of tumors reduced the serum CD19$^+$ EVs in gastric and colon tumor patients. Thus, CD19$^+$ EV holds high potential in tumor diagnosis and prognosis.

O340

Genetically defined mutations in primary immunodeficiencies by new generation sequencing targeted gene method: results of 200 patients from one center in Western Turkey

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Primary immunodeficiencies (PID) are heterogeneous inherited diseases that are often caused by monogenic disorders. Turkey has a 21% rate of consanguineous marriages, prevalance of all PID cases being 30.5/100.000 within two centers in the West. With the development of the new generation DNA sequencing (NGS) methods in clinical practice, definition rate of disease-causing mutations is increasing. Targeted-sequence-analysis panels where many genes can be analyzed at the same time have been widely used and to date, more than 350 different genes have been shown to cause primary immunodeficiency. In this study, we aimed to find disease-causing mutations by Ion AmpliSeq ™ Primary Immunodeficiency Panel based on NGS in 200 patients with unknown primary immunodeficiency. The panel used is targeted for 264 genes that may be associated with primary immunodeficiencies. Ion Torrent´s Ion S5 ™ Sequencer system is used for sequencing. At least one mutation (homozygous, heterozygous, or compound heterozygous) relevant with clinical presentation was detected in 40% of subjects. Autosomal recessive mutation rate was 38%. The most common mutations were BTK (n = 11), STAT3 (n = 5), CD40LG (n = 4), CYBB (n = 4) and NOD2 (n = 4), LRBA (n=3). Mutations like SPPL2A, MALT1, IL21R, TRNT1, ADA2, VPS45 and BLNK genes were found as single cases. The definition of a molecular genetic defect is crucial for appropriate follow-up and treatment. The 264 gene panel is a reliable and fast method for the investigation of genetic defects in PID patients.

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DOI: 10.1002/eji.201970400

O341

Circulating antibodies as novel cancer biomarkers

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B cell differentiation and the production of tumour-specific antibodies are dynamic processes influenced by changing tumour antigen profiles and microenvironmental as well as systemic signals. Circulating antibody characteristics and dynamics can thus provide insight into bi-directional engagement between the immune system and the tumours. The ability to simultaneously assay a significant proportion of the systemic tumour antigen-specific antibody repertoire would thus provide a unique snapshot of the tumour at a given time point, while circumventing the influence of inter- and intra- tumour heterogeneity that affects tissue-based techniques, thereby offering an attractive route to the discovery of novel blood-based biomarkers. Furthermore, unlike tumour antigens which are rapidly degraded upon entry into circulation, specific antibody titres are relatively stable.
We have developed a custom cancer microarray platform that can readily measure circulating antibodies against over 100 full-length correctly-folded cancer-specific and -associated antigens with high sensitivity. When screening healthy individuals on the array, no antibodies are detected, highlighting it's specificity to cancer. In addition, the antigen content of the array is not restricted to a single cancer type. Thus, we aimed to investigate the translational uses of the array to detect novel diagnostic, prognostic and therapeutic monitoring biomarkers. To date, we have investigated the array's use in melanoma, prostate cancer, breast cancer and colon cancer, yielding promising results. Although the role of B cells in cancer is incompletely understood, the measurement of circulating tumour-specific antibodies represents a promising avenue in the search for cancer biomarkers.

O342

Novel immune micro-lens imaging system is developed for detection of TORCH antigen and antibody^

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Traditional and classical methods for detecting antigen and antibody encounter many problems, such as time-consuming, high cost and low accuracy. Here, a novel immune micro-lens imaging system was developed to test TORCH antigens or antibodies to TORCH invading pregnant women through changes of refractive index before and after antigen-antibody reaction. This system consists of a parallel light source, a high resolution cooling CCD, an intelligent analysis software, an autofocus and a temperature controller accompanied with a special multi-well micro-lens sample test plate, and can achieve multi-channel detection of antigen or antibody, ensuring the greatly reduced errors in the process of measurement. It can quickly perform qualitative and quantitative detection on antigen-antibody reactions without any labeling, pre-modification, post-washing and enzyme-substrate. The

DOI: 10.1002/eji.201970400

standard curves for ten items of TORCH were established for the first time by this system and the critical threshold values for clinical diagnosis were confirmed. Compared to clinical diagnostic reports, the data from the tested clinical samples of TORCH-noninfectious pregnant women and TORCH-infected pregnant women showed that the negative coincidence rate was 91.7%, the positive coincidence rate reached 100%, and the total coincidence rate exceeded 90%. However, the results from ELISA used to test the same clinical samples displayed that the total coincidence rate was about 81%, the positive coincidence rate reached 89%, and the negative coincidence rate was only 78%, which indicates that the accuracy and sensitivity for this immune imaging system is significantly superior to ELISA, and that it is a promising one applied to clinical diagnosis.
Funding: This work was supported by the Guangzhou City Science and Technology Program Synergistic Innovation Major Project (grant number: 201604020146)

LB036

The diagnostic and prognostic value of new urine based leishmania antigen detection test

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Background: Definitive diagnosis of visceral leshmaniasis is by parasitological demonstration of amastigate in splenic, lymph node or bone marrow aspirates, which are collected using invasive methods and unsuitable in the field. There is no satisfactory test of cure to monitor the efficacy of treatment. This study aimed to evaluate the diagnostic and prognostic the new urine based ELISA test and the newly developed rapid diagnostic test (RDT) for diagnosis of VL and monitoring the response to treatment.
Methods: The newly developed urine ELISA test and new urine based rapid test were evaluated using urine samples. The confirmed VL patients ware followed during the treatment period, and urine samples were collected at day8, day17, day30, and day90 for detection of parasite antigen.
Results: The sensitivity of urine lateral flow assay was 72.2%. The test identified 5(7%) out of 71 of smear negative participants as positive reactors, and exhibited a specificity of 93.42%. The sensitivity of urine ELISA reached 83.33 % and specificity 95.05%. The leishmania antigen ELISA became negative 2 months after completion of treatment in 78 out of 83 patients. Only two SSG-treated remained positive at the end of treatment. On the other hand, 3 patients combination-treated (SSG+PM) remained positive at the end of treatment (day 17).
Conclusion: Urine based antigen tests have the advantages of non-invasive sampling and accurate diagnosis of infected patients. The two tests showed high potential to improve the diagnosis of VL and to monitor the response of patients to treatment.

LB037

Nanostructured lipid carriers (NLC) delivering Punica granatum seed oil with antimicrobial activity against *Staphylococcus epidermidis*: production, characterization and *in vitro* studies

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DOI: 10.1002/eji.201970400

Staphylococcus epidermidis has been recognized as the most common cause of device-related infections that occur in hospitals and is thus recently known as an essential opportunistic pathogen. On the other hand, the antimicrobial effect of NLC associated products is an attractive topic for finding more effective agents. The main aim of this study was the preparation and characterization of nanostructured lipid carriers (NLCs) encapsulated with P. granatum seed oil and assessment of antimicrobial effect of this combination against *Staphylococcus epidermidis*.
Particle size distribution, morphology, as well as physical stability of the optimized formulation, was organized by particle size analysis, X-ray diffraction (XRD), scanning electron microscopy, gas chromatography and hot melt homogenization method. MIC (Minimum inhibitory concentrations) of P. granatum seed oil -loaded NLCs were assessed and compared to seed oil emulsion of P. granatum against *Staphylococcus epidermidis*. NLCs loaded with seed oil of P. granatum were spherical shaped nanosize (102.1 nm) particles having a narrow size distribution (PDI = 0.268) and good physical stability following storage for 3 months. The results from XRD revealed that the seed oil of P. granatum had an amorphous form within nanoparticle matrix. Antibacterial assay showed that P. granatum seed oil-loaded NLCs displayed a significant in vitro antimicrobial activity than seed oil emulsion of P. granatum.
In conclusion, NLCs may be a favorable carrier for the development of new antimicrobial agents.

LB074

Profiling inflammatory response in active lesion of cutaneous leishmaniasis patients using a non-invasive sampling method and a high-throughput protein detection assay

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Background: Cutaneous leishmaniasis (CL) is an infection caused by *Leishmania* protozoa transmitted through the bite of sand flies, and elicits an inflammatory response in the skin. Previously, invasive sampling of blood and skin along with low-throughput methods were used for determination of inflammatory response in CL patients. Here, we report a novel approach based on a non-invasive sampling combined with a powerful multiplexing technique called proximity extension assay (PEA) for profiling 92 inflammatory cytokines, chemokines and surface molecules in CL patients' lesions infected with *L.tropica*.
Method: Sample collections were done non-invasively by using adhesive tape-discs from lesion and normal skin of 33 *L.tropica* positive patients.
Result: Out of 92 inflammatory proteins, the level of 34 proteins was significantly increased in the lesion of CL patients. This includes the chemokines CCL2, CCL3, CCL4, CXCL9, CXCL10, CXCL1, CXCL11 and CXCL5, together with the interleukins IL-18, IL-8, IL-6, LIF and OSM, surface molecules and receptors CD40, CDCP1, 4E-BP1, TNFRSF9, CD5, IL-18R1 and OPG as well as 16 other cytokines and proteins (MMP-1, CSF-1, VEGFA, uPA, EN-RAGE, LAP, HGF, MMP-10, CASP-8, TNFSF14, STAMPB, ADA, TRAIL and ST1A1). Furthermore, 13 proteins showed an increasing trend in the CL lesions, including TGF-α, CCL23, MCP-2, IL-12B, CXCL6, IL-24, FGF-19, TNFβ, CD6, TRANCE, IL10, SIR2 and CCL20.
Conclusion: We established a novel approach based on a non-invasive sampling method combined with the high-throughput protein detection method PEA for profiling proteins involved in the inflammatory response in CL lesions, which may have implications for other skin infections and disorders.

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DOI: 10.1002/eji.201970400

LB075

Bioengineered polyester beads coated with *Mycobacterium tuberculosis* antigens enable enhanced diagnosis of tuberculosis

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Despite recent advances in prevention and diagnosis, tuberculosis (TB) remains among the top 10 causes of death. Diagnosis of latent TB (LTBI), a major reservoir for acute TB, remains challenging. Here, we bioengineered polyester beads to present *Mycobacterium tuberculosis* (Mtb) proteins (ESAT6, CFP10, MTB7.7) as stimulating antigens for the interferon-γ release assay (IGRA), a blood test for diagnosis of Mtb infection. The beads-based assay (BIGRA) showed a 10% increased sensitivity for diagnosis of pulmonary tuberculosis (PTB) when compared with the commercially available Qantiferon-TB Gold in-tube (QFT) assay. In healthy Tuberculin Skin Test (TST) negative and positive individuals the specificity was decreased by 3-fold when compared to QFT. BIGRA showed an about 2-fold increased sensitivity for LTBI diagnosis in healthy TST positive individuals when compared to QFT. The bioengineered antigen-displaying polyester beads represent a new materials platform for immunodiagnostic tests such as enhanced IGRA for diagnosis of Mtb infection.

LB111

The emergence of arboviruses changes the profile of viral meningitis in Salvador, Bahia

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Background: Recently, different arboviruses became endemic in Brazil mostly causing acute febrile illnesses, however neurological manifestations have also been reported. This study aimed to investigate which viruses were involved in the meningitis etiology and the contribution of the circulating arboviruses in Salvador, Bahia, Brazil.
Methods: From June, 2014, to February, 2016, 170 patients with suspected viral meningitis were identified in Couto Maia Hospital, Salvador-BA, Brazil. Their CSF samples were investigated for possible viral etiology by RT-PCR for different arboviruses: DENV, ZIKV and CHIKV, and for the EV; and PCR for the HHV1-5 complex (HSV1-2, VZV, EBV and CMV). Also, ELISA was performed to detect IgM antibodies against DENV, ZIKV and CHIKV and NS1 antigen of DENV.
Results: Thirty-nine patients were PCR or ELISA positive for at least one of the studied viruses (overall prevalence 22.9%), from which arboviruses accounted for 69.2%. DENV was the agent most frequently detected (13 cases; 33.3%). We also detected 6 (15.4%) cases of CHIKV and 8 (20.5%) of ZIKV. Among the non-arboviral meningitis, the most common etiology was EV (11 cases; 28.2%). Only one (2.6%) sample was confirmed to be Varicella zoster virus (HHV3 or VZV). Among those, four cases represented of viral co-infection: DENV1+CHIKV, DENV1+EV, DENV4+ZIKV, and CHIKV+ZIKV. **Conclusions:** Arboviruses accounted for the majority of identified viruses among patient with confirmed viral meningitis. In areas where they are endemic it is crucial to increase viral surveillance and consider them in the differential diagnosis of meningitis.

DOI: 10.1002/eji.201970400

P0566

Anti-double-stranded DNA isotypes and anti-C1q antibody improve the diagnostic specificity of systemic lupus erythematosus

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Objectives: *We aimed to evaluate the value of immunoglobulin (Ig) G, IgM, and IgA isotypes of anti-dsDNA and anti-C1q antibody in diagnosing systemic lupus erythematosus (SLE) patients and elucidate their association with disease activity and lupus nephritis.*
Methods: Blood samples were obtained from 96 SLE patients, 62 other autoimmune disease patients, and 60 healthy blood donors. Anti-dsDNA IgG, IgM, and IgA isotypes and anti-C1q antibody were measured by enzyme-linked immunosorbent assay. Disease activity of SLE patients was assessed according to the SLE Disease Activity Index score.
Results: The prevalence of anti-dsDNA IgG (p = 0.002), anti-dsDNA IgA (p = 0.028), and anti-C1q antibody (p = 0.000) in active cases was significantly higher than those in inactive ones. In addition, the presence of anti-C1q antibody was associated with renal involvement (p = 0.032). Anti-dsDNA IgM showed no significant association with disease activity but it was inversely linked with lupus nephritis (p = 0.005). When anti-dsDNA IgG and IgA and anti-C1q were combined to evaluate SLE disease activity, the specificity reached the highest level (90%). When anti-C1q positive was accompanied by anti-dsDNA IgM negative, the specificity of diagnosing lupus nephritis was up to 96%. **Conclusions:** This study demonstrated the role of anti-dsDNA IgG, IgM, and IgA isotypes and anti-C1q antibody alone or combination in diagnosing SLE. Anti-dsDNA IgG and IgA and anti-C1q were shown to be associated with disease activity, while anti-dsDNA IgM and anti-C1q were associated with lupus nephritis. When the related antibodies were comblied, the diagnostic specificity was significantly higher.

P0567

High mobility group box 3 as an emerging biomarker in diagnosis and prognosis of hepatocellular carcinoma

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Purpose: High mobility group box 3 (HMGB3) is associated with hepatocytes malignant transformation by our previous work. We continued to investigate the diagnostic and prognostic values of HMGB3 for hepatocellular carcinoma (HCC).
Patients and methods: Circulating HMGB3 levels were quantitatively detected in a cohort of 225 patients with chronic liver diseases by ELISA and compared with alpha-fetoprotein by the receiver operating characteristic curve. HMGB3 expression in tissues of 170 HCC was detected by tissue microarray and immunohistochemistry. Relationship between HMGB3 level and HCC prognosis was evaluated by the Kaplan-Meier curves and Cox regression model.
Results: The incidence of serum HMGB3 >2.0 ng/mL was 75.6% in HCC (96/127), 20.8% in liver cirrhosis (10/48), 16.0% in chronic hepatitis (8/50), and none in healthy controls (0/49). Significant difference (P< 0.001) of circulating HMGB3 level was found between HCC and benign liver diseases. Total diagnostic sensitivity of serum HMGB3 plus alpha-fetoprotein was up to 89.0% for HCC. Higher

DOI: 10.1002/eji.201970400

HMGB3 expression was confirmed to be 73.5% in HCC tissues (125/170) >30.6% in their paracancerous tissues (52/170). HMGB3 expression was closely related to tumor size, TNM stage, poor survival, and high recurrence, suggesting an independent prognosis factor for HCC.
Conclusion: HMGB3 with aberrant expression could be a novel diagnostic and prognostic marker for HCC.

P0568

Development and validation of anti-ADA2 single chain antibodies coupled to alkaline phosphatase for the diagnosis of pleural tuberculosis

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Background: To determine the diagnostic utility of the adenosine deaminase (ADA)-2 isoenzyme measured by a new method in diagnosing tuberculous pleuritis and to compare its performance with that of the total ADA.
Methods: A retrospective study of 41 patients with tuberculous pleuritis and 47 with non-tuberculous etiologies (23% of them with elevated total ADA in pleural fluid). ADA-2 isoenzyme levels were determined by a quantitative assay with anti-ADA2 single-chain antibodies fused to alkaline phosphatase.
Results: Pleural fluid total ADA > 35 U/L and ADA-2 > 300 ng/mL yielded respective sensitivities of 98%, specificities of 77% and 91%, positive likelihood ratios (LR) of 4.2 and 11.5, and negative LR of 0.03 for the diagnosis of pleural tuberculosis. Eleven (6 parapneumonic and 5 malignant pleural effusions) and 4 (1 parapneumonic and 3 malignant pleural effusions) patients presented falsely elevated levels of total ADA and ADA-2, respectively.
Conclusion: Pleural fluid ADA-2, using this new method, improves the specificity of the total ADA by 14% in the diagnosis of pleural tuberculosis.

P0569

A combination of ATAC-seq and RNA-seq was used to study the downstream genes of FOXQ1 transcription factor in colorectal cancer

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Objective: Forkhead box Q1 (FOXQ1) gene is highly expressed in colorectal cancer(CRC).In this study, we tried to find the downstream gene of FOXQ1 transcriptional regulation in CRC cells, so as to provide a new drug target for the treatment of CRC.
Methods: ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) and RNA-seq were combined to analyze the sequencing of two CRC cell lines with knock down FOXQ1 gene. According to overlapping analysis, conjoint analysis, KEGG analysis to find the potential downstream target genes of FOXQ1 transcriptional regulation in CRC.
Results: (1) Conjoint analysis: The results showed that there were 973 up-regulated genes in the

DOI: 10.1002/eji.201970400

chromatin open enhancement region and 1975 down-regulated genes in the chromatin open attenuation region of DLD1 cell line. There were 752 up-regulated genes in the chromatin open enhancement region and 581 down-regulated genes in the chromatin open attenuation region of SW480 cell line. (2) Overlapping analysis: In DLD1 and SW480 cell lines, there were 61 genes with both enhanced and up-regulated chromatin open regions, and 70 genes with both decreased chromatin open regions and down-regulated chromatin open regions. (3) Pathway analysis: We will focus on the inflammatory bowel disease , which is significant enrichment of the up-regulated SMAD3 and TLR2 genes . The p53 signaling pathway , which is significantly enrichment down-regulated CCND1, EI24 , and CDK6 genes. Inflammatory mediator regulation of TRP channels, which is significantly enrichment down-regulated the MAP2K3 and PRKCD genes.

P0570

A novel lateral flow test strip based on latex microspheres and colloidal gold composite nanoparticles for influenza viruses detection

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Lateral flow assays (LFAs) are highly attractive for point-of-care testing (POCT) for clinical diagnosis, food safety, and many other medical uses because of its simplicity, rapidity, and low cost. However, these designs often suffer from only capable of single analyte detection, low-signal intensity and poor sensitivity. Therefore, different kinds of nanoparticles (NPs) has been exploited as a simple and feasible strategy to overcome these challenges and improve the detection sensitivity of LFAs. Here, We report a simple and effective alternative approach that involves binding a large number of individual colloidal gold onto larger latex microspheres nanoparticles to obtain a composite gold-nanoparticle-decorated latex microspheres (G-MNPs) probes for higher sensitively lateral flow test. With utilizing the good biocompatibility, convenient surface modification, and the big binding capacity of latex microspheres the new composite G-MNPs were used for SIV H3N2 subtype detection. Under the optimal detection conditions, the visual detection limit based G-MNPs was as low as 1/16 (HAU), which has 64- fold improvement than the colloidal gold-labelled lateral-flow strips (4 HAU). Same as traditional colloidal gold test paper, the new probe strip has no nonspecific or cross-reaction in the detection of other influenza virus subtypes (H1, H5, H7, H9). The method are not only can be used for SIV sensitivity and early Clinical diagnosis, but also has a potential application for other targets.
Keywords: Gold-nanoparticle-decorated latex microspheres; Lateral flow assay; Influenza Viruses; Sensitive detection; Nanotechnology

DOI: 10.1002/eji.201970400

P0571

Development of a lateral flow immunoassay for the rapid diagnosis of invasive candidiasis

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Early and accurate diagnosis of invasive candidiasis (IC) is very important. In this study, a lateral flow immunoassay (LFIA) was developed to detect antibody against *Candidaalbicans* enolase (Eno). Colloidal gold particle labeled mouse anti human IgG (1.0mg/L) was used as the detector reagent. Recombinant enolase (rEno,1.0mg/L) and goat anti IgG (1.0mg/L) were immobilized in test and control lines, respectively, of a nitrocellulose membrane, acting as the capture reagents. The LFIA was used to detect anti Eno in 38 sera from clinically proven IC patients, as well as in 50 healthy control subjects. Compared with an indirect ELISA designed as a reference test, the specificity and sensitivity of the LFIA were 98.2 and 84.8%, respectively. Excellent agreement between the results obtained by ELISA and the LFIA (k=0.851) was observed in this study. Inaddition, the agreement between the blood culture results and LFIA test is strong (k=0.658). The data presented in the study indicate that the LFIA test is a suitable tool for the serological surveillance of IC in the field or in poorly equipped laboratories.

P0572

Nucleic acid-unextracted, isothermal and discriminative detection of classical swine fever virus virulent and vaccine vtrain by CRISPR/Cas13a

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Classical swine fever (CSF) is an OIElist A porcine infectious disease, causinggreateconomiclosses to the swineindustryworldwide during the past decades.Discrimination of CSFV infected and vaccinated animals is urgent for eradication of CSF.In this study,an isothermal molecular diagnostic method SHERLOCK(Specific High Sensitivity Enzymatic Reporter UnLOCKing) was introduced in combination of recombinasepolymerase amplification(RPA) and CRISPR/Cas13acollateral RNase assay. With screened CSFV universal primer pairs and strain-specific CRISPR RNAs, SHERLOCK achieveddiscriminative detection of Shimen and HCLV viral RNAs as fluorescent signals.In addition, with the use of heat and chemical reduction, HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases) treatment was also introducedto avoid extractionof viral nucleic acids. HUDSON-SHERLOCK assay can detect Shimen and HCLV at the titer level up to 10^4 $TCID_{50}$/mL and 10^2 $TCID_{50}$/mL respectively, exhibiting an equal or higher sensitivities compared with qPCR and IDEXX CSFV antigen ELISA.Meanwhile, HUDSON-SHERLOCKshowed no cross-reactivity with other viral cell cultures includingBovine viral diarrhea virus(BVDV), Porcine reproductive and respiratory syndrome virus(PRRSV), Porcine epidemic diarrhea virus(PEDV), Pseudorabies virus(PRV) and Porcine circovirus 2 (PCV 2). Positive fluorescent readouts were also obtained when detectingthe spleen of Shimen-infected pigs, indicating the potential applications of HUDSON-SHERLOCK for field clinical samples.
Keywords: Classical Swine Fever Virus, Recombinase Polymerase Amplification, CRISPR/Cas13a, Discrimination of Infected and Vaccinated Animals

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DOI: 10.1002/eji.201970400

P0573

Expression of prostate-specific membrane antigen in tumor-associated vasculature predicts poor prognosis in hepatocellular carcinoma

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Objective: To investigate prostate-specific membrane antigen (PSMA) expression in the tumor-associated vasculature of hepatocellular carcinoma (HCC), and to analyze the association between PSMA expression and other clinicopathological features and prognosis.
Methods: Tissue samples of HCC were collected and tissue slides were used for the detection of CD31 and PSMA expression by immunohistochemical staining. Patients' information was collected and the association between vascular PSMA expression and clinicopathological features and prognosis were analyzed.
Results: Among the 103 cases, 27 (26%) cases showed PSMA expression in more than 50% of tumor-associated vasculature; 49 (48%) cases showed PSMA expression in less than 50% of vasculature; while 27 (26%) cases did not have detectable PSMA expression. Vascular PSMA expression was associated with several clinicopathological features, such as tumor stage, tumor differentiation, lymph node metastasis and Ki-67 index. Furthermore, high vascular PSMA expression was also associated with poor prognosis in HCC patients.
Conclusion: PSMA was expressed in tumor-associated vasculature of HCC. Vascular PSMA expression is closely associated with the clinicopathological features and prognosis of patients with HCC, which may be used as a novel prognostic marker and a vascular therapeutic target for HCC.

P0574

z-Movi: a high throughput cell-cell avidity screening and fractionating acoustic force-based technology

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z-Movi, uses acoustic forces to assess the overall strength of binding or 'avidity' between immune cells and their targets, as a parameter reflecting interaction efficiency, and to collect enriched cell population fractions. Our technology can analyze thousands of cells in parallel with controllable and non-invasive forces in a label free manner, enabling us to perform further analysis, such as sequencing, mass spectrometry, flow cytometry, and functional (cytotoxicity) assays. This has great potential to disrupt immunology applications like Immunotherapy, where one of the main obstacles has been the generation of immunotherapies promoting effective and long-lasting immune responses, due to the lack of tools measuring avidity. Here, we show our availability to identify the T cells with the highest avidity for melanoma cells and to measure an increased interaction strength of patient derived T cells to specific tumor targets cells in the presence of Bi-specific T-cell engagers (BiTEs). The z-Movi is also a useful tool to obtain insights into the cell adhesion process and we demonstrate how we can resolve the adhesion forces and kinetics of CD4+ T lymphocytes to fibronectin.
z-Movi is not only applicable on immunotherapies and cell adhesion but more in general in the antigen presentation field, as well as for other applications like vaccination and autoimmunity.

DOI: 10.1002/eji.201970400

P0575

High-throughput and high-dimensional profiling of single antigen specific T cells

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Identification of correlated antigens recognized by T cell and its T cell receptor (TCR) sequences at single cell level is extremely useful to the understanding and treating immune-related diseases. Although CyTOF has pushed the number of antigenic peptides that can be interrogated at once to around 100, the destructive nature of CyTOF prohibits linking pMHCs recognized by a T cell with its TCR receptor sequences.
We previously developed a new technology, **Tet**ramer associated **TCR seq**uencing (TetTCR-Seq), that is capable of de novo generating a large amount of peptides and mapping antigen recognition with single cell TCR sequences by DNA barcoded tetramers. It involves three major steps: 1) de novo peptide generation, 2) DNA barcoded tetramer generation, and 3) single cell sequencing of tetramer DNA barcode, TCR sequences and gene expression.
Further, we expanded the dimensions that TetTCR-Seq is capable of profiling and developed TetTCR-SeqHD. We applied TetTCR-SeqHD on high throughput single cell gene expression analysis platform, to simultaneously profile T cell antigen specificities, TCR repertoire, transcriptional and proteogenomic profiles from thousands of single cells and demonstrated the application of TetTCR-Seq to reveal phenotypic and TCR usage differences of distinct antigen specific T cells.
In summary, TetTCR-SeqHD enables one to quickly survey T cell repertoire at single cell level for a large number of peptides. It is a useful technology to provide a comprehensive evaluation of antigen-specific T cell repertoire under various immune-related diseases, and provide an approach to the development of better prognosis and treatment decision metrics.

P1163

Acute myeloid leukemia patients with DNMT3A mutation: a single-institution study

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Background: DNA methyltransferase 3α (DNMT3A) plays a vital role in *de novo* methylation, and mutations in the *DNMT3A* gene contribute to cancer progression. This study aimed to investigate the laboratory features of acute myeloid leukemia (AML) with mutated *DNMT3A* and to determine the correlation between *DNMT3A* mutation and other laboratory characteristics in AML patients.
Methods: Bone marrow cells were collected from 112 patients with primary AML at The Affiliated Cancer Hospital of Zhengzhou University, China. Gene mutations were detected by next-generation sequencing, immunophenotyping was carried out by multi-parameter flow cytometry, gene fusion was detected by real-time polymerase chain reaction, and karyotype analysis was performed by RHG chromosome banding. Patients were divided into two groups according to the presence or absence of *DNMT3A* mutations.
Results: *DNMT3A* mutations were found in 11.61% of AML patients. Patients with mutations had higher white blood cell (WBC) counts (73.75 ± 23.203 vs 34.10 ± 5.748, p=0.029), a higher frequency of nucleophosmin gene (*NPM1*) mutations (38.5% vs. 8.1%, p=0.007), more frequent expression of CD7 (53.8% vs. 22.2%, p=0.037) and CD4 (61.5% vs 13.1%, P< 0.001), and were more likely to have acute monocytic leukemia (84.6% vs. 15.4%, p=0.003) subtypes compared with patients with wild-type *DNMT3A*.
Conclusion: AML patients with *DNMT3A* mutation are more likely to have increased WBC counts, express CD7 and CD4, and have co-existing *NPM1* mutations, which may explain the poor prognosis of this group of patients.

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DOI: 10.1002/eji.201970400

P1164

Analysis of Immunophenotypic features of childhood acute myeloid leukemia

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Objective: To investigate the immune phenotypic characteristics of childhood acute myeloid leukemia(AML) and its clinical significance.
Methods: The immune phenotype in the 127 cases with childhood AML were detected by four color flow cytometry with CD45 / SSC double parameters scatter plot setting as door, and then the antigentic expressions were analysed.
Results: In 127 patients with childhood AML, the Myeloid antigen of CD33、CD13、CD117 showed highly expressed. The positive rate was 95.3%、90.6%、90.6%。Hematopoietic stem/progenitor cell marker antigen CD34, HLA-DR and CD38 were expressed in AML with the rate of 53.5%, 71.6% and 97.6%. The lymphoid antigen was positive in 65.4%of cases, with the highest frequency of CD56(38.6%)followed by CD7(21.3%). The expression of CD34+ and HLA-DR+ patients in LymAg+ group were significantly higher than LymAg- group (P＜0.05).
Conclusion: The immunophenotyping played an important role in diagnosis and classification of childhood AML.
Key words:acute lymphoblastic leukemia；childhood; immunophenotype；flow cytometry

P1166

New applications of RabMAb® pairs to multiple immunoassay platforms in cytokine measurement

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Cytokines are vital intercellular communication molecules which play various roles in regulation immunity, inflammation and hematopoiesis. Many of them are considered as important circulating biomarkers in immunology, oncology and neurology. Usually picogram level of cytokines secrete into body fluids. Sensitivity therefore, is the main challenge in cytokine biology. Differ from other quantification methods, immunoassay is a powerful tool to detect low abundant targets. A higher sensitivity is always intriguing for the accuracy in cytokine detection and quantification.
Rabbit Monoclonal Antibodies (RabMAb®) are well known for the specificity and higher affinity (can reach K_D10^{-10} to 10^{-12}), compared to commonly used mouse monoclonal antibodies (K_D10^{-7} to 10^{-9}). Taking these advantages, Abcam has implemented a specialized pipeline to develop best-in-class RabMAb® pairs against the key cytokines. All the RabMAb® antibody pairs were fully validated and published as SimpleStep ELISA®. 80% immunoassays meet or beat benchmarks and exhibit high specificity, robust reproducibility with high sensitivity.
Furthermore, Abcam implemented those highly validated RabMAb® pairs into multiplex assay platform, called FirePlex immunoassay. It combines innovative FirePlex particle technology along with optimized RabMAb® pairs for analyte detection. This platform provides an easy and efficient solution to accelerate and scale up the cytokine profiling directly from crude biofluids, while minimizing workload.
From biomarker profiling to thoroughly quantification till routine screening, Abcam immunoassays

DOI: 10.1002/eji.201970400

provide a full spectrum of robust tools based on the top-class RabMAb® pairs. As an example, we pictured how researchers can be benefit from FirePlex Human Key Cytokines - Immunoassay Panel (ab229791).

P1167

CCL22 and Foxp3 are key mediators of the immunosuppressive properties of cancer-associated inflammatory signatures in pancreatic tumor microenvironment

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Pancreatic adenocarcinoma (PDA) is characterized by a highly suppressive tumor microenvironment, which leads to poor clinical outcomes. We performed a comprehensive study integrating immune cellular-molecular analyses from the tumor tissues and validating on human immune system(HIS) mice with patient derived Pancreatic adenocarcinoma tissues. In paired analyses of adenocarcinoma and adjacent normal tissues from patients, we found an increased frequency of immune suppressive FOXP3 Treg cells in conjunction with the local infiltrated high immune checkpoint molecule PD-L1 expressed cells. Subsequently, patient subgroups were identified with high densities of FOXP3 Treg and with low CD8/FOXP3 and low CD8/PD-L1 ratios were correlated with poor survival. Transcriptome analysis of heterogeneity in epithelial cells, stromal cells, and T cell subsets obtained from pancreatic tumor samples from patients revealed a distinct gene expression signature associated FOXP3 with overexpression of CXCL9, CXCL10, CCL18 and CCL22 found upregulated in cancerous epithelium/stromal. In addition, CCL22 favors promoted Terg migration but did not covert T cells to Treg. Treg also regulated IL35-mediated TIM3, leading to CD8 T cell exhaustion. Additionally, among human immune system(HIS) mice with implanted patient derived pancreatic adenocarcinoma tissues of high level FOXP3 and CCL22, administration of anti-CCL22 was sufficient to significantly slow tumor growth, which correlated to the reduced the FOXP3 mediated by CCL22, and increased CD8 T cells for antitumor immunity. Our results offer a proof of concept to use humanized mouse to validation the prognostic value of FOXP3 as a biomarker associated with unique gene expression signature of cytokine, chemokines in pancreatic tumor microenvironment.

P1168

3D culture system mediated by self assembled polypeptide biomaterials enhanced the amplification of mouse hematopoietic stem cell and progenitor cell

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To explore that the 3D induction system mediated by the self assembling peptide biomaterial will do help to provide a good platform for in vitro research of the HSC generation from PSC, we further evaluated that whether the 3D induced system is effective for the expansion of murine fetal liver or bone marrow derived hematopoietic stem cell and progenitor cell compared with 2D culture system or not. We used 3D self assembling peptide hydrogel biomaterials combined with hematopoietic cytokines and small molecular compounds to expand the ckit+ cells derived from bone marrow. We found that,comparing with 2D same culture conditions, 3D self assembling peptide hydrogel combined with cytokines and small molecular compounds can better amplify the ckit^{+} cells and maintain

DOI: 10.1002/eji.201970400

hematopoietic stem cell and progenitor cell. In 3D culture system LSK cells owned the higher ratio compared with 2D under the same condition. CFU assay showed that the total number of CFU in 3D condition is higher than 2D culture system with significantly differences.We got the conclusion that 3D culture system mediated by self assembling peptide biomaterial is more conducive to the maintenance of bone marrow derived hematopoietic stem cell and progenitor cell compared with 2D culture system. Meanwhile, 3D culture system is used to the growth of hematopoietic downstream lineage cells. Comparing with 2D, 3D system can better simulate the nich of HSC to provide a theoretical basis for exploring the HSC differentiation from PSC.

P1169

Development and application of diphtheria toxin antigen detection system

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Objective: To develop a detection system for the quantification of diphtheria toxin (DT).
Methods: The checkerboard titration method was used to determine the concentration of coated antibody and enzyme-labeled antibody, and double antibody sandwich ELISA (DAS-ELISA)was established to determine the linear detection range and verify the specificity, repeatability, accuracy and precision of the method.
Results: By checkerboard method, the optimal concentration of coating antibody was 5 µg/mL, and the working dilution of HRP-labeled antibody was 1:2000. When DT was detected in an range 0.0027 Lf/mL to 0.0429 Lf/mL, the detection showed a good linearity with a correlation $R^2 > 0.98$. The assay also showed excellent repeatability, precision and specificity with the requirements of the routine quality control. The method was used to determine the content of diphtheria toxin at each stage of the diphtheria production process, and compared with the flocculent unit method, there was a highly correlation between the two methods (Pearson´s correlation = 0.897).
Conclusion: The developed ELISA provided an effective quality control method for the production process of diphtheria vaccine and the evaluation of the adsorption rate of diphtheria toxoid in the associated combined vaccine.

P1170

Assessment of antibody functional affinity using ImmunSpot®

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Studies of B cell immunity often relay upon serologic methodologies that primarily assess antibody specificity. However, functional affinity of the antigen-specific antibody repertoire is a key determinant of protective efficacy. Moreover, conventional assessments of serum antibody occur at the population level and may not accurately capture the spectrum of functional affinities. At present, detailed assessment of single B cell/antibody functional affinity is labor-intensive and low-throughput. Therefore, we sought to evaluate the hypothesis that B cell Fluorospot would enable distinction between B cells with differential functional affinity since fluorescent intensity is directly proportional to antibody abundance in this assay. Furthermore, since antibodies with increased functional affinity for the antigen should be efficiently captured in close proximity to the secreting cell, whereas low-affinity antibody would be more prone to diffusion, the Fluorospot morphology is also anticipated to reflect

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DOI: 10.1002/eji.201970400

upon differential functional affinity. To test our hypothesis, murine B cell hybridomas (P65C6-2, 36-65 and 36-71) secreting monoclonal antibodies (mAb) with differential affinity for the p-azophenylarsonate (Ars) hapten were utilized as model antibody-secreting cells (ASC) and resulting Fluorospots evaluated using the Fluoro-X™ suite. Differential functional affinity of Ars-specific ASC resulted in highly reproducible Fluorospot morphology. Additionally, using an anti-idiotypic mAb (17-63) specific for the high-affinity anti-Ars mAb (36-71), unambiguous segregation of Ars-specific ASC was confirmed. Collectively, our data support the notion that fluorescent ImmunoSpot® assays can be used to assess the functional affinity of antigen-specific B cells, and that this platform is well-suited for detailed assessment of humoral immunity.

P1171

Flow-based multiplex assay panels for quantifying immune effector molecules from leukocytes

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Leukocytes including T and B cells, macrophages, neutrophils, dendritic cells, NK cells, and the soluble factors they produce are the driving force and/or regulators of immune responses, which coordinate host defense, tissue repair, and immunoregulatory functions. In addition, these cells and the factors they produce are also critically involved in various disease states including infection, autoimmune disorders, asthma, diabetes, and cancer. Therefore, quantification of these molecules in a cell type-specific context will help achieve in-depth understanding of both immune responses and disease processes.
We have developed multiplex bead-based assay panels targeting specific leukocytes, which are suitable for use on common flow cytometers. The human panels collectively allow for the detection of IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, IL-17F, IL-21, IL-22, IL-23, IFN-γ, TNF-α, CCL17/TARC, CXCL10/IP-10, Arginase, APRIL, BAFF, sCD40L, TNF-β, sFAS, sFAS ligand, Granzyme A, Granzyme B, Perforin and Granulysin, while the mouse panels for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, IL-17F, IL-18, IL-21, IL-22, IL-23, IFN-γ, TNF-α, CCL17/TARC, CCL22/MDC, CXCL1/KC, G-CSF, BAFF, sBCMA, sCD40L and TGF-β1.
These panels have been optimized for assay specificity, sensitivity, accuracy and reproducibility, and validated by detecting expected expression level changes of analytes in biological samples cultured under various stimulation conditions. These high quality, low cost, and easy to use panels will be useful tools for the study of the specific cells involved in innate and adaptive immune responses as well as relevant diseases.

P1760

Detection of gene rearrangement in DLBCL by next generation sequencing

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Purpose: To explore the value of Next Generation Sequencing (NGS) in the auxiliary diagnosis of diffuse large B cell lymphoma (DLBCL).
Methods: Of 20 cases, 15 were diagnosed as diffuse large B cell lymphoma (DLBCL) and 5 as lymphoid tissue reactive hyperplasia (LRH). Routinely blocks were cut and dewaxed, DNA were extracted, amplified purified and pooled, and lastly sequenced as the protocol of clonality detection of IgH FR2 and TRG on the platform of PGM.

DOI: 10.1002/eji.201970400

Results: All 5 reactive hyperplasia cases showed polyclonal in the PGM clonality detection. 11 DLBCL cases (73.3%, 11/15) showed monoclonal in PGM IgH FR2 clonality detection. Interestingly, additional 4 cases (case 2, 5, 8, 15), which were shown polyclonal in the PGM IgH FR2 clonality detection, were demonstrated IgH monoclonal in the conventional PCR gel electrophoresis clonality detection. Meanwhile, of 3 cases (case 6, 7 and 14), which were shown monoclonal in PGM TCRG clonality detection, only one case (case 7) showed monoclonal in the PCR gel electrophoresis clonality detection.
Conclusion: Although PGM high-throughput sequencing clonality detection method is highly specific, sequences obtainable, its detection sensitivity is still need to be improved in the future.

P1761

Analysis of serum antinuclear antibody test results in 7016 cases

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Objective: To analyze three methods of ANA detection, namely ANA qualitative, ANA quantification and ANA spectrum quantitative results.
Methods: Randomly selecting 7016 serum samples which were detected qualitative and quantitative of ANA by the three methods respectively.
Results: The positive rate of ANA quantification, ANA quantification and ANA spectroscopy in 7016 serum samples were 14.9%, 13.5% and 9.8% respectively. The sensitivity of ANA qualitative plus quantitative detection was highest (88%), missed diagnosis rate (12%) lowest, negative predictive value (97.5%) highest and Youden index (87.2%) highest, ANA qualitative + ANA spectrum quantitative detection specificity (100%), the misdiagnosis rate (0.03%) was the lowest, the positive predictive value (99.8%) was the highest, and the odds ratio (8753.8) was the largest. Comparing the three test results, the overall distribution difference was statistically significant (both $P < 0.05$); the Kappa values of the two comparisons were better (Kappa values were 0.776, 0.883, and 0.824, respectively). When the ANA was qualitatively negative, the coincidence rates of ANA quantification and ANA spectrum quantitative results were 99.16% and 99.97% respectively.
Conclusion: Selecting a single method for ANA detection easily lead to missed diagnosis, and joint detection is indispensable. In the early stage of disease screening and health examination, ANA qualitative and quantitative combination can be selected to save cost and prevent missed detection. In the diagnosis and curative effect observation of specific diseases, ANA qualitative plus ANA spectrum quantification should be selected.

P1762

Development and optimization of a TDAR quantitative method for BALB/c mice using KLH as a specific antigen

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Although TDAR with KLH as the specific antigen had many advantages, most experiments conducted with this formulation are qualitative. We hypothesized that if the results could be quantitative, the sensitivity and reproducibility of TDAR assays could be greatly improved. In this study, we obtained KLH-immunized serum from BALB/c mice, and optimized the formula to detect serum antibodies by indirect ELISA. According to our results, the optimal coating concentration of KLH was 80 µg/mL; the optimal dilution range of the serum at an immunization dose of 5 mg/Kg was 1:200 to 1:800; and the

DOI: 10.1002/eji.201970400

optimal dilution of the goat anti-mouse IgG/HRP-labeled conjugated antibody was 1:16000. Using this formula, we performed methodological verification in an indirect ELISA, and obtained a regression model of y = 144.16x + 0.9815 and a correlation coefficient of R^2 = 0.9571, which indicated very good linearity. The intragroup precision was determined as 7.51, 9.40 and 8.33 percent, and the intergroup coefficient of variation was 9.83, 14.22 and 14.10 percent. The lower limit of quantitative detection was 0.1385. Our results showed that the established indirect ELISA method exhibited very good linearity, accuracy, and precision.

P1763

Evaluation of Csa2 as a serodiagnosis marker differentiating *Candida albicans* colonization and infection by ELISAs

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Candida albicans is an opportunistic fungus which has both yeast and hyphal form. It is difficult to differentiate colonization and infection of *Candida albicans*. One of important virulence factors is to transform the yeast into hyphal form. Csa2 specifically exists in membrane of the hyphal form. In this study, we evaluate Csa2 as a potential biomarker for differentiation of colonization and infection. A double-antibody sandwich ELISA was developed to detect Csa2 antigen. Csa2 was detected specifically in the culture supernatant of *Candida albicans* hyphal form and had no cross-reaction with other pathogenic fungi of Candida. By 23 patients sera of *Candida albicans* urine tract infection as test group and 324 health sera and 7 patients sera of other pathogenic fungi of Candida urine tract infection as control group, the sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of this antigen assay were 21.74%(5/23), 99.09%(328/331), 71.43%(5/7) and 94.80%(328/346). Meanwhile, a double-antigen sandwich ELISA was also developed to detect Csa2 antibody in sera of test and control groups. The sensitivity, specificity, PPV and NPV of this antibody assay were 65.22%(15/23), 99.70%(330/331), 97.75%(15/16), 97.63%(330/338). **Csa2 is a potential biomarker differentiating *Candida albicans* colonization and infection. Csa2 antibody assay has higher sensitivity and PPV compared with its antigen assay.**

P1764

Clinical value of quantitative PCR technology for detection of lymph node tuberculosis in FFPE tissue

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Objective: To explore the application of fluorescent quantitative PCR (FQ-PCR) and its clinical value for histopathological detection of mycobacterium tuberculosis DNA (TB-DNA).
Methods: The paraffin blocks from 43 cases with lymph node tuberculosis were detected by quantitative PCR technique with TB-DNA in real time, and acid-fast staining and histopathological examinations were conducted at the same time.
Results: Among 43 cases with the clinical diagnosis of lymph node tuberculosis in paraffin-embedded tissues, there were 30 cases(69.78%)screened positive by fluorescence quantitative PCR, 12 cases (27,91%) screened positive by acid-fast staining examination, and 24 cases (55.81%) screened positive by histopathological examination. The fluorescence quantitative PCR detection was of the

DOI: 10.1002/eji.201970400

highest positive rate. Among 20 cases from fine needle aspiration biopsy, the FQ-PCR and the acid-fast staining shared the same positive rate,50%. While Among 23 cases from excisional biopsy, the fluorescence quantitative PCR detection was of the highest positive rate. **Conclusion:** The fluorescence quantitative polymerase chain reaction technology is simple, efficient, and of sensitivity and high specificity. Since it is of high clinical value, it can be used as a pathological diagnostic method for tuberculosis molecular detection.

The study was supported by Hainan Provincial Health Department medical research key project foundation 2012ZD-05 and Hainan Natural Science Foundation 309036.

P1765

A robust human immunophenotyping workflow using Maxpar Direct Immune Profiling Assay coupled with Maxpar Pathsetter, an automated data analysis solution for mass cytometry

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Immune monitoring is a key application in translational and clinical research, used in the investigation of chronic inflammation, infectious disease, autoimmune diseases, and cancer. The diversity of immune populations demands a high-parameter approach to fully and efficiently quantify these changes. Mass cytometry, utilizing CyTOF® technology, is a single-cell analysis platform that uses metal isotope-tagged antibodies to resolve over 40 markers in a single tube of sample without the need for compensation. It is an ideal solution for routine enumeration of immune cell populations.
We have developed a sample-to-answer solution for human immune profiling using mass cytometry. It includes an optimized 30-marker immune profiling panel provided in a dry, single-tube format, validated SOPs for human whole blood and PBMC staining, an instrument data acquisition template, instructions for data acquisition on a Helios™ system, and automated software for data analysis. The software automatically reports cell counts, percentage calculations, and staining intensity and produces graphical elements such as histograms, dot plots, and a Cen-se′™ (t-SNE variant) graph for 37 immune cell populations.
Here we present analytical validation data on repeatability and reproducibility for the Maxpar® Direct™ Immune Profiling Assay™ and on the precision and accuracy of Maxpar Pathsetter™ software using whole blood and PBMC. We also demonstrate comparative performance between the dry panel and a liquid panel of the same antibodies. This assay provides a robust, complete solution for broad immune profiling using mass cytometry that reduces sources of variability and subjectivity in sample preparation and data analysis.

P1766

Discover cell signaling pathways using antibody arrays

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Cell signaling is comprised of complex networks that regulate homeostasis and immune response to diseases. The analyses of such pathways would improve our understanding of the molecular mechanism of human disease, immune response and direct drug development. However, it remains a

DOI: 10.1002/eji.201970400

great challenge to study pathways using traditional methods. Thus, a high-content and high-throughput method for simultaneous detection of multiple targets is necessary. Using a sandwich-based ELISA format, we have developed an antibody array technology which can simultaneously identify relative expression levels or phosphorylation levels of major signaling pathway proteins. This array-based system features a nitrocellulose membrane or glass slide solid support, each spotted with antibodies targeting 182 classical signaling pathway proteins, including RTK, EGFR, MAPK, AKT, Apoptosis, TGFb, JAK/STAT, NFkB and Insulin Receptor pathways. We employed our antibody arrays to investigate how the anti-cancer drugs, Camptothecin and phorbol 12-myristate 13-acetate (PMA), alter protein phosphorylation in Jurkat and HeLa cells, respectively. Our array data suggest that Camptothecin-treatment induced DNA double-strand breaks in Jurkat cell activate the DNA damage pathways ATM and Chk2, and then further induces apoptosis through Caspase 3 and BAD. PMA induces the MAPK pathway in HeLa cells through the activation of Erk, CREB, and RSK1. These array results are consistent with previous studies using traditional methods, and were validated with western blotting. Our studies demonstrate that pathway antibody arrays provide a rapid, efficient, and multiplexed approach for profiling phosphorylated proteins. This technology will facilitate studies on molecular mechanisms of human diseases, drug resistance and biomarker discovering.

P2354

Rapid and quantitative analysis of exosomes by a chemiluminescence immunoassay using superparamagnetic iron oxide particles

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Since the discovery of exosomes, their potential diagnostic value has been the focus of considerable research. However, the lack of a rapid and simple technique for the quantitative analysis of exosomes greatly limits the application of exosomes in clinical research.In this study, we describe a newly developed one-step chemiluminescence immunoassay for the rapid quantitative analysis of exosomes from biofluids.Our new technique, named ExoNANO, adopts a double-antibody sandwich strategy using anti-CD63 antibody-conjugated superparamagnetic iron oxide particles (SIOPs) and acridinium ester (ACE)-labeledanti-CD9 antibodies.SIOPs have narrow size distribution and high magnetic susceptibility, and ACEhas excellent chemiluminescent properties such as low background signal and no need for a catalyst.We demonstrated that ExoNANOallows the quantitative analysis of exosomes in the range of $2.92 \times 10^5$to 2.80×10^8particles/µL, with a limit of detection of 2.63×10^5particles/µL. UsingExoNANO, we quantified exosomes incell culture mediumand clinical biofluids such as serum, saliva, ascitic fluid, and cerebrospinal fluid.We believe that ExoNANOmight pave the way for the rapid isolation and quantitative analysis of exosomes for routine clinical applications.

P2355

Assessment of antigen-specific B cells using multiplex Immunospot®

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Despite the broad utility of classical serologic techniques, assessment of serum antibody occurs at the population level and may not fully capture the diversity present within the antigen-specific memory B cell compartment. Specifically, the antigen-specific B cell response could encompass a variety of immunoglobulin (Ig) classes (IgM/IgG/IgA/IgE) and/or subclasses (IgG1/IgG2/IgG3/IgG4), each

DOI: 10.1002/eji.201970400

endowed with distinct effector functions such as complement fixation, opsonization and antibody-dependent cellular cytotoxicity. Importantly, half-lives of individual Ig classes and IgG subclasses are variable and relatively short in plasma, and maintenance of antibody levels requires continuous replenishment. The Immunospot® platform is suitable for assessment of antigen-specific B cell immunity; however, traditional single-color enzymatic assays are still limited since they do not enable simultaneous dissection of the antibody response into the respective Ig class and IgG subclass components. To overcome this shortcoming and highlight the utility of multi-color Immunospot® for detailed assessment of antigen-specific B cells, healthy human donors with evidence of B cell reactivity against seasonal influenza following polyclonal stimulation were characterized. Amongst the antigen-specific B cells, heterogeneity in Ig class (IgM/IgG/IgA) and subclass (IgG1/IgG2/IgG3/IgG4) was observed for these donors and was inline with their distinct immunologic histories. Furthermore, parallel detection of Ig classes and IgG subclasses significantly reduced the number of donor cells, reagents and labor required for data generation. Collectively, the multi-color FluoroX™ Immunospot® assay platform enables unambiguous segregation of Ig class and subclass and opens the door to comprehensive *ex vivo* assessment of antigen-specific B cells that will refine our understanding of protective humoral immunity.

P2356

SET domain containing 1B gene (SETD1B) is mutated in primary hepatic neuroendocrine tumors

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Primary hepatic neuroendocrine tumors (PHNETs) are extremely rare NETs originating from the liver. These tumors are associated with heterogeneous prognosis, and few treatment targets for PHNETs have been identified. Because the major genetic alterations in PHNET are still largely unknown, we performed whole-exome sequencing of 22 paired tissues from PHNET patients and identified 22 recurring mutations of somatic genes involved in the following activities: epigenetic modification (*BPTF*, *MECP2* and *WDR5*), cell cycle (*TP53*, *ATM*, *MED12*, *DIDO1* and *ATAD5*) and neural development (*UBR4*, *MEN1*, *GLUL* and *GIGYF2*). Here, we show that *TP53* and the SET domain containing the 1B gene (*SETD1B*) are the most frequently mutated genes in this set of samples (3/22 subjects, 13.6%). A biological analysis suggests that one of the three *SETD1B* mutants, A1054del, promotes cell proliferation, migration and invasion compared with wild-type *SETD1B*. Our work unveils that *SETD1B* A1054del mutant is functional in PHNET and implicates genes including *TP53* in the disease. Our findings thus characterize the mutational landscapes of PHNET and implicate novel gene mutations linked to PHNET pathogenesis and potential therapeutic targets.

DOI: 10.1002/eji.201970400

P2357

Screening, identification of tropomyosin, a candidate vaccine molecule of schistosoma japanicum

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Objective: We envisaged utilizing the specific immune recognition ability of SjscFv of *S. japonicum* to find the targeting components of the adult worm antigen, to provide a new way on vaccine research of the *Schistosoma japonicum* natural molecules.
Methods: Construction and identification of pET28a(+)/SIEA26-28 kDa-scFv recombinant plasmids, and expression, purificatiion the fusion protein；The *S. japonicum* adult worm antigen was fully separated by different range immobilized pH gradient two-dimensional gel electrophoresis, and the targeting points immunoreaction with the fusion protein were confirmed by Western blot; NanoLC-ESI-MS/MS mass spectrometry analysis and database retrieves of the specific binding of proteins.
Results: Narrow range strips pH 4~7 can isolate protein better than pH 3~10 strips by two-dimensional gel electrophoresis. 2DE-WB immune targeting anynisis present three specific spots of *Sj*AWA and pET28a (+)/SIEA26-28 kDa-scFv;3. The three spots identified by NanoLC-ESI-MS/MS mass spectrometry analysis, the most marked spot (protein 2) consist of *S. japonicum* tropomyosin uniquely, which we think that is the targeting of *Sj*scFv.
Conclusions: 1. It is further confirmed that SIEA26-28 kDa-scFv can specifically identify adult *S. japonicum* antigen in 26-28 kDa band; 2. The *SjscFv* targeting components of *Sj*AWA were muscle structural proteins (paramyosin, myosin, tropomyosin and actin) of *S. japonicum;* 3. Tropomyosin of *S. japonicum* is an expectant vaccine candidate by structural and functional analysis. The study represents the first attempt to use 2DE-WB combined with NanoLC-ESI-MS/MS technology screening and identification of *S. japonicum* antigen on the vaccine candidate molecules.

P2358

Development of a real-time PCR reaction lyophilized powder to detect African swine fever virus in blood samples of domestic pigs in China

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A novel, highly specific and sensitive Real-time fluorescent quantitative PCR Lyophilized Powder for accurate detection of African swine fever virus (ASFV) was developed. A fluorescent probe and primers were designed based on 54 isolated gene sequences in GenBank which encode ASFV structural protein p72. Optimal conditions for the Real-time PCR assay were established. A total of 146 blood samples of domestic pigs were employed to assess the powder. Coefficient of variation (C_T) of repeatability was less than 1.20%. Sensitivity was 10 copies plasmids, and the specificity was 100% by detecting five others swine viruses and ASFV plasmid. Compared with commercial kit,sensitivity and specificity of this powder were 98.37% and 78.26%, respectively. Coincidence rate was 95.21% and Kappa value was 0.809, P< 0.0001. In this study, a time-saving, reliable and convenient Taqman Real-time quantitative PCR lyophilized powder has been prepared for laboratory routine diagnosis of ASFV.
The study was partly supported by National Key Research and Development Program(2018YFC0840405)
Keywords: African swine fever virus (ASFV); Taqman probe; Real-time PCR; laboratory diagnosis

DOI: 10.1002/eji.201970400

P2359

Attomolar quantification of antibody subtypes for autoimmune diseases using plasma microarrays

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Objective: To develop a high-throughput plasma microarray platform that can quantify hundreds of clinical samples simultaneously, with which we would like to investigate changes of plasma antibody subtypes in autoimmune diseases.
Methods: We developed a high-throughput microarray platform that can assay antibody concentrations in hundreds of plasma samples within 30 minutes. Then we screened eight antibody subtype concentrations from 100 Behcet´s disease (BD) cases, 30 Takayasu arteritis (TA) cases, 30 ANCA-associated vasculitis (AAV) cases, 30 Sjogren´s syndrome (SS) cases and 30 healthy controls (HC) and analyzed the differential expression of antibody subtypes in these diseases.
Results: The microarray platform shows high reproducibility with r correlation of 0.96 and high sensitivity (~attomolar) in plasma antibody detection. The median (Q1-Q3) of antibody concentrations (mg/ml) in HC were 2.512 (1.767-2.886) for IgA, 1.723 (0.540-2.302) for IgA1, 0.428 (0.395-0.520) for IgA2, 29.288 (24.661-35.762) for IgG, 22.806 (17.145-31.314) for IgG1, 0.245 (0.038-0.484) for IgG2, 0.008 (0.006-0.055) for IgG3, 0.006 (0.005-0.015) for IgG4, respectively. Except for IgG3 and IgG4, antibody subtype was significantly different between the following disease pairs($P< 0.05$), IgA: AAV/HC, AAV/BD, AAV/SS, AAV/TA, SS/BD; IgA1: AAV/BD, AAV/SS, AAV/TA; IgA2: SS/HC, SS/TA; IgG: BD/HC, AAV/HC, SS/HC, SS/BD, AAV/SS, AAV/TA, SS/TA; IgG1: SS/HC, SS/BD, SS/AAV, SS/TA; IgG2: BD/HC, AAV/HC, SS/HC, TA/HC.
Conclusion: Our high throughput microarray platform has great reproducibility and sensitivity with only a minute amount of clinical samples, which has great potential to detect plasma or serum protein biomarkers in future.

P2360

Multiple epitope fusion of Tp47 protein alleviate the false-positive of syphilis serological test

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Background: Anti-TP screening tests in both ELIA and CLIA have false positive rates, especially in low-prevalence epidemics. Multiple epitope fusion might be an ideal strategy to improve the specificity and alleviate the false positive reaction
M**ethod:** Fusion protein which include the three fragments containing multiple major B cell epitope of Tp47 protein was obtained from prokaryotic expression system. This multiple epitope fusion protein as an antigen was subsequently used to establish ELISA to detect 96 syphilis positive serum(1＜S/CO＜

DOI: 10.1002/eji.201970400

10).Using TPPA results as a gold standard, The sensitivity and specificity of the multiple epitope fusion protein was compared with Tp47.
Result: The sensitivity and specificity of were found to be 90.32%（56/62）and 94.12%（32/34）respectively. The Tp47 protein was found to have sensitivity 93.55%（58/62）and a lower specificity 79.41%（27/34）. The coincidence rate of TPPA results, the multiple epitope fusion was 91.67% higher than 88.54% of TP47.
Conclusion: Multiple epitope fusion could effectively improve the specificity of syphilis sera diagnosis. This study provided a feasible strategy to develop new syphilis diagnosis assay with higher specificity.
Key words: B cell epitope, syphilis, false positive reaction, Tp47, ELISA

P2361

Clinical validation of GMP grade AfuPEPLISA assays for detection of A. fumigatus specific IgG and IgE antibodies in patients with bronchial asthma or pulmonary tuberculosis

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Aspergillus fumigatus, an opportunistic fungus, causes a spectrum of allergic manifestations depending upon the immune status of the host. Prevalence of aspergillosis complicating asthma and pulmonary tuberculosis varies from 5-20% and 26-27% respectively in Indian population. Serodiagnosis of *A. fumigatus* specific IgG and IgE antibodies is an excellent tool to screen aspergillus infection early on to combat its harmful implications. We had previously developed and patented AfuPEPLISA assays for specific IgG and IgE, based on the synthetic peptide epitope of 12 amino acid of Asp f 1, an 18 kD major allergen/ antigen. In the present study, the AfuPEPLISAs were optimized and manufactured at a licensed GMP facility. The assays were independently validated at PGIMER and VPCI hospitals with serum samples (n=1212) from suspected bronchial asthma patients in comparison with currently used ImmunoCAP assay. The diagnostic specificity and sensitivity was found to be 95.7% and 89.8% for IgG and 94.2% and 70% for IgE AfuPEPLISA respectively and was significantly not different from ImmunoCAP assay. The assays were used to screen the suspected patients of pulmonary tuberculosis (PTB) at RBIPMT Hospital for the presence of *A. fumigatus* specific IgG and IgE antibodies. A total of 154 out of 500 suspected PTB patients (30.8%) were seropositive in coherence with the existing reports. The study validated AfuPEPLISA assays for efficient diagnosis of allergic aspergillosis in patients with suspected bronchial asthma or PTB.

P2362

Comparison between PPD and ELISA method fo diagnosis and follow up of latent and active Tb in diabetic patients

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DOI: 10.1002/eji.201970400

Introduction: There is an increase in cases between diabetes and tuberculosis (DM2/Tb), which has caused great mortality throughout the world, for this reason is important to detect Mtb infection in time.
Methodology: 193 subjects divided into four groups: 50 diabetic patients type 2 (DM2), 46 patients with diabetes and pulmonary tuberculosis (DM2 / Tb), 48 patients with pulmonary tuberculosis (Tb) and 49 healthy subjects, the samples were analyzed using the patent ELISA technique 285260; glycemia levels and PPD were also tested.
Results: The average glycemia for DM2/TBP was 160mg/dl, DM2 144mg/dl, TBP 101mg/dl and Healthy 96mg/dl. When using the ELISA test for the diagnosis of Tb, we found the following: in the DM2/TBP group 52% tested positive for IgM, 48% positive for IgG, and 87% positive for PPD; in the DM2 group, 20% tested IgM/IgG negative, 48% tested positive for IgM, 56% tested positive for IgG and 32% positive for PPD; In the TBP group, 63% tested positive for IgM, 100% tested positive for IgG and 63% tested positive for PPD; in the healthy group 44% were IgM/IgG negative, 2% IgM positive, 40% IgG positive and 43% positive PPD. The odds ratio was calculated and the value increased from 2.22 to 15.04.
Conclusion: In the study we observed that the ELISA test is useful in the evaluation of diabetic patients, especially when these are contacts. The results allow us to establish a prioritization of the medical attention of the contacts of a patient with tuberculosis, especially those who are diabetic.

P3253

Detection of hepatoma serum marker CD105 with graphene/hemin/aptamer nanoprobe

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The commonly used serum marker of liver cancer is mainly alpha fetoprotein (AFP). CD105 also known as endoglin, is a cell membrane antigen of proliferation-associated endothelial cells, which is not expressed or weakly expressed in the vascular endothelial cells of normal tissuesbut is highly expressed in tumor neovascular endothelial cells. A stable detection platform based on HGNs-apt was obtained by optimizing the proportion among graphene, hemin, and aptamer during the preparation of HGNs-apt, and by optimizing the specific detection conditions during the detection of CD105. The detection platform enables the sensitive detection of CD105 with a limited concentration down to 5.5 ng/mL can be measured. This experiment innovatively constructs a detection platform based on the graphene-hemin-aptamer complex (HGNs-apt) probe, which can specifically detect serum hepatoma marker CD105 with high sensitivity and a larger detection range. It provides an important basis for the early diagnosis of hepatoma, alleviating patient's suffering, mental and economic burdenand also providing a quantitative indicator for the prognosis of hepatoma.

P3254

Biosensor study of detecting tumor marker AFP based on graphene oxide/aptamer/hemoglobin nanozyme probe

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A graphene oxide / aptamer/hemoglobin (GHNs-apt) nanozyme detection probe was constructed to develop a convenient, low-cost and high-sensitivity biosensor for the detection of liver cancer marker AFP. Aptamers that specifically bind to the detection target AFP bind to GHNs through π-π bonds, and 96-well plates can also bind tightly to the detection target AFP through antigen-antibody interactions

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DOI: 10.1002/eji.201970400

after coating the antibody. Therefore, in the presence of the target AFP, the GHNs-apt, the detection target AFP, and the antibody-coated 96-well plate constitute a "sandwich sandwich structure." Because GHNs have a strong peroxidase-like activity, the substrate TMB can be catalyzed by the presence of hydrogen peroxide. The degree of coloration is positively correlated with the concentration of the target. Therefore, detection of the detection target AFP can be achieved. Through the characterization of various materials, the nanozyme probe (GHNs-apt) was successfully prepared. GHNs-apt, which has peroxidase-like activity and specific recognition, can detect AFP protein in the range of 32.25-1000 ng/mL with a limit of detection (LOD) of 0.1696 ng/mL. The relative standard deviations of 0.18%-9.02% were observed in 19 serum samples compared with the clinical chemiluminescence method. This nano-enzyme probe based on graphene oxide/aptamer/hemoglobin (GHNs-apt) is expected to be used for the detection of AFP in clinical blood samples.

P3255

Detection of anti-SMP30 antibody for diagnosis of hepatocellular carcinoma based on antibody-modified gold nanoparticle probe

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In this study, we designed a signal-amplification detection strategy for highly sensitive detection of anti-SMP30 antibodies using gold nanoparticle probe-based enzyme-linked immunosorbent assay (ELISA). The probe is highly loaded with HRP-conjugated goat anti-human IgG antibody on the surface of gold nanoparticle, and has good stability and easy preparation. During the detection process, firstly, genetically engineered bacteria were used to express and purify soluble hepatocellular carcinoma-associated antigen SMP30. Using this protein as an antigen, the anti-SMP30 antibody levels in hepatocellular carcinoma, hepatitis, liver cirrhosis, benign liver tumors and normal human serum were detected. The detection limit of this probe was lower than that of the traditional ELISA method. It could increase the positive rate of anti-SMP30 antibody in serum of patients with hepatocellular carcinoma to 45.76%.And the combination of anti-SMP30 antibody and AFP could increase the diagnostic rate of hepatocellular carcinoma. Thus, anti-SMP30 antibody is expected to become one of the serum diagnostic indicators of liver cancer.

P3256

Development of a dual signal amplification biosensor based on hemin functionalized graphene nanosheets for the detection of leukaemia

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Objective: Diagnostic blood cell counting is of limited use in monitoring minimal number of leukaemia cells, warranting further research to develop more sensitive and reliable techniques to detect leukaemia cells in circulation. In this study, a dual signal amplification biosensor was developed by functionalizing graphene oxide nanosheets with hemin which has peroxidase mimetic activity.
Methods: Hemin-conjugated graphene oxide nanosheets (HGNs) were prepared by hydrazine reduction using graphene oxide and hemin. A Sgc8 aptamer, which recognizes a specific target on leukaemia cells, was loaded onto HGNs and biotinylated. Cancer cells associated with the functional nanocomposite were then enriched by magnetic beads coated with avidin. The performance of this biosensor was characterized using blood samples from leukaemia patients.

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DOI: 10.1002/eji.201970400

Results: The functional nanocomposites were made successfully as nanosheets averaged 4 nm thick. Sgc8 aptamer was loaded onto HGNs and confirmed by ultraviolet spectrum, and its modification efficiency for magnetic beads was measured to be 64.7%. The ratio between graphene oxide and hemin was optimized to be 1 to 5, and the optimal concentration of magnetic capture probe was 240 nM. Under optimal condition, the biosensor allowed detection of 10 to 10^5 CEM cells. Analysis of patient samples showed a reduction of signals with the treatment time, correlating with results of white blood cell counting.
Conclusion: A biosensor constructed by grafting HGNs with peroxidase-mimicking hemin was developed, optimized, and tested in patient blood samples. It can be used in monitoring the minimal residual disease during the treatment of leukaemia.

P3257

An activatable aptamer probe for detection of leukemia cells

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Objective: The highly sensitive and specific detection of cancer cells is significant for the early diagnosis of leukemia. In the present study, we developed an activatable aptamer probe based on conformation alteration (AAPC) to detect cancer cells.
Results: The AAPC consists of two fragments: sequence of aptamer sgc8 that targets CEM cell line cells, and two short extending complementary DNA sequences at the 5´- and 3´- ends, with a fluorophore and a quencher attached at either terminus. The specificity and sensitivity of the AAPC were assessed using flow cytometry and fluorescence microscopy. These results revealed that fluorescent signals were not detected when there were no cancer cells, however the fluorescent signals were activated while the AAPC bound to target cancer cells.
Conclusion: In conclusion the AAPC could detect CEM cells quickly and efficiently. Furthermore, as a simple, rapid, highly sensitive and specific molecular probe, it has a certain application value in the detection of cancer cells.

P3258

Generation and characterization of novel monoclonal antibodies against DCLK1

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Doublecortin-like kinase 1 (DCLK1) is considered a putative tumor stem cell (TSC) marker and a promising therapeutic target, as DCLK1+ progeny cells exhibit high expression in tumors. However, the biological function of DCLK1+ cells in tumorigenesis and tumor progression remains unclear. We generated rabbit monoclonal antibodies (mAbs) against DCLK1, DCLK1-42, and DCLK1-87 mAbs, using a novel chip-based immunospot array assay on a chip system. First, the specificity of two mAbs to DCLK1 was con rmed by Western blot, which were bound to DCLK1-long in normal colon cells and to DCLK1-short in a cancer cell line as well as colorectal cancer (CRC) cells. Precise localization analysis using immunofluorescence revealed that both mAbs had cytoplasmic signal and exhibited a high degree of overlap with microtubules. Furthermore, bacterial display technology indicated that the antigenic epitope region of DCLK1-87 mAb was consistent with that of a commercial anti-DCLK1 polyclonal antibody. In addition, DCLK1-42 mAb has the common polyclonal antibody characteristic of binding to more than one site on DCLK1. By immunohistochemistry, we found that DCLK1-87 mAb was more specific for DCLK1+ cell labeling than a commercial anti-DCLK1 polyclonal antibody.

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DOI: 10.1002/eji.201970400

DCLK1 labeled with DCLK1-87 mAb might be a potential TSC marker because the tissue expression site covers the ALDH1 area in CRC tissues. Our results indicated that we successfully generated an efficient tool for the precise detection of DCLK1+ cells in cancer tissues.

P3259

Combined electrochemiluminescent and electrochemical immunoassay forinterleukin 6 based on the use of TiO2 mesocrystal nanoarchitectures

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Interleukin 6 (IL-6) is a important cytokine involoved in immune regulation. A dual-responsive sandwich-type immunosensor is described for the **hypersensitibity detection of IL-6 by combining electrochemiluminescent (ECL) and electrochemical (EC) detection based on the use of two kinds of TiO2 mesocrystal nanoarchitectures**. A composite was prepared from TiO2 (anatase) mesocages (AMCs) and a carboxy-terminated ionic liquid (CTIL) and then placed on a glassy carbon electrode (GCE). In the next step, the ECL probe Ru(bpy)3(II) and antibody against IL-6 (Ab1) were immobilized on the GCE. Octahedralanatase TiO2 mesocrystals (OAMs) served as the matrix for immobilizing acid phosphatase (ACP) and secondary antibody (Ab2) labeled with horse radish peroxidase(HRP) to form abioconjugate of type Ab2-HRP/ACP/OAMs. It was self-assembled on the GCE by immunobinding. 1-Naphthol, which is produced in-situ on the surface of the GCE due to the hydrolysis of added 1-naphthyl phosphate by ACP, is oxidized by HRP in the presence of added H2O2. This results in an electrochemical signal (typically measured at 0.4 V vs. Ag/AgCl) that **increases linearly in the 10 fg· mL^{-1} to 90 ng·mL^{-1}** IL-6 concentration range with **a detection limit of 0.32 fg·mL^{-1}**. Secondly, the oxidation product of 1-naphthol quenches the ECL emissionof Ru(bpy)32+. This leads to a decrease in ECL intensity which is linear in the 10 ag·mL^{-1} to 90 ng·mL^{-1} concentration range, with a detection limit of 3.5 ag·mL^{-1}. The method exhibits satisfying selectivity and good reproducibility which demonstrates its potential in clinical testing and diagnosis.

P3260

Diagnostic value of the quantitative level of hepatitis B core antibody in patients with HBV infection

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With the aggravation of liver fibrosis, the level of hepatitis B core antibody is gradually increased, and the level of hepatitis B core antibody is positively correlated with liver fibrosis stage. According to the quantitative level of hepatitis B core antibody, liver tissue fibrotic lesions can be reflected.

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DOI: 10.1002/eji.201970400

P3261

Establishment and validation of an LC/MS peptide mapping method for the characterization of anti-TNF-α monoclonal antibody

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Objective: To establish a LC/MS peptide mapping method for specific identification of anti-human TNF-α monoclonal antibody (TNF-α mAb).
Methods: After denatured by guanidine hydrochloride, reduced by DTT, the free cysteine sulfhydryl of TNF-α mAb was alkylated and the excessive alkylating agents were neutralized by DTT. Finally, digestion buffer was replaced by ultrafiltration, and the digestion reaction was terminated by FA. An Waters UPLC column was adopted using water（containing 0.1% FA）as the mobile phase A and acetonitrile（containing 0.1% FA）as the mobile phase B. Gradient elution of mobile phase B was started from 5 min to 120 min followed with changing from 2% to 45%. Data were obtained with electrospray ionization and positive ionization, and the acquisition range was m/z 100- 1 990.
Results: Six complementary CDRs of the heavy and light chains of TNF-α mAb were identified by mass spectrometry. The HC CDR2 and HC CDR3 were co-eluted. Extent of variation of this method such as specificity, precision, robustness and stability of digested sample were evaluated by the relative retention time（RRT）of CDR to reference peak.
Conclusion: Based on CDR related peptides identification, LC/MS peptide method could qualitative identify TNF-α mAb. Method validation results showed that the established method could be applied to specificity identification of anti-human TNF-α mAb and used for quality control and batch release test.

P3262

A novel DT40 antibody library for the generation of monoclonal antibodies for the diagnosis of emerging viral infections

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Early etiological diagnosis which is very important to the control of sudden viral infectious diseases and requires antibodies with high sensitivity and high specificity. Traditional antibody preparation technologies have limitations, such as a long and arduous cycle, complicated operation, and high cost. A chicken lymphoma cell line, DT40, produces IgM-type antibodies and undergoes gene conversion and somatic mutation in the variable region of the immunoglobulin gene during culture. Thus, the DT40 cell line has been developed to produce antibody libraries and for rapid antibody preparation in vitro. Hypermutation in DT40 cells was regulated by the activation-induced cytidine deaminase (AID) gene. Therefore, AID expression must be controlled to fix the Ig sequence by stopping mutation or to improve affinity by resuming mutation after antibodies are selected. In this study, we generated a novel AID-inducible DT40 cell line (DT40-H7), in which the endogenous AID gene was knocked out using the CRISPR/Cas9 genome editing system, and an inducible AID gene based on the Tet-Off expression system was stably transfected. The expression of AID was controlled in DT40-H7 cells in a simple and efficient manner. Gene conversion and point mutations were observed only when AID was expressed. Using the antibody library generated from this cell line, we successfully obtained monoclonal antibodies against the NS1 protein of Zika virus. The DT40-H7 cell line represents a useful

DOI: 10.1002/eji.201970400

tool for the selection and evolution of antibodies and may also be a powerful tool for the rapid selection and generation of diagnostic antibodies for emerging infectious diseases.

P3263

Development of a relative potency test using ELISA for human rabies vaccines

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The NIH potency test for human rabies vaccines has disadvantages for use, especially in developing countries where rabies is endemic and prophylaxis needs ample, rapid, and reliable vaccine supplies. In China, 60-75 million doses of human rabies vaccines are administered each year. Vaccine quality control is of paramount importance, as is the release of potency-validated vaccines. We intended to design an alternative to the NIH *in vivo* method, and developed a relative potency test using an ELISA. Using Pearson's correlation analysis, we found a close relationship between the rabies vaccine glycoprotein content *in vitro* and the potency values *in vivo*. We suggest the relative potency test developed here as a simplified method for human rabies vaccine quality control in China and a possible alternative to the NIH method.

P3264

Plasmonic ELISA for sensitive detection of disease biomarkers with a smart phone-based reader

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Serum myoglobin is one of the earliest markers for the diagnosis of acute myocardial infarction. It is, therefore, critical to develop a point-of-care testing technology for myoglobin detection. In this work, we reported a sensitive plasmonic immunoassay-based on enzyme-mediated localized surface plasmon resonance change of gold nanorods for the point-of-care testing detection of myoglobin. In addition, we developed a novel plasmonic immunoassay reader using the ambient light sensor of smart phone to increase the accessibility and utility of the plasmonic immunoassay. The linear detection range of gold nanorods-based plasmonic immunoassay for myoglobin detection was 0.1-1000 ng $mL^{-1}$and the limit of detection was 0.057 ng mL^{-1}. Myoglobin in serum samples was also analyzed by the plasmonic immunoassay. The results were significantly correlated with those of conventional enzyme-linked immunosorbent assay. The plasmonic immunoassay, coupled with smart phone-based reader, could be widely used for point-of-care testing application of acute myocardial infarction, especially in the regions with limited technological resources.

P3265

IP-10 and RANTES as biomarkers for pulmonary tuberculosis diagnosis and monitoring

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DOI: 10.1002/eji.201970400

Objective: We aimed to determine whether IP-10 and RANTES plasma levels can be used in diagnosis and monitoring of pulmonary tuberculosis (PTB).
Methods: Plasma levels of cytokines/chemokines were measured using a Bio-Plex ® multiplex cytokine assay system in a cohort containing 457 clinically suspected PTB patients including a training set (n = 41) and two independent test sets A (n = 242) and B (n = 174).
Results: Plasma levels of IP-10 and RANTES were signifificantly higher in PTB patients than healthy controls´ in both training and independent test sets (P < 0.05). Compared with other combinations, the combination of IP-10 and RANTES had the best performance with an AUC of 1.0 in training set. The performance characteristic of this model was successfully validated in independent test set A although this combination only resulted in a slightly improvement of AUC value in independent test set B. Plasma IP-10 and RANTES levels were weakly and positively correlated with blood glucose concentrations. Moreover, IP-10 levels were positively correlated with CRP and ESR in PTB patients. Furthermore, in response to therapy, both IP-10 and RANTES levels signifificantly decreased over the period of 6 months (P < 0.001).
Conclusions: Taken together, combination of IP-10 and RANTES could be potentially used as diagnostic and monitoring biomarker in PTB management.

P3266

Comparative study on clinical detection methods of Clostridium difficile in patients with diarrhea

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Objectives: This study aimed to evaluate the clinical application of three methods for detecting *Clostridium difficile* infection(CDI) in fecal samples.
Methods: 150 cases of fecal specimens were collected and tested by three methods: The toxigenic culture(TC): culturing and isolation of *C.difficile* using three agars, detection of DNA from *C.difficile* positive strains by multiplex PCR test for detection of *C.difficile* toxin. The Vidas enzyme immunoassay(EIA): the Vidas *C.difficile* glutamate dehydrogenase (GDH) assay and toxin A/B assay was used to detect *C.difficile* GDH antigen and A/B toxin. The GeneXpert *C.difficile* PCR assay was used to detect *C.difficile* toxin gene.
Results: The positive rate of toxin-producing *C.difficile* was 17.33% (26/150). Compared with the anaerobic culture, the sensitivity, specificity, positive predictive value(PPV), and negative predictive value(NPV) of the Vidas *C.difficile* GDH assay of 150 specimens were 100.0%, 97.4%, 91.9%, and 100.0%, respectively. Compared with TC, the sensitivity, specificity, PPV and NPV of the GeneXpert *C.difficile* PCR assay of 1~79 specimens were 100.0%, 96.8%, 88.9%, and 100.0%, respectively; The Vidas EIA of 1~79 specimens were 55.6%, 100.0%, 100.0%, and 88.4%, respectively. The area under the GeneXpert *C.difficile* PCR assay and the Vidas EIA curve were 0.984 and 0.813. There was no statistical difference between the area test under the GeneXpert *C.difficile* PCR assay and TC (Z= 1.426, *P*>0.05).
Conclusion: The Vidas GDH assay is a good indicator for the initial screening of CDI. The GeneXpert *C.difficile* PCR assay is a simple and rapid method and has good clinical application value.

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DOI: 10.1002/eji.201970400

P3267

A novel electrochemiluminescence sensing platform for precise analysis of multiple LTBI markers

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Tuberculosis (TB), an infectious bacterial disease, kills almost two million people annually and is an international public health priority. According to the World Health Organization, an estimated third of the world's population is infected with Mycobacterium tuberculosis (M. tb). The enormous reservoir of individuals with latent tuberculosis infection (LTBI) is one of the major contributing factors for the high incidence of TB patients, but the sensitivity and accuracy of the existing diagnosis methods in the diagnosis of LTBI are suboptimal. Multiple immunoregulatory cytokines are produced by immune cells upon stimulation with M. tb, and they have been found to play crucial roles in regulating immune response and can serve as biological markers for LTBI diagnosis. Here we reported a novel electrochemiluminescence (ECL) sensing platform for the precise analysis of multiple LTBI markers, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-2. In this approach, luminol and self-preparedcarbon quantum dots and CdS quantum dotswere integrated onto gold nanoparticles (AuNPs), which were further enriched on the surface of magnetic bead (MB) to create three solid-phase ECL nanoprobes for improving the detection sensitivity efficiently. The ECL intensity depended linearly on the content of INF-γ, TNF-α and IL-2 in the range from 0.01 to 1000 pg mL-1 with a low detection limit of 10 fg mL-1. The proposed ECL sensing platform is simple, sensitive, accurate, reliable, and specific to the detection of INF-γ, TNF-α and IL-2 in human serum, and provides a valuable protocol for facilitating fast and precise diagnosis of LTBI.

P3268

CRISPR-Cas13a system combined with magnetic beads chemiluminescence for detection of H7N9 virus

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Early diagnosis can effectively reduce the harm of avian influenza A (H7N9) flu. Therefore, a reliable, sensitive and fast H7N9 virus detection system was developed, which was tactfully combined CRISPR-Cas13a system with magnetic beads (MBs) chemiluminescence. The Cas13a protein was successfully expressed and purified. Cas13a has RNA nuclease activity after verification. The H7N9 HA/NA gene transcribed H7N9 HA/NA RNA was used as the target RNA and the corresponding specific crRNA was designed. RNA probe was synthesised by modification of midogen and biotin. MB-CO-NH-RNA-Biotin-SA-AP complex was preparated with carboxylated MBs, RNA probe and streptavidin (SA) labed alkaline phosphatase (AP). When crRNA guided LwCas13a is activated by target RNA, the prepared MB-CO-NH-RNA-Biotin-SA-AP complex would be non-specifically cleaved and releases AP. The chemiluminescence intensity after AP reaction with the substrate was used as a signal to verify the presence of target RNA. After optimization, the optimal probe concentration for H7N9 detection in 1 mg MBs was 0.4 nM, the reaction temperature was 25 °C, and the reaction time was 20 min. H7N9 RNA can be detected by the system combined with magnetic beads chemiluminescence in just 30 min. The sensitivity of using non-amplified products was as low as 100 pM. In addition, the high efficacy was not obtained at the expense of specificity. This study was the first to develop a CRISPR-Cas13a combined magnetic beads chemiluminescence H7N9 virus RNA detection system, which can rapidly detect the RNA of H7N9 virus.

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DOI: 10.1002/eji.201970400

P3269

Screening of differentially expressed genes and identification of *NUF2* as a prognostic marker in breast cancer

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The aims of this study were to screen differentially expressed genes (DEGs) in breast cancer (BC), and investigate *NUF2* as a prognostic marker of BC. Four BC microarray datasets, downloaded from GEO and TCGA databases, were used to screen DEGs. A total of 190 DEGs with the same expression trends were identified in the 4 datasets, including 65 upregulated and 125 downregulated DEGs. Functional and pathway enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery. The upregulated DEGs were enriched for 10 Gene Ontology terms and 7 pathways, and the downregulated DEGs were enriched for 10 GO terms and 10 pathways. A protein-protein interaction network containing 149 nodes and 930 edges was constructed using the Search Tool for the Retrieval of Interacting Genes, and functional modules were identified using the MCODE plugin of Cytoscape. Based on an in-depth analysis of module 1 and literature mining, *NUF2* was selected for further research. Oncomine database analysis and reverse transcription-quantitative PCR showed that *NUF2* is significantly upregulated in BC tissues. In analyses of correlations between *NUF2* and clinical pathological characteristics, *NUF2* was significantly associated with the malignant features of BC. Using 5 additional datasets from GEO, we found that *NUF2* has a significant prognostic role in both ER-positive and ER-negative BC. A Gene Set Enrichment Analysis indicated that *NUF2* may regulate breast carcinogenesis and progression via cell cycle-related pathways. Our results demonstrated that *NUF2* is overexpressed in BC and is significantly associated with its multiple pathological features and prognosis.

P3270

Study on newcastle disease virus immunosensor based on 81° tilted fiber grating

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A type of newcastle disease virus (NDV) immunosensor based on 81°tilted fiber grating (81°-TFG) was proposed. The basic principle and sensing properties of the 81°-TFG were simply analyzed. Staphylococcus aureus protein A (SPA) was used to modify the surface of 81°-TFG, then highly purified NDV monoclonal antibodies (MAbs) were immobilized on 81°-TFG surface through SPA molecules, so as to form the immunosensor specific to NDV. The experimental results show that, the immunosensor has a lowest limit of detection to NDV of between 0.1ng/ml and 0.2ng/ml and become saturated at ~1.0ng/ml, and has good linearity with R2 of ~0.982 and sensitivity of ~342pm/(ng/ml) in the concentration range of 0~1.0ng/ml. In addition, this immunosensor shows good reusability and highly specificity to NDV, and also
it could be used in clinical test for NDV. Compared with the traditional biochemical methods such as immunofluorescence technique, enzyme-linked immunoabsorbent assay, etc., the technique proposed in this work has the advantages of label free, easy to operate, fast detection, and so forth.

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DOI: 10.1002/eji.201970400

P3271

Development of an up-converting phosphor technology-based lateral flow assay as a point-of-care test for diagnosis of brucellosis

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Objective: Brucellosis is a serious infectious zoonotic disease. Here, an up-converting phosphor technology-based lateral flow (UPT-LF) assay, named the BP26-UPT-LF assay, was developed for the rapid and qualitative detection of human anti-*Brucella* antibody.
Methods: Various performance aspects of this assay were evaluated, including accuracy, stability, tolerance, and specificity.
Results: Validation of its efficacy for clinical application was performed via comparison with the standard tube agglutination test, the results of which were used to determine the BP26-UPT-LF assay cut-off value of 0.08. Both the intra-assay and inter-assay coefficient of variation values were lower than 15%, indicating that the BP26-UPT-LF has good accuracy. Following assay storage at 37 °C for 8 days, the coefficients of variation were also below 15%; therefore, this assay will likely be stable for at least 12 months if stored at room temperature. Common interfering substances, such as triglyceride (2.0 mg/ml), hemoglobin (10 mg/ml), and bilirubin (0.15 mg/ml), had little influence on qualitative detection. The BP26-UPT-LF assay also showed excellent specificity for brucellosis when assessed using serum samples from patients infected with other Gram-negative bacteria. Compared with standard tube agglutination test, the specificity and sensitivity were 82% and 95%, respectively, showing a strong agreement between these assays (kappa value, 0.773).
Conclusion: These results indicate that this new UPT-LF assay for the qualitative detection of IgG antibodies against *Brucella* is rapid, sensitive, and meets the requirement for Chinese clinical trials of brucellosis detection; therefore, it may provide an efficient alternative point-of-care testing method for the diagnosis of human brucellosis.

P3272

Aptamer-based fluorometric assay for determination of alphafetoprotein based on deoxyribonuclease I-aided target recycling and signal amplification using graphene oxide as a quencher

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Alphafetoprotein (AFP) is an indicator of liver cancer. Hence, a new method for AFP detection that is sensitive and selective is urgent for early clinical diagnosis. In this work, the authors describe an aptamer based fluorometric assay for the determination of AFP. It is based on deoxyribonuclease I (DNase I)-aided target recycling and signal amplification. The DNA probe consists of two regions (sequences) that represent the capture probe and the signal probe, respectively. In the absence of AFP, the probe is adsorbed by the surface of graphene oxide (GO) via π-stacking interactions. This results in quenching of the fluorescent label (carboxyfluorescein) and protects it from being cleaved by DNase. Upon adding AFP, the probe will be repelled by GO because AFP binds to the aptamer. This triggers an increase in fluorescence as measured at excitation/emission wavelengths of 480/514 nm. The detection limit is as low as 0.01 nM, and the calibration plot is linear in the 0.1 to 100 nM AFP concentration range. In addition, good results are also achieved in clinical samples analysis. Therefore, the assay is specific and sensitive, and in our perception has a large potential in terms of detecting other tumor biomarker molecules.

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DOI: 10.1002/eji.201970400

P3273

The correlative study of carbohydrate antigen 125 detected between multiplex suspension bead arrays and chemiluminescence

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Objective: to observe the difference and correlation of carbohydrate antigen 125 (CA125) between Siemens Chemiluminescence Instrument CP (Chemiluminescence Instrument CP) and liquid phase chip Luminex200.
Methods: The serum concentration of CA125 in 51 clinical specimens was detected by Chemiluminescence Instrument CP or Luminex200. The differences and correlations between the two methods were compared.
Results: The concentration CA125 detected by Luminex200 was 160±112 kU/ml, significantly higher than that detected by Chemiluminescence Instrument CP (46 kU/ml) ($P< 0.05$). Furthermore, the positive rate of CA125 detected by Luminex200 significantly higher than that detected by Chemiluminescence Instrument CP (100% vs. 64.71%, $P< 0.05$). However, it indicated that the results of CA125 detected by two methods were significantly correlated ($P< 0.05$).
Conclusion: The result of CA125 detected by Luminex200 is related to that detected by Chemiluminescence Instrument CP, but the CA125 concentration of Luminex200 is significantly different from that of Chemiluminescence Instrument CP. Different reference intervals need to be established in two methods.

P3274

Multiplexed immunoassay based serum cytokine profiling for potential prognosis predictors in patients with metastatic breast cancer

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Background: Although patients of metastatic breast cancer (MBC) experienced considerably wide survival range, reliable prognostic biomarker remained poorly identified. This study attempted to profile serum cytokine candidates that potentially predict the prognosis of MBC patients.
Methods: By using ProcartaPlex Multiplex Immunoassay, totally 45 cytokines were assayed in sera from 18 MBC patients before treatment.
Results: Thirty-three cytokines were detectable in more than 50% of the patients and were acceptable for further analysis. Univariate analysis uncovered ten serum cytokines that were significantly associated with the death of MBC patients. Among them, IL-8, FGF2, HGF and VEGFA were previously reported, and six cytokines including NGF-β, IFN-γ, IP-10, IL-18, MCP-1 and MIP-1β were identified for the first time as MBC death-correlated serum factors.
Conclusions: Identification of these cytokines would facilitate developing novel serum biomarkers for MBC, favoring multiplexed serum cytokines profiling as a promising method in predicting the outcome of MBC patients.

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DOI: 10.1002/eji.201970400

P3275

Circulating tumor cell clusters from gastric cancer patients co-cultured in label-free microfluid system promotes cancer metastasis

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Many studies have been dedicated to developing circulating tumor cells (CTC) as non-invasive cancer liquid biopsy, but the capture and detection of CTCs of gastric cancer (GC) patients is still in its infancy. We employed a microfluidic-based approach and co-cultured CTC clusters and immune cells from the same patient blood sample using minimal preprocessing and no need for prior enrichment of CTCs. Then we profiled genome wide expression of CTC clusters co-cultured with immune cells using single-cell RNA sequencing. Compared to single CTCs, CTC clusters from GC patients had differential expression of Sox2, Survivin9 and Notum, which were the stemness and proliferation-associated characteristics of CTC clusters. Genome wide expression of CTC clusters also showed intra-patient heterogeneity of cancer cells upon selection pressures of therapeutic drugs. Our data demonstrate the ability of CTCs and immune cells to form clusters has been linked to increased metastatic potential. Our work will gain insights into the biology of CTC clusters of GC, and provide potential markers for CTC clusters of GC patients. These results will lead to high resolution analyses of GC and improve personalized therapy for patients.

P3276

Reverse transcription recombinase polymerase amplification with lateral flow dipsticks for detection of influenza A virus and subtyping of H1 and H3

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Three reverse transcription recombinase polymerase amplification assays with lateral flow dipsticks (RT-RPALFD) were developed for identification of the matrix and hemagglutinin (HA) genes to detect influenza A virus and distinguish subtypes H1 and H3. Assessment of the assays' specificity showed that there was no crossreactivity
with other targets. Their limits of detection were 123.6 copies per reaction for the matrix gene, 677.1 copies per reaction for the H1 HA gene, and 112.2 copies/reaction for the H3 HA gene. Of 111 samples tested by RT-RPA-LFD assays, 27 were positive for influenza A virus, 14 were positive for H1, and 10 were positive for H3.Compared to the results obtained from real-time RT-PCR assays, the sensitivity of RT-RPA-LFD assays was 75%,93.33% and 71.43% for the matrix, H1, and H3, with 100% specificity. The sensitivity of RT-RPA-LFD assays is lower than that of real-time RT-PCR, comparable or better than that of conventional RT-PCR, and much better than that of RIDTs. In conclusion, these assays offer an efficient and reliable tool for identification and subtyping of influenza A virus (subtype H1 and H3) in the resource-limited setting.

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DOI: 10.1002/eji.201970400

P3277

Prediction of hematopoietic stem-cell transplantation associated bleeding after transplantation using a machine learning algorithm: a Chinese group for blood and marrow transplantation retrospective mining study

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Background: Hematopoietic stem-cell transplantation (HSCT) associated bleeding after transplantation is a life-threatening complication after transplantation. However, limited studies have showed the prediction model of HSCT associated bleeding.
Methods: We did a retrospective analysis of data from patients with hematological diseases after with HSCT from May 1, 2004 to April 1, 2012. We analyzed the association between outcomes and bleeding class, recipient-related variates, and donor- or treatment-related variates. A training dataset was randomly selected from the cohort and used to derive a bleeding score.
Findings: 711 patients were included. HSCT patients with accentuating bleeding class had higher cumulative incidence of overall mortality (log-rank $p< 0.0001$). Evaluation of time-dependent effect showed a prominent and stable improvement of AUC for predicting overall mortality and NRM by bleeding class beyond 24 months. We further assessed reclassification after adding bleeding class to a conventional risk model. Consistently, bleeding class improved the ability of predicting overall mortality and NRM. Optimization of the model yielded 5 highest rated covariates, including sex, leukocyte reconstitution, urine occult blood, gvhd stage, and organ injury. After model simplification, a finalized SLUGO score was derived as summarized in Figure 3F. Furthermore, AUC for the risk of bleeding showed that the new SLUGO score had better concordance over conventional bleeding scores including Hasbled, Hemorr2, and mobri.
Interpretation: HSCT patients with accentuating bleeding class had higher cumulative incidence of overall mortality. SLUGO score we derived could make a prediction of HSCT associated bleeding and help doctors to interfere in advance.

Gene-editing in Immunity

O343

Off-target recombination in the Foxp3$^{YFP\text{-}Cre}$ mouse line resulting in conditional knockout gene leads to a completely deletion

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The Cre-LoxP conditional knockout strategy has been used extensively to study gene function in a specific cell-type. Foxp3-Cre mice are used to study the conditional knockout of a target gene in regulatory T cells (Treg). To study the function of Dbc1 in Treg cells, we generated a transgenic

DOI: 10.1002/eji.201970400

mouse strain carrying a loxP-flanked (floxed) Dbc1 allele and crossed this mouse with Foxp3$^{YFP\text{-}Cre}$ mice. However, we found that some mice are completely Dbc1 gene knocked out. Since the germline recombination can cause that, so we tested the expression of Foxp3 in testis and used various mouse breeding strategy to verify that. Even though we tested the Foxp3 expression in the testis, but we failed to obtain Dbc1 knockout mice when Cre and LoxP alleles were co-inherited from the male parent, so as the female parent. Interestingly, we noted that Dbc1 allele in some mice was partially recombined. When we used the male mice with chimerical Dbc1 allele to cross with wild type female mice, **we gained some Dbc1 gene knockout mice. Additionally, we found that the Foxp3 was expressed in the ovary and fetus. This is likely that the expression of Foxp3 in the ovary or fetus results in the chimerical Dbc1 allele in male mice, while some Dbc1 knock out in germ resulted in Dbc1 knockout offspring**. While Cre-loxP is considered to be a powerful technique to study gene function in specific cell type, a caution must be made to exclude the out of control of a gene conditional recombination.

O344

Systemic discovery of anti-CRISPR genes that target RNA editing

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Since 2012, CRISPR-Cas9 has garnered intense interests due to its powerful gene editing ability; however, its gene editing potential is dampened by the off-target effects and potential harm to mammalian cells. Cas13 is an RNA endonuclease that only targets RNA and may have broad utility for RNA editing, nucleic acid detection, and disease diagnosis. Our newly discovered AcrVI proteins may help control the targeting specificity and potency to allow optimal RNA editing by diminishing off-target effects. We also found that *acrIC1*$_{Rca}$ exhibits dual inhibitory effects on both type I and VI CRISPR-Cas systems. These CRISPR inhibitors significantly dampen RNA editing potency of Cas13a in human cells, for example the dCas13a RNA Editing Programmable A to I Replacement (REPAIR). Manipulating RNA benefits human health as it does not interfere with the mammalian genome for alleviation and/or cure of various genetic and acquired diseases. Our AcrVIAs extend our toolkits for avoiding side effect when using Cas13 for RNA editing.

LB038

Combined data driven computational tool and B cell maturation assay for new target identification in systemic lupus erythematosus

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Background and Aim: SLE is a heterogeneous systemic disease driven by autoimmune reactions involving autoantibodies, with an unmet medical need. We aim to identify novel targets by combining a computational tool for druggable target selection and a B cell maturation assay for target validation. **Methods:** The top 1% genes (~150 genes) ranked by Priority Index (Pi), a computational tool integrating genomic and network information to prioritize disease-relevant genes (Fang et al, Nature Genetics, 2019), were further narrowed down by the following filters: (i) network genes; (ii) gene

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DOI: 10.1002/eji.201970400

targets with druggable pockets; (iii) genes highly ranked in other immune disease similar to SLE; and (iv) genes that do not interfere with the B cell stimulation cocktail, i.e. sCD40L, IL4, IL10, IL21 and CpG OND2006. Accordingly, 11 genes were selected for validation by knockdown using siRNA in PBMCs: IFNGR1, IL-2, IRF4, IL-12A, IL-12B, VCAM1, ATF6B, RELA, IKBKG, CHUK, MAPK14. Induced maturation of B cells (CD19+CD27+) and cell viability was analyzed by flow cytometry at day 6 post transfection, and IgG secretion was analyzed on day 3 and 6.
Results and conclusions: We experimentally validated most of the genes selected by the Pi computational tool, including IFNGR1, IL2, IL12A, MAPK14, IRF4, CHUK, ATF6B, IKBKG, and RELA. There were differences between the effects of validated target knockdown on IgG secretion versus B cell maturation suggesting differences in the mechanistic roles of these genes. Therefore, further studies of these targets for drug development in SLE is granted.

LB112

HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells

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A small number of HIV-1 infected individuals develop broadly neutralizing-antibodies to the virus (bNAbs). These antibodies are protective against infection in animal models. However, they only emerge 1 - 3 years after infection, and show a number of highly unusual features including exceedingly high levels of somatic mutations. It is therefore not surprising that elicitation of protective immunity to HIV-1 has not yet been possible. We show that mature, primary mouse and human B cells can be edited *in vitro* using CRISPR/Cas9 to express mature bNAbs from the endogenous *Igh* locus. Moreover, edited B cells retain the ability to participate in humoral immune responses. Immunization with cognate antigen in wild type mouse recipients of edited B cells elicits bNAb titers that neutralize HIV-1 at levels associated with protection against infection. This approach enables humoral immune responses that may be difficult to elicit by traditional immunization.

P0576

Establishment of a novel gene-editing mouse bioreactor to ubiquitously express the anti-CD20 monoclonal antibody

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Large-scale production of human recombination functional antibody by transgenic animal bioreactors is a “hot topic” in the field of biotechnology in recent years. However, this technology has two defects: Firstly, the use of random transgenic technology affected the sustained and stable expression of

DOI: 10.1002/eji.201970400

foreign genes, because of the random insertion of foreign protein genes, multiple copies, positional effects and gene silencing etc.; Secondly, commonly used transgenic animals bioreactor such as mammary glands, blood and salivary glands are independent and have some disadvantages that the transgenic animals born are only of specific gender, the specific lactation period and specific tissue sources (blood, breast or salivary glands) can be used for the production of the recombination functional proteins. These disadvantages greatly limit the utilization of transgenic animals' bioreactor. We hope to overcome the above shortcomings and to establish a whole body expression system to produce monoclonal antibodies (MAbs) by the large animals in future. Based on our previously report that we produced the anti-CD20 monoclonal antibody by the transgenic cattle mammary bioreactor. Here, we generated a new gene-editing mouse bioreactor which ubiquitously express the anti-CD20 monoclonal antibody by the Cas9-mediated mouse *ROSA26* "safety locus" gene knock-in technique. Both the individual health of the mouse and the continuous expression of the anti-CD20 monoclonal antibody in the blood, saliva and milk of the mouse were detected. In summary, our work provides an important theoretical and technical foundation for the establishment of gene-editing large animal bioreactor for the large-scale preparation of monoclonal antibodies in future.

P1172

CRISPR/Cas9 editing and ultrasound microbubble-mediated knockout of *C-erbB-2* in HEC-1A cells

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Objective: Epidermal growth factor receptor 2 (C-erbB-2) is one of the most frequently mutated oncogenes in human tumors. We aimed to evaluate the knockout efficiency of clustered regularly interspaced short palindromic repeat (CRISPR) technology using ultrasound microbubble transfection to target *C-erbB-2* in human endometrial cancer (HEC)-1A cells.
Methods: Three single guide RNAs (sgRNAs) targeting *C-erbB-2* were designed and used to construct CRISPR/ CRISPR-associated (Cas)9-C-erbB-2 plasmids. The constructed plasmids were transfected into HEC-1A cells using ultrasound microbubbles. C-erbB-2 knockout cloned cells were identified by green fluorescence. C-erbB-2 mRNA and protein expression was measured by reverse transcription (RT)-PCR and western blotting, respectively.
Results: RT-PCR showed that C-erbB-2 mRNA expression was significantly lower in sgRNA1-transfected cells (0.57±0.06) than in blank (1.00±0.09) and negative-control groups (1.02±0.12). Western blotting revealed C-erbB-2 protein expression to be significantly lower in sgRNA1-transfected cells (0.269 ± 0.033) than in blank (0.495±0.059) and negative-control groups (1.243±0.281). However, there was no significant difference in C-erbB-2 protein and mRNA expression in sgRNA2- and sgRNA3-transfected cells compared with controls.
Conclusion: Ultrasound microbubbles can mediate plasmid transfer into HEC-1A cells to interfere with gene expression and knockout *C-erbB-2*.
This work was financially supported by Hainan Provincial Natural Science Foundation of China(No. 817313), Major Science and Technology Program of Hainan Province (No. ZDKJ2017007).

DOI: 10.1002/eji.201970400

P1769

The effects of knock-out BLM gene on proliferation and apoptosis in human triple-negative breast cancer MDA-MB-231 cells by using CRISPR /Cas9 technology

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Bloom syndrome helicase (BLM) participates in cell metabolism including DNA replication, repair, recombination, telomere maintenance, and plays key roles in proliferation of cancer cells. In the study, the CRISPR/Cas9 gene editing technique was used to construct the human triple-negative breast cancer MDA-MB-231 cell line with removal of *BLM* gene, and the effect of knockout of *BLM* gene on human breast cancer MDA-MB-231 cells was preliminarily investigated. The positive clones with high targeting efficiency were cultured by monoclonal culture, and the expression of BLM in breast cancer MDA-MB-231 cell lines with *BLM* knockout were further detected with Western Bloting. The off-targeting of *BLM* knockout cell lines was detected by using CruiserTM endonuclease. The proliferation and apoptosis of the *BLM* knockout breast cancer cell was detected by using MTT, Hoechst 33258 staining, and FCM method. The apoptosis-related protein expression in BLM-KO cells was detected with Western Bloting. The Results show that: 1. Two gRNA were designed for targeting the 3rd exon in *BLM* Gene, and the sequencing results showed that the gRNA recombinant plasmid was successfully constructed. 2. It was verified that the gRNA2 target was more efficient than another. A cell line with significantly reduced BLM protein was identified. It was verified that the cell line was not off target. 3.The proliferation of the BLM knockout breast cancer cell was inhibited and their cells apoptosis was induced. 4. As compared with control, expression of BRCA1,RAD51,BCL-2 protein was downregulated and expression of P53,BAX,CASPSAE-3,CASPASE-8 protein was upregulated in BLM knokout breast cancer cell.

P2363

Exploiting a novel immunoglobulin diversification mechanism to engineer primary human B cells

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The ability of antibodies to recognize various foreign antigens is generated by two classical diversification mechanisms during maturation, V(D)J recombination and somatic hypermutation. In previous work, our laboratory identified an novel, additional antibody diversification mechanism that induced opsonization of erythrocytes infected by different *P. falciparum* isolates. This antibodies achieved broad neutralization ability through a large DNA fragment insertion, encoding for the entire extracellular domain of the LAIR-1 receptor, creating an additional immunoglobulin-like domain in the elbow of the antibody proven to be the crucial binding element. Subsequent sequencing of healthy Europeans donors revealed the presence of diverse templated switch region insertions, but solely in memory B cells, indicating that the template insertions occur during AID mediated class switching. Employing this novel findings allowed the invention of an *in vitro* engineering system to induce genomic insertions of DNA templates in primary human B cells using only their endogenous AID nuclease activity. Applying this technique allowed the successful engineering of primary human B cells to express domains of foreign proteins involved in pathogen cell entry, within the antibody's elbow. Targeting conserved receptor binding sites of pathogens reduces the likelihood of their evasion. Furthermore we utilized this technique to insert templates encoding for VHH domains of known

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DOI: 10.1002/eji.201970400

antigen specificity in the antibody's elbow. In summary, we present a novel method to engineer primary human B cells utilizing only their intrinsic AID activity to express a new functional class of antibodies. This novel technology represents a pioneering approach of personalized adaptive humoral immune therapy.

P3278

Anti-lung cancer effects of recombinant H9N2 subtype avian influenza virus expressing hGM-CSF

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The present study aimed to investigate the killing effect of recombinant virus 14GM on human lung adenocarcinoma cell line A549 in vitro and in vivo by constructing recombinant virus carrying human colony-stimulating factor (hGM-CSF) through reverse genetics technology. The recombinant virus 14GM plasmid was obtained by PCR, then rescued in an eight-plasmid transfection system by the co-transfection of MDCK cells and 293T cells with liposomes. The anti-lung cancer effect of recombinant virus 14GMwas assayed by MTT method and flow cytometry in vitro, and further confirmed in an in vivo xenograft model by immunohistochemistry and TUNEL. The TCID50 of recombinant virus 14GM was$10^{-6.74}$. MTT assay showed that 14GM had a significant anti-proliferation effect on A549 cells. Flow cytometry analysis showed thatthe apoptoticcells treatedwith14GM was significantly increased compared with SH14 virus. In vivo anti-tumor experiments indicated that compared with SH14 wild-type group, 14GM significantly inhibited and reduced the growth of lung cancer A549 cells and tumor size in nude mice, respectively. These results found that recombinant H9N2 subtype avian influenza virus 14GM carrying hGM-CSF gene can inhibit the proliferation of lung adenocarcinoma cells A549in vitro and in vivo, which may bea potential drug for the treatment of lung cancer.

P3279

Precise and rapid validation of candidate gene by allele specific knockout with CRISPR/Cas9 in wild mice

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The wild-derived mouse strains harbor enormous genetic variations. However, validating the genetic variation in non-classical strains was extremely difficult, until the advent of CRISPR/Cas9 genome editing tools. In this study, we first describe a T cell phenotype in both wild-derived PWD/PhJ parental mice and F1 hybrids, from a cross to C57BL/6 (B6) mice, and we isolate a genetic locus on Chr2, using linkage mapping and chromosome substitution mice. Importantly, we validate the identification of the functional gene controlling this T cell phenotype, *Cd44*, by allele specific knockout of the PWD copy, leaving the B6 copy completely intact. Our experiments using F1 mice with a dominant phenotype, allowed rapid validation of candidate genes by designing sgRNA PAM sequences that only target the DNA of the PWD genome. We obtained 10 animals derived from B6 eggs fertilized with PWD sperm cells which were subjected to microinjection of CRISPR/Cas9 gene targeting machinery. In the newborns of F1 hybrids, 80% (n = 10) had allele specific knockout of the candidate gene *Cd44* of PWD origin, and no mice showed mistargeting of the B6 copy. In the resultant allele-specific

DOI: 10.1002/eji.201970400

knockout F1 mice, we observe full recovery of T cell phenotype. Therefore, our study provided a precise and rapid approach to functionally validate genes that could facilitate gene discovery in classic mouse genetics. More importantly, as we succeeded in genetic manipulation of mice, allele specific knockout could provide the possibility to inactivate disease alleles while keeping the normal allele of the gene intact in human cells.

P3280

Potent gene transfer in human primary T lymphocytes using interleukin-2-displaying lentiviral vectors

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Adoptive immunotherapy using T cells modified with T cell receptor or chimeric antigen receptor is an emerging and powerful therapeutic approach for the treatment of patients with refractory cancer. However, the clinical application of this therapy has been limited by the low gene transfer rate of viral vectors into T lymphocytes. Here, with the aim of improving the efficiency of T cell transduction, we constructed a novel packaging plasmid for chimeric glycoproteins consisting of interleukin-2 (IL2) fused to the N-terminus of the vesicular stomatitis virus G protein (VSV-G) (IL2-VSV-G) to produce IL2-displaying lentiviruses. The yields of lentiviral particles pseudotyped with varying ratios of IL2-VSV-G and wild-type VSV-G (WT-VSV-G) were compared using immunoblotting and ELISA, and 20% IL2-VSV-G was selected as the optimal proportion. The capacity of EGFP-encoding IL2-displaying lentiviruses to infect T lymphocytes from healthy donors were tested in combination with spinoculation and the adjuvant F108 using flow cytometry assays. The IL2-modified lentiviruses exhibited a significantly improved gene transduction efficiency compared to the WT-VSV-G pseudotyped vectors (89.2% *vs.* 40.2%). Our results demonstrate that IL2-displaying lentiviral particles have a greatly enhanced transduction efficiency in human T lymphocytes when combined with spinoculation and a poloxamer-based adjuvant. Therefore, this system may be more suitable for the establishment of high-efficiency and clinically oriented manufacturing methods for genetically modified T cells.

Imaging of Immune System

O027

High-dimensional analysis of the immune system in mycosis fungoides

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Mycosis Fungoides (MF) is the most common type of cutaneous T-cell lymphoma. Despite earlier immunohistochemical studies the composition and functional characteristics of the cutaneous lymphocytic infiltrate has not been fully characterized in MF. Here, we applied a 42 antibody panel and high-dimensional single-cell mass cytometry to identify and characterize disease-associated immune subsets. For this we optimized the protocol for isolation of cells from skin tissue and verified that this protocol yielded sufficient cells and was compatible with the subsequent mass cytometry analysis. After labelling with the 42 antibody panel, cells were analysed with a Helios mass cytometer. To determine the heterogeneity and expression profiles of immune subsets in skin and matched PBMC samples a tSNE-ACCENSE based analysis pipeline was used. Unbiased hierarchical clustering of

DOI: 10.1002/eji.201970400

subsets revealed clear separation of distinct CD4, CD8 and myeloid cell lineages. The tSNE analysis further showed that subset distribution between samples from non-lesional and lesional skin of MF was similar with a predominance of CD4 and CD8 T cells. In contrast, substantial differences were found between normal and MF samples with a dominant presence of myeloid cell subsets in normal skin. To characterize the spatial distribution of immune subsets in the tissue context we performed imaging mass cytometry using a 36 antibody panel on skin sections from a MF patient and controls. Results from this analysis revealed colocalization of proliferating $CD4^+$ and $CD8^+$ T cells with antigen-presenting cells in MF, potentially indicative of antigen-driven expansion. This work may offer guidance to improve therapy of MF.

O028

Skint1 clustering and interactions with γδ T cells in mouse epidermis revealed by super-resolution fluorescence microscopy

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Mammalian epidermis is populated by a large number of immune cells. In mouse these include immune monitoring γδ T cells, also known as dendritic epidermal T cells (DETC), which form a dense network in parallel to the network of Langerhans cells. A transmembrane protein Skint1 expressed by keratinocytes was suggested to play a pivotal role in molecular interactions between γδ T cells and surrounding epithelial cells. Here we applied a super-resolution fluorescence microscopy approach to investigate the role of Skint1 in defining tissue homeostasis and cell functionality. Using Structured Illumination Microscopy, we were able to visualize and quantitate Skint1 clustering in vicinity of γδ T cell receptor (TCR) clusters at dendrite contact points of DETC in tissue at 120 nm resolution. The clustering of Skint1 on epithelial cell surface was further characterised in a number of mouse lines where Skint1 was mutated or overexpressed, as well as in γδ TCR and other Skint family protein knockout lines. The role of interplay between Skint1 and Skint2 and their potential dimerization was investigated by analysis of Skint1 clustering in Skint2 knockout mice and by quantitating Skint2 clustering in Skint1 knockout mice. Further, we applied multi-colour Single Molecule Localization Microscopy approach revealing Skint1-TCR interactions in tissue at 15 nm isotropic resolution. These super-resolution fluorescence imaging data provide support for direct interactions of Skint1 with γδ TCR, and for Skint1-mediated immune sensing in epithelial tissues.

P0577

Intravital imaging to evaluate the tumor killing capacity of cytotoxic T Cells in vivo

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As the main effector cells for killing tumor cells, cytotoxic T lymphocytes (CTLs) play a key role in eliminating tumor. CTLs kill tumor cells is a rapid and efficient process in vitro. However, it's not clear the efficiency of CTLs killing tumor metastasis cells in vivo, and how the organs and tumor growth process effect on CTL killing capability. Here, we used intravital imaging to dynamically monitor the process of CTLs killing liver metastatic cells, in which the death of tumor cells was reflected by fluorescence resonance energy transfer (FRET) signal of caspase-3 or calcium sensor. On average, each CTL kills 0-7 tumor cells per day in liver. FRET imaging of calcium signal in liver metastatic cells revealed that CTL killing tumors is heterogeneous. CTLs in spleen kill tumor cells more efficiently than

DOI: 10.1002/eji.201970400

in liver. In addition, CTLs killing tumor cells at the early stage of growth were more efficiently than that in the late growth stage. In a word, our study quantitative calculation and comparison of CTL killing capacity to tumor cells in vivo, and evaluates the effects of different organs and tumor microenvironments on the killing capability of CTLs.

P0578

Visualizing monocyte migration and activation in acquired cutaneous immunity

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Mobilizing the interstitial migration of leukocytes and the subsequent organization of cooperation among these leucocytes in inflamed microenvironment are critical for intelligent and effective immune response to foreign antigen challenges. When infiltrated into the dermal interstitial space, monocyte/macrophages (Mon/Mφ) quickly migrate to sense inflammatory mediators and communicate with the dermal microenvironment. Therein, they can be activated to eliminate invasive pathogens or mediate tissue damages. Although the migration strategies of neutrophils (e.g., directional migration) and T cells (e.g., Lévy walk) within interstitial space have been thoroughly studied, the behavior of the Mon/Mφ is still far from fully investigated till date. Here, we establish a new method to synchronously monitor migration and activation of Mon/Mφ through imaging the behavior of Mon/Mφ, cell-cell contact, and the molecular dynamic in Mon/Mφ in a multicolor-labeled contact hypersensitivity (CHS) model. We find that epicutaneous antigen challenge induces IFN-γ release, which mobilizes Mon/Mφ migration towards epidermis and induces Mon/Mφ cluster formation at the dermal-epidermal junction. This presents a change in the spatial distribution of Mons/Mφs in the interstitial space. In this process, hair follicle shapes the inflammatory microenvironment for communication among Mon/Mφs, keratinocytes, and effector T cells in the cutaneous immunity. Furthermore, we investigate the strategy of Mon/Mφ migration and activation through quantitatively characterizing the migration parameter and activation feature of Mon/Mφ. These findings reveal the cascade of inflammatory responses in contact hypersensitivity, which provide real and intuitive evidence for the pathogenetic of allergic contact dermatitis.

P0579

Two-photon imaging of the *in vivo* dynamics of immune cells and cellular pathophysiology in influenza virus-infected lung

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Influenza virus is a respiratory pathogen that causes pandemics and seasonal epidemics. The *in vivo* pathophysiology and dynamics of immune cells in influenza virus-infected lungs are not fully understood. Here, we established an *in vivo* imaging system that employs two-photon excitation microscopy and fluorescent influenza viruses of different pathogenicity (backbones of H5N1 A/Vietnam/1203/2004 or A/Puerto Rico/8/34 [H1N1; PR8]). This approach allowed us to monitor and correlate several parameters and pathophysiological changes including the spread of infection, pulmonary permeability, speed of pulmonary perfusion, the number of pulmonary vascular neutrophils,

DOI: 10.1002/eji.201970400

and neutrophil motion in the lungs of live mice. Several pathophysiological changes were larger and occurred earlier in mice infected with a highly pathogenic H5N1 influenza virus compared with those in mice infected with a PR8 strain. Time-lapse imaging analysis revealed that in influenza virus-infected lung, the number of pulmonary vascular neutrophils temporally increased and neutrophil movement changed to longer periods of slow motion after the climax of neutrophil recruitment. We also made real-time observations of cell-cell interactions, with morphological changes, between infected cells and alveolar macrophages infiltrating the alveoli of the infected lungs. These findings demonstrate the potential of our *in vivo* imaging system to provide novel information about the pathophysiological consequences of virus infection and the immune response to influenza virus.

P1173

Intravital imaging of local photothermal therapy with an immunostimulant induced antitumor immune response for melanoma

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Melanoma is one of the deadliest and hardly-treated malignancy with high risk of relapse and metastasis. Photothermal therapy combined with immunoadjuvants is advantageous in the personalized cancer immunotherapy. As photothermal treatment in the local tumor offers complete antigen to the immune system which has been stimulated by immunoadjuvants and induce strong antitumor immune response to prevent tumor recurrence and inhibit tumor metastases. Here, we developed a laser immunotherapy (LIT) strategy which combined 980 nm laser and an immunoadjuvant to treat the B16 tumor. We found that this combination treatment can eliminate primary tumors, reject rechallenged tumors, and inhibit tumor metastases. By western blot detection of damage associated molecular patterns (DAMPs), ELISA detection of cytokines, flow cytometry analysis of immune cells, we clarified the functions of key immune cells spatially and temporally after LIT treatment. Furthermore, we used the intravital imaging to observe the LIT-elicited immunoreactions in a rechallenged tumor microenvironment through window chamber on the mice. The results showed that, compared with control mice, in the LIT treated mice, there are much more endogenous tumor infiltrating T cells infiltrated into the tumor areas and keep high speed to search the target tumor cells and then successfully eradicate them. Our findings provide personalized vaccine approaches for treatment of metastatic cancers.

P1174

Spatial and phenotypic immune profile predicts clinical outcomes of gastric cancer

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The purpose of this study is to address the relationship between the immunological microenvironment and clinical outcomes of Gastric Cancer (GC) patients. The local inflammation is suggested to polarize Tumor-Associated Macrophage (TAM) immunity. Therefore, a comprehensive evaluation of the composition and distribution of TAM elements and T-cell infiltration is necessary to delineate their roles. Here we develop a novel computational imaging technology for the simultaneous evaluation of seven distinct markers for cytokeratin (Tumour cells), immune checkpoint molecule (PD-L1),

DOI: 10.1002/eji.201970400

CD8(Cytotoxic T cell), FOXP3(Regulatory T cell), CD68 and CD163 (TAMs) to allow characterized immune infiltration for spatial analysis of distinct populations within the same section. We report a heterogeneous population of infiltrating T lymphocytes. Spatial distribution of CD8+ T cells in proximity to cancer cells correlates with increased overall patient survival. We also found an increased density of tumor TAMs in the tumor core, strikingly correlated with prolonged survival in GC patients. Furthermore, CD8+T cell densities were elevated which correlate with those of increased CD163. This co-existence of CD163 and CD8+T cells independently predicted prolonged patient survival. Furthermore, gene expression from the TCGA database indicates that CSF1/CSF1R is strikingly associated with CD8, CD68 and CD163, suggesting that CSF1 is a key mediator regulating $CD8^+$T cells and TAMs. Collectively, our study demonstrates that prognostic value of the intercellular spatial distribution is better than cell density in the tumor microenvironment. The potential synergy of $CD163^+$TAMs and $CD8^+$T cells which help us to better stratify the gastric cancer patients for immune-modulatory therapies.

P1175

Multiplex immunofluorescence of bone marrow core biopsies: visualizing the bone marrow immune contexture in human plasma cell malignancy

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Multiple myeloma (MM) is an incurable malignancy of clonal plasma cells (PC). MM is preceded by the asymptomatic precursor conditions termed monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM). We hypothesize that the BM immune contexture (in situ immune cell nature, density, functional orientation, and spatial relationships to abnormal PCs) plays a significant role in determining whether MGUS/SMM patients will remain stable and asymptomatic or progress to MM. Indeed, the ability to visualize and quantify the spatial arrangement and geographic proximity of immune cells to tumor cells provides valuable insight into the complex mechanisms underlying cancer biology and progression. Multiplexing, which involves immunofluorescence labeling and the visualization of multiple epitopes within formalin-fixed paraffin embedded tissue sections, is a methodology that has been increasingly employed in recent years. Despite the power of immunofluorescence multiplex analysis, a major obstacle to the application of this technology to archival paraffin embedded bone marrow core biopsies has been high levels of intrinsic autofluorescence. In this study we first identified a blocking strategy that quenched autofluorescence. Next, we employed a multiplex strategy that uses a simple stripping solution to remove primary and secondary antibodies prior to subsequent rounds of staining. This method was found to be highly efficient and did not significantly alter antigenicity or tissue integrity. Our studies illustrate for the first time that immunofluorescence multiplexing is achievable in BM core biopsies and will provide a novel opportunity to analyze the role of the immune contexture in disease progression of the monoclonal gammopathies.

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DOI: 10.1002/eji.201970400

P1770

Radioimmunoimaging of ^{125}I-anti-CD93 mAb in non-small cell lung cancer mice model

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Purpose: Lung cancer is the most common malignant tumor and the leading cause of death from malignancies. Angiogenesis is frequent and is vital for non-small-cell lung cancer (NSCLC). Recently CD93 was reported to be a key regulator of tumor angiogenesis by regulating adhesion between cells and the cell-matrix. This study is to estimate the value of CD93 in visualized monitoring of angiogenesis as a novel non-invasive tumor molecular targets.
Methods: The CD93 expression of three kinds of NSCLC was measured. NSCLC carcinoma xenograft mice were intravenously injected with ^{125}I-antiCD93 McAb. Biodistribution and whole-body phosphor autoradiography were were conducted dynamically. Histology and immunohistochemical staining of CD93 were carried out to prove CD93 expression.
Results: A549 cells and H460 cells showed the relatively higher expression of CD93 in Western Blot and RT-PCR. Biodistribution studies showed the T/NT ratio of A549 group and H460 group were higher than the H520 group. In vivo radioimmunoimaging showed tumor tissues clearly both in A549 group and H460 group. Pathological sections of tumor tissues showed that malignant proliferation of tumor cells with neovascularization, and the positive rate of CD93 immunohistochemical staining in A549 group and H460 group was higher than that in H520 group, consistent with the result of dynamic radioimmuoimaging.
Conclusion: CD93 was highly expressed in lung adenocarcinoma and large cell carcinoma cells, compared with the lower expression in squamous cell carcinoma cells. ^{125}I--antiCD93McAb could provide a noninvasive profiling of the NSCLC in terms of angiogenesis characteristics, particularly in the identification of the histologic subtypese.

P1771

Toward validation of quantitative analysis of multiplex fluorescent immunohistochemistry data

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Multiplex immunohistochemistry (mIHC) can examine the number and location of cell subpopulations within the tumor microenvironment. Using mIHC, we described a Cumulative Suppression Index (CSI), which examines the number of $CD8^+$ T cells located in the invasive margin of tumor combined with the number of $FoxP3^+$ or PD-$L1^+$ cells within 30 mm of $CD8^+$ T cells. CSI correlated with overall survival in a cohort of patients with HPV^- oral squamous cell cancer (Feng 2017). Future application of CSI requires a validated assay. Here we systematically compared three methods of enumerating mIHC cellular phenotypes across multiple users. Analysis of variation within each user across the three different analysis methods demonstrated tight correlation for the principle phenotypes of the CSI, namely $CD8^+$PD-$L1^-$, $CD8^+$PD-$L1^+$, $FoxP3^+$, and PD-$L1^+$ cells (Spearman Rank Correlation $p< 0.05$). The intraclass correlation coefficient demonstrated consistency across all phenotypes for all users using 2 of 3 analysis methods ($p< 0.05$). Most analysis software require feature-based cell selection (i.e. fluorescence intensity values) for downstream classification that exclusively relies on the observer and his/her perception of a cell being “positive” or “negative” for a given feature. Global application of mIHC for tailoring combination immunotherapy will be helped by developing standards and running proficiency panels. Our group has produced formalin-fixed cell pellet arrays with flow cytometry-characterized expanded human T cell populations and documented reproducible assessment of

DOI: 10.1002/eji.201970400

critical markers using mIHC. Sections from this T cell Activation Marker Proficiency Panel (TAMPP) array are being distributed to groups around the world to evaluate reproducibility.

P2364

TLR5 is a new reporter for triple-negative breast cancer indicated by radioimmunoimaging and fluorescent staining

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Purpose: Earlier diagnosis and treatment are very important for triple-negative breast cancer (TNBC) which with high mortality and lack of target-therapy. Recently enhanced Toll-like receptor 5 (TLR5) expressions was reported in TNBC, but how TLR5 acts on TNBC and whether it could be used as a noninvasive monitoring target are not fully understood.
Methods: TLR5$^-$ 4T1 cell line was established with Lentivirus-shRNA-TLR5 (named TLR5$^-$ 4T1) and effect TNCB invasive activates was detected. ^{125}I-anti-TLR5 and isotype IgG were prepared and identified, injected through tail veins to TLR5$^{+/-}$ 4T1-bearing mice models. Biodistribution, whole-body phosphor-autoradiography and optic imaging were performed. Furthermore, tumor HE and immunochemistry staining were carried out to prove TLR5 expression.
Results: TLR5 expression on 4T1 was stably knocked down which showed higher proliferation, migration, invasion and high expression of MMP2/9. ^{125}I-anti-TLR5 could bind to TLR5$^-$ and TLR5$^+$ 4T1 with high affinity and specificity. Tumor images in both groups could be observed early in 24h, more obviously in 48h after tracer injection. In tumor tissues, radioactivity was positively related with TLR5 expression. In vivo optical imaging successfully showed tumor tissues clearly both TLR5$^+$ and TLR5$^-$ 4T1 mice. The optical image position is consisted with radioactivity accumulation in tumor. Immunochemistry staining showed that TLR5 expression in 4T1 tumor was indeed down-regulated in TLR5$^-$ 4T1 mice.
Conclusion: TLR5 was high expressed in TNBC, TLR5-down regulation increased its invasive activities; ^{125}I-antiTLR5 McAb is an ideal agent for noninvasive imaging of TLR5$^+$ tumors. TLR5 may be as a novel molecular target for TNBC diagnosis and treatment.

P2365

Morphological observations using image cytometry for the comparison of trypan blue and fluorescence-based cellular viability

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Determining cell viability is an essential component in many biological experiments ranging from standard cell culture to immunology and oncology research. The traditional method for cell viability measurement is the trypan blue exclusion assay, which utilizes a hemacytometer to manually enumerate stained cells. However, this assay can be time-consuming, tedious, and have user-dependent variation. Studies have also shown that long incubation with trypan blue can induce cytotoxicity. With the increase in the availability of fluorescence detection systems, a number of fluorescence-based viability measurement methods have been adopted. In this work, we compared cellular viability measured using trypan blue and acridine orange (AO)/propidium iodide (PI). The results showed that trypan blue produced higher viability measurements when viability decreased to below 70%. Utilizing image-based cytometry, morphological changes of trypan blue stained cells were

DOI: 10.1002/eji.201970400

observed, which may explain the differences between the two methods. Results showed that some dying cells displayed large blue haze when stained with trypan blue that may be missed during manual cell counting. In addition, multiple concentrations of trypan blue were tested. At low concentrations, cells were not properly stained, but at concentrations, many cells turned into large blue haze, which may generate artificially higher viability measurements. In conclusion, using image-based cytometry, we were able to observe morphological changes in the cells, which could have attributed to the differences between trypan blue and fluorescence staining. In addition, it showed that the trypan blue assay can potentially only function properly in a certain range of cell viabilities.

P3281

Endoglin aptamer screening and in vivo imaging studies

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Tumor vascular endothelial cells are the basis of tumor angiogenesis and play an important role in tumor growth and metastasis. Endoglin is recognized as a specific marker for tumor angiogenesis and neovascularization. The nucleic acid aptamer sequence which can specifically recognize human Endoglin molecule was screened in vitro, and its biological function was identified, so that it can display good tumor vascular targeting ability in vivo and in vitro. 5 nucleic acid aptamers that specifically bind to target cell cell293T-hE were obtained, named ENG-1, ENG-2, ENG-3, ENG-4 and ENG-5. Each aptamer could bind to target cell 293T-hE at 4 °C and 37 °C. The results of the mouse tumor in vivo test also confirmed that the aptamer has good tumor targeting in vivo. This study successfully screened the nucleic acid aptamer sequence with high affinity and high specificity binding to the tumor neovascular surface molecule Endoglin, which provides a new potential tool for the diagnosis and treatment of tumors targeting tumor neovascularization.

Omics and Bioinformatics in Immunity

O401

Alternative splicing profiling of Aire-sensitive genes in the thymic epithelium revealed increased splice event exclusion

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Aire induces medullary epithelial cells (mTECs) to express and present a large number of tissue-restricted self-antigens (TRAs) whose recognition by developing thymocytes triggers immunological tolerance. Although it is well established that mTECs present a high transcript diversity, the impact of splicing activity in AIRE-sensitive genes remain unclear. Therefore, we analyzed the inclusion ratio of Alternative Splicing Event (ASE) inferred from the mouse mm10 annotated transcripts and found a dramatic increase of the proportion of excluded ASE in Aire- sensitive versus neutral genes in both WT and *Aire*-KO mTECs. Thus, this specific feature of Aire-sensitive genes is in place in *Aire*-KO mTECs and conserved upon upregulation by Aire. We also showed that the Aire-sensitive genes

DOI: 10.1002/eji.201970400

present a higher proportion of ASE exclusion in mTECs than in their normal tissues of expression, therefore pointing to a loss of coding sequence expression in mTECs. Among all RNA-binding proteins expressed in mTECs, we identified Raver2 as the only splicing factor significantly upregulated in these cells and tightly correlated with the levels of splicing activity across peripheral tissues. Raver2 is a PTB binding protein known to interact with H3K36me3 that triggers splicing in some models. Analysis of H3K36me3 Chip-seq data in mTECs revealed that the Aire-sensitive genes are deprived of this mark and that H3K36me3 is associated with increased ASE in mTECs. Thus, our results showed that Aire-sensitive genes escape the Raver2-driven enhancement of ASE inclusion occurring in mTECs, suggesting a hole in the self-antigenic peptides repertoire induced by Aire and presented to developing thymocytes.

O402

Deciphering the complexity of immune repertoires using systems immunology and machine learning

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The adaptive immune system recognizes antigens via an immense array of antigen-binding antibodies and T-cell receptors, the immune repertoire. The interrogation of immune repertoires is of high relevance for understanding the adaptive immune response in (autoimmune) disease and infection. We and others have recently shown that immune repertoires are much more predictable than previously thought, which is of incredible importance for the precise manipulation and prediction of adaptive immunity. However, as of yet, we lack the computational methods that enable us to understand the construction rules according to which immune repertoires are assembled. Specifically, (i) on the repertoire-level, we lack mathematical methods to measure repertoire similarity across individuals. We will show results of a network approach that allows quantifying immune repertoire similarity based on the multidimensional combination of immunological features that cover the full dimensionality of the repertoire space. Our networks-based similarity definition explicitly allows many-to-many repertoire comparisons, which implies that we can now quantify a repertoire´s deviation from the population norm. (ii) On the sequence level, we lack machine learning methods that enable modeling immune-receptor-antigen binding in the absence of extensive 3D data. Therefore, we trained a collection of machine learning models on large synthetic antibody sequence datasets with varying pattern complexities, which simulate antibody-epitope binding. We tested the models in terms of pattern prediction accuracy and recovery. Taken together, we have developed computational tools that pave the way towards understanding the molecular construction rules of adaptive immunity on the repertoire and single sequence level.

O403

A system of immune cell subpopulation subtle subdivision based on gene plasticity

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The immune system is highly complex, which is mainly manifested in the complex and diverse immune molecules, immune cells and their interactions, and so on. There are many kinds of immune cells, which not only contain a variety of immune cell lineages, but also have different morphology and

DOI: 10.1002/eji.201970400

function. Moreover, the phenotypes and functions of immune cells of the same lineage are also very complex and diverse, and can be further divided into different subsets. However, whether immune cell subpopulations could be predicted and whether there are some common rules to subdivide immune cell subpopulations have not been well addressed. According to our previous studies and recent omic big data analysis, herein we proposed a system for immune cell Subpopulation Subtle Subdivision based on gene plasticity, or called three ´S´ system. The basic connotation of the classification system includes: 1) genes have expressional plasticity, which contains two features, the change in the level of expressional amount and the change in the level of rank expression; 2) highly plastic genes can be suited for the labeling of immune cell subpopulations; 3) based on gene plasticity, subtle subpopulations can be classified from the greater subpopulations; 4) phenotypic plasticity is closely related to gene plasticity; 5) external phenotypes of immune cells that are usually measured are constrained by internal phenotypes. The internal phenotype is used to describe the intrinsic and restrictive relations among genes, such as strong expressional correlation and repulsion. The unrelated expression of genes is a special case of internal phenotypes.

P0580

Single-cell RNA-seq reveals the heterogeneity of liver-resident immune cells in human

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The liver plays a critical role in both immune defense and tolerance in the body. The liver-resident immune cells (LrICs) may determine the immune properties, but the unique composition and heterogeneity of these cells are incompletely understood. Here, we dissect the heterogeneity and functional diversity of LrICs by a comprehensive transcriptomic profiling. Unbiased single-cell RNA-sequencing (scRNA-seq) was performed on $CD45^+$ immune cells isolated from the paired liver perfusion, spleen and peripheral blood as references from 3 transplant donors. Identified immune cell subsets were validated by RT-PCR and flow cytometry. A total of 63, 195 cells were profiled and 25 discrete cell populations comprising T and natural killer (NK) cells, B cells, antibody-secreting cells and myeloid cell subsets were identified in these tissues. We further characterized the tissue distribution of these cell subsets and uncovered elevated metallothionein pathway in the liver. Three populations of $CXCR6^+$ T and NK cells and two populations of $MerTK^+$ monocytes and macrophages were identified to be existed specially in the liver, showing the unique composition of the LrICs. Finally, we resolved the evolving trajectory and relationships among immune cell lineages for LrICs and provided novel markers, genetic modules and scRNA transcriptomics datasets to define each distinct subset. Thus, we identify five subsets of LrICs and confirm their heterogeneity. This study presents the first comprehensive landscape of human LrICs and will enable further study on their roles in various human diseases.

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DOI: 10.1002/eji.201970400

P0581

Integrative analysis identifies DNMTs against immune-infiltrating neutrophils and dendritic cells in colorectal cancer

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Molecular characterizations, including microsatellite instability (MSI) and the CpG island methylator phenotype (CIMP) showed strong associations in colorectal carcinoma (CRC) and provided a deeper understanding of the etiology of disease. However, the global relationship between epigenetic alternations and changes in mRNA expression in CRC remains largely undefined, especially regarding the roles of DNA methyltransferases (DNMTs). **Here, we conducted a systematic network comparison to explore the global conservation between co-expressed and co-methylated modules. We successfully identified immune-related modules that were regulated by DNMTs and had strong associations with immune-infiltrating neutrophils and dendritic cells in CRC**. Moreover, we found that genes in those modules were prognostic for CRC, with 97.1% (168/173) being significantly influenced by DNMTs. Thus, this study resolved an interaction between DNA methylation and mRNA expression through DNMTs. Additionally, we provided evidence that DNMTs control the global hypomethylation of oncogenes, including *ALOX5AP* and *CSF3R* that otherwise have high methylation in normal colons. Such genes were also more sensitive to DNMT changes, such as in CRC. Collectively, our analyses provided a systems biology approach to investigate the association among different molecular phenotypes in diseases.

P0582

Replacing alpha-fetoprotein with alpha-fetoprotein-L3 increases the sensitivity of prenatal screening for trisomy 21

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This study aimed to investigate the serum concentration of alpha-fetoprotein (AFP)-L3 in midterm pregnancies and its potential application in prenatal trisomy screening. The serum samples from 27 women with trisomy 21 fetuses and 800 women with normal fetuses were examined to measure the concentrations of AFP, AFP-L3, human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin-A. The screening results of various tests consisting of these markers were analyzed. In normal pregnancies within 15-20 weeks of gestation, the medians of serum AFP-L3 were 4.63, 5.70, 5.78, 6.58, 7.03, and 7.25 pg/mL. The median of AFP-L3 MoM in the trisomy 21 group was 0.46, which was significantly lower than the value of 1 in the normal group ($P< 0.05$).When using a cutoff value of 1/270, the sensitivity of the triplemarker test (AFP, hCG, uE3) was improved from 74% to 81% by replacing AFP with AFP-L3, with the false-positive rate slightly increased from 5.4% to 6.8%. Similarly, the sensitivity of the quad marker test (AFP, hCG, uE3, inhibin-A) was improved from 81% to 89% by replacing AFP with AFP-L3, with the false-positive rate slightly increased from 4.6% to 5.6%. Serum AFP-L3 concentration increases along with more weeks of gestation in the midterm pregnancies. Trisomy 21 screening tests with AFP replaced by AFP-L3 have higher sensitivities at the expense of slightly increased false-positive rates. This improvement in screening may help to better prepare the parents and caregivers for the special needs of newborns with trisomy 21.

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DOI: 10.1002/eji.201970400

P0583

Time-course transcriptome analysis of medullary thymic epithelial cells in the early phase of thymic involution

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Degeneration of the thymic epithelium is believed to be the primary cause of age-associated thymic involution. In order to investigate the molecular events during the early phase of thymic involution, RNA-seq was performed to gain the transcriptional profiles of medullary thymic epithelial cells (mTEC) from mice of 2, 6 and 10 weeks of
age. We confirmed and extended the previous observation of declined expression of cell cycle-related genes and diminished E2F3 activity during thymic involution, showing that it occurred as early as 2-6 weeks after birth. Moreover, we demonstrated that mTEC aging was coupled with augmented expression of inflammatory chemokines
and cytokines, reminiscent of the senescence-associated secretory phenotype. Impaired cell cycling and proinflammatoty response therefore represent two predominant transcriptional signatures during the very early phase of thymic involution. Taken together, the present study provides not only complimentary information about, but also new insight into the molecular mechanisms underlying age-related degeneration of thymic epithelial cells.

P0584

APEC: An accesson-based method for single-cell chromatin accessibility analysis

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The development of sequencing technologies has promoted the survey of genome-wide chromatin accessibility at single-cell resolution; however, comprehensive analysis of single-cell epigenomic profiles remains a challenge. Here, we introduce an accessibility pattern-based epigenomic clustering (APEC) method, which classifies each individual cell by groups of accessible regions with synergistic signal patterns termed “accessons”. By integrating with other analytical tools, this python-based APEC package greatly improves the accuracy of unsupervised single-cell clustering for many different public data sets. APEC also identifies significant differentially accessible sites, predicts enriched motifs, and projects pseudotime trajectories. Furthermore, we developed a fluorescent tagmentation- and FACS-sorting-based single-cell ATAC-seq technique named ftATAC-seq and investigated the per cell regulome dynamics of mouse thymocytes. Associated with ftATAC-seq, APEC revealed a detailed epigenomic heterogeneity of thymocytes, characterized the developmental trajectory and predicted the regulators that control the stages of maturation process. Overall, this work illustrates a powerful approach to study single-cell epigenomic heterogeneity and regulome dynamics.

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DOI: 10.1002/eji.201970400

P0586

iReceptor plus facilitates sharing and analysis of AIRR-seq data

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The iReceptor Plus Consortium, composed of 20 partners from 9 countries, will promote human immunological data storage, integration and controlled sharing for a wide range of clinical and scientific purposes. The four-year project will expand iReceptor, an innovative platform that integrates massive distributed repositories of Adaptive Immune Receptor Repertoire sequence (AIRR-seq) data (antibody/B-cell and T-cell receptor data).
Most AIRR-seq data are currently stored and curated by individual labs, using a variety of tools and technologies. The project will support the sharing of public AIRR-seq data across labs, diseases and institutions, guided by the protocols and standards developed by the AIRR Community. iReceptor Plus will develop and link to new analysis tools, integrate AIRR-seq data with other types of clinical and "omics" data, including single-cell data, and offer an advanced and collaborative immune-profiling hub. The project will add advanced security during transfer of public data, and provide a mechanism for users to protect private data when such protection is necessary. The platform's software will be free through open-source licensing, making it possible for the research community to extend and adapt the tools and technologies used in the project. The iReceptor Plus project will lower the barriers to access, integrate and analyze large AIRR-seq datasets, in order to advance our understanding of immune responses, and thus provide new targets for immunotherapies and new methods for monitoring therapeutic efficacy.
Funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 825821 and the Canadian Institutes of Health Research (CIHR).

P1176

Perturbation of ephrin receptor signaling and glutamatergic transmission in the hypothalamus of depression using proteomics integrated with metabolomics

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Hypothalamic dysfunction is a key pathological factor in inflammation-associated depression. In the present study, isobaric tags for relative-absolute quantitation (iTRAQ) combined with mass spectrometry and gas chromatography-mass spectrometry (GC-MS) were employed to detect the proteomes and metabolomes in the hypothalamus of the lipopolysaccharide (LPS)-induced depression mouse, respectively. 187 proteins and 27 metabolites were differentially expressed against the control group. Following integration of bi-omics data, pertinent pathways and molecular interaction networks were further identified. The results indicated the altered molecules were clustered into Ephrin receptor signaling, glutamatergic transmission, as well as inflammation-related signaling included the LXR/RXR activation, FXR/RXR activation, and acute phase response signaling. As the firstly discovered in the hypothalamus, the Ephrin receptor signaling significantly regulated N-methyl-D-aspartate receptors (NMDARs)-predominant glutamatergic transmission, and further acted on AKT signaling that contributed to the changes in hypothalamic neuroplasticity. The Ephrin type-B receptor 2 (EPHB2), a transmembrane receptor protein in Ephrin receptor signaling, was significantly elevated and interacted with the accumulated NMDAR subunit GluN2A in the hypothalamus. In addition, molecules involved in synaptic plasticity regulation, like the hypothalamic postsynaptic density protein-95 (PSD-95), p-AKT and brain-derived neurotrophic factor (BDNF), were significantly altered in the LPS-induced depressed group. It might be an underlying pathogenesis that EPHB2-GluN2A-AKT cascade regulates synaptic plasticity in depression. EPHB2 could be a potential therapeutic target in correction of glutamatergic transmission dysfunction. To sum up, our findings point to previously undiscovered molecular

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DOI: 10.1002/eji.201970400

underpinnings of the pathophysiology in the hypothalamus of inflammation-associated depression and offer potentially targets for the development of antidepressants.

P1177

PromPDD, a web-based tool for the prediction, deciphering and design of promiscuous peptides that bind to HLA class I molecules

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Promiscuous T cell epitopes that can be presented by multiple human leukocyte antigens (HLAs) have great potential for the development of vaccines with wide population coverage. However, no 'one-step' method for the prediction of promiscuous peptides that bind to HLA molecules beyond HLA supertypes is yet available. In this study, we re-evaluated and characterized four matrix-based prediction models that have been extensively used in the prediction of HLA-binding peptides based on benchmark datasets, and found that the prediction models generated based on the average relative binding (ARB) matrix shared a consistent and conservative threshold for all well-studied HLA class I alleles. Evaluations on the datasets of HLA supertype-specific peptides with various cross-binding abilities and peptide mutant analogues indicated that the ARB-based binding matrices could be used to decipher and design promiscuous peptides that bind to multiple HLA molecules. A web-based tool called PromPDD was developed using the ARB matrix-based models and enables the prediction, deciphering and design of promiscuous peptides that bind to multiple HLA molecules within or across HLA supertypes in a simpler and more direct way. Furthermore, we expanded the application of PromPDD to HLA class I alleles with limited experimentally verified data by generating pan-specific matrices using a derived modular method, and 2641 HLA molecules encoded by HLA-A and -B genes are available in PromPDD. PromPDD is the first tool for deciphering and design of promiscuous peptides that bind to HLA class I molecules, and is freely available at http://www.immunoinformatics.net/PromPDD/.

P1178

LPAR1, a potential prognostic biomarker, is correlated with immune infiltrates in prostate cancer

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Background: Lysophosphatidic acid receptor 1 (LPAR1) is an important gene that mediates diverse biologic functions, including proliferation, chemotaxis, inhibition of neuroblastoma cell differentiation, and tumor cell invasion. However, the correlation between tumor-infiltrating lymphocytes and the impact of LPAR1 to prognosis in prostate cancer remain unclear.
Method: LPAR1 expression analysis was based on R studio and Oncomine database. The influence of LPAR1 on prognosis was analyzed by using survival package in R. We evaluated the correlation between LPAR1 and immune infiltrates in Tumor Immune Estimation Resource (TIMER) site. Additionally, we used MCPcounter package to elucidate the critical role of LPAR1 and its effect on immune infiltrates.
Result: The Cancer Genome Atlas (TCGA) data set showed that LPAR1 was significantly down-regulated in patients with prostate cancer. Low LPAR1 expression was associated with poorer overall survival (OS) in prostate cancer (HR=0.51, P=0.00462). Interestingly, LPAR1 expression was positively correlated with the abundance of several immune cell types, including CD8+ T cells, CD4+ T

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DOI: 10.1002/eji.201970400

cells, macrophages, B cells, neutrophils and dendritic cells. In addition, we found that patients with high LPAR1 expression had a higher tumor mutational burden (TMB).
Conclusion: These findings suggest that LPAR1 is a potential prognostic biomarker in patients with prostate cancer and plays a critical role in immune cell infiltration. It means that patients with high LPAR1 expression have better antitumor immune responses. Besides, those patients also have higher TMB ,which might predict favorable outcome to immunotherapy in prostate cancer.
Keywords: LPAR1, tumor-infiltrating, lymphocytes, prostate cancer

P1179

Characteristics of the tumor microenvironment in Chinese minimal invasive adenocarcinoma

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Lung cancer is the leading cause of cancer death, and comprehending the immune characteristics in pre-cancer is vital for understanding the mechanism of neoplastic formation. In this study, we performed RNA-seq for matched tumor and tumor-adjacent normal samples from 59 Chinese minimal invasive adenocarcinomas (MIA) patients. We investigated mutation profiling, immune cell components, immune repertoire, and immune-related signals to understand tumor environment. We found that universal metabolism pathway, mucin family protein activation, and cytokines interaction enriched in MIA tumors. Individually, many Chinese MIAs were driven by EGFR mutations (~30%), and MIA tumors had higher B cell, CD4+ T cell infiltration, and lower CD8+ T cell infiltration than normal lungs. Not only the different immune cell components in tumor and normal tissues also the distinct immune signature patterns among patients revealed the tumor heterogeneity. After assembling T cell, B cell receptors from RNA-seq reads, we found more clonal expanded T and B cells in MIA tumors, indicating strong adaptive immune responses. Besides, cytokines and chemokine receptors were associated with immunoglobulin production in two patterns. We also uncovered the evidence of maintaining ectopic lymphoid structure in Chinese MIA, which was supported by the homing of B cells, CD4+ T cells and follicular dendritic cells to germinal center. Our study gives a comprehensive insight into the understanding of the complex interactions between cancer cells, immune cells and immune-related cytokines in the pre-cancer development.

P1180

Low-input transcriptomics library construction through EASY RNA-seq

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High-throughput sequencing for transcriptome profiling is an increasingly accessible and important tool for biological research. However, accurate profiling of small cell populations remains challenging due to issues with gene detection sensitivity and experimental complexity. Here we describe a streamlined RNA-seq protocol (EASY RNA-seq) for sensitive transcriptome assessment starting from low amount of input materials. EASY RNA-seq is technically robust enough for sequencing small pools of

DOI: 10.1002/eji.201970400

homogenous and heterogeneous cells, recovering higher numbers of genes and with a more even expression distribution pattern than other commonly used methods. Additionally, it bypasses template switching mechanism to synthesize second strand cDNA, yielding full-length RNA information, resulting in less PCR bias in RNA-seq library construction. EASY RNA-seq can detect more than 15,000 of protein-coding genes, enhancing both sensitivity and accuracy of distinguishing subgroups from rare cells, and aiding in feature selection of cell subsets for development of novel biomarkers. It may also allow for easier application of deconvolution techniques for characterizing subpopulations of cells within pooled populations as a result of the advantages offered by the more linear distribution pattern of transcript expression compared to other existing techniques.

P1181

Investigation of the whole blood transcriptome using RNAseq to find biomarkers associated with resilience in a natural polymicrobial disease challenge model

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Commercial pigs may be exposed to multiple pathogens such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), influenza virus and *Mycoplasma hyopneumoniae*. Pigs that are able to maintain “normal” productive performance during polymicrobial challenges may be disease resilient. In this study, we investigated the whole blood transcriptome to identify biomarkers associated with resilience in a natural disease challenge model. We grouped 58 pigs into 4 groups (Resistant, Resilient, Susceptible and Early-death) according to pre-slaughter weight, number of medical treatments and mortality during the challenge. Resistant pigs did not receive individual treatment while the other groups were treated. The Resilient and Susceptible groups weighed greater than or less than 120 kilograms respectively at 184 days of age and the Resistant animals weighed 110-145kg. Total blood RNA was extracted before pathogen challenge and sequenced for differential expression analysis. Only two significant DETs (differential expressed transcripts) were identified between the Susceptible and the other three groups (q value < 0.1): both DETs are novel transcripts. The first DET contains exon 1 of *Immunoglobulin Heavy Constant Alpha 2-Like*and two additional exons. The second is a novel transcript of *Signaling Lymphocytic Activation Molecule Family Member 9*(*SLAMF9*). An alternative approach (Ingenuity Pathway Analysis) using the top 100 DETs (with lowest p value) identified pathways associated with thyroid hormones, S-Adenosyl methionine (SAM) synthesis, virus entry and signaling in cytotoxic T lymphocyte. These results suggest pig disease susceptibility may be dependent on both the neuroendocrine and immune system.

DOI: 10.1002/eji.201970400

P1182

Multi-omic single cell analysis of T cells reveals comprehensive information on transcriptome, immune repertoire and T cell interactions

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Advancement in single-cell technology has allowed the study of every cell type in the human body at high resolution. For immunology and immunotherapy applications, it enables the integration of simultaneous measurements of cell-surface protein, transcriptome, immune repertoire and antigen specificity of the same single immune cells.
Using the 10x Genomics Single Cell Immune Profiling Solution with Feature Barcoding technology along with oligo-conjugated antibodies and peptide-MHC (pMHC) Dextramer reagents, we performed multi-omic characterization of PBMCs from cytomegalovirus (CMV) seronegative and seropositive patients. Full length, paired TCRα/β sequences with specificity to known CMV antigens were identified in the seropositive donor, but not in the seronegative donor. A large Epstein Barr Virus (EBV) pMHC specific T cell expansion was identified in the CMV seronegative donor, suggesting an active EBV response. Moreover, the combination of transcriptomic and cell surface protein information resulted in an increase in resolution of cell type identification. This workflow allowed the identification of enriched amino acid motifs within the TCR sequences that contained novel and known CDR3 amino acid sequences specific to CMV.
We scaled this technology with 14 oligo-conjugated antibodies and 50 pMHC Dextramer reagents spanning different CMV, EBV, Influenza, HIV, and Cancer antigens and performed a comprehensive characterization of ~200,000 CD8+ T cells from four MHC-matched donors. In addition to identifying novel and known TCR-pMHC specificity, we also observed TCR alloreactivity. These technological advancements provide new biological insights that are critical for progress in the field.

P1772

Longitudinal analysis reveals a dynamic antibody repertoire response to Zika virus infection in convalescent human based on next generation sequencing

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Zika virus (ZIKV) is an emerging mosquito-transmitted flavivirus threatening human health. Here, we analyzed the dynamic B cell response to ZIKV infection in a Chinese donor, Pt1, from early acute phase to late convalescent phase by next-generation sequencing (NGS). To develop prophylactic and therapeutic agents against ZIKV, we also investigated a panel of monoclonal antibodies (mAbs) previously isolated from Pt1 and tested the protective potential in one of the most stringent mouse models of microcephaly. The most potent neutralizing antibody tested, ZK2B10, provided a complete protection in pre-exposure treatment and partial protection in post-exposure therapy against ZIKV infection with markedly reduced tissue pathology. We then characterized the maturation pathways for antibodies (mAbs) from Pt1. Distinct lineage patterns were uncovered for mAbs ZK2B10 and others, which differ in their recognition of envelope glycoprotein (E) and degree of flavivirus cross-reactivity. NGS-derived somatic variants were analyzed for neutralization and in vivo protection.

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DOI: 10.1002/eji.201970400

P1773

Changes in TCR β repertoire and gut *E.coli* and evidence of molecular mimicry in primary biliary cirrhosis

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Molecular mimicry for an extrinsic protein of an infectious agent has long been suggested as a possible initiating event in primary biliary cholangitis (PBC). However, the stimulus responsible for the breakdown of immunological tolerance and the development of autoimmune responses against this short sequence of the pyruvate dehydrogenase E2 complex (PDC-E2) is still unclear. We utilized immune repertoire sequencing for a comprehensive analysis of the T cell receptor β-chain (TCRβ) repertoire of peripheral $CD4^+/CD8^+$ memory T cells in PBC patient and healthy volunteers and found that there were distinctive differences of immune repertoire in PBC patients: (1) The diversity of TCRβ repertoires of PBC patients was relatively low compared to healthy volunteers. (2) The length of TCRβ complementarity-determining region 3s (CDR3) was statistically significant reduction, and the degree of overlap was significantly higher in PBC patients compared with healthy control. Of note, the shorter CDR3s arise independently of positive/negative selection. (3) There was a skewed and restricted TRBV segment usage and an altered insertion patterns during TCRβ rearrangement in PBC patients. (4) The TCR CDR3 amino acid sequences were variable among patients. However, some conserved amino acid sequences (motifs) presented in the CDR3 of the TCR β-chain. Furthermore, we show that *E.coli* is a triggering event in the breakdown of self-tolerance in PBC patients. In conclusion, the TCRβ CDR3 immune repertoire of CD4+/CD8+ memory T cells is abnormal in PBC patients. *E.coli* is a key factor leading to the loss of self-tolerance in PBC patients.

P1774

Methionine enkephalin(MENK) regulates transcriptome of $CD8^+$T cells in tumor-bearing mice

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The goal of this work was to investigate how MENK could affect the transcriptome of $CD8^+$ T cells in spleen of C57BL / 6 mice S180 subcutaneous tumor-burdened model. High-throughput RNA transcriptome sequencing was performed to analyze the effect of MENK on lncRNA and mRNA of $CD8^+$ T cells in spleen. Based on transcriptome splicing results, a total of 845 genes with noncoding potential were obtained after careful screening. Subsequently the cluster analysis and GO and KEGG enrichment analysis were performed on differentially expressed transcripts. After MENK treatment, there were 8 up-regulated genes and 5 down-regulated genes in differentially expressed lncRNA; there were 3 up-regulated genes and 7 down-regulated genes in differentially expressed TUCP; for differentially expressed mRNA, there were 301 up-regulated genes and 240 down-regulated genes. The differentially expressed genes were confirmed by real-time PCR. The differentially expressed mRNAs were bcl-2, Bax, Rap1b, Pak2, Rasa3, Bad, Pten, Rasgrp2, MOR, DOR, in which the up-regulated mRNAs were bcl-2, Pak2, MOR, DOR, while the down-regulated mRNAs were Bax, Rap1b, Rasa3, Bad, Pten, Rasgrp2; the differentially expressed lncRNAs were GM27008 and Pvt1 and both of them were verified for up-regulation. Our currently data indicated that MENK treatment could increase the proportion of $CD8^+$T cells in spleen and lymph nodes in tumor-bearing mice. MENK treatment could significantly affect the expressions of transcriptome mRNA and lncRNA of $CD8^+$ T cells in the spleen of tumor-bearing mice.
Keywords: Methionine enkephalin; $CD8^+$ T cells; Immune regulation; Opioid receptor ; RNA-sequence

DOI: 10.1002/eji.201970400

P1775

Tumor-infiltrating immune cells prognostic model and immune feature gene analysis in human breast cancer based on gene expression profiles

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Aim: This study integrated public breast cancer transcriptome datasets and established a prognostic model based on the immune cells landscape.
Methods: We screened the GEO for independent breast cancer transcriptome datasets using microarray. Relative abundance of TILs subpopulations was estimated by applying CIBERSORT. Prognostic model based on immune cells were established with GEO datasets using the least absolute shrinkage and selection operator (LASSO) Cox regression model and validated at the TCGA-BRCA and METABRIC cohorts.
Results: Among the 22 immune cell subsets estimated by CIBERSORT, 7 cell subsets were selected for breast cancer immunotyping, and classified breast cancer into immunotype A and Immuntype B. Immuntype A was characterized as B_cell ^{high} NK ^{high} CD8^{+}_T ^{high} CD4^{+}_memory_T_activated ^{high} γδT ^{low} Mast_cell_activated ^{low} Neutrophils ^{low} and immunotype B was B_cell ^{low} NK ^{low} CD8^{+}_T ^{low} CD4^{+}_memory_T_activated ^{low} γδT ^{high} Mast_cell_activated ^{high} Neutrophils ^{high}. The 5-year OS (85.7% vs. 73.4%, p = 0.0003 ***) and 10-year OS (75.60% vs. 61.73%, p = 0.0006 ***) of immunotype A breast cancer were significantly higher than immunotype B, as well as the recurrence free survival (RFS), disease-free survival (DFS), and distant metastasis free survival (DMFS). Multivariate survival analysis suggested that breast cancer immunotype was a new prognostic factor independent of pathological staging and PAM50 molecular typing.
Conclusion: Breast cancer immunotypes established based on the immune cell fractions indicated to be a new predictor of clinical prognosis.
Keywords: breast cancer; tumor infiltrating immune cells; microarray; bioinformatics

P1776

Integrating SWIFT tools to enhance flow data analysis, exploration and visualization in ImmPort

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High-dimensional flow cytometry datasets are difficult to analyze manually. In response, several automated algorithms have been developed for primary analysis, including our high-resolution SWIFT clustering algorithm. However, even the processed data can be highly complex, e.g. hundreds of sub-populations in thousands of samples. We have developed analysis and visualization tools to assist biologists to rapidly examine and compare data with objective methods, and also to explore these complex data, using their domain knowledge and intuition to develop new models.
The interface for the user-friendly tools consists of a simple GUI to select the tools to be applied, plus an Excel spreadsheet containing all the choices for filtering data and specifying analysis parameters. MATLAB is used for backend processing. Current tools include cluster-wise comparison of groups in volcano plots; versatile plotting of cluster data; Quality Control plots for granular analysis; t-SNE plots assessing either sample or cluster similarities; heatmaps comparing samples. Within each tool are many output choices, including sample filtering, cluster filtering, parameters to be analyzed, analysis methods, normalization, plot styles.
The Registration tool is particularly useful for minimizing the inevitable variability of samples analyzed

DOI: 10.1002/eji.201970400

in multiple batches. This tool uses the high-resolution SWIFT cluster template to align sub-populations between batches. Notably, this tool minimizes batch variation, while preserving biological variation, thus improving the ability to discern differences between experimental groups.
To facilitate use of the tools by online users on various datasets, the tools are currently being incorporated in the Galaxy interface of the large ImmPort database.

P2366

Quantitative screening of hepatocellular carcinoma biomarkers in serum by reverse phase protein arrays

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Screening non-invasive serum for disease biomarkers to improve patient care has always been of great interest. The novel antibody-antigen reaction based technology reverse phase protein array (RPPA) can measure sets of proteins from thousands of patients´ samples in parallel, and has been widely used for the detection of signaling molecules. Here, we make the assay more robust and accurate in application of serum sample detection by simplifying the experimental procedure and optimizing multiple parameters of RPPAs, including the support matrix, negative control, data analysis, and antibody validation. We then measured the expression levels of 10 cancer-related proteins quantitatively in serum from 132 hepatocellular carcinoma (HCC) patients and 78 healthy volunteers. Six of these proteins were significantly increased in HCC patients; these proteins included Alpha-Fetoprotein, Beta 2 Microglobulin, Golgi Protein 73, Growth Differentiation Factor 15, Insulin-Like Growth Factor Binding Protein 2, and Osteopontin. ROC curve analysis of individual proteins to distinguish HCC from healthy controls revealed diagnostic accuracies ranging from 0.617 to 0.908. The accuracy increased to 0.951 and 0.955 with linear discriminant analysis of the training and validating data sets, respectively, when all six proteins were combined as a specific HCC signature. Our work highlights the potential of RPPAs in biomarker discovery using serum samples.

P2367

MPPA-ML: a machine-learning program for multi-feature prediction of protective antigens

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Reverse vaccinology wherein vaccine antigens are directly predicted and screened from their genomes has proved to be highly effective in rational vaccine designs for pathogens since its first application for Group B meningococcus vaccine in the early 1990s. Recently, several scoring and machine learning-based methods have been developed for predicting protective antigens. However, these methods used vast features and particular algorithms for prediction, which may result in limited accessibility to non-expert users. Herein, we proposed a publicly accessible machine-learning program for the multi-feature prediction of protective antigens called MPPA-ML. It was designed in Python using an ensemble strategy for protective antigen prediction that extracts several pivotal biological features automatically, including subcellular localization, antigen similarity, antigenicity, epitope,

DOI: 10.1002/eji.201970400

virulence, adhesion and physicochemical property. The ensemble strategy integrates multiple representative machine learning models, such as Support Vector Machine, Neural Network, Bayesian Classification and Decision Tree, which overcome the shortcomings of a structuring classifier using a single algorithm. The prediction accuracy of this program is 0.91 in 10-fold cross-validation testing, which is superior to the reported accuracies of other programs. In addition, we applied this program on *Brucella spp.*, which causes zoonotic Brucellosis in humans and various animals; consequently, multiple protective antigens were predicted and showed protective abilities against *B. abortus* infections in BALB/c mouse models. This study provides a convenient and accurate machine-learning software program, MPPA-ML, for automatic protective antigen prediction that can provide potential targets for bacterial vaccine designs and help shorten the vaccine development process.

P2368

Immune composition dominates over tumor cell phenotype to stratify patients with DLBCL

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Diffuse large B-cell lymphoma (DLBCL) is notorious for its clinical and biological heterogeneity. The current research uses single-cell mass cytometry consisting of two 40-target panels, and a multiplex array of 13 T cell-related cytokines and cytolytic molecules, to perform deep proteomic profiling of tumor cells and immune cells simultaneously in 54 cases of DLBCL and 31 reactive lymph node (rLN) controls.
Unsupervised clustering of malignant populations in DLBCL samples revealed striking heterogeneity in tumor cell expression profile, with each individual tumor population occupying a unique region in phenotypic space, independent of cell-of-origin classification.
Phenograph clustering of non-malignant cells revealed 37 unique populations, including 11x $CD8^+$ populations, 20x $CD4^+$ populations, and a number of rare populations like NK and NKT cells. Clustering of samples by decomposition into these populations revealed two groups of DLBCL samples. One group of samples closely resembled rLN controls, with high abundances of naive $CD4^+$ and $CD8^+$ T cells. The other group of samples displayed a pattern of highly activated immune responses, with very few naive cells and correspondingly high abundance of T_{FH} and T_{REG} $CD4^+$ cells, and polyfunctional and exhausted $CD8^+$ cells, which typically predicts a superior clinical outcome. These findings demonstrate that tumor cell phenotype in DLBCL displays overwhelming heterogeneity between patients, while detailed analysis of the immune microenvironment may offer a more robust manner through which outcome can be predicted, and by which patients may be identified as candidates for a range of different immunotherapies like checkpoint inhibition or bi-specific T cell engagers (BiTEs).

P2369

EIF2AK2 selectively regulates the transcription of genes in immune response

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PKR, also known as EIF2AK2, is an IFN-stimulated gene (ISG) with multiple effects on cells. However, the mechanistic link between EIF2AK2 and immune response is poorly understood. Here we reported that EIF2AK2 selectively regulates the transcription of genes related to immune response in HeLa cells. We overexpressed EIF2AK2 to study EIF2AK2-regulated genes in HeLa cells using RNA-seq technology and analyzed targets of transcriptional factors (TFs) regulated by EIF2AK2.

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DOI: 10.1002/eji.201970400

Overexpression of EIF2AK2 promotes apoptosis and inhibits cell proliferation in HeLa cells. Strikingly, EIF2AK2 selectively repressed the transcription of a large set of histone protein genes in SLE pathway according to the KEGG analysis. Furthermore, genes whose transcription were upregulated by EIF2AK2 were enriched in Influenza A KEGG pathway, and in the GO functional terms including response to virus, signal transduction, and T cell receptor signaling pathway. We further used motif analysis approach to show that EIF2AK2 regulated the expression of 9 transcription factors whose binding motifs were enriched in the promoters *EIF2AK2*-regulated genes. **Our results suggest that the dsRNA-dependent kinase EIF2AK2 selectively regulates the transcription of immune response, and such a specific regulatory network is likely to be operated by EIF2AK2-targeted transcription factors.**

P2370

Immunoinformatics approach to design the best probable B and T cell epitopes-based vaccine against adhesin PSTS1 of Mycobacterium tuberculosis

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Tuberculosis is a contagious disease caused by the Koch bacillus (*Mycobacterium tuberculosis*: M.tb) that usually attacks the lungs. This disease has been on the rise for some fifteen years. BCG (Bacille Calmette-Guérin) is currently the only vaccine used to protect against tuberculosis but it does not protect reliably adult and immunodeficiency patients. So, the development of a safe vaccine that is more effective than BCG in eradicating tuberculosis in adults is a major goal of the public health. In view that infection caused by M.tb has proved recently to stimulate strongly the humoral immunity against adhesin. Therefore, in the first step of our work we use the immunoinformatic tools to predict the best B cells epioptes from PstS1 adhesin of Mtb. Further, we designed the best T cells epitopes from pstS1. Four best potential linear B cell epitopes (“PSGSPET”, “NNRQKDA”, “SDGSGDT” and GENGNGG) were found to fulfill all the criteria for becoming an ideal and safe B cell epitope. However, an epitope “PPSGSPE” seems to present a risk of autoimmunity and must be avoid from the load of all TB vaccine. The retrospective validation of the four best B epitopes was evaluated using in silico techniques. Sixty one T cell epitopes were also predicted to have the highest probability to be the best T cell epitopes and which contain promiscuous epitopes. Our computational study allows us to predict and design the best probable B and T candidates epitopes-based vaccines from PstS1 adhesin.
Keywords: Tuberculosis, *Mycobacterium tuberculosis*, PstS1, immunoinformatic, Vaccine design

P2371

The adaptive immune-receptor repertoire (AIRR) community of the Antibody Society

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The first high-throughput DNA sequencing of B- and T-cell receptor repertoires (adaptive immune-receptor repertoires (**AIRRs**)) was accomplished in 2009. This AIRR sequencing (**AIRR-seq**) technology promised the characterization of AIRRs at depths that were previously impossible; however, it soon became obvious that different labs were using different standards for annotating and storing their AIRR-sea data, making it difficult for one to analyze AIRR-sea data from multiple labs.

DOI: 10.1002/eji.201970400

Thus, in the fall of 2014, a planning meeting was held in Vancouver, BC, Canada, to address this problem. From it came an international meeting the following spring, where the attendees decided to form the "AIRR Community", based on the principle that the value of AIRR-seq data is directly related to its ability to be shared. This poster describes the AIRR Community its 7 Working Groups and 3 Subcommittees, as well as its progress, including 5 publications recommending standards for annotating, representing, storing, sharing and analyzing AIRR-seq data. Anyone who is interested in AIRR-seq data is welcome to join this grassroots Community of volunteers by joining The Antibody Society (https://www.antibodysociety.org) and participate in any of its Working Groups.

P3282

HLA-DRB1/DQB1 alleles in Chinese patients with early onset solid cancer

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Aim: To explore relationships between the early-onset solid cancer and HLA-DRB1/DQB1 allelic polymorphism and mechanisms involved in the pathogenesis of early-onset solid cancer.
Methods: Patients, meanly with gastric cancer, liver cancer or lung cancer, were included in this study. Cancer patients with less than 55 years old were recruited as an early-onset cancer group and whose age more than 65 years old were recruited as a non-early-onset cancer group. The control population was nonagenarians from the same geographical area. The samples were typed using an HLA-DR/DQ "low resolution" PCR-SSP typing kit including 13 allele-specificity for HLA-DRB1 locus and 5 allele-specificity for HLA-DQB1 locus.
Results: Frequency of DRB1*03 was 13.6% in early-onset cancer group as well as 3.8% in control population and significant difference was obtained (P< 0.05). Frequency of DRB1*13 was recorded in 11.8% of non-early-onset cancer group and 3.2% of control population and significant difference was also obtained (P< 0.05). Significant differences were not found in other HLA-DQB1/DQB1 locus.
Conclusions: HLA-DRB1 alleles may be involved in the pathogenesis of early-onset solid cancer. The anti-cancer mechanisms with the instinct may be a common pathway for different solid cancer and associated with immune system and HLA allelic polymorphism.

P3283

A novel cross-validation design for identification of disease-related hla alleles, illustrated by association analysis for *HLA-DRB1* and solid cancers

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Aim: A new study design based on cross-validation of the age at the onset of cancer and the differences between the frequency distributions of relevant genes in 2 groups was developed for identification of disease-related HLA.
Methods: Patients with different solid cancers were recruited. On the basis of their age at the time of the first surgery, 70 patients were divided into the following 7 age groups (< 50 years, 50-54 years, 55-59 years, 60-64 years, 65-69 years, 70-74 years, and 75-79 years). The samples were typed using an HLA-DRB1 PCR-SSP typing kit. Allele frequencies were compared with control population. The mean age of patients with and without the alleles was compared.
Results: The frequency values of *HLA-DRB1*01* and *HLA-DRB1*03* were higher in the cancer group than in the control population (p< 0.05). The frequency of *HLA-DRB1*09* was lower in the cancer group than in the control population (p< 0.05). The median ages of the subjects with and without *HLA-A*01* were 51.5 years and 61.0 years, respectively, and a significant difference was observed between

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DOI: 10.1002/eji.201970400

the ages for these groups ($p < 0.05$). The cross-validation of the 2 abovementioned analytical results showed that a statistically significant difference was noted for *HLA-DRB1*01* ($p < 0.05$), whereas no such statistically significant difference was observed for *HLA-DRB1*03* and *HLA-DRB1*09*.
CONCLUSIONS: HLA-DRB1*01 allele was related to cancer susceptibility. The new analysis method may be an efficient and reliable approach for the identification of disease-related HLA.

P3284

Immune infiltration in renal cell carcinoma

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Immune infiltration of tumors is closely associated with clinical outcome in renal cell carcinoma (RCC). Tumor-infiltrating immune cells (TIICs) regulate cancer progression and are appealing therapeutic targets. The purpose of this study was to determine the composition of TIICs in RCC and further investigate the independent prognostic values of TIICs. CIBERSORT, an established algorithm, was applied to estimate the proportions of 22 immune cell types based on gene expression profiles of 891 tumors. Cox regression was used to evaluate the association of TIICs and immune checkpoint modulators with overall survival (OS). We found that CD8+ T cells were associated with prolonged OS (hazard ratio [HR] = 0.09, 95% confidence interval [CI] 0.01-0.53; p = 0.03) in chromophobe carcinoma (KICH). A higher proportion of regulatory T cells was associated with a worse outcome (HR = 1.59, 95% CI 1.23-0.06; p < 0.01) in renal clear cell carcinoma (KIRC). Moreover, the immunomodulator molecules CTLA4 and LAG3 were associated with a poor prognosis in KIRC, and IDO1 and PD-L2 were associated with a poor prognosis in renal papillary cell carcinoma (KIRP). This study indicates TIICs are important determinants of prognosis in RCC meanwhile reveals potential targets and biomarkers for immunotherapy development.

P3285

Investigation of candidate long noncoding RNAs and messenger RNAs in microglial cells after spinal cord injury based on gene expression profiles

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Spinal cord injury (SCI) is a serious devastating condition and it has a high mortality rate and morbidity rate. Inflammation after spinal cord injury plays a key role in the pathological development of spinal cord injury. Microglia cells are the executors of inflammation in spinal cord tissue. However, the roles of lncRNAs and messenger RNAs (mRNAs) in microglial cells after spinal cord injury are not clear. We examined the expression of mRNAs and lncRNAs in a rat model's microglial cells after SCI and identified the differentially expressed lncRNAs (DE lncRNAs) and differentially expressed mRNAs (DE mRNAs) using microarray analysis. 810 DE lncRNAs and 1054 DE mRNAs were identified in spinal cord samples in the microglial cells following SCI compared with control samples. Moreover, Gene Ontology (GO) term annotation results showed that cellular response to interleukin-1 (IL-1), monocyte chemotaxis, macrophage differentiation, CXCR chemokine receptor binding, neutrophil apoptotic process and neutrophil migration were the main significantly enriched GO terms. The results of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that the DEGs were enriched in toll-like receptor signaling pathway, p53 signaling pathway, MAPK signaling pathway and Jak-STAT signaling pathway. IL6, MBOAT4, FOS, TNF, JUN, STAT3, CSF2, MYC, CCL2 and FGF2

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DOI: 10.1002/eji.201970400

were the top 10 high-degree hub nodes and may be important targets of SCI Inflammatory regulation. The current study provides novel insights into how lncRNAs and mRNAs regulate the inflammatory pathogenesis of SCI.

P3286

Identification of the co-expressed genes of Myh-11 related to spermatogenesis by bioinformatics analysis

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Myosin has been involved in spermatogenesis and plays an important role in acrosome formation, nuclear morphogenesis, mitochondrial translocation and sperm individualization. Myosin heavy chain MYH-11, a marker of the smooth mucle cells, affects the sperm tube wall contraction and sperm movement process, leading to the male infertility and other related diseases. Therefore, it is important to identify the co-expressed genes of MYH-11 to explore the MYH-11 function and the molecular mechanism underlying male infertility. In this study, bioinformatics analysis of Myh-11 (mus musculus) co-expression network was performed. The predicted Myh-11 co-expression genes were identified and classified by GO analysis, KEGG pathway and litterature study. As a result, Actg2 is considered as a major co-expressed genes with Myh-11, and may play an important role in male sterility. Further study needs to be done to confirm it.

P3287

Molecular evolutionary patterns of B7 family and their receptors

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The B7 family consists of several positive and negative costimulatory molecules, which played different roles during T cell activation. Recently, B7 family has expanded to ten members, and most of the receptors have already been identified, there are still some receptors for B7-H3, B7-H4 and B7-H5 need to be identified. In this work, we searched all known B7 molecules in various species from fishes to mammals in public database and systematically analyzed the evolution of those molecules, found that B7-H3/H5/H6/H7 molecules first appeared in elasmobranches, which was earlier than other molecules. We also mapped the evolution of all the known B7 receptors (CD28, CTLA4, PD1, ICOS, NKp30 and CD28H), and found that ligands and receptors co-evolved during species evolution. For each molecule, the amino acid sites involved in ligand-receptor contact were more conserved than other sites, showing the conserved interaction mode of B7 family and their receptors among different species during evolution. These findings could threw a new light to understand the evolution of B7 family, and may help us to identify the unknown receptors, such as B7H3, B7H4 and B7H5.

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DOI: 10.1002/eji.201970400

P3288

Mapping the spatial distribution of T cells in repertoire dimension

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T cells play a vital role in the processes of microorganism infection, tumor eradication and autoimmunity. The T-cell receptor (TCR), located on the surface of T cells is responsible for the recognition of the antigen-major histocompatibility complex. Currently, TCR deep sequencing allows for global analysis of the TCR repertoires and provides significant insights into the function of T cell in immune surveillance and immune tolerance. However, little work has been reported on systematic distribution of T-cell clonotypes in different lymphoid tissues and non-lymphoid tissues in repertoire dimension. In this study, we analyzed the CDR3 sequences of TCR beta chain to map the organization of T cells in the different lymphoid tissues and non-lymphoid tissues. According to similarity index between the tissues, we found that T cells were classified into three compartments: skin, gastrointestinal tract, lymph nodes and blood vessel associated organs. Interesting, the TCR repertoire of gastrointestinal tract exhibited the highest similarity among other tissues within a mouse, but the lowest similarity in different mice. Furthermore, public T-cell clonotypes were found more easily in lymph nodes in individual mice.These data suggests a reshape of T cells distribution by various environmental and/or self-antigens in each mouse, and implicates that individual genetic factors had little impact on the overlap of TCR repertoires.

P3289

Identification of core genes and potential molecular mechanisms in breast cancer using bioinformatics analysis

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Background: Breast cancer is the most frequently diagnosed cancer in women worldwide. This study aimed to elucidate the potential key candidate genes and pathways in breast cancer.
Methods: The gene expression profile dataset GSE65212 was downloaded from GEO database. Differentially expressed genes (DEGs) were obtained by the R Bioconductor packages. The Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs were performed using DAVID database. The protein-protein interaction (PPI) network was then established by STRING and visualized by Cytoscape software. Module analysis of the PPI network was performed by the plug-in Molecular Complex Detection (MCODE). Then, the identified genes were verified by Kaplan-Meier plotter online database and quantitative real-time PCR (qPCR) in breast cancer tissue samples.
Results: A total of 857 differential expressed genes were identified, of which, the upregulated genes were mainly enriched in the cell cycle, while the downregulated genes were mainly enriched in PPAR signaling pathway. Moreover, six hub genes with high degree were identified, including TOP2A, PCNA, CCNB1, CDC20, BIRC5 and CCNA2. Lastly, the Kaplan-Meier plotter online database confirmed that higher expression levels of these hub genes were related to lower overall survival. Experimental validation showed that all six hub genes had the same expression trend as predicted.
Conclusion: These results identified key genes, which could be used as a new biomarker for breast cancer diagnosis and treatment.

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DOI: 10.1002/eji.201970400

P3290

Identification of potential critical genes in lung squamous cell carcinoma by bioinformatic analysis

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Objective: To identify potential biomarkers by analyzing differentially expressed genes between lung squamous cell carcinoma(SCC) and para-carcinoma.
Methods: SCC microarray was obtained from Gene Expression Omnibus (GEO).The original data was preprocessed,differentially expressed genes(DEGs)were identified based on limma package of R software. GO and KEGG analysis were performed by clusterProfiler of R.DEGs-associated protein-protein interaction (PPI) network was analyzed by STRING online software, and Cytoscape was used to filter the critical genes. The expression of hub genes were validated based on GEPIA.
Results: 734 DEGs including 290 up-regulated genes and 444 down-regulated genes were identified. **GO analysis showed DEGs were mainly enriched in extracellular structure organization, regulation of body fluid levels and other biological processes. KEGG analysis showed DEGs were enriched in complement and coagulation cascades, cell cycle and DNA replication. Hub genes filtered from PPI network including PCNA, FOS, PTPRC, MMP9, CDK1, CCL2, IL6, GMPS, CCNB1, TOP2A.PCNA, MMP9, CDK1, GMPS, CCNB1 and TOP2A were proved to be increased in lung squamous cell carcinoma. FOS, PTPRC, CCL2 and IL6 were decreased in cancer.**
Conclusion: The DEGs and signal pathways obtained from bioinformatic analysis may contribute to the molecular mechanism of lung squamous cell carcinoma,the critical genes could be diagnostic markers and therapeutic targets for SCC.
Keywords: SCC; microarray;differentially expressed genes;bioinformatics

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DOI: 10.1002/eji.201970400

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DOI: 10.1002/eji.201970400

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DOI: 10.1002/eji.201970400

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DOI: 10.1002/eji.201970400

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