The role of microorganisms in atopic dermatitis

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Summary

Atopic dermatitis (AD) is a common, fluctuating skin disease that is often associated with atopic conditions such as asthma and IgE-mediated food allergy and whose skin lesions are characterized by a Th-2 cell-mediated response to environmental antigens. The increasing prevalence and severity of atopic diseases including AD over the last three decades has been attributed to decreased exposure to microorganisms during early life, which may result in an altered Th-1/Th-2-balance and/or reduced T cell regulation of the immune response. Patients with AD exhibit defects in innate and acquired immune responses resulting in a heightened susceptibility to bacterial, fungal and viral infections, most notably colonization by *S. aureus*. Toxins produced by *S. aureus* exacerbate disease activity by both the induction of toxin-specific IgE and the activation of various cell types including Th-2 cells, eosinophils and keratinocytes. Allergens expressed by the yeast *Malazessia furfur*, a component of normal skin flora, have also been implicated in disease pathogenesis in a subset of AD patients.

Microorganisms play an influential role in AD pathogenesis, interacting with disease susceptibility genes to cause initiation and/or exacerbation of disease activity.
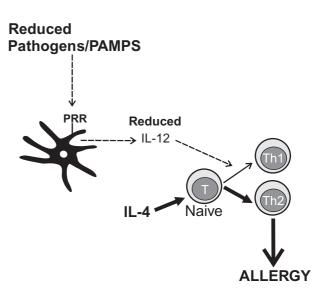
Introduction

Atopic dermatitis (AD) is a common, chronic fluctuating skin disease with prevalence in children of between 7 and 17%. The disease presents commonly within the first 2 years of life, and in approximately two-thirds of cases will persist into adulthood. A high proportion of infants with AD have a positive family history of AD, asthma or allergic rhinitis in one or both parents, and will go on to develop further atopic complications later in childhood. AD is a multifactorial disease in which both hereditary and environmental factors play a role. Four chromosomal regions of linkage to the disease, which differ from atopy-associated loci, have been identified in genome scans of children with AD [1,2]. Interestingly, these linkage sites closely correspond to four loci reported to contain susceptibility genes for psoriasis suggesting that they may contain genes that control skin inflammation and immunity in both diseases [2]. In addition, several candidate genes coding for proteins with immunological function, such as the high affinity IgE receptor present on mast cells, Th-2 cytokines, RANTES and IRF2 [3–8], and receptors involved in the response to microorganisms,

CARD4/Nod1, CARD15/Nod2, TLR2 and TLR4 [9–11] have been implicated in AD. Environmental factors that interact with susceptibility genes in AD, inducing the production of IgE antibodies and activation of Th-2 cells, include foods, house dust mite (HDM) allergens, secondary microbial infections and stress. This review focuses on the role of microorganisms in AD, and discusses the mechanisms involved in the prevention, initiation and exacerbation of disease activity by microbial components.

The hygiene hypothesis

Over the last 30 years there has been a continuously increasing prevalence of AD and other atopic allergic disorders, especially in western industrial countries where it can be as high as 20–37% of the population [12]. Environmental factors such as increased air pollution, indoor exposure to HDM antigens in less ventilated modern homes, and dietary changes have been implicated, but there is little consistent evidence that these factors account for the observations. A more relevant factor appears to be a reduction in the exposure to infections during early childhood, which may result

from modern lifestyle factors such as the use of antibiotics, a reduction in family size (allergic sensitization is higher in the first-born, but is less frequent in children from large families), and an increase in hygiene and living standards [13]. The relationship between decreased exposure to microbial antigens associated with a western lifestyle and the increasing severity and prevalence of atopic diseases has become known as the 'hygiene hypothesis' [14]. Innate immune cells, such as macrophages and dendritic cells, express pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) associated with microorganisms; activation of these receptors induces a Th-1 type response. It was therefore suggested that a lack of microbial antigen-induced immune deviation from the Th-2 cytokine profile that predominates at birth to a Th-1 type profile could explain the development of enhanced Th-2 cell responses to allergens (Fig. 1) [15]. However, this explanation did not did not take into account the fact that chronic parasitic worm (helminth) infections which induce strong Th-2 responses and high IgE levels are not associated with allergy, or that the prevalence of Th-1-associated autoimmune diseases have also increased at the same time as allergic diseases [16].

Subsequently, a second mechanism was proposed for the switch to an atopic phenotype; a defect in the stimulation of dendritic cells by nonpathogenic microorganisms in gut-associated lymphoid tissue leading to reduced production of IL-10-producing regulatory T cells (Fig. 1) [16,17]. Differences in the bacterial colonization of the gut have been reported in children with AD; *Enterococci* and *Bifidobacteria* were reduced, and *Clostridia* and *Staphylococcus aureus* numbers increased [18,19]. These changes have been attributed, at least in part, to a frequent use of antibiotics, which has consistently been shown to markedly increase the risk of developing AD, even into the antenatal period [20]. This has led to the use of diet supplementation with *Lactobacillus* (marketed as probiotics) in both the prevention and treatment of AD [20]. A randomised, controlled trial in which oral *Lactobacillus* CG supplementation was given prenatally to mothers with an allergic family history, and postnatally for 6 months to their offspring, resulted in an almost 50% reduction in AD frequency [21]. Furthermore, a significant reduction in SCORAD (disease severity scoring of atopic dermatitis) after one month of *Lactobacillus* GG supplementation was observed in a randomised controlled trial of 31 infants with AD and cow's milk intolerance [22]. It was subsequently demonstrated that *L. reuteri* and *L. casei* prime monocyte-derived dendritic cells, via DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing nonintegrin), to drive the development of IL-10-producing regulatory T cells [23]. These findings suggested a possible mechanism for the beneficial effects of *Lactobacillus* administration in AD, and provided support for the proposal that reduced T cell regulatory activity may be responsible for the increased prevalence of allergy.
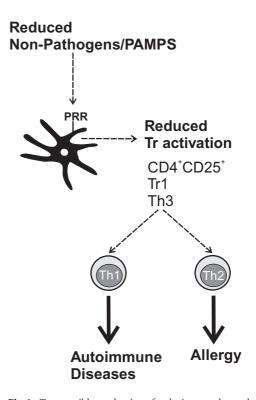

Fig. 1. Two possible mechanisms for the increased prevalence of allergy as a result of decreased exposure to microbial antigens.

Although a recent review of 64 studies published between 1966 and August 2004 failed to find any convincing evidence that exposure to a specific infection such as tuberculosis reduces the risk of AD, there was, in contrast, some evidence

that common viral and bacterial childhood infections were positively associated with an increased risk of AD expression [20]. However, a protective effect was associated with endotoxin exposure in a farming environment, day care attendance or having a dog during infancy, situations in which chronic (nonpathological) microbial stimulation may take place. Thus it is possible that the effects of infections in early childhood on the development of AD may depend partly upon the nature and degree of exposure, rather than infection *per se*. Clearly further research into the early priming of a child's immune system by microorganisms is necessary in order to establish the factors required to induce the development of AD.

Microbial colonization of AD skin

Patients with AD are highly susceptible to certain cutaneous bacterial, fungal and viral infections [24]. AD patients have an increased incidence of warts caused by the human papillomavirus, and of cutaneous fungal infections such as that caused by *Trichophyton rubrum*. They are particularly susceptible to severe infections caused by herpes simplex type 1 virus (eczema herpeticum or Kaposi's varicelliform), vaccinia virus (eczema vaccinatum) coxsackieA virus and molluscum contagiosum virus. These viral infections can represent serious complications in AD, and if not treated promptly have the potential to be life threatening. However, bacterial colonization with *Staphylococcus aureus* is the most common skin infection in AD (> 90% of patients compared to 5% of normal individuals) and occurs on both lesional and, to a lesser extent, nonlesional AD skin [25,26]. Furthermore, there appears to be a causative relationship between the numbers of bacteria present on the skin and the severity of disease in AD patients, whilst treatment-induced removal of the bacteria is associated with improvement in skin lesions in most cases [27,28]. Various factors are involved in the altered skin colonization by *S. aureus* in AD including an altered epidermal barrier, increased bacterial adhesion, defective bacterial clearance, and decreased innate immune responses.

S. aureus are tightly attached to the uppermost corneocytes, and can penetrate the epidermis via the intercellular spaces probably as a result of lipid deficiencies in AD skin. In AD, the average pH of the skin is slightly more alkaline, and sphingosine levels are decreased in both lesional and nonlesional stratum corneum [29,30]. In addition, the dryness and cracking of AD skin, as a result of transepidermal water loss caused by altered lipid content, may facilitate bacterial colonization. Furthermore, Th-2 cytokines such as IL-4 in atopic skin increase expression of fibronectin and fibrinogen, receptors that mediate the adhesion of *S. aureus* to stratum corneum [31]. In a proportion of AD patients who respond poorly to anti-inflammatory treatment, persistent *S. aureus* colonization is associated with higher total IgE levels suggesting that IgE may contribute to an increased susceptibility to infection [28]. Indeed, IgE has been shown to inhibit neutrophil adhesion, phagocytosis and respiratory burst, which may affect clearance of microorganisms from the skin [32]. A major factor in increased *S. aureus* colonization of AD skin is a defective cutaneous innate immune response, involving decreased production of antimicrobial peptides and the expression of functional variants of the TLR and Nod/CARD receptors for microbial components (Fig. 2).

Defective innate immunity in AD: antimicrobial peptides

The innate immune system of the epidermis is the first line of defence against invasion by microorganisms, which gain entry after the skin is damaged. Anti-microbial peptides form part of this defence system, three of which are triggered by injury or inflammation of the skin: the β defensins HBD-2 and HBD-3, and a cathelicidin, hCAP18/LL-37 [33]. HBD-2 shows microbicidal activity against predominately Gram-negative organisms such as *E. coli* and *P. aeruginosa*, and yeasts, but is relatively ineffective against Gram-positive bacteria such as *S. aureus* [34]. In contrast, HBD-3 and hCAP18/LL-37 are more potent, broad-spectrum antibiotics that kill both Gram-positive and Gram-negative organisms and the yeast *C. albicans* [35,36]. In addition, HBD-2 can enhance the innate immune response of the epidermis, and provide a link with the acquired immune response by inducing up-regulation of costimulatory molecules and the maturation of immature dendritic cells in a TLR4-dependent manner [37]. Beta-defensins can also act as chemoattractants for immature dendritic cells and memory T lymphocytes via the chemokine receptor CCR6 [38].

Immunostaining, measurement of specific mRNA by real-time reverse-transcriptase-PCR or GeneChip microarray analysis for HBD-2, HBD-3 and hCAP18/LL-37 in acute and chronic lesions from patients with AD showed a significant decrease in expression as compared to that of psoriasis patients [39,40]. IL-4 and/or IL-13, has been shown to suppress the TNF-α or IFN-γ-induced up-regulation of HBD-2 and HBD-3 mRNA in keratinocytes and normal skin explants suggesting that the reduced levels of antimicrobial peptides may be explained by the predominance of Th-2 type cytokines in AD skin lesions [40]. Clinical isolates of *S. aureus* from AD patients can be killed by a combination of HBD-2 and hCAP18/LL-37 at the concentrations found in psoriatic lesions, but the levels present in AD skin are too low to be effective.

The innate skin defence system of patients with AD is further compromised by a deficiency of dermcidin-derived antimicrobial peptides in sweat, which correlates with infectious complications [41]. Dermcidin, a peptide with no homology to other antimicrobial peptides, is specifically and constitutively expressed in sweat glands in the dermis of skin,
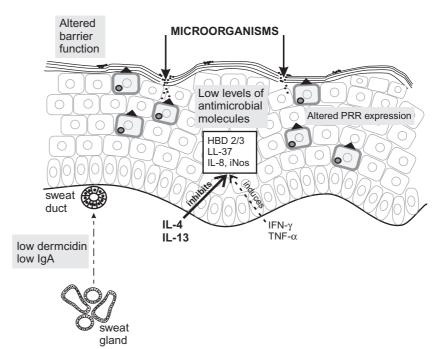
Fig. 2. Defective innate immunity in AD skin. Altered intracellular and extracellular PRR expression, and decreased antimicrobial molecule production (partly due to inhibition by Th-2 cytokines) results in an impaired innate immune response to microorganisms, which gain entry to the epidermis as a result of an altered barrier function. HBD-2/HBD-3, human β-defensin-2/3; LL-37, a member of the cathelicidin family; iNos, induced nitric oxide synthetase.

secreted into sweat and transported to the epidermal surface [42]. In common with HBD-3 and hCAP18/LL-37, dermcidin has a broad spectrum of activity against a variety of pathogenic microorganisms. In healthy individuals, a significant reduction in viable bacterial cells on the skin surface occurs after sweating, but this is not the case in patients with AD [41]. Furthermore, the rate of sweat production, and the secretion of IgA in sweat are reduced in AD patients contributing to the impaired innate immune response [43,44].

The expression of the innate immune response genes, IL-8 (CXCL8) and induced nitric oxide synthetase (iNos) was also decreased in AD compared to psoriatic skin [40]. IL-8 is a chemokine that attracts polymorphonuclear leucocytes into the skin where they phagocytose and kill bacteria, whilst iNos can kill viruses, bacteria and fungi through production of nitric oxide. In common with the antimicrobial peptides, production of IL-8 and iNos is also inhibited by Th-2 cytokines.

Defective innate immunity in AD: TLR and Nod/CARD proteins

Toll-like receptor 2 (TLR2) and TLR4 are members of a family of PRRs that recognize various conserved microbial components or PAMPs. TLR2 recognizes components of Gram-positive bacteria and yeasts, such as lipotechoic acid, peptidoglycan (although recent evidence suggests that this may be contaminating lipoteichoic acid [45]), lipoproteins or zymosan, whilst the PAMPs detected by TLR4 include the Gram-negative bacterial component, LPS [46]. Recognition of PAMPs by TLRs initiates a signalling cascade that results in the production of proinflammatory cytokines, chemok-

ines, antimicrobial peptides and inducible enzymes in the skin, via activation of transcription factors, activator protein (AP)-1 and nuclear factor (NF)-κB [47]. Two single nucleotide polymorphisms (SNPs) have been described for each of the receptors, which result in changes in amino acid sequences. One of the TLR2 polymorphisms (Arg753Gln), which is located within the intracellular part of the receptor and has been particularly associated with *S. aureus* infections, was found to be present in a higher frequency in AD patients compared to controls [11]. The subgroup of AD patients carrying this polymorphism had increased disease severity characterized by markedly elevated IgE antibodies to *S. aureus* superantigens and HDM allergens. In addition, a further subgroup of AD patients expressed a higher frequency of the two TLR4 polymorphisms, Asp299Gly and Thr399Ile, than controls [11]. These cosegregating polymorphisms, located in the extracellular domain of the receptor, have previously been reported in patients with septic shock, particularly that induced by Gram-negative bacteria, and are linked to LPS hyporesponsiveness [48].

Intracellular PRRs are represented by the Nod (nucleotide-binding oligomerization domain) family, which includes Nod2/caspase recruitment domain containing protein (CARD) 15 and the closely related Nod1/CARD4 protein. Both proteins detect peptidoglycan, the major component of the bacterial cell wall, although the specificities of the two receptors are distinct and nonoverlapping [49]. Polymorphisms in the Nod2/CARD15 gene that result in changes in peptidoglycan recognition have been reported to be associated with susceptibility to Crohn's disease, a Th-1-mediated inflammatory disease of the bowel [50,51]. Three of these Crohn's-associated Nod2/CARD15 polymor-

phisms have been investigated in children with asthma and allergy by PCR-based restriction enzyme assays [10]. Children with the polymorphic C2722 allele had a more than 3-fold risk to develop allergic rhinitis and an almost 2-fold risk for AD. More recently, an association of Nod1/CARD4 polymorphisms with AD have been reported in a study that examined the effects of 11 SNPs, covering the complete gene, on atopy phenotypes [9]. One Nod1/CARD4 haplotype and three polymorphisms (rs2907748, rs2907749, rs2075822) were significantly associated with AD in a population-based cohort, case-control population, and/or family–based association analysis. These polymorphisms were also associated with asthma and total serum IgE levels, but not with allergic rhinoconjunctivitis or specific sensitization.

Peptidoglycan from *S. aureus* has been shown to induce the production of various keratinocyte-derived mediators including GM-CSF, a cytokine that is overproduced in AD skin lesions [52]. It remains to be established whether this occurs via PRR stimulation; however, keratinocytes are known to express several members of the TLR family [47], and intracellular Nod/CARD receptors may also be present in these cells.

Thus the reduced production of antimicrobial peptides and other innate immune factors, together with an impaired recognition of microbial antigens as a result of functional polymorphisms in the genes coding for PRRs, are major factors contributing to the susceptibility to infection and resulting exacerbation of disease activity in patients with AD.

Cellular activation by staphylococcal superantigens

The main consequence of increased colonization of AD skin by *S. aureus* is exacerbation of the inflammatory immune response, which is largely mediated by the release of staphylococcal enterotoxins (SE), such as SEA, SEB and toxic shock syndrome toxin (TSST)-1, also referred to as 'superantigens' [53] (Table 1). SEB applied to intact normal skin or the non-lesional skin of patients with AD can induce erythema and dermatitis, and, in some AD patients, a flare of their disease in the elbow flexure of the same arm to which the toxin was applied [54]. Furthermore, 14 of 68 patients recovering from toxic shock syndrome caused by TSST-1, but no patients recovering from Gram-negative sepsis, developed chronic eczematous dermatitis [55]. These findings suggest that superantigens can initiate, exacerbate and maintain inflammation associated with AD.

The major effects of staphylococcal superantigens in AD are likely to be mediated via the polyclonal activation of superantigen-specific TCR Vβ families of T cells. T cells expressing Vβ chains specific for the superantigen accumulate selectively in superantigen-treated skin, but not in skin treated with sodium lauryl sulphate, supporting this hypothesis [56]. In the peripheral blood of AD patients whose skin is colonized by superantigen-secreting *S. aureus*, a relevant skewing of superantigen-reactive Vβ families was observed in CD4+ and CD8+ T cells coexpressing cutaneous lymphocyte antigen (CLA), a skin homing receptor [57]. Superantigens up-regulate CLA expression by T cells via stimulation of IL-12 production, thus promoting their homing to the skin [58].

T cell activation by superantigens may be further augmented by an inhibitory effect (at least by SEB) on the immunosuppressive activity of circulating CLA+CD4+ CD25+ regulatory T cells, which, surprisingly, are increased in patients with AD [59]. Furthermore, SEB-reactive (Vβ3+, Vβ12+ or Vβ17+) CD4+ T cells producing Th-2 cytokines in

Table 1. Role of staphylococcal superantigens in AD.

Superantigen-induced effects	Functional consequences
Application of SEB to uninvolved skin	Induces local erythema and dermatitis
	Sometimes causes disease flare
Superantigen-specific IgE production	Release of histamine by mast cells and basophils
	Facilitates toxin presentation by Langerhans cells to T cells
	Correlates with disease severity
SEC1-specific IgG$_2$ production	Decreased in AD; may affect phagocytosis of *S. aureus* by polymorphonuclear leucocytes
Selective Vβ family expansion in lesional skin and blood	Activation of Th-2 cells in a Vβ-specific manner
CLA expression on activated Th-2 cells, circulating and in lesional skin	Induces homing of T cells to skin
Apoptosis of superantigen-reactive T cells	SEB-reactive Th-2 cells in AD are apoptosis-resistant, thus helping to maintain information
Inhibition of CLA+CD4+CD25+ regulatory T cell activity	Increases inflammation
Dermal eosinophils	Inhibits apoptosis, increases expression of surface activation antigens and enhances oxidative burst
Langerhans cells and macrophages	Stimulates production of IL-1, TNF-α and IL-12
HLA-DR+ keratinocytes	Transient Ca^{2+} mobilization
	TNF-α production
	Presentation of superantigen to Th-2 cells

AD patients are more resistant to SEB-induced apoptosis than corresponding SEB-reactive Th-1 cells from healthy individuals [60].

In addition to T cells, superantigens can also mediate effects on other cell types such as eosinophils, Langerhans cells, macrophages and keratinocytes. During flares of AD, eosinophils are recruited to the skin by chemoattractants such as RANTES (regulated on activation, normal T expressed and secreted) and eotaxin, where they are activated and undergo degranulation and cytolytic degeneration, the products of which promote inflammation and tissue damage [61,62]. It has been demonstrated that SEB in AD skin lesions is localized predominately to eosinophils in the dermis, as well as to a lesser extent on Langerhans cells and IgE-bearing cells [63]. Superantigens modulate the effector function of eosinophils, and probably the course of AD, by inhibiting eosinophil apoptosis, increasing expression of activation antigens on the eosinophil surface, and enhancing oxidative burst of eosinophils *in vitro* [64]. They also bind to HLA-DR on Langerhans cells and macrophages and stimulate them to produce IL-1, TNF-α and/or IL-12. These cytokines either up-regulate the expression of adhesion molecules on endothelial cells, or increase CLA expression on T cells, respectively, thus facilitating the recruitment of CLA$^+$ memory T cells to the skin. In addition, keratinocytes that have been induced to express MHC Class II molecules by stimulation with IFN-γ can interact with superantigens, resulting in transient intracellular Ca^{2+} mobilization and the release of proinflammatory TNF-α [65,66]. HLA-DR$^+$ keratinocytes can also present superantigens to T cells; because KCs do not synthesize IL-12, this results in the activation of Th-2 rather than Th-1 cells [67]. Two other *S. aureus* products, staphylococcal protein A and α-toxin also induce the production of TNF-α by keratinocytes, with additional cytotoxic effects exerted by the latter [68].

Superantigen-specific IgE and IgG$_2$ antibodies

Superantigen-secreting *S. aureus* have been isolated from over 50% of AD patients, and many of these patients produce IgE antibodies specific for the toxins found on their skin [69,70]. In contrast, although SEA, SEB or TSST – secreting *S. aureus* have also been isolated from the lesional skin of psoriasis patients, their sera did not contain IgE antibodies to the toxins [69]. Basophils and mast cells from AD patients with antitoxin IgE antibodies release histamine on exposure to toxins, but only those toxins against which they have raised specific IgE antibody [69]. Furthermore, there is a correlation between the presence of IgE antibodies specific for staphylococcal superantigens and both the severity of AD, and total serum IgE levels [71]. Thus toxins produced by *S. aureus* also exacerbate AD by activating mast cells, basophils and other Fcϵ-receptor bearing cells carrying the relevant antitoxin IgE antibody.

Conversely, a subgroup of patients with AD showed a deficiency in the production of IgG$_2$, but not of IgG$_1$ or IgG$_4$ antibodies against toxin SEC$_1$, which was associated with a severe disease phenotype [71]. This appeared to be specific for SEC$_1$ as levels of IgG$_2$ antibodies against SEB, or another bacterial antigen, pneumococcal capsular polysaccharide, were normal in these patients. IgG$_2$ antibodies are poor complement activators, but can effectively mediate polymorphonuclear leucocyte phagocytosis of *S. aureus* via the FcγRIIa receptor on the cell surface [72]. Although the functional role of anti-SEC$_1$ IgG$_2$ antibodies in AD pathogenesis remains unclear, it is possible that such a selective decrease in some patients with AD may contribute to the persistence of SEC$_1$-producing *S. aureus* on lesional skin and the resulting exacerbation of disease activity.

Malassezia-induced IgE- and Th-2 cell-mediated responses

Malassezia (formerly known as *Pityrosporum orbiculare/ovale*) is part of the normal human skin flora and is most abundant at sites of high sebum production such as the scalp, chest and back where it colonizes the stratum corneum. Most healthy individuals have developed IgG antibodies to *Malassezia*, but in 30–80% of AD patients, IgE and/or T cell reactivity to the organism is present [73]. Patients with AD affecting mainly the head and neck region appear to be more likely to produce *Malassezia*-specific IgE antibodies, coinciding with the higher levels of yeast colonization in these areas, than patients with AD located elsewhere on the body [74]. *Malassezia*-specific IgE antibodies are rarely produced by atopic patients whose skin is unaffected [75,76]. This, together with the high prevalence of type I hypersensitivity to *Malassezia* as compared to other fungi in AD suggests that *Malassezia*-specific antibodies are pathogenically significant [77,78]. Histamine release tests have confirmed the biological activity of circulating *Malassezia*-specific IgE antibodies in approximately 70% of AD patients, supporting a role for these antibodies in the disease process [78].

The defective skin barrier in AD may allow the whole *Malassezia* yeast cells and their allergens, nine of which have been isolated and cloned so far, to enter the skin and be taken up by Langerhans cells. *In vitro* studies have shown that the process of internalization of *Malassezia* causes maturation of dendritic cells and the production of proinflammatory and immunoregulatory cytokines, but not IL-12, thereby favouring the induction of a Th-2 type response [79]. Indeed, higher blood and skin Th-2 type responses to *Malassezia* have been demonstrated *in vitro* in AD patients compared to that of normal controls [80]. Furthermore, it has been demonstrated that a positive atopy patch test to *Malassezia in vivo* correlates with a Th-2-like peripheral blood mononuclear cell response in AD patients *in vitro* [81].

Malassezia also exerts other proinflammatory effects such as activation of the alternative complement pathway, and the

stimulation of keratinocytes to produce a variety of inflammatory cytokines such as IL-6, IL-8 and TNF-α, which may contribute to its role in AD pathogenesis [82,83]. However, antifungal treatment studies have so far proved inconclusive as to the clinical relevance of *Malassezia*-induced allergy in AD. This may be explained by a lack of selection of relevant patient subgroups and the use of inappropriate measures of clinical outcome [84].

Conclusion

AD is a chronic inflammatory skin disease whose initiation and clinical activity is modified by exposure to, and interaction with, microorganisms. In genetically predisposed individuals a lack of chronic exposure to microbial antigens in early life increases the risk of disease. Once manifested, defects in skin immune surveillance mechanisms such as decreased production of antimicrobial peptides and the expression of functionally altered PRRs result in exacerbation of disease activity by skin colonizing microorganisms, particularly *S. aureus* and *Malassezia furfur*. Manipulation of the innate immune response to microorganisms could provide a novel approach to the treatment of AD, as suggested by the beneficial effects of administration of probiotic bacteria in preliminary studies. TLR ligands are also being tested for their ability to shift an allergen-specific Th-2 immune response to that of protective Th-1 immunity, although the long-term effects of such a strategy are, at present, unknown [85].

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