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REVIEW

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The emerging role of epigenetics in the immune response to vaccination and infection: a systematic review

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ABSTRACT
Extensive research has highlighted the role of infection-induced epigenetic events in the development of cancer. More recently, attention has focused on the ability of non-carcinogenic infections, as well as vaccines, to modify the human epigenome and modulate the immune response. This review explores this rapidly evolving area of investigation and outlines the many and varied ways in which vaccination and natural infection can influence the human epigenome from modulation of the innate and adaptive immune response, to biological ageing and modification of disease risk. The implications of these epigenetic changes on immune regulation and their potential application to the diagnosis and treatment of chronic infection and vaccine development are also discussed.

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KEYWORDS
Vaccination; infectious diseases; immune regulation

Introduction

Recognition of the factors that influence the human epigenome has expanded in parallel with understanding of the molecular mechanisms of epigenetic regulation. Many intrinsic and extrinsic influences can mark the epigenome, including age, sex, smoking and diet [1–3]. Maternal factors, including stress and famine, have persistent effects on the epigenome of subsequent generations supporting the notion that epigenetic modifications are heritable [3].

There is growing evidence that vaccination and natural infection alter the epigenome, modulating both the initial immune response and longitudinal disease risk. (Figure 1) Much of the current understanding of the impact of infection on the human epigenome comes from studies of carcinogenic viruses and bacteria, including human papilloma virus (HPV), Epstein Barr virus (EBV), hepatitis C virus (HCV), and *Helicobacter pylori* [4]. Induction of epigenetic modifications, particularly aberrant DNA methylation, is considered the primary mechanism by which viral and bacterial infections lead to cancer development.

Despite significant research into the role of infection-induced epigenetic changes in the development of cancer, less is known about the non-carcinogenic effects of infections on the human epigenome. Even less is known about the impact of vaccinations on the epigenetic landscape. One area in which epigenetics may play a role is in the increasingly recognized heterologous ('non-specific') effects of vaccines. Heterologous effects are additional effects exerted by the vaccine beyond the specific protection afforded against the targeted disease [5]. The immunological mechanisms underlying these heterologous effects are only beginning to be understood but there is emerging evidence that epigenetic mechanisms are involved.

This systematic review focuses on vaccine and infection-induced epigenetic modifications that do not directly influence the development of cancer.

Methods

Search strategy and selection criteria

Original studies investigating the non-carcinogenic effects of (i) vaccinations and (ii) bacterial, viral, fungal and parasitic pathogens or their constituents on the human epigenome were identified using two independent search strategies.

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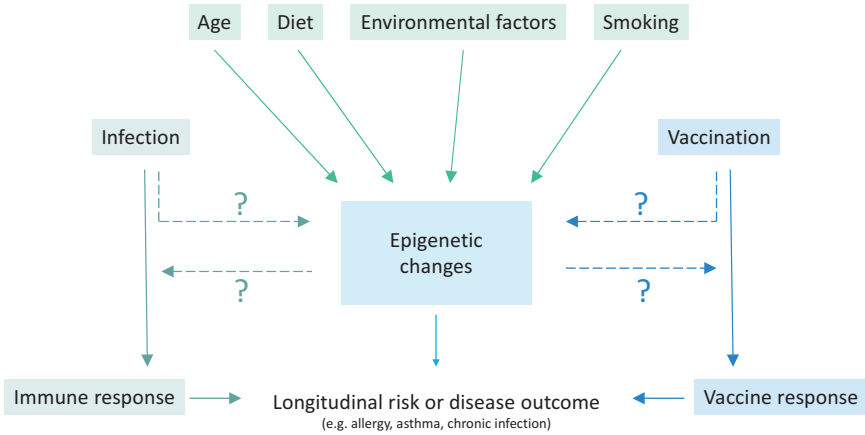

Figure 1. Influences on the human epigenome and role in disease.

In August 2019, MEDLINE (1946 to present) and Embase (1947 to present) were searched using the Ovid interface. Additional publications were identified through hand-searching of reference lists of relevant retrieved articles. Results were limited to English language and studies in humans. The search thesaurus terms and keywords together with the histories are listed in Figure 2(a,b). Studies were excluded if: the experiments were done in animals or plants; the study pertained directly to carcinogenesis; the epigenetic modification occurred in the genome of the infecting pathogen; the study assessed only modifying enzymes such as DNA methyltransferases (DNMT) or histone deacetylases (HDAC); or the identified epigenetic change preceded infection.

Systematic review results

The first search, identifying studies of vaccine-induced epigenetic changes, identified 259 studies, of which 28 full-text articles were assessed for relevance and 11 were included in the review (Figure 2(a)). One additional reference was identified during the second search resulting in a total of 12 studies. These investigated five different vaccines: Bacillus Calmette-Guérin (BCG) vaccine, yellow fever virus (YFV) vaccine, influenza A virus (IAV) vaccine, hepatitis B virus (HBV) vaccine, tetanus toxoid vaccine and modified *Vaccinia* Ankara 85A vaccine (MVA 85A). (Table 1) These studies all investigated the epigenetic effects of *in vivo* vaccination of human subjects, except two studies that exposed human monocytes to BCG vaccine or MVA 85A vaccine *in vitro*. Three studies investigating the epigenetic effects of BCG vaccine used chromatin immunoprecipitation (ChIP) to detect histone modifications in monocytes and the fourth used the Illumina Infinium HumanMethylation450 BeadChip array to measure genome-wide methylation in CD4 + T cells. The study of MVA 85A also used ChIP to assess histone modifications in monocytes. The remaining seven studies investigated DNA methylation in whole blood, peripheral blood mononuclear cells (PBMC) or CD8 + T cells using either the Illumina Infinium HumanMethylation450 BeadChip array to measure genome-wide methylation or bisulphite sequencing to detect site-specific CpG methylation in regulatory regions. One study examining the epigenetic effects of YFV vaccine in CD8 + T cells also used Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) to assess chromatin accessibility across the genome. (Table 2)

The second search, identifying studies of pathogen-induced epigenetic changes, identified 943 articles, of which 106 full-text articles were assessed and 73 were included in the review (Figure 2(b)). Five additional relevant articles were identified through hand searching of reference lists and one article was retracted, resulting in a total of 77 studies.

The articles were grouped according to whether they investigated epigenetic effects in human subjects (38 studies) or in human-derived cells or cell lines (41 studies), with some articles investigating both. Of the studies done in human subjects, eight focused on bacteria with six assessing pathogenic bacteria, including *Mycobacterium tuberculosis*

(a)

Search strategy 1: MEDLINE

1	Epigenomics/
2	Epigenesis, Genetic/
3	DNA methylation/
4	vaccines/ or exp bacterial vaccines/ or fungal vaccines. Or exp protozoan vaccines/ or exp toxoids/ or vaccines, attenuated/ or exp vaccines, combines/ or vaccines, contraceptive/ or exp vaccines, inactivated/ or vaccines, marker/ or exp vaccines, subunit/ or exp vaccines, synthetic, live, unattenuated/ or exp viral vaccines/
5	exp Immunization/
6	(1 or 2 or 3) and (4 or 5)
7	exp animal/ not humans*.sh.
8	6 not 7
9	exp neoplasms/
10	8 not 9
11	limit 10 to english language

Search strategy 1: Embase

1	epigenetics/
2	genetic epigenesis/
3	DNA methylation/
4	exp vaccination/
5	exp immunization/
6	(1 or 2 or 3) and (4 or 5)
7	exp animals/ not humans*.sh.
8	6 not 7
9	exp neoplasms/ or plant*1.hw
10	8 not 9
11	limit 10 to english language

Search strategy 1 flow chart

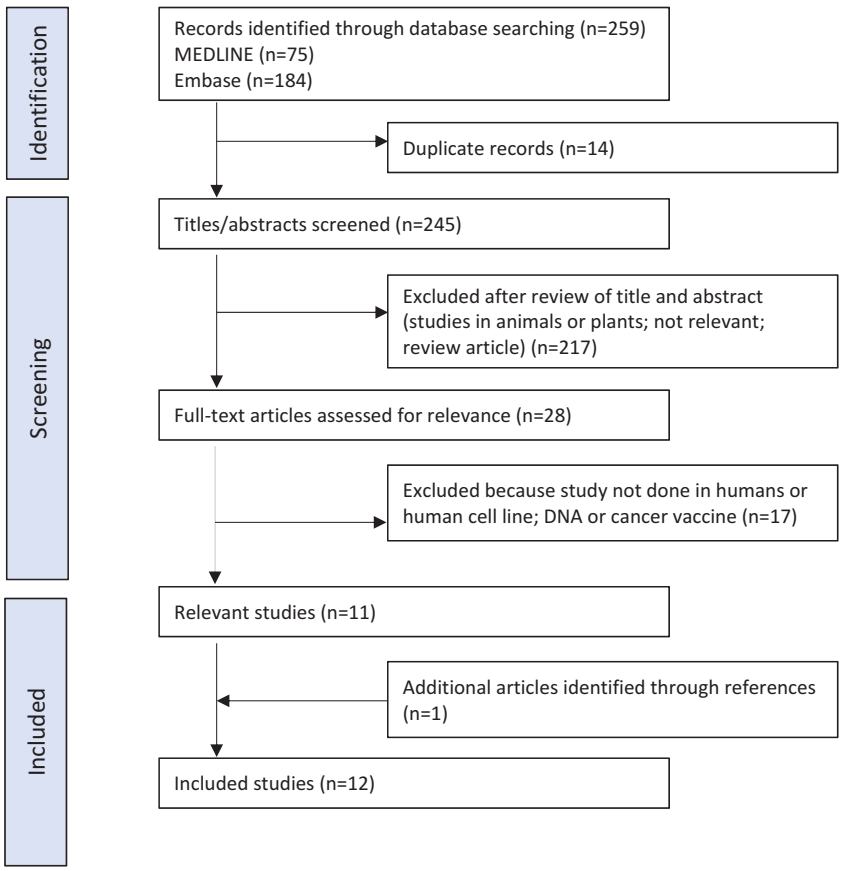

Figure 2. (a) Search strategy 1. (b) Search strategy 2.

(b)

Search strategy 2: MEDLINE

1	Epigenomics/
2	Epigenesis, Genetic/
3	DNA methylation/
4	(infection* or bacteria or parasite* or virus).tw,kw,hw
5	(im or ph or mi).fs.
6	(*Epigenomics/ or *Epigenesis, Genetic/ or *DNA Methylation/) and 4 and 5
7	exp animal/ not humans*.sh.
8	6 not 7
9	exp neoplasms/ or plant*1.hw.
10	8 not 9
11	limit 10 to english language

Search strategy 2: Embase

1	epigenetics/
2	genetic epigenesis/
3	DNA methylation/
4	1 or 2 or 3
5	(infection* or bacteria or parasite* or virus).tw,kw,hw
6	4 and 5
7	exp animals/ not humans*.sh.
8	6 not 7
9	exp neoplasms/ or plant*1.hw
10	8 not 9
11	exp immune response/
12	10 and 11
13	limit 12 to english language

Search strategy 2 flow chart

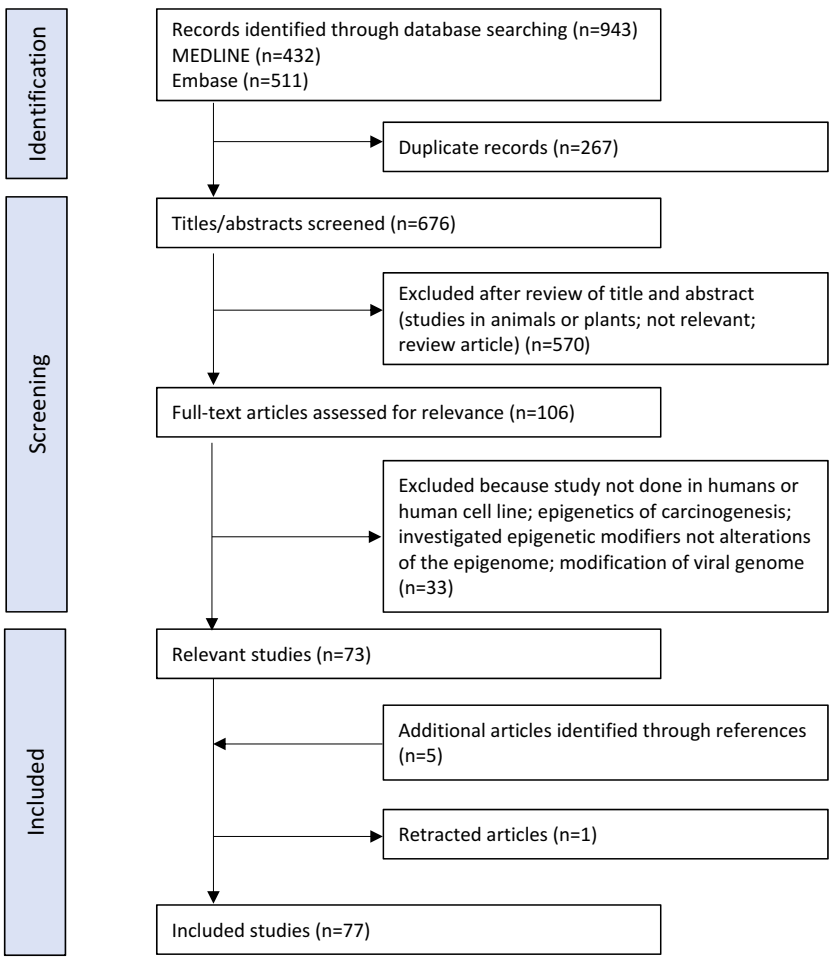

Figure 2. (Continued).

Table 1. Host epigenetic modifications of infectious pathogens and vaccines.

Infectious pathogens (n = 77)		Vaccines (n = 12)	
Studies in human cells or cell lines (n = 41)	Studies in humans (n = 38)	Studies in human cells (n = 2)	Studies in humans (n = 10)
Bacteria			
• *Mycobacterium tuberculosis* [6,83,86,97,98] • *Burkholderia pseudomallei* [7] • *Escherichia coli* [104] • *Anaplasma phagocytophilum* [82] • *Listeria monocytogenes* [8,9,85] • *Chlamydia trachomatis* [90] • *Porphyromonas gingivalis* [87,89] • *Fusobacterium nucleatum* [89] • *Bifidobacterium breve* [10] • *Lactobacillus rhamnosus GG* [10] • *Acinetobacter baumanni* [11] • *Pseudomonas aeruginosa* [11]	• *Mycobacterium tuberculosis* [12,96–98] • *Helicobacter pylori* [13,14] • Gut microbiota (*Bacteroides, Proteobacteria, Firmicutes*) [111] • Placental microbiota [101]	• Bacillus Calmette-Guérin [43] • *Vaccinia* Ankara MVA85A [47]	• Bacillus Calmette-Guérin [15,44,45]
Viruses			
• Human immunodeficiency virus-1 [16–20,70,78,79] • Influenza A virus [21–25] • Epstein Barr virus [26] • Human rhinovirus [27,113] • Respiratory syncytial virus [11] • Human herpesvirus-6B [80] • Dengue virus [28]	• Human immunodeficiency virus-1 [29,72,75,94,95,107,108] • Human T-cell lymphotropic virus-1 [71] • Cytomegalovirus [63–65,76,109] • Epstein Barr virus [76] • Hepatitis B virus [30,66,81,92,99] • Hepatitis C virus [66,110] • Respiratory syncytial virus [31] • Dengue virus [91]		• Influenza A virus [32,53,54] • Hepatitis B virus [55] • Yellow fever virus [76,77]
Parasites			
• *Leishmania donovani* [84]	• *Leishmania braziliensis* [33] • *Schistosoma haematobium* [93] • *Ascaris lumbricoides* [93] • *Plasmodium falciparum* [34,62] • *Trypanosoma cruzi* [35]		
Pathogen constituents	**Clinical infections**		**Toxins**
• β-glucan (*Candida albicans*) [36,59,61] • Lipopolysaccharide (*Porphyromonas gingivalis, Escherichia coli, Fusobacterium nucleatum*) [37] • BaSET (*Bacillus anthracis*) [38] • 2-aminoacetophenone (*Pseudomonas aeruginosa*) [68]	• Neonatal sepsis [102,103] • Periodontitis (*Porphyromonas gingivalis*) [87,88] • Chorioamnionitis [100]		• Tetanus [58]

some studies examined more than one pathogen or conducted experiments both in humans and human cells or cell lines.

and *Helicobacter pylori*, and two assessing commensal bacteria in the gut and placental microbiota. There were 21 studies involving viral pathogens, including human immunodeficiency virus-1 (HIV), human T-cell lymphotropic virus-1 (HTLV), cytomegalovirus (CMV), EBV, hepatitis B virus (HBV), HCV, respiratory syncytial virus (RSV) and dengue virus. Five studies looked at parasites including *Leishmania braziliensis, Schistosoma haematobium, Ascaris lumbricoides, Plasmodium falciparum* and *Trypanosoma cruzi*. The remaining five articles considered syndromes of clinical infection, including neonatal sepsis, periodontitis and chorioamnionitis. (Table 1)

Of the 41 studies done in human cells or cell lines, 16 assessed bacteria with 12 investigating pathogenic bacteria, including *M. tuberculosis, Burkholderia pseudomallei, Escherichia coli, Anaplasma phagocytophilum, Listeria monocytogenes* and *Chlamydia trachomatis*, and four

Table 2. Analysis technique by cell type.

	CPG methylation															
	Whole genome										*Site-specific*					
	Illumina BeadChip array*	MethylC-seq	ELISA-based methods**	DNA digestion and gel	Pyrosequencing of genome wide Alu elements	Methylated DNA sequencing	EpiTect Methyl II PCR	MeDIP ± CpG island array	RRBS global methylation analysis	RP-HPLC	Bisulphite sequencing	Bisulphite pyrosequencing	MSP	MS-HRM	MALDI-TOF	Southern blot
Blood																
Whole blood	[30,55,58,108–110]		[103]			[111]				[34]	[34]	[31]	[91,92,102]			
Umb cord blood	[99]											[99]				
Myeloid cells																
PBMC	[32,53,54]		[95]	[79]				[29]			[29,94]	[72]	[29,71,81]			
Monocytes	[62,96]															
Macrophages								[97,98]					[97,98]			
Granulocytes	[96]															
Dendritic cells		[86]										[86]				
THP-1 cells	[84]										[6,97,98]	[84]	[97,98]			
Lymphoid cells																
NK cells	[63]								[65]		[63–66]					
B cells	[26]											[26]				
T cells	[50,93]		[20]				[93]	[77]			[70,75–77]		[71,76]			[18]
Molt-3 T cells	[80]											[80]				
A3.01^ cells																
Hut78 cells																[18]
NK3.3 cells																[18]
SUPT1 cells																
Respiratory																
Nasal epith cells	[27,113]				[27]							[27,113]				
HGEC											[87]		[87–89]			
TERT cells													[89]			
NOK-SI cells																
Gastric mucosa											[13]		[13,14]			
Colonic mucosa												[72]				
Huh7.5 cells																
HT-29/B6|T84 cells#			[10]													
Bronch epith cells															[11]	
U937 cells	[7]															
A549 cells	[25]										[21–24]		[22]			
Genitourinary																
Podocytes											[17]		[17]			
293 T cells											[17]		[17]			
5637												[104]				
HeLa																
Other																
Brain	[108]															
Conj epithelial cells												[90]	[90]	[90]		
HUVEC																
Placental tissue	[100,101]															
Cutan biopsy							[33]									
Myocardium	[35]											[35]				

	Histone PTMs											ncRNA	
	ChIP	ChIP-seq	ChIP-qPCR	Western blot	ELISA	Immunofluorescence	Mass spectrometry	Immuno-blotting ± IP	Immunohisto-chemistry + IF	ATAC-seq	miRNA microarray	qRT-PCR	lncRNA microarray
Blood													
Whole blood													
Umb cord blood													
Myeloid cells													
PBMC				[79]							[28]	[28]	
Monocytes	[36,43,44,47,59]	[45,61]											
Macrophages													
Granulocytes													
Dendritic cells		[86]											
THP-1 cells	[68,83]		[82]	[83]	[68]			[68]					
Lymphoid cells													
NK cells													
B cells													
T cells			[12]							[77]			[12]
Molt-3 T cells													
A3.01^ cells		[78]											
Hut78 cells													
NK3.3 cells													
SUPT1 cells							[19]						
Respiratory													
Nasal epith cells													
HGEC					[89]								
TERT cells					[89]								
NOK-SI cells									[37]				
Gastric mucosa													
Colonic mucosa													
Huh7.5 cells													
HT-29/B6|T84 cells#				[10]									
Bronch epith cells													
U937 cells													
A549 cells	[21]			[25]	[25]		[25]						
Genitourinary													
Podocytes	[17]												
293 T cells	[17]			[16]		[16]		[38]					
5637													
HeLa				[9,85]									
Other													
Brain													
Conj epithelial cells													
HUVEC	[8]			[8]									
Placental tissue													
Cutan biopsy													
Myocardium													

Illumina BeadChip array, Illumina Infinium HumanMethylation450 BeadChip array*; -seq, -sequencing; ELISA, enzyme-linked immunosorbent assay; MeDIP, methylated DNA immunoprecipitation; RRBS, reduced representation bisulphite sequencing; RP-HPLC, reverse-phase high performance liquid chromatography; MSP, Methylation specific polymerase chain reaction; MS-HRM, methylation-sensitive high resolution melting; MALDI-TOF, matrix assisted laser desorption/ionization time-of-flight; ChIP-(qPCR), chromatin immunoprecipitation-(quantitative polymerase chain reaction); IP, immunoprecipitation; IF, immunofluorescence; ATAC-seq, Assay for Transposase-Accessible Chromatin using sequencing; miRNA, micro RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction; lncRNA, long non-coding RNA; Umb, umbilical; NK, natural killer; epith, epithelial; HGEC, human gingival epithelial cells; NOK-SI, normal oral keratinocytes-spontaneously immortalized; Bronch, bronchial; Conj, conjunctival; HUVEC, human umbilical vein endothelial cells; Cutan, cutaneous.

*one study used the Illumina Infinium HumanMethylation27 BeadChip array [26].

**ELISA based methods: Colorimetric ELISA (HT-29 cells), 5⊠-methylcytosine DNA ELISA (whole blood, PBC), Imprint Methylated DNA quantification kit (CD4 + T cells).

^includes the HIV–infected T cell lines NCHA1, NCHA2, ACH2.

#cell lines co-cultured with human PBMC.

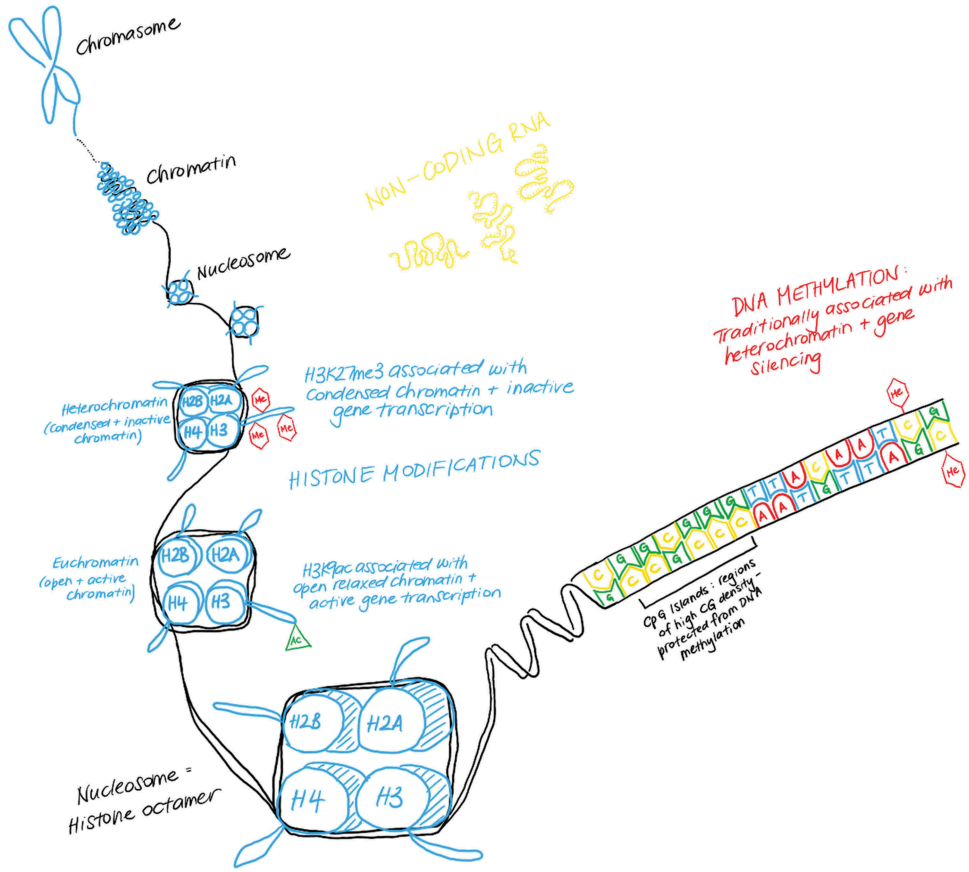

Figure 3. Chromatin structure.

investigating specific bacteria of the oral, airway and gut microbiota. One article investigated the effects of the parasite *Leishmania donovani* and 19 investigated viruses including HIV, IAV, dengue virus, EBV, human rhinovirus (HRV), RSV and human herpesvirus-6B (HHV-6B). Six articles investigated pathogen constituents, including β-glucan derived from the cell wall of *Candida albicans*, lipopolysaccharide (LPS) from *Porphyromonas gingivalis, E. coli* and *Fusobacterium nucleatum, Bacillus anthracis* suppressor-of-variegation, enhancer-of-zeste, trithorax protein (BaSET) and 2-aminoacetophenone (2-AA) from *Pseudomonas aeruginosa*. (Table 1)

Most of the studies measured either DNA methylation or histone modifications, with only three investigating non-coding RNA. (Figure 3) Genome-wide DNA methylation was analysed in 19 articles, site-specific DNA methylation was analysed in 45 articles and histone modifications were analysed in 21 articles. The studies included in the review were heterogeneous in design with a broad range of human cells, tissues and human-derived cell lines investigated using a variety of laboratory techniques (Table 2). Some studies analysed epigenetic changes in more than one cell type using more than one analysis technique.

Results and discussion

The results of the systematic review are summarized in Table 3–5. Despite the variation between studies in design, methodology and outcome, several themes emerge that highlight the many and diverse ways in which vaccines and infectious pathogens modify and regulate the human epigenome.

The influence of vaccination on the epigenome

Immune modulation

Neonatal BCG vaccination confers a significant survival benefit in high-mortality settings above and beyond its protective effect against tuberculosis. This

Table 3. Epigenetic effects of vaccines.

Vaccine Strains (vaccine type)	Study design (No. of replicates/participants)	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Bacillus Calmette-Guérin Statens Serum Institut, Copenhagen, Denmark (live attenuated)	*In vitro* 'training' of human monocytes (n = 8) with ● live BCG ● γ-irradiated-BCG ● RPMI control	Monocytes	ChIP	Histone PTMs (site specific)	Increased H3K4me3 at: ● TNF-α promoter ● IL-6 promoters ● greater effect observed for PBMC trained with live BCG vs γ-BCG ($p < 0.05$)	[43]
Bacillus Calmette-Guérin Statens Serum Institut, Copenhagen, Denmark (live attenuated)	Adults (n = 3) ● pre-vaccination ● 3mo post-vaccination	Monocytes	ChIP	Histone PTMs (site specific)	H3K4me3 increased 3mo post-vaccination at the level of: ● *TNF-α* promoter ($p < 0.05$) ● *IL-6* promoter ($p < 0.05$) ● *TLR4* promoter	[44]
Bacillus Calmette-Guérin Statens Serum Institut, Copenhagen, Denmark (live attenuated)	Adults (n = 7) ● pre-vaccination ● 28d post-vaccination	Monocytes	ChIP-seq	Histone PTMs (whole genome)	Genome-wide changes in H3K27ac distribution with: ● 646 differential peaks (456 upregulated and 190 downregulated) ● Increased H3K27ac in several important signalling and inflammatory related pathways ● lower viraemia following administration of YFV vaccine ($p < 0.05$)	[45]
Bacillus Calmette-Guérin Connaught	Female adults with type 1 diabetes mellitus (n = 3) ● pre-vaccination ● 8w post-vaccination (2 doses of BCG administered at 0 and 4w)	CD4 + T cells	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Analysis of 6 'signature' Treg genes: ● Foxp3, TNFRSF18, IL2RA, IKZF2, IKZF4 and CTLA4 ● After BCG treatment majority of the targets of all 6 signature genes showed demethylation of most of the methylation control sites ● BCG-associated demethylation at the gene level correlated with increased mRNA of the Treg signature genes	[50]
Yellow fever YFV-17D (live attenuated)	Adults ● 14d post-vaccination ● 90d post-vaccination	CD8 + T cells	Bisulphite sequencing	CpG methylation (site specific)	*PDCD1*-conserved region: ● naïve CD8 + T cells: >70% methylation ● YFV-17D-specific effector CD8 + T cells: complete demethylation (14d post-vaccination) YFV-17D-specific memory CD8 + T cells: recovery of a substantial level of methylation (90d post vaccination after the contraction phase of the CD8 + T cell response to vaccination)	[76]

(*Continued*)

Table 3. (Continued).

Vaccine Strains (vaccine type)	Study design (No. of replicates/participants)	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Yellow fever YFV-17D (live attenuated)	Adults ● 14d post-vaccination ● 120–180d post-vaccination ● >8y post-vaccination	Naïve CD8 + T cells YFV-specific CD8 + T cells ● effector (14d) ● Early memory (120–180d) ● Long-lived memory (>8y)	MeDIP sequencing (n = 1) Bisulphite sequencing (n = 5) ATAC-seq (n = 8)	CpG methylation (whole genome + site-specific) Chromatin accessibility	As CD8 T cells differentiate from naïve to effector cells: ● More than 25,000 regions show *de novo* methylation (30% remain methylated in memory cells) ● 8,000 regions are demethylated (50% remain demethylated in memory cells ● CpG sites near the *GZMB* and *PRF1i* loci are demethylated in effector and memory cells compared with naïve cells Effector and memory CD8 T cells have similar chromatin maps on principle component analysis that are distinct from naïve cells	[77]
Influenza A virus Trivalent A/California/7/2009 H1N1-like, A/Perth/16/2009 H3N2-like, B/Brisbane/60/2008-like (inactivated virus)	Adults (n = 158) ● pre-vaccination ● post-vaccination	PBMC	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Influenza A virus vaccine associated with: ● minimal alterations to global DNA methylation profile ● strong negative correlation between *IL32* promotor methylation and *IL32* gene expression ($r = -0.61$, $p = 2.7x10^{-17}$) ● coordinated hypomethylation of a specific group of CpG sites associated with lower humoral immune response	[32,53]
Influenza A virus Monovalent A/California/7/2009 H1N1-like Trivalent A/California/7/2009 H1N1-like, A/Perth/16/2009 H3N2-like, B/Brisbane/60/2008-like (inactivated virus)	Adults ● vaccine responders (n = 23) ● Aged ≥50y (n = 11) ● vaccine non-responders (n = 21) ● Aged ≥50y (n = 12) ● pre-vaccination ● 4–6w post-vaccination	PBMC	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Vaccine response associated with: ● 83 differentially methylated CPG sites ● 305 differentially methylated CPG sites in subgroup of older participants (≥50y) ● 142 differentially methylated CPG sites in subgroup of younger participants (<50y) Differentially methylated probes mapped in genes involved in immunosenescence (*CD40*) and innate immunity responses (*CXCL16, ULK1, BCL11B, BTC*)	[54]
Hepatitis B virus (recombinant DNA)	Infants with ● poor vaccine response (n = 13) ● adequate vaccine response (n = 12)	Whole blood	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Poor vaccine response associated with: ● 146 differentially methylated loci ● several hypomethylated loci corresponding to *RNF39* within MHC class I region ● hypermethylation of *SULF2*	[55]

(*Continued*)

Table 3. (Continued).

Vaccine Strains (vaccine type)	Study design (No. of replicates/participants)	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Tetanus (toxoid)	Adults (18y) ● tetanus vaccine between 10–18y (n = 269) ● controls (n = 101)	Whole blood	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Tetanus vaccination associated with: ● decreased methylation of cg14472551 associated with decreased risk of asthma at 18y (p = 0.5x10^{5}) ● increased methylation of cg01669161 associated with decreased risk of asthma (p = 0.0007) and decreased serum IgE levels at 18y	[58]
Vaccinia Ankara MVA85A (live attenuated)	*In vitro* 'training' of human monocytes (n = 8) with ● MVA85A ● RPMI control	Monocytes	ChIP	Histone PTMs (site specific)	MVA priming of cells associated with: ● Non-significant increase in H3K4me3 on the IL-6 promoter ● No difference in H3K4me3 on the TNF-α promoter	[47]

has been attributed to heterologous protection against mortality from neonatal sepsis, pneumonia, and diarrhoeal disease [41]. These heterologous effects of BCG vaccine are proposed to be the result of epigenetic reprogramming of innate immune cells and the development of innate immune memory, termed 'trained immunity' [42].

In vitro 'training' of human monocytes with live or gamma-irradiated BCG vaccine invokes a strongly potentiated pro-inflammatory cytokine response upon re-stimulation with LPS [43]. This is accompanied by the upregulation of the activating histone mark H3K4me3 at the level of the TNF-α and IL-6 promoters. These *in vitro* findings are consistent with human studies in which monocytes isolated from healthy adults 3 months after BCG vaccination demonstrate increased production of IFN-γ, TNF-α and IL-1β following exposure to mycobacterial and non-mycobacterial stimuli [44]. ChIP analysis and sequencing also showed genome wide changes in H3K27ac and enrichment of H3K4me3 at the TNF-α and IL-6 promoters in monocytes following BCG vaccination [44,45]. In a controlled human infection model, administration of BCG vaccination one month before vaccination with the live attenuated YFV vaccine resulted in lower levels of YFV viraemia in BCG-vaccinated subjects [45]. This demonstrates a correlation between the induction of trained immunity and protection against an unrelated viral infection.

MVA85A is a recombinant strain of modified *Vaccinia* Ankara expressing the immunodominant MTB protein 85A [46]. It is being investigated as a booster vaccination for BCG vaccine to protect against tuberculosis [46]. *In vitro* priming of human monocytes with *Vaccinia* virus induces trained immunity similar to the effect observed with BCG priming. In contrast, monocytes primed with MVA85A show decreased heterologous IL-6 and TNF-α responses suggestive of induced innate immune tolerance. While MVA85A did not induce any changes in H3K4me3 in the IL-6 or TNF-α promoters in monocytes, the tolerising effect of MVA85A was reversed by the addition of a histone methyltransferase inhibitor. This suggests the induction of innate immune tolerance may also be epigenetically mediated [47].

Table 4. Epigenetic effects of infectious pathogens: studies conducted in human cells or cell lines.

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Bacteria						
Mycobacterium tuberculosis MTB H37Rv	*In vitro* infection of human cell line (n ≥ 10)	THP-1 cell line	Bisulphite sequencing	CpG methylation (site-specific)	MTB infection associated with: ● demethylation of *NLRP3* promoter region ● increased NLRP3 expression ($p < 0.01$) ● increased IL-1β ($p < 0.001$) and IL-18 production ($p < 0.05$)	[6]
Mycobacterium tuberculosis MTB H37Rv	*In vitro* infection of human cell line (n = 3)	THP-1 cell line	Western blot ChIP	Histone PTMs	Infection with live MTB associated with the following changes at 24 hrs post-infection compared with heat-killed infected or uninfected cells ($p < 0.05$): ● HDAC1 recruitment to the promoter of *IL-12B* at 24 hrs post-infection ● decreased H3ac ● downregulation of *IL-12B* gene	[83]
Mycobacterium tuberculosis	*Ex vivo* infection of human monocytes (n = 13)	Human monocyte-derived dendritic cells	MethylC-seq (n = 6) Bisulphite pyrosequencing (n = 5) ChIP-seq (n = 2)	CpG methylation (whole genome + site-specific) Histone PTMs	3271 DMRs in infected cells ($p < 0.01$): ● 48% hypermethylated ● 52% hypomethylated 21 CpG sites further assessed using methylation-sensitive pyrosequencing: ● 10/10 hypomethylated CpG sites validated ● 0/11 hypermethylated CpG sites validated MTB infection associated with: ● increase in active chromatin marks (H3K27ac, H3K4me1, H3K4me3) co-localizing with hypomethylated DMRs and regions of open chromatin Gene ontology analysis: ● DMRs enriched near genes known to play a role in immune regulation processes including regulation of transcription, signal transduction and cell apoptosis	[86]
Mycobacterium tuberculosis MTB H37Rv 15 clinical isolates	*In vitro* infection of human cell line	THP-1 cell line	MSP Bisulphite sequencing	CpG methylation (site-specific)	Infection with MTB H37Rv associated with: ● hypermethylation of *IL12B* and *IL4 R* promoters ● intermediate methylation of *CCL25, IL4 R* and *IL13Ra* promoters 72hrs post infection Infection with Beijing/W MTB strains associated with: ● hypermethylation of *IL17RA* ($p < 0.027$), *IL15RA* ($p < 0.024$), *IL4 R* ($p < 0.024$), *IL6 R* ($p < 0024$) and *IL6ST* ($p < 0.024$) Infection with non-Beijing/W strains associated with: ● hypermethylation of *IL17RA* ($p < 0.048$), *IL6 R* ($p < 0.048$) and *IL6ST* ($p < 0.024$) with non-Beijing/W strains MTB strains isolated from patients with pulmonary disease compared with disseminated disease induced: ● higher methylation of *IL12A* ($p < 0.048$) and *IL7* ($p < 0.048$) ● intermediate methylation of *IL13RA1* ($p < 0.048$)	[97,98]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Burkholderia pseudomallei	*In vitro* infection of human cell line (n = 2)	U937 cell line	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Infection associated with: ● 388 DMRs ● 76 genes containing DMRs mapped to a subset of 2604 genes known to be differentially expressed in patients with septicaemic meliodosis and involved in immune system process, response to stress and inflammatory response Comparison to known epigenetic changes in MTB infected dendritic cells identified 121 genes containing DMRs common to both *B. pseudomallei* and MTB [86]	[7]
Escherichia coli Uropathogenic *E. coli* UT189 (UPEC)	*In vitro* infection of human cell line (n = 3)	5637 cell line	Bisulphite pyrosequencing	CpG methylation (site-specific)	UPEC infection associated with: ● increased methylation of *CDKN2A* exon 1 ($p < 0.05$) ● downregulation of *CDKN2A* mRNA expression ($p < 0.01$) ● de novo DNMT activity ($p < 0.005$) ● increased DNMT1 RNA expression ($p < 0.05$)	[40]
Anaplasma phagocytophilum	*In vitro* infection of human cell line (n = 2)	THP-1 cell line	ChIP-qPCR	Histone PTMs	*A. phagocytophilum* infection associated with: ● decreased H3ac in 9/11 defence gene promoters (*CYBB, MPO, DEFA1, DEFA4, DEFA6, BPI, LYZ, AZU1)* ● increased H3me in 11/11 defence gene promoters (the above plus *GNLY and DCD)* ($p < 0.05$ for all except *DEFA1, DEFA6* and *DCD*) ● increased HDAC1 binding to promoters of all 11 defence genes	[82]
Listeria monocytogenes serotype 1/2a strain (EGD)	*In vitro* infection of human cell line (n = 3)	HUVEC cell line	Western blot ChIP	Histone PTMs	Intracellular *L. monocytogenes* induced: ● global H4K8ac, H3S10p and H3K14ac 1–3hrs post infection ● *IL-8* promoter H3S10p and H3K14ac detected at 1 hr but undetectable at 3hrs ● *IL-8* promoter H4K8ac detected at 2–3hrs Inhibition of HDAC increased *Listeria*-induced expression of IL-8	[8]
Listeria monocytogenes	*In vitro* infection of human cell line (n = 3)	HeLa cell line	Western blot	Histone PTMs	*L. monocytogenes* infection associated with: ● transient increase of H3S10p at 1 hr post infection followed by dramatic decrease after 3hrs ● decreased H3ac and H4ac at 3hrs ● Histone PTMs correlate with reduced transcriptional activity of a subset of host immunity genes ● dephosphorylation mediated by listeriolysin O toxin (LLO) CDC toxins from unrelated bacteria (perfringolysin O from *C. perfringens* and pneumolysin of *S. pneumoniae*) found to induce dephosphorylation of H3S10p in HeLa cells to levels comparable to those induced by LLO	[85]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Listeria monocytogenes WildtypeEGD	*In vitro* infection of human cell line	HeLa cell line	Western blot	Histone PTMs	*L. monocytogenes* infection induced: ● deacetylation of H3K18 via sirtuin 2 (a host histone deacetylase) within 3hrs ● levels continued to decrease up to 24hrs ($n \geq 3$, $p < 0.05$)	[9]
Porphyromonas gingivalis	*Ex vivo* infection of human gingival cells (n = 4)	Human gingival epithelial cells (HGECs)	MSP	CpG methylation (site-specific)	'Dysregulated' HGECs stimulated with *P. gingivalis* demonstrate: ● blunted IL-1β production ($p < 0.001$) ● lower TLR2 expression ($p < 0.0001$) ● higher percentage methylation of *TLR2* promoter compared with 'normal' HGEC Chronic *P. gingivalis* infection induced de novo methylation of the *TLR2* promoter in human gingival cells	[87]
Porphyromonas gingivalis *Fusobacterium nucleatum*	*Ex vivo* infection of human cell line (n = 2) and human gingival cells (n = 2)	TERT cell line Human gingival epithelial cells	MSP ELISA	CpG methylation (site-specific) Histone PTMs	*P. gingivalis* infection of TERT cells associated with: ● increased methylation of *CD276* ($p < 0.005$), *elastase 2* ($p < 0.01$), *INHBA* ($p < 0.01$), *GATA3* ($p < 0.005$), *TLR2* ($p < 0.005$), and *IL-12A* ($p < 0.005$) gene promoters ● decreased methylation of *ZNF287* ($p < 0.05$) and *STAT5A* ($p < 0.005$) gene promoters *P. gingivalis* infection of HGECs associated with: ● decreased H3K4me3 in HGECs ($p < 0.01$) *F. nucleatum* infection of TERT cells associated with: ● increased methylation of *MALT1* gene promoter ● decreased methylation of *GATA3* ($p < 0.01$) and *elastase 2* ($p < 0.005$) gene promoters	[89]
Bifidobacterium breve *Lactobacillus rhamnosus GG*	*In vitro* exposure of a 3D enterocyte-PBMC coculture model to LPS ± *B. breve* and *LGG* (n = 4)	HT-29/B6 or T84 cell lines co-cultured with human PBMC	Western blot Colorimetric ELISA	Histone PTMs CpG methylation (whole genome)	*B. breve* and *LGG* stimulation: ● inhibited LPS-induced H4ac, H3S10p and H3K4ac in both HT-29/B6 and T84 cells co-cultured with PBMC ● inhibited LPS-induced NF-κβ transcription activity ($p < 0.05$) and expression of IL-17 and IL-23 mRNA ($p < 0.05$) ● slightly enhanced the DNA methylation in untreated cells (NS) ● significantly restored LPS-reduced DNA methylation in untreated cells ($p < 0.05$) *B. breve* stimulation: ● decreased the accumulation of H4ac in the untreated and LPS-treated cells ($p < 0.05$), *LGG* stimulation: ● inhibited only the LPS-induced accumulation of H4ac ($p < 0.05$)	[10]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Acinetobacter baumanni *Pseudomonas aeruginosa* *Respiratory syncytial virus* Strain A2	*In vitro* infection of human cell line (n = 3)	Human bronchial epithelial cells	MALDI-TOF	CpG methylation (site-specific)	*P. aeruginosa* infection associated with: ● increased methylation of *NODAL* promoter ($p = 0.0045$) ● decreased Nodal mRNA by 4.14 fold ($p < 0.01$) *A. baumanni* infection associated with: ● increased methylation of *NODAL* promoter ($p = 0.0054$) ● decreased Nodal mRNA by 2.8 fold ($p < 0.01$) RSV infection associated with: ● decreased methylation of *NODAL* promoter ($p = 0.0037$) ● increased Nodal mRNA by 9.8 fold ($p < 0.01$)	[11]
Chalmydia trachomatis	*In vitro* infection of human conjunctival epithelial cells (n = 4)	Human conjunctival epithelial cells	MSP Bisulphite sequencing MS-HRM	CpG methylation (site-specific)	*C. trachomatis* infection induced an increase in DNA methylation in the CpG island of the CDH1 promoter from 12.8% in control cells to 21.8% in infected cells	[90]
Viruses						
Human immunodeficiency virus-1	*In vitro* infection of human cell line (n = 3)	293 T cell line	Immunofluorescence Western blot	Histone PTMs	Vpr (HIV-encoded protein) induced: ● displacement of HP1 from chromatin ● H3K9ac at sites of primary chromosome constriction ● premature chromatid separation	[16]
Human immunodeficiency virus-1	*In vitro* infection of human podocytes (n = 3)	239 T cell line Human podocytes	MSP Bisulphite sequencing ChIP	CpG methylation (site-specific) Histone PTMs	HIV infection associated with: ● increased methylation of *VDR* promoter ($p < 0.05$) ● increased H3K4me3 of *SNAIL* promoter	[17]
Human immunodeficiency virus-1	*In vitro* comparison of human cell line to HIV-latency cell lines	A3.01 cell line NCHA1, NCHA2 and ACH2 (HIV-1 latency) cell lines	ChIP-seq	Histone PTMs (whole genome)	HIV infection associated with: ● minimal changes in histone PTMs ● H3K4me3 downregulated at 387 sites and upregulated at 451 sites ● H3K9ac downregulated at 493 sites and upregulated at 962 sites ● most histone PTMs in promoter regions not at viral integration sites	[78]
Human immunodeficiency virus-1 NL4-3 NL(AD8)	*Ex vivo* infection of human Tregs (n = 20)	Regulatory T cells (CD4 + CD25 + $CD127^{low}$)	Bisulphite sequencing	CpG methylation (site-specific)	HIV infection associated with: ● increased methylation of the *FOXP3* promoter and CNS2 ($p = 0.043$) ● decreased Foxp3 expression ($p = 0.018$) ● increased DNMT3b expression ($p < 0.05$) ● decreased production of TGF-β ($p = 0.028$) ● increased production of IL-4 ($p = 0.018$)	[70]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Human immunodeficiency virus-1 NL3-4 HXB-2	*Ex vivo* infection of human T lymphocytes and human T cell line (n = 3)	Human CD4 + and CD8 + T cells Hut78 cell line NK3.3 cell line Control: NK3.3 cell line infected with HTLV-1 or uninfected THP-1 cells	Southern blot	CpG methylation (site-specific)	HIV infection of primary T cells and T cell lines associated with: ● increased genomic methylation ● specific de novo methylation of *IFNG* gene promoter ● increased DNMT expression ● downregulation of expression of IFN-γ HTLV-1 infection of NK3.3 cells also associated with increased methylation of *IFNG* promoter	[18]
Human immunodeficiency virus-1 NL4-3	*Ex vivo* infection of human PBMC (n = 5)	PBMC	DNA digestion and gel electrophoresis (n = 3) Western blot (n = 2)	CpG methylation (whole genome) Histone PTMs	HIV infection associated with: ● increased global 5⊠-methylcytosine in cellular genome at 12 hr ($p < 0.01$) and 36 hr ($p < 0.001$) post infection ● increased H3K9me3 at 12 hr ($p < 0.01$) and 24hr ($p < 0.05$) ● increased H3K27me3 at 24hr ($p < 0.01$) and decreased at 36 hr ($p < 0.05$) ● decreased H3K4me3 at 12 hr ($p < 0.05$)	[79]
Human immunodeficiency virus-1 LAI	*In vitro* infection of human cell line (n = 3)	SUP-T1 cell line Control: UV–inactivated HIV	Mass spectrometry	Histone PTMs	HIV infection associated with: ● major changes in histone H3 and H4 PTM abundances ● large fluctuations in mRNA expression of associated chromatin enzymes ● few differences between infected and UV–inactivated infected cells	[19]
Human immunodeficiency virus-1 NL4-3	*Ex vivo* infection of human PBMC (n = 12)	CD4 + T cells	Imprint Methylated DNA Quantification kit	CpG methylation (whole genome)	In resting infected CD4 + T cells DNA methylation percentage increased with culture time	[20]
Influenza A virus H3N2	*In vitro* infection of human cell line (n = 3)	A549 cell line	Bisulphite sequencing ChIP	CpG methylation (site-specific) microRNA	*In vitro* influenza A virus infection associated with: ● demethylation of the -57 CpG in the *COX2* promoter ● reduction in the binding of DNMT3a and DNMT3b at this site ● increased miR29b expression and suppression of DNMT activity ($p < 0.05$) Increased expression of miR29b confirmed in PBMC from influenza patients compared with healthy controls (n = 10, $p < 0.01$)	[21]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Influenza A virus H5N1 H1N1 H1N1 (2009) vaccine strain H5N1	*In vitro* infection of human cell line (n = 3)	A549 cell line	MSP Bisulphite sequencing	CpG methylation (site-specific)	Influenza A virus infection associated with: ● significant change in the promoter methylation levels of 7/24 inflammatory genes including: ○ pro-inflammatory cytokine genes *CXCL14, CCL25, CXCL6* ○ interleukin genes *IL13, IL17 C, IL4 R* ● increased expression of *IL17 C, IL13* and *CXCL6* genes Analysis of *IL17 C* promoter region resulted in identification of a demethylated CpG site within the Retinoid X receptor-alpha transcription factor binding site-specifically in H5N1-infected cells but not in cells infected with other strains of influenza	[22]
Influenza A virus H5N1 H3N2 H1N1	*In vitro* infection of human cell line (n = 3)	A549 cell line	Bisulphite sequencing	CpG methylation (site-specific)	Influenza A virus infection associated with: ● hypomethylation of the *IL32* promoter with demethylation of the CREB binding site ● increased binding of CREB to the *IL32* promoter ● increased activation of *IL32* transcription	[23]
Influenza A virus H5N1	*In vitro* infection of human cell line or treatment with dsRNA (n = 3)	A549 cell line	Bisulphite sequencing	CpG methylation (site-specific)	Influenza A virus infection and dsRNA treatment associated with: ● demethylation of IL-6 promotor at -162 CpG (CREB binding site) *In vitro* methylation resulted in decreased activity of the *IL-6* promoter ($p < 0.01$)	[24]
Influenza A virus PR8hv	*In vitro* infection or mock-infection of human cell line (n = 3)	A549 cell line	Illumina Infinium HumanMethylation450 BeadChip array ELISA Mass spectrometry Western blot	CpG methylation (whole genome) Histone PTMs	Influenza infection associated with: ● minimal differences in DNA methylation profiles between infected and uninfected ● increased H3K79me and H4K20me2 ● reduced H3K36me H4K79ac and H4K16ac ● reduced lysine acetylation of histone 3 and 4	[25]
Dengue virus DENV2	*Ex vivo* infection of human PBMC (n = 2)	PBMC	miRNA microarray qRT-PCR	microRNA	DENV2 infection associated with: ● altered levels of multiple miRNAs involved with biological regulation, cell response to stimulus, signal transduction and metabolism (fold change>1.5; $p < 0.05$ ● upregulation of 11 miRNAs ● downregulation of 4 miRNAs	[28]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Human rhinovirus HRV-16	*In vitro* infection of human nasal epithelial cells	Primary nasal epithelial cells from asthmatics (n = 10) and controls (n = 7)	Pyrosequencing of genome wide Alu elements Illumina Infinium HumanMethylation450 BeadChip array (n = 9) Bisulphite pyrosequencing	CpG methylation (whole genome + site-specific)	Global DNA methylation analysis revealed: ● 389 loci influenced by either asthma or HRV infection ● a CpG site in SNORA12 was methylated in both infected asthmatic and control cells HRV infection associated with: ● increased global DNA methylation in asthmatic cells only ($p = 0.04$) ● significantly increased SNORA12 methylation in control cells ($p = 0.04$) ● increased SNORA12 gene expression in asthmatic cells ($p = 0.01$)	[27]
Human rhinovirus HRV-16	*In vitro* infection and mock-infection of human nasal epithelial cells	Primary nasal epithelial cells from children with (n = 10) and without (n = 7) asthma	Illumina Infinium HumanMethylation450 BeadChip array Pyrosequencing	CpG methylation (whole genome + site-specific)	HRV infection associated with: ● Genome-wide differences in DNA methylation in asthmatics ● 27,517 CpGs with significant differences in DNA methylation ● 471 CpGs in 268 genes identified to have modified DNA methylation and mRNA expression in asthmatics	[113]
Epstein Barr Virus	*Ex vivo* EBV-mediated transformation of human resting B lymphocytes to lymphoblastoid cell lines (n = 6)	Human resting B lymphocytes	Illumina Infinium HumanMethylation27 Assay Bisulphite pyrosequencing	CpG methylation (whole genome + site-specific)	EBV-transformation of resting B lymphocytes to lymphoblastoid cells associated with: ● hypomethylation of 256 genes (fold change >2; $p < 0.05$) ○ Bisulphite pyrosequencing of CpG sites for 20 genes with most significant changes in methylation confirmed at least a 2-fold significant decrease in all genes ○ Promoter hypomethylation associated with upregulation of genes relevant for the phenotype of proliferating lymphoblasts ● no hypermethylated genes	[26]
Human herpesvirus-6B Z29	*In vitro* infection of human cell line (n = 3)	Molt-3 T cells	Illumina Infinium HumanMethylation450 BeadChip array Bisulphite sequencing	CpG methylation (whole genome + site-specific)	HHV-6B infection associated with: ● 406 CpG sites with significant methylation changes 3 days post-infection of which 86% were located <1 Mb from chromosomal ends and all were hypomethylated in HHV-6-infected cells ● CpG hypomethylation of chromosome 17p13.3 2 days post-infection ● Upregulation of TET2	[80]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Parasites						
Leishmania donovani	*In vitro* infection of human cell line (n = 3)	THP-1 cells	Illumina Infinium HumanMethylation450 BeadChip array Bisulphite pyrosequencing	CpG methylation (whole genome + site-specific)	Infection with live promastigote vs. heat killed treated or uninfected macrophages associated with: ● 443 CpG sites with significant methylation changes ($p < 0.05$) ● 51.47% hypomethylation ● 48.53% hypermethylation ● 315 CpG sites mapped to an annotated gene involved multiple immune processes including the chemokine signalling pathway, the calcium signalling pathway, the Notch signalling pathway and natural killer cell mediated cytotoxicity ● pyrosequencing confirmed the increased methylation of CpG sites in the *IRAK2* and *LARS2* genes ($p < 0.01$)	[84]
Pathogen constituents						
β-glucan *Candida albicans* β-1,3-(D)-glucan	*Ex vivo* training of human monocytes with β-glucan (n = 8)	Human monocytes	ChIP	Histone PTMs	'Training' of monocytes with β-glucan resulted in: ● genome-wide changes in H3K4me3 and H3K27ac ● associated upregulation of genes involved in innate immune and signalling pathways, glycolysis and mTOR pathway ● subsequent stimulation of monocytes with LPS, Pam3Cys, *S. aureus* and *E. coli* resulted in a strongly potentiated pro-inflammatory cytokine response of TNF-α and IL-6 ($p < 0.05$)	[59]
β-glucan *Candida albicans* β-1,3-(D)-glucan	*Ex vivo* training of human monocytes with β-glucan (n = 5–8)	Human monocytes	ChIP	Histone PTMs	'Training' of monocytes with β-glucan resulted in: ● global increase in H3K4me3 with minimal change in H3K27me3 'Training' of monocytes with *C. albicans* resulted in: ● increased production of TNF-α and IL-6 upon re-stimulation with LPS, Pam3Cys, *C. albicans* and *M. tuberculosis* ($p < 0.05$)	[60]
β-glucan *Candida albicans* β-1,3-(D)-glucan	*Ex vivo* training of human monocytes with β-glucan	Human Monocytes	ChIP-seq	Histone PTMs	β-glucan exposure induced 2688 dynamic changes in H3K27ac	[61]
Lipopolysaccharide *Porphyromonas gingivalis, Escherichia coli* *Fusobacterium nucleatum*	*In vitro* stimulation of human cell line with LPS from *P. gingivalis, E. coli* and heat-inactivated *F. nucleatum*	NOK-SI cell line	Immunohistochemistry and immunofluorescence	Histone PTMs	LPS stimulation associated with increased H3K9ac: ● LPS from *P. gingivalis* induced H3K9ac within 15 mins ($p < 0.05$) ● LPS from heat-inactivated *F. nucleatum* induced H3K9ac (<0.05) within 15 mins ● LPS from *E. coli* induced H3K9ac within 6hrs ($p < 0.001$) ● Histone acetylation associated with accumulation of p300/CBP ($p < 0.05$) and nuclear accumulation of NF-κβ ($p < 0.01$)	[37]

(*Continued*)

Table 4. (Continued).

Pathogen Strains	Study design (No. of replicates)	Cell type	Analysis techniques	Epigenetic change	Main results	Ref
Bacillus anthracis *Ba*SET	*In vitro* transfection of human cell line with FLAG-*Ba*SET plasmid (n = 3)	293 T cell line	Immunoblotting and immunoprecipitation	Histone PTMs	Dose-dependent expression of *Ba*SET associated with: ● enhancement of H1Kme2 and H1Kme3 ● downregulation of NF-κβ functions ● repression of transcription of inflammatory genes	[38]
2-aminoacetophenone *Pseudomonas aeruginosa*	*In vitro* treatment of THP-1 cells (n = 3) or human primary macrophages (n = 3)	THP-1 cell line Macrophages	Immunoblotting Colorimetric assay ChIP	Histone PTMs	First exposure to 2-AA associated with: ● global increase in H3K18ac after 1 hr ● unaltered H3K9ac, H3K9me3, H3K14ac, H3K56ac Subsequent exposure to 2-AA: ● attenuation of enrichment of H3K18ac at TNF promoter locus ($p < 0.05$)	[68]

CNS2, conserved non-coding sequence 2; CBP, CREB-binding protein; *Ba*SET (suppressor-of-variegation, enhancer-of-zeste, trithorax protein from *B. anthracis*)

There is emerging evidence that BCG vaccine may also protect against the progression of certain autoimmune diseases including diabetes and multiple sclerosis [48,49]. In females with type 1 diabetes mellitus administration of BCG vaccination results in improved glycaemic control and demethylation of the methylation control sites in six central T-regulatory genes [50].

Vaccine response

Predicting the degree to which an individual will mount an adequate immune response to vaccination and develop sustained immunity poses an ongoing challenge to clinicians and researchers [51]. In the elderly, a poor humoral immune response to influenza vaccination is attributed to immunosenescence [52]. In a study in healthy 50–74 year olds, although the global DNA methylation profile underwent minimal changes, a specific group of CpG sites, when coordinately hypomethylated, was associated with lower humoral immune response to influenza vaccination [53]. A study comparing influenza vaccine responses in older and younger populations showed larger epigenetic remodelling in vaccine responders aged over 50 years [54]. The differentially methylated probes mapped to genes involved in immunosenescence. Similarly, hypomethylation of *RNF39* (Ring Finger Protein 39), a transcription factor in the major histocompatibility complex (MHC) class I region, is associated poor HBV vaccine responses in infants [55].

Disease risk

The role of epigenetics in the ability of vaccines, including BCG and pertussis, to modulate allergic disease risk is poorly understood [56,57]. In a whole population birth cohort on the Isle of Wight in 1989, tetanus vaccination was associated with differential DNA methylation and a reduced risk of asthma in adolescence [58].

The influence of infection on the epigenome

Innate immune system modulation

Similar to the studies of BCG vaccine-induced trained innate immunity, exposure of human monocytes to β-glucan, the main cell wall constituent of *C. albicans*, results in a genome-wide increase in H3K4me3 and increased production of TNF-α and IL-6 upon re-stimulation with *C. albicans* and unrelated innate immune stimuli [59,60]. β-glucan 'training' also results in dynamic genome-wide changes in H3K27ac in monocytes [61]. The Fulani and Mossi, sympatric ethnic groups in Burkina Faso, are known to have different susceptibility to malaria. The Fulani group have lower rates of malaria infection and demonstrate a 'high alert' immune state similar to that of trained immunity. Monocytes from *Plasmodium falciparum* infected and uninfected Fulani and Mossi males showed stable DNA methylation profiles between disease states and ethnic groups but significantly different transcriptional responses in the malaria infected Fulani with increased expression of chromatin factors involved in H3K4me [62].

CMV has also been shown to contribute to innate immune memory. CMV infection induces subsets of 'adaptive' natural killer (NK) cells that lack expression of B cell and myeloid cell-related signalling proteins and display epigenetic diversification compared to naïve NK cells. These subpopulations of NK cells possess a global methylation signature similar to cytotoxic T lymphocytes and undergo expansion in response to CMV–infected cells in an antibody-dependent manner [63–65]. Study of these adaptive NK cells in chronic viral hepatitis shows that these cells preferentially expand in CMV seropositive chronically HBV–infected patients and that their distinct methylation pattern of the FCER1 G and IFNG promoter regions is conserved [66].

At the other end of the spectrum of innate immune memory, tolerance results in dampening of host inflammatory responses and facilitates pathogen persistence [67]. Pre-treatment of human macrophages with 2-AA, a quorum-sensing molecule excreted by *Pseudomonas aeruginosa*, reduces H3K18ac at the TNF promoter on re-exposure to 2-AA and results in attenuated transcription of pro-inflammatory cytokines [68].

Adaptive immune system modulation

Studies have also focused on the epigenetic modulation of the adaptive immune system in response to infection, in particular T cell dysregulation in the setting of acute and chronic viral infection. Regulatory T cells (Tregs) are required for peripheral immune tolerance and Foxp3 is the major transcription factor essential for maintaining the suppressive function of Tregs. Demethylation of a

CpG island in the *FOXP3* locus, the Treg-specific demethylated region (TSDR), is associated with stable Foxp3 expression during Treg development in the thymus [69]. *In vitro* HIV infection modifies the phenotype and function of Tregs isolated from healthy donors through altered methylation of the *FOXP3* gene. Increased methylation of the *FOXP3* locus is associated with loss of Treg suppressive capacity and an altered cytokine profile that may contribute to heightened immune activation in HIV infection [70]. Increased methylation of the *FOXP3* TSDR and decreased functional suppression was also found in Tregs isolated from patients with HTLV associated myelopathy/tropical spastic paraparesis (HAM/TSP) [71]. In contrast, the *FOXP3* promoter is significantly demethylated in colonic mucosa and PBMC from HIV–infected patients [72].

Chronic viral infection is associated with persistent antigen presentation and progressive T cell dysfunction [73]. Sustained expression of the inhibitory programmed death-1 (PD-1) receptor on exhausted CD8 + T cells is associated with lack of viral control and correlates with disease progression in HIV [74]. HIV-specific CD8 + T cells isolated from donors with acute HIV infection and viraemia demonstrate demethylation of the PD-1 transcriptional regulatory region compared to donor matched naïve CD8 + T cells. The PD-1 transcriptional regulatory region remains demethylated in the chronic stages of HIV infection, even following spontaneous or pharmacological reduction in viral load [75]. Persistent demethylation of the PD-1 regulatory region is also observed in other chronic viral infections including CMV and EBV [76]. In a model of acute viraemia, YFV-specific CD8 + T cells isolated from donors 14 days after YFV vaccination show transient expression of PD-1 and near complete demethylation of the PD-1 regulatory region. After contraction of the YFV-specific CD8 + T cells 90 days post-vaccination, a functional population of memory CD8 + T cells emerges with re-methylation of the PD-1 regulatory region [76]. Using a similar controlled human infection model, deuterium labelling of CD8 + T cells that proliferate in response to YFV vaccine shows that long-lived memory CD8 + T cells retain epigenetic marks of their effector history and are distinct from naïve cells [77].

Host evasion and latent infection

Invading pathogens can also use epigenetic modification as a strategy to promote viral latency and host evasion. A genome wide analysis of histone modifications in an *in vitro* model of latent HIV infection revealed minimal overall changes in histone modifications, but several regions within gene promoters showed changes in H3K4me3 and H3K9ac signal. These genes include the cell cycle regulatory genes *CDKN1* and *CCND2*, which may play a role in the maintenance of HIV latency [78]. *In vitro* HIV infection also increases global DNA methylation in the cellular genome [79]. HHV-6B infection induces hypomethylation *in vitro* in regions close to telomeres that may facilitate viral integration and promote latency [80].

Epigenetic manipulation of the host immune system may also play a role in chronic infection with HBV. Increased methylation of a CpG island in a promoter of the *CIITA* gene, which regulates expression of major histocompatibility complex (MHC) class II, is associated with persistent HBV infection [81].

A. phagocytophilum infection of THP-1 cells results in epigenetic silencing of host defence genes through histone modifications that promote intracellular survival of the bacterium [82]. *M. tuberculosis* infection of THP-1 cells is also associated with histone modifications through the recruitment of HDAC1 to the *IL-12B* promoter and histone deacetylation. This results in downregulation of *IL-12B* gene expression, which is known to play a role in Th1 responses, and may contribute to the mechanisms by which *M. tuberculosis* subverts the host immune system [83]. Genome-wide DNA methylation profiling following infection with *L. donovani* identified a group of over 400 CpG sites with altered methylation corresponding to genes involved in host immune defence [84]. *In vitro L. monocytogenes* infection induces a dramatic dephosphorylation of histone 3 and deacetylation of histone 4 that is mediated by the toxin listeriolysin O. These histone modifications are associated with reduced transcriptional activity of a subset of host immunity genes. Cholesterol-dependent cytolysins (CDC) from other bacterial pathogens also induce a similar degree of histone dephosphorylation, suggesting that these bacterial toxins regulate the host immune response

via epigenetic manipulation [85]. Comparison of the epigenetic changes induced by infection with *B. pseudomallei* and *M. tuberculosis* reveal a subset of over 100 genes containing differentially methylated regions (DMR) common to both pathogens. This demonstrates that there are both pathogen-specific and pathogen-common epigenetic changes that occur in response to microbial infection [86].

Disease pathogenesis and progression

Periodontitis, a chronic inflammatory disease predominantly driven by *P. gingivalis*, is associated with blunted Toll-like receptor (TLR) expression and signalling. The *TLR2* promoter is hypermethylated in gingival epithelial cells isolated from periodontitis-affected tissue [87,88]. Consistent with this finding, *in vitro* infection with *P. gingivalis* induces hypermethylation of several gene promoters, including *TLR2* [89]. *C. trachomatis* infection of human conjunctival epithelial cells increases methylation of the CDH1 promoter and downregulates expression of E-Cadherin, which is hypothesized to contribute to the conjunctival fibrosis that causes blindness from trachoma [90]. Global DNA methylation analysis of myocardium from patients with chronic Chagas cardiomyopathy shows differential methylation of more than 7000 CpG sites [90]. These sites correlate with the differential expression of pathogenically relevant cardiovascular and immune system genes. The cytokine storm of severe dengue virus infection is characterized by the overexpression of proinflammatory cytokines, including TNF-α and IFN-γ. Individuals with dengue virus infection have reduced methylation of the TNF-α promoter and increased TNF-α messenger RNA (mRNA) in whole blood compared with uninfected controls but no difference in methylation of the IFN-γ promoter [91].

In a cohort of patients with chronic HBV, those with acute on chronic HBV liver failure had higher methylation of the *GSTM3* gene promoter compared with those without liver failure. The *GSTM3* gene codes for glutathione-s-transferase M3 that mediates oxidative stress in the liver. Hypermethylation of the *GSTM3* promoter may correlate with disease severity and progression in chronic HBV [92]. In a cohort of tuberculosis (TB) exposed children in Swaziland, helminth co-infection with *S. haemotobium* was associated with increased global DNA methylation that persisted six months after successful eradication therapy [93]. These DNA methylation changes were associated with a decrease in the proliferation of TB-specific CD4 + T cells, supporting the hypothesis that helminth co-infection perturbs the immune response to *M. tuberculosis* and increases the risk of tuberculosis progression.

The modifying effect of long-term antiviral therapy on the epigenome has also been assessed. Acute untreated HIV-1 infection is associated with demethylation of the *CCR5* intron and increased expression of CCR5 [94]. Suppression of viral replication, either spontaneously or pharmacologically, is correlated with increased methylation of the *CCR5* intron [94]. An inverse correlation between DNA methylation and active viral replication is also seen in the setting of long-term antiretroviral therapy for HIV [95].

Disease biomarker

The use of DNA methylation signatures as biomarkers for latent and occult infection has been explored for several pathogens and clinical infection syndromes. The DNA methylation profiles of granulocytes and monocytes isolated from patients with active TB and latent TB show multiple DMR enriched in several immune regulatory pathways [96]. Macrophages from patients with active TB and latent TB also show different methylation profiles that could potentially be used as diagnostic biomarkers [97,98]. Additionally, *in vitro* infection of THP-1 cells with *M. tuberculosis* strains isolated from patients with pulmonary or disseminated TB show distinct methylation signatures [97,98].

The differential methylation of more than 500 genes in umbilical cord blood from infants born to mothers with active HBV infection suggests a possible role for DNA methylation status at birth as a biomarker of prenatal HBV exposure [99]. Similarly, differential DNA methylation was found in placental tissue from preterm infants born in the setting of chorioamnionitis compared with preterm infants without significant pathology. The placentas with chorioamnionitis showed altered DNA methylation signatures that were not observed in the absence of inflammation [100]. These changes were hypothesized to reflect both increased number and altered function of innate immune cells in the placenta. The composition

Table 5. Epigenetic effects of infectious pathogens: studies conducted in humans.

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Bacteria						
Mycobacterium tuberculosis	Active MTB (n = 6)	CD8 + T cells (CD244- or CD244+)	lncRNA microarray ChIP-qPCR	Non-coding RNA Histone PTMs	Active MTB infection associated with: ● increased levels of lncRNA-BC050410 in CD244+ CD8 + T cells (p = 6.6E-09) lncRNA-BC050410 mediates H3K27me3 at *INFG/TNFA* promoters in CD244 + CD8 + T cells resulting in: ● a repressive chromatin state ● inhibition of IFN-γ and TNF-α expression	[12]
Mycobacterium tuberculosis	Active MTB (n = 3) LTBI (n = 3) Controls (n = 4)	Macrophages	MSP MeDIP + CpG island array	CpG methylation (site-specific)	● Hypomethylation of *FADD* ($p < 0.023$) and *IL17RA* ($p < 0.049$) in active MTB infection ● 514 CpG sites differentially methylated between active MTB and LTBI ● IL17 signalling pathway particularly identified to contribute to the inflammatory response against primary MTB infection ○ hypermethylation of *IL17RA* in active MTB and LTBI (p = 0.046) ○ hypomethylation of *IL17D* in active MTB and LTBI (p = 0.010–0.046) ○ *IL17RD* (p = 0.004) and *IL17 C* (p = 0.021) differentially methylated between active MTB and LTBI	[97,98]
Mycobacterium tuberculosis	Active MTB (n = 8) LTBI (n = 8)	Granulocytes Monocytes	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Differential CpG methylation between active MTB and LTBI cohorts: ● DMRs significantly enriched in 5 blood transcriptional modules including B-cell surface signature, cell cycle and transcription, cell cycle, extracellular matrix and MHC-TLR7-TLR8 cluster ● 4 DMRs in the MHC-TLR7-TLR8 cluster ○ CpG in HLA-DQB1 hypermethylated in active MTB compared with LTBI ○ CpGs in HLA-F promoter hypomethylated in active MTB compared with LTBI	[96]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Helicobacter pylori	HP+ children (n = 27) HP- children (n = 20) HP+ adults (n = 22) HP- adults (n = 16)	Gastric mucosa	MSP Bisulphite sequencing	CpG methylation (site-specific)	In HP+ compared with HP- paediatric samples: ● Average number of methylated genes higher (3.4 vs 0.3, $p < 0.001$) ● 7 genes showed significantly higher methylation levels (*CDH1, DAPK1, CRABP1, GRIN2B, TIMP3, CALCA, TWIST1*) (all $p < 0.05$) In HP+ compared with HP- adult samples: ● Average number of methylated genes higher (7.6 vs 0.9, $p < 0.001$) ● 6 of the above genes (all except for *TWIST1)* showed significant hypermethylation (all $p < 0.001$)	[13]
Helicobacter pylori	HP+ (n = 158) HP- (n = 190)	Gastric mucosa	MSP	CpG methylation (site-specific)	HP infection associated with: ● significant overmethylation in body and/or antrum of stomach of multiple housekeeping and stomach specific genes including *CDH1, ARRDC4, PPARG, SHH, MMP2, CDK2NA, RUNX2, RUNX3, PGA, TFF2, ATP4B, PGC, TFF1, TFF3* (all $p < 0.05$) ● concurrent methylation of transitional CpG sites and CpG islands in HP infected stomachs that may be used as a clinical marker for infection	[14]
Gut microbiota *Bacteroidetes* *Proteobacteria* *Firmicutes*	Pregnant women Predominance of *Bacteroidetes* and *Proteobacteria* (n = 4) Predominance of *Firmicutes* (n = 4)	Whole blood	Methylated DNA sequencing	CpG methylation (whole genome)	Women with predominance of *Firmicutes* had: ● increased methylation of 568 gene promoters (fold change 1.5, $p \leq 0.05$) ● decreased methylation of 245 gene promoters (fold change 1.5, $p \leq 0.05$) Pathway analysis demonstrated gut microbiota composition differentially effects the DNA methylation status of genes linked to cardiovascular diseases, inflammatory response, metabolic pathways and gastrointestinal diseases including cancer ($p < 0.05$)	[111]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Placental microbiota 16 bacterial species*	Biopsies from placentas of infants born <28/40 (n = 84)	Placental tissue	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	1789 CpG sites corresponding to 1079 genes displayed differential methylation in relation to the presence of placental microorganisms ● Altered genes encode for proteins involved in immune/inflammatory responses, specifically the NF-κB signalling pathway ● GBS associated with the greatest number of differentially methylated CpG sites (1257) of which 98% were hypomethylated ● Methylated probes were unique to the bacterial species present	[101]
Viruses						
Human immunodeficiency virus-1	Monozygotic twins HIV+ (n = 1) HIV- (n = 1) Validation cohort HIV/AIDS (n = 8) Controls (n = 8)	PBMC	MeDIP Bisulphite sequencing MSP	CpG methylation (whole genome + site-specific)	HIV+ twin had: ● 4679 unique DMRs with significantly higher peak values ($p < 0.001$) ● DMRs in CpG promoters associated with biological process, molecular function and cellular component categories on GO classification Selected genes validated in CEM-174 cell line and HIV/AIDS patients: ● *IGFBP6* and *SATB2* significantly downregulated in HIV infected cells and HIV/AIDS patients ($p < 0.05$) ● associated hypermethylation of gene promoters	[29]
Human immunodeficiency virus-1	Acute HIV (n = 2) Chronic HIV (n = 3) HIV elite controllers (n = 7)	HIV-specific and naïve CD8 + T cells	Bisulphite sequencing	CpG methylation (site-specific)	PD-1^{hi} HIV-specific T cells demonstrate: ● demethylation of PD-1 receptor transcriptional regulatory region ($p < 0.0001$) ● persistent PD-1 receptor transcriptional regulatory region demethylation following reduction of viral load by ART or spontaneously in elite controllers	[75]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Human immunodeficiency virus-1	HIV+ (n = 10) Controls (n = 10)	Colonic mucosa PBMC	Bisulphite pyrosequencing	CpG methylation (site-specific)	HIV+ patients had: ● significant demethylation of FOXP3 in both colon tissue and PBMC ($p < 0.0001$) ● comparable methylation levels in both specimens from the same subject ● negative correlation of methylation level with relative gene expression in colon tissue ($p = 0.0438$) ● downregulation of DNMT1 ($p = 0.0095$) with a positive correlation to FOXP3 promoter methylation ($p = 0.0254$)	[72]
Human immunodeficiency virus-1	Controls (n = 18) HIV+ pre and post-ART (n = 29) Elite/viraemic controllers (n = 42) CD4+ <800cells/μL (n = 22) CD4+ ≥800cells/μL (n = 10)	PBMC	Bisulphite sequencing	CpG methylation (site-specific)	● Demethylation of *CCR5* cis-regions (Pr2 and intron 2) associated with HIV infection ($p < 0.001$) ● Changes in methylation status of *CCR5* intron 2 negatively correlated with CCR5 levels ($p = 0.04$) ● Suppression of viral replication, spontaneously or by ART, and normalization of CD4+ count (≥800 cells/μL) associated with increased methylation compared with acute untreated HIV ($p < 0.001$) but lower methylation than HIV negative subjects	[94]
Human immunodeficiency virus-1	HIV+ on ART (n = 137) Controls (n = 44)	Whole blood	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	HIV infection associated with: ● global dysregulation of methylome with >80,000 CpG sites affected ● altered methylation of 26,927 CpG sites with a known association with biological age ($p < 10^{-8}$) ● an average biological age advancement of 4.9 yrs ($p < 10^{-8}$) ● specific hypomethylation of HLA locus ($p < 10^{-10}$) with degree of hypomethylation correlated with CD4+/CD8 + T cell ratio	[107]
Human immunodeficiency virus-1	Fresh frozen brain specimens from deceased HIV+ subjects (n = 99) and controls (n = 31) Whole blood from HIV+ subjects (n = 24) and controls (n = 67)	Whole blood Brain tissue (occipital lobe, cerebellum, frontal lobe)	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	HIV+ subjects had: ● advanced biological age as measured by the 'epigenetic clock' ● accelerated epigenetic age by an average of 7.4 yrs in brain samples ($p = 8x10^{-8}$) and 5.2 yrs in blood specimens ($p < 0.0048$)	[108]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Human Immunodeficiency virus-1 F1	HIV+ on ART (n = 81) Controls (n = 18)	PBMC	5-mc DNA ELISA	CpG methylation (whole genome)	Increased DNA methylation: ● inversely correlated with pro-viral DNA and active replication ● correlated with lower number of gag copies/106 cells (rho = -0.52; p = 0.03) and less time with detectable HIV viraemia in HIV-undetectable study participants (rho = -0.46; p = 0.03) ● observed in controls compared with HIV–infected subjects but not significant (14.5% vs 9%; p = 0.23)	[95]
Human T-cell Lymphotropic Virus-1	HAM/TSP (n = 9) Controls (n = 10)	PBMC CD4 + T cells CD4+ CD25 + T cells	MSP	CpG methylation (site-specific)	Demethylation of FoxP3 intron 1 Treg-specific demethylated region (TDSR) in CD4+ (p < 0.0018) and CD4+ CD25 + T cells (p < 0.001) compared with PBMC in all patients HAM/TSP patients had: ● reduced demethylation of TDSR in CD4 + CD25 + T cells (p = 0.0339) ● correlation between reduced demethylation and reduced Treg suppressive function (p = 0.0041)	[71]
Cytomegalovirus	CMV+ (n = 6)	NK cells (early mature $CD3^{-}CD56^{dim}CD57^{-}$; late mature $CD3^{-}CD56^{dim}CD57^{bright}EAT\text{-}2^{+}$; adaptive $CD3^{-}CD56^{dim}CD57^{bright}EAT\text{-}2^{-}$)	Illumina Infinium HumanMethylation450 BeadChip array (n = 4) Bisulphite sequencing (n = 2)	CpG methylation (whole genome + site-specific)	CMV induces: ● subsets of 'adaptive' NK cells lacking specific markers ([64]) 'Adaptive' NK cells display: ● a distinct global methylation signature that more closely resembles effector T cells than naïve NK cells. ● specific hypermethylation of the *FCER1 G* and *SH2D1B* gene promoters that is associated with reduced expression of FCεR-γ and EAT-2 respectively	[63]
Cytomegalovirus	Healthy donors with unknown CMV serological status (n = 62)	NK cells ($CD3^{-}CD56^{+}FcR\gamma^{+}$; $CD3^{-}CD56^{+}FcR\gamma^{-}$)	Bisulphite sequencing (n = 3)	CpG methylation (site-specific)	60% of NK cell pool were SYK-deficient: ● with associated hypermethylation of *SYK* promoter ● all donors with SYK-deficient NK cells had previous CMV exposure (p < 0.005) ● Nearly all SYK deficient NK cells also deficient in FcR-γ and other proteins including EAT-2, PLZF and DAB2 ● FcR-γ deficient NK cells undergo expansion in response to CMV–infected cells in an antibody-dependent manner	[64]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Cytomegalovirus	CMV+ (n = 4) CMV- (n = 2)	NK cells ($CD3^{-}CD56^{dim}CD57^{+}NKG2\ C^{-}$; $CD3^{-}CD56^{dim}CD57^{+}NKG2\ C^{+/hi}$)	Reduced representation bisulphite sequencing global methylation analysis Bisulphite sequencing	CpG methylation (whole genome + site-specific)	NKG2 C^{-} and NKG2 C^{+} NK cell subsets from both CMV+ and CMV- donors showed hypermethylation of the CNS1 of the *IFNG* locus NKG2 C^{hi+} NK cells showed: ● demethylation and an open configuration of CNS1 of the *IFNG* locus similar to memory Th1 cells ● enhanced *IFNG* transcriptional activity of NK cells upon triggering of NKG2C ● global methylation profile similar to CD8 + memory and CD4+ Th1 cells	[65]
Cytomegalovirus	122 nonagenerians CMV+ (n = 116) CMV- (n = 6) 21 young controls CMV+ (n = 12) CMV- (n = 9)	Whole blood	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	CMV associated with: ● Accelerated epigenetic age ● Nonagenerians: median epigenetic age 76.0 yrs CMV+ vs. 70.0 yrs CMV- ($p < 0.01$) ● Young controls: median epigenetic age 26.5 yrs CMV+ vs. 24.0 CMV- ($p < 0.02$)	[109]
Cytomegalovirus *Epstein Barr Virus*	Healthy donors	CD8 + T cells	MSP	CpG methylation (site-specific)	Antigen-experienced PD-$1^{hi}CD8^{+}$ T cells generated in response to EBV or CMV infection showed complete demethylation of the *PDCD1*-conserved region relative to naïve CD8 + T cells which showed >70% methylation	[76]
Hepatitis B virus	Acute HBV (n = 26) Chronic HBV (n = 65) Cirrhosis + HBV (n = 45) Hepatocellular carcinoma (n = 21) Controls (n = 95)	PBMC	MSP	CpG methylation (site-specific)	HBV infection associated with: ● increased rate of methylation of CpG island in the pIV promoter region of *CIITA* gene (encodes for MHC-II) ● significant differences observed between ○ HBV–infected participants vs. controls ($p < 0.01$) ○ persistent vs. acute infection ($p < 0.01$)	[81]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Hepatitis B virus	Acute on chronic HBV liver failure (n = 10) Chronic HBV (n = 30) Controls (n = 10)	Whole blood	MSP	CpG methylation (site-specific)	Acute on chronic HBV liver failure (ACHBLF) associated with: ● higher methylation of *GSTM3* (glutathione-s-transferase M3) promoter (30%) compared with chronic HBV patients (6.7%) and controls (0%) (p = 0.02) ● higher malondialdehyde (p = 0.011), model for end-stage liver disease (MELD) scores (p = 0.029) and mortality rate (p = 0.032) in the methylated group compared with the unmethylated group of ACHBLF patients	[92]
Hepatitis B virus	Neonates with HBsAg+ mothers (n = 12) Controls (n = 12)	Umbilical cord blood	Illumina Infinium HumanMethylation450 BeadChip array Pyrosequencing	CpG methylation (whole genome + site-specific)	Maternal HBV infection associated with: ● no significant differences in the average level of global DNA methylation ● differential methylation of 663 CpG sites associated with 534 genes of which 357 had decreased methylation and 306 had increased methylation ● 4 significantly differentially methylated CpG sites in the KLHL35 gene and additional CpGs in the CPT1B gene	[99]
Hepatitis B virus	Chronic HBV on viral suppressive therapy (n = 54) Healthy matched controls (n = 288)	Whole blood	Illumina Infinium HumanMethylation450	CpG methylation (whole genome)	Chronic HBV with viral suppression associated with: ● Age acceleration of 3.5 yrs compared with controls (p = 6.8x10-7)	[30]
Hepatitis B virus *Hepatitis C virus*	Chronic HBV (n = 14–49) Chronic HCV (n = 10–45) Healthy controls (n = 10–51)	NK cells	Bisulphite sequencing	CpG methylation (site-specific)	Adaptive NK cells (FCεRIγ- CD56dim): ● expand in CMV+ chronically HBV–infected patients ● display a distinct methylation pattern of the FCER1 G and IFNG promoter regions	[66]
Hepatitis C virus	HCV (n = 32) HCV/HIV co-infection of antiviral therapy (n = 31) Healthy controls (n = 253)	Whole blood	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Age acceleration (compared to controls): ● 5.02 yrs in HCV/HIV co-infection with hepatic fibrosis (p = 0.029) ● 4.51 yrs in HCV/HIV co-infection without hepatic fibrosis (p = $4.3x10^{-5}$) ● 1.84 yrs in HCV with hepatic fibrosis (p = 0.011) Not significant in HCV without hepatic fibrosis (-0.84 yrs, p = 0.63)	[110]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Respiratory syncytial virus	Children with a history of hospitalization for severe RSV disease in first 2 yrs of life (n = 43) Controls (n = 43)	Whole blood	Bisulphite pyrosequencing	CpG methylation (site-specific)	History of RSV infection associated with: ● lower levels of DNA methylation at 2 CpG loci (E1 and E2) of the *PRF1* proximal enhancer compared with controls (E1 69.5% vs. 79.0%; E2 65.0% vs. 75.0%) ($p < 0.001$) ● lower methylation at E1 in cases with a diagnosis of persistent wheeze prior to hospitalization ($p = 0.04$) ● lower methylation at E2 in cases with a history of maternal asthma ($p = 0.04$)	[31]
Dengue virus	Infected adults (n = 52) Uninfected controls (n = 37)	Whole blood	MSP	CpG methylation (site-specific)	Dengue virus infection associated with: ● increased frequency of demethylation in the TNF-α promoter ● absence of CpG island methylation in the TNF promoter in 32.6% of dengue virus infected subjects compared with 8.1% of uninfected subjects ($p = 0.013$) ● no difference in methylation of the IFN-γ promoter	[91]
Parasites						
Leishmania braziliensis	Skin biopsies taken from lesions and normal skin (n = 8) of subjects with cutaneous leishmaniasis (CL)	Cutaneous biopsy	EpiTect Methyl II PCR	CpG methylation (site-specific)	CL associated with: ● reduced methylation of friend leukaemia virus integration 1 (FLI1) promoter in biopsies from CL lesions compared with normal skin from the same donor ($p = 0.001$) Lesion size and duration: ● negatively correlated with FLI1 promoter methylation ● positively correlated with expression of FLI1 at the mRNA level	[33]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Schistosoma haematobium *Ascaris lumbricoides*	Asymptomatic children with recent intense TB exposure with helminth co-infection (n = 8) and without helminth co-infection (n = 18)	CD4 + T cells	Illumina Infinium HumanMethylation450 BeadChip array EpiTect Methyl II PCR	CpG methylation (whole genome + site-specific)	Active helminth co-infection associated with: ● 751 differentially methylated probes of which 72% were hypermethylated ● DNA methylation perturbation in the immune system in the IL-4 and IFN-γ response, regulation of cytokine production, cell proliferation, mTOR signalling, and glycolysis ● Persistently elevated DNA methylation profiles 6 months after successful eradiation therapy in children with *S. haematobium* infection but not A. lumbricoides infection ● Increased frequency of TB-specific CD4 + T cells producing IL-4 ● Decreased frequency of TB-specific CD4 + T cells producing IFN-γ and TNF	[93]
Plasmodium falciparum	Complicated malaria (n = 20) Uncomplicated malaria (n = 20) Uninfected controls (n = 20)	Whole blood	RP-HPLC Bisulphite sequencing	CpG methylation (whole genome + site-specific)	Lower global DNA methylation associated with: ● High parasitaemia ● Complicated malaria ● Before treatment (compared with after treatment) Decreased methylation of 30 CpG sites in the in the *ABCB1* promoter in malaria patients compared with controls (22.41% vs. 42.35%, $p < 0.05$)	[34]
Plasmodium falciparum	Adolescent males from the Fulani and Mossi ethnic groups with malaria (n = 3 per group) and without malaria (n = 3 per group)	Monocytes	Illumina Infinium HumanMethylation450	CpG methylation (whole genome)	DNA methylation relatively stable between ethnic groups and infected and uninfected subjects	[62]
Trypanosoma cruzi	Chronic Chagas cardiomyopathy (n = 14–25) Organ donors with no underlying cardiac disease (n = 7)	Left ventricular myocardium	Illumina Infinium HumanMethylation450 BeadChip array (n = 14) Pyrosequencing (n = 25)	CpG methylation (whole genome + site-specific)	Chronic Chagas cardiomyopathy associated with: ● No difference in mean methylation compared with controls ● 7595 differentially methylated sites located in 4720 genes of which 65% were hypermethylated and 35% hypomethylated ($p < 10^{-7}$) Pyrosequencing validated 79% of the assays	[35]

(*Continued*)

Table 5. (Continued).

Pathogen	No of participants	Cell type	Analysis techniques	Epigenetic change	Main result	Ref
Clinical infections						
Neonatal sepsis	Clinical sepsis (n = 51) (Culture positive = 36, negative = 15) Controls (n = 37)	Whole blood	5⊠-methylcytosine DNA ELISA	CpG methylation (whole genome)	Neonatal sepsis associated with: ● higher percentage of methylated genomic DNA compared with controls (2.4% vs. 2.07%; $p < 0.0001$) ● higher methylation in cases with a positive blood culture (2.47% vs. 2.23%; $p < 0.05$)	[103]
Neonatal sepsis	Early onset bacterial sepsis (EOS) (n = 8) Early and late onset bacterial sepsis (ELS) (n = 10) Late onset bacterial sepsis (LOS) (n = 10) Isolated infection (n = 5) Controls (n = 16)	Whole blood	MSP	CpG methylation (site-specific)	Bacterial sepsis associated with altered DNA methylation of CpG sites in the *CALCA* gene promotor: ● partial methylation of CpG -769 in EOS and LOS (both gram positive and gram negative organisms) ● Demethylation of 8 CpGs in gram negative EOS and ELS ● Demethylation of 7 CpGs in gram positive EOS and ELS	[102]
Periodontitis *Porphyromonas gingivalis*	Periodontitis (n = 4)	Gingival epithelial cells	Bisulphite sequencing	CpG methylation (site-specific)	Periodontitis associated with increased *TLR2* promoter methylation in affected compared with healthy gingival tissue	[87]
Periodontitis *Porphyromonas gingivalis*	Periodontitis (n = 20) Controls (n = 20)	Gingival epithelial cells	MSP	CpG methylation (site-specific)	● hypermethylation of *TLR2* promoter ($p \leq 0.001$) and low gene expression ($p = 0.003$) in periodontitis samples ● negative correlation between methylated DNA frequency and *TLR2* transcript level in all samples x($p = 0.01$) ● positive correlation between *TLR2* methylation frequency and inflammatory cell numbers in all samples ($p < 0.023$)	[88]
Chorioamnionitis	Preterm chorioamnionitis cases (n = 12) Preterm births without other pathology (n = 12)	Chorionic villi	Illumina Infinium HumanMethylation450 BeadChip array	CpG methylation (whole genome)	Chorioamnionitis associated with: ● 18 differentially methylated sites, the majority of which were associated with immune system genes including Orosomucoid 1	[100]

HP, *Helicobacter pylori*; GBS, Group B streptococcus; AIDS, acquired immunodeficiency syndrome; GO, gene ontology; ART, antiretroviral therapy; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HBsAg, Hepatitis B surface antigen.

and diversity of the placental microbiome also influences DNA methylation patterns in placental tissue. A study examining 16 bacterial species in the placentas of 84 preterm infants found methylation signatures unique to the particular bacterial species present [101]. The differentially methylated genes are involved in immune and inflammatory responses and are enriched in the NF-κB signalling pathway that is critical for foetal development.

In preterm infants, altered DNA methylation patterns within the *CALCA* gene promoter, that codes for calcitonin, are associated with early and late onset neonatal sepsis [102]. A clinical diagnosis of neonatal sepsis is also associated with increased methylated genomic DNA [103]. Uropathogenic *E. coli* infection results in increased methylation of *CDKN2A* exon 1, that may have clinical utility as a biomarker for urinary tract infection susceptibility and recurrence [104].

Biological ageing

The 'epigenetic clock' is a validated biomarker of ageing that comprises a linear combination of 353 CpG sites. It is applicable to most human cell and tissue types and is strongly correlated with chronological age throughout life [1]. Advanced 'epigenetic age' is associated with reduced physical and mental fitness and is prognostic of all-cause mortality in later life [105,106]. Chronic viral infection has been shown to induce biological age acceleration, as measured by the epigenetic clock. Chronic treated HIV infection is associated with global dysregulation of the methylome in blood and an average biological age advancement of approximately 5 years [107]. In brain specimens from HIV subjects, this advancement is more than 7 years [108]. Similarly, CMV infection is associated with accelerated epigenetic age [109]. In chronic HCV infection age acceleration is only seen in the presence of fibrosis or co-infection with HIV [110]. Using a peripheral blood immunophenotyping model to infer epigenetic age, slowing of age acceleration was observed in HBV or HCV mono-infection with sustained virological response on therapy [110]. In the study examining the influence of chronological age on influenza vaccine responses there was no difference in the epigenetic age of vaccine responders and non-responders [54].

Disease risk

The modulation of long-term disease risk by infection or bacterial colonization may be epigenetically mediated in several disease processes. Whole genome methylation analysis of pregnant women with different gut microbiota profiles found an association between predominant gut bacterial phyla and methylation patterns. A predominance of *Firmicutes* was associated with differential methylation of gene promoters linked to cardiovascular disease and metabolic syndrome [111].

Epigenetic variations may also play a role in susceptibility to malaria and efficacy of anti-malarial drugs. Analysis of whole blood from healthy controls and *P. falciparum* infected subjects with varying severities of clinical disease found lower global DNA methylation is associated with untreated disease, complicated malaria and higher parasitaemia. Malaria affected subjects also had specific demethylation of the *ABCB1* promoter which codes for P-glycoprotein, an efflux protein that is proposed to modulate the effect of antimalarial drugs on intracellular parasites.

Early life viral respiratory infections, including RSV and HRV, are implicated in the development of childhood asthma [112]. Children with a history of severe RSV bronchiolitis in infancy have decreased methylation of CpG sites within the proximal enhancer of the *PRF1* gene. This gene codes for perforin, which is involved in the cellular immune response to viral infection. Decreased methylation of these CpG sites is also independently associated with persistent wheeze and maternal asthma. This methylation signature may be a potential biomarker for the development of complications of severe RSV infection, including persistent wheeze and asthma. *In vitro* HRV infection of nasal epithelial cells from children with and without asthma showed genome-wide differences in DNA methylation. These differences were associated with altered transcription of genes involved in the host immune response to viral pathogens and asthma pathogenesis [113]. These findings support the theory that HRV infection may contribute to the persistence and progression of asthma via epigenetic mechanisms.

Conclusion

Our understanding of the complex interaction between vaccines and infectious pathogens, and

modification of the human epigenome is rapidly evolving. Until recently, epigenetic research has focused predominantly on epigenomic profiling with functional inference made through correlation with modifying enzymes and gene transcription. New technological advances enable the manipulation and 'editing' of epigenomic features that allow causality and function to be directly established [114]. Refined methods of epigenomic profiling will more clearly define the role of vaccines and pathogens in the dynamic regulation of the epigenetic landscape. A more comprehensive understanding of this interplay will identify potential targets to modulate the immune system and novel therapies to treat chronic infection. This is particularly important in vaccine development where the potential heterologous effects of vaccines may have significant implications on immune regulation. Future research needs to focus on the epigenetic impact of vaccines in early childhood, when the epigenome is particularly susceptible to modification and the consequences on immune maturation and development are most significant. This will both inform the development of new vaccines and guide global vaccine policy.

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Authors' Contributions

SB formulated the research question, performed the literature search, conducted data analysis and drafted and edited the manuscript. NM contributed to data analysis and interpretation and revision of the manuscript. BN contributed to drafting and revision of the manuscript. NC formulated the research question and contributed to drafting and editing of the final manuscript. All authors read and approved the final manuscript.

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