

Microbial colonization and atopic dermatitis

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Purpose of review

Atopic dermatitis is a chronic relapsing, pruritic inflammation of the skin, affecting 10–20% of children and 1–3% adults worldwide, with increasing prevalence in highly industrialized countries. Here we review relevant studies, published since June 2002, about immunological triggers in atopic dermatitis, with emphasis on the role of microbial colonization.

Recent findings

During the past 2 years there has been considerable interest in the mechanisms and trigger factors underlying the increased microbial colonization of atopic skin. *Staphylococcus aureus* appears to play a significant role as it leads to a worsening of disease severity by producing superantigens that induce a strong proliferation of T cells and favour a T helper type 2-like cytokine profile. In addition, different *Malassezia* species seem to elicit and maintain skin inflammation after sensitization, but the precise immunological pathway has not yet been described. All these microorganisms are not only perceived as aetiological factors but also as agents responsible either for sustained disease activity or resistance to therapy by modulation of the immune response.

Summary

New insights into the important role of microorganisms and their key immunomodulatory pathways in atopic dermatitis may have important implications from a therapeutic point of view because patients with atopic dermatitis may benefit from more than just anti-inflammatory treatment in the future.

Keywords

atopic dermatitis, *Malassezia*, microbial colonization, *Staphylococcus aureus*, superantigen

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Abbreviations

AD	atopic dermatitis
APC	antigen-presenting cell
APT	atopy patch test
SPT	skin prick test

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Introduction

Atopic dermatitis (AD) is a chronic relapsing, highly pruritic inflammation of the skin, with a worldwide prevalence in children of 10–20% and in adults of 1–3% [1]. During the past few years an increased prevalence in highly industrialized countries has been recognized, particularly in upper social classes and urban regions [2].

The manifestations of AD result from a complex interaction between environmental factors, pharmacological abnormalities, skin barrier dysfunction, susceptibility genes and immunological phenomena, which have been confirmed in numerous experimental and epidemiological studies.

Among several contributing factors, cutaneous hyper-reactivity, inappropriate immune response to various bacteria, fungi and other microorganisms, and secondary infections appear to play an important role, not only in the underlying pathology but also as a factor responsible for sustained disease activity.

Erythema, serous exudation and excoriation characterize the acute eczematous lesions, whereas lichenification and dry, fibrotic papules are a chronic manifestation of AD. Clinical signs of secondary infected AD are impetiginization and superficial pustules.

As AD often leads to asthma and allergic rhinitis (so-called ‘atopy march’) [3], an anti-inflammatory therapy of microbial colonization is of utmost importance in the management of AD and the development of new treatment approaches.

Staphylococcus aureus

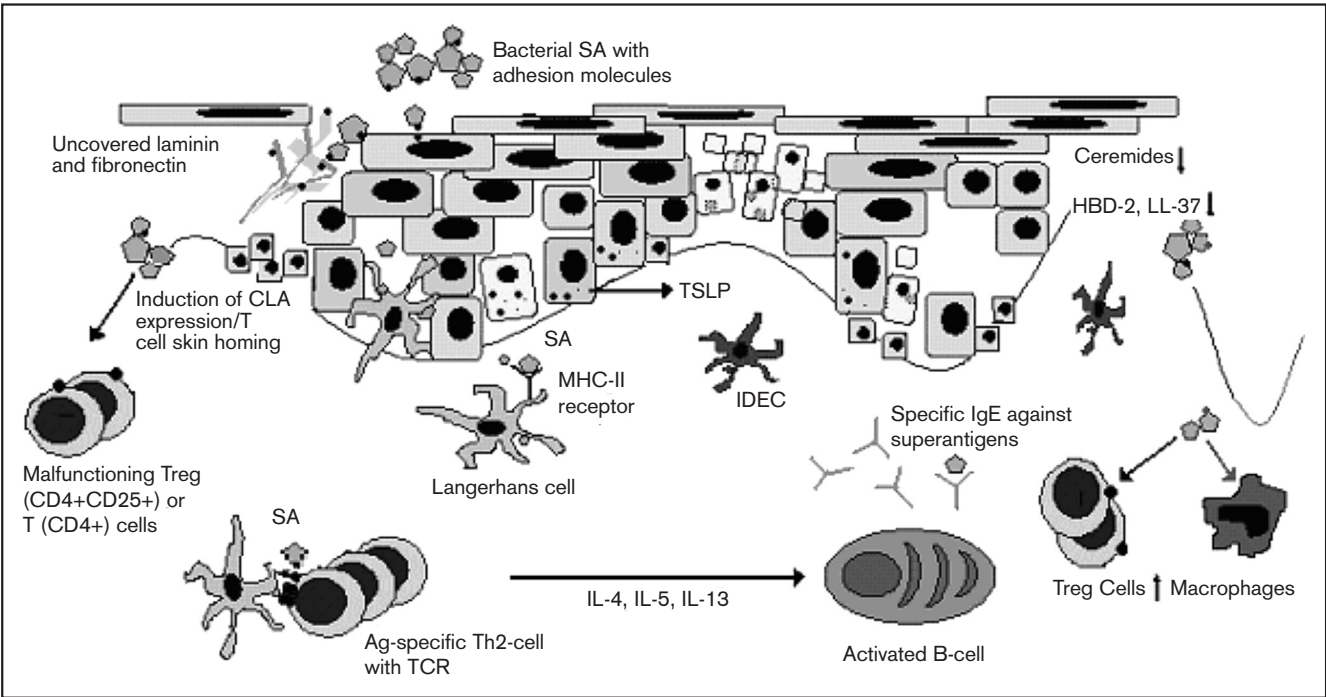
As a result of an altered skin structure characterized by skin barrier dysfunction, the inflammation of the upper dermis and immunodeficiencies, *Staphylococcus aureus* is one of the predominant bacteria in patients with AD. Many studies have shown that the extent of *S. aureus* colonization correlates with AD disease activity [4]. In more than 90% of AD patients a massive skin colonization with *S. aureus* (up to 10^7 colony-forming units per square centimetre of lesional skin) can be found [5]. This is an impressive finding, knowing the prevalence of *S. aureus* of approximately 5% in healthy individuals [6]. *S. aureus* can be isolated from lesional skin, especially from intertrigous regions, the nose (‘nasal carriage’), as well as unaffected atopic skin. Next to mechanical triggers (scratching), predominantly alkaline pH and decreased IgA secretion through sweat production, there

are several important pathophysiological features leading to the disruption of the primary skin defence system [7,8]. Various factors contributing to increased numbers of *S. aureus* in atopic skin and the perpetuation of inflammation have recently been discovered: altered lipid composition within the stratum corneum; exposed extracellular matrix adhesins; changes in immune response; bacterial superantigens and increased specific IgE production (see Fig. 1 and Table 1).

Defective lipid layer and upregulated expression of molecular adhesins

As a result of the reduced activity of acid ceramidase and decreased levels of ceramides, there are decreased levels of sphingosine in the stratum corneum in patients with AD. This favours colonization with *S. aureus*, because sphingosine, under normal circumstances, exerts a potent antimicrobial effect on *S. aureus*. Arikawa *et al.* [10] reported an approximately 200-fold increase in *S.*

Figure 1. Influences of bacterial superantigens on atopic skin



Ag, antigen; CLA, cutaneous lymphocyte-associated antigen; HBD-2, β -defensin 2; IDEIC, inflammatory dendritic epidermal cell; LL-37, cathelicidin; SA, superantigen; TCR, T-cell receptor; Treg, T regulatory cell; TSLP, human thymic stromal lymphopoietin. Modified from Fischer *et al.* [9], with permission.

Table 1. Immunomodulatory causes and consequences of microbial colonization in atopic dermatitis

	Bacterial agents	Fungal agents
Example	(1) <i>S. aureus</i>	(1) <i>M. sympodialis</i>
Pathophysiology	(2) Skin barrier ↓	(2) Skin barrier ↓
	(3) LL-37 and HBD-2 ↓	(3) LL-37 and HBD-2 ↓
	(4) Ceramide levels ↓	(4) APCs ↑ (IgFce ↑)
	(5) T cell and macrophage activity ↑	(5) T-cell reactivity ↑
	(6) Adhesin molecules ↑	(6) IL-10 and TGF- β 1 ↑
	(7) Superantigen	
	(8) IL-4, IL-5 and IL-13 ↑	(7) IL-4, IL-5 and IL-13 ↑
	(9) IgE production	(8) IgE production
	(10) Bacterial biofilm ↑	
	(11) CLA ↑	
	(12) Cytotoxic α -toxin	
	(13) Anti-infectious treatment (proved positive effect on AD)	(9) Anti-mykotic treatment (positive effect on AD reported in single cases)
Therapy	(14) Anti-inflammable treatment (bacterial colonization ↓)	

AD, Atopic dermatitis; APC, antigen-presenting cell; CLA, cutaneous lymphocyte-associated antigen; HBD-2, β -defensin 2; LL-37, cathelicidin; TGF- β , transforming growth factor beta.

aureus colonization in atopic skin in comparison with healthy controls. There is evidence that *S. aureus* itself stimulates the hydrolysis of ceramides by bacterial-derived ceramidase in atopic skin [11].

Furthermore, the cell membrane of *S. aureus* contains adhesins for epidermal and dermal laminin and fibronectin. They are uncovered in lesional skin, which enhances *S. aureus* cutaneous adherence [4]. The precise mechanism by which *S. aureus* gains access to the dermis is unknown, but it is suggested that IL-4, produced by T helper type 2 cells, induces fibronectin synthesis, which in combination with plasma exudation of fibrinogen, allows *S. aureus* to bind to the skin. *S. aureus* is able to encase itself in a kind of biofilm composed of a hydrated matrix of polysaccharides (glycocalyx) and proteins, which supports cell adhesion [12].

Changes in the immunological profile

As the affinity of *S. aureus* to inflammatory, atopic skin is greater than to normal skin, in cases in which inflammation is mediated by T helper type 2 cells, it has been speculated that in AD the skin fails to respond to microbial invasion with an appropriate local immune response. Therefore, once having gained access to atopic skin, colonies of *S. aureus* grow in an uncontrolled manner because of the missing, naturally occurring, antimicrobial peptides such as β -defensin 2 and cathelicidin that are expressed by keratinocytes. Ong *et al.* [13] demonstrated that samples taken from inflammatory skin lesions of patients with AD and analysed immunohistochemically, by immunodot and Western blot analysis as well as by reverse transcriptase–polymerase chain reaction assays were deficient in cathelicidin and β -defensin 2 and expressed significantly lower levels of cathelicidin messenger RNA and β -defensin 2 mRNA.

Bacterial superantigens

S. aureus produces immunomodulatory toxins such as staphylococcal enterotoxins A and B and toxic shock syndrome toxin-1 in up to 65%, which are high-molecular-weight proteins with superantigenic properties [14]. They cause a number of diseases, some of which are followed by fever and shock. The potent immunostimulatory properties of superantigens are a direct result of their simultaneous interaction with the V_{β} domain of the T-cell receptor on lymphocytes and the MHC class II molecules on the surface of antigen-presenting cells (APCs) and macrophages [6]. Superantigens do not only activate T cells and macrophages, but induce T-cell skin homing by an upregulation of cutaneous lymphocyte-associated antigen and differentiation through cytokine production (IL-4 and IL-10), which may contribute to the induction or enhancement of skin inflammation [15,16]. In a very recent study [17^{*}], it was demonstrated that T regulatory cells (CD4⁺CD25⁺) are substantially

increased in AD, but lose their immunosuppressive activity by stimulation by bacterial antigens. Such T regulatory cells might thus not function properly in skin colonized by superantigen-producing *S. aureus*, which could in addition increase other T-cell activation and the inflammatory reaction in AD.

Superantigens can also induce glucocorticosteroid insensitivity, which encompasses a variety of mechanisms by which superantigens increase AD severity and may be an explanation for the difficulties in the management of AD [18].

Another virulent product of *S. aureus* is the thermolabile α -toxin that is cytotoxic for keratinocyte cell lines [19].

Specific IgE production

S. aureus induces the synthesis of specific IgE antibodies directed against staphylococcal superantigens in approximately 57% of AD patients. These have been shown to be positively correlated with disease severity in contrast to healthy individuals, in whom these IgE antibodies are extremely rare [20]. The strong impact as a 'conventional' allergen on disease activity has been shown in earlier studies, performing clinical assessment and determining eosinophil protein mediators in serum and urine as in-vitro parameters of inflammation [21].

Therapeutic long-term options and anti-inflammatory approaches

Long-term management with topical steroids alone should be avoided because of their numerous side-effects and the high risk of *S. aureus* developing glucocorticoid insensitivity. In combination with tacrolimus or other anti-inflammatory treatment, steroids reduce colonization with *S. aureus* efficiently. The newer calcineurin inhibitors (tacrolimus and pimecrolimus) inhibit the transcription of various T helper types 1 and 2 cytokine genes, and seem to block *S. aureus* superantigen-triggered T cells, so far without significant side-effects [22–24]. Topical fucidic acid reduces the prevalence and population density of *S. aureus* without increasing fucidic acid-resistant *S. aureus* [25^{*}]. A new approach to AD treatment are silver-coated textiles [26^{*}]. They are currently of interest because a highly significant reduction in *S. aureus* was demonstrated after only a few days of textile treatment. The mechanisms of antibacterial activity of silver are not yet fully understood, but silver ions seem to cause a detachment of the cytoplasmic membrane from the bacterial cell wall and may have an influence on DNA replication and protein activity.

Fungal colonization: the role of *Malassezia*

Malassezia species are lipophilic yeasts commonly found on the body surface in humans, typically predominating in seborrheic skin sites such as the head and neck.

Because of their defect in the synthesis of fatty acids they rely on the exogenous delivery of fatty acids [27].

Malassezia species, in particular *Malassezia sympodialis*, *Malassezia globosa* and *Malassezia restricta*, occur in up to 90% of patients with AD, but they are also found in 34% of normal cutaneous flora [28,29]. More interestingly, Johansson *et al.* [30*] showed that only AD patients (67% of 132 patients) and none of 33 healthy controls were sensitized to *M. sympodialis*, demonstrated by either a positive skin prick test (SPT), positive atopy patch test (APT) or specific IgE antibodies to *Malassezia*. Therefore, IgE production seems to be exclusively AD-specific, as it only appears in AD patients and is missing in the healthy control group. Interestingly, a positive APT reaction was found in approximately the same number of patients with and without head/neck type dermatitis. This contradicts earlier evidence of *Malassezia* allergy occurring mainly in AD patients with head/neck type dermatitis on the grounds that it colonizes sebum-rich skin [27].

To date, *Malassezia* extracts have been shown to contain 11 different IgE-binding components (Mal s1–11), eight of which have been produced by recombinant technology for diagnostic purposes (rMal s1, rMal s5–11) [31–34]. As the efficiency of detecting sensitized AD patients between the capsulated hydrophilic carrier polymer (CAP) method for specific serum IgE, SPT and APT differs enormously, it has been suggested that there are different molecular structures of *Malassezia* responsible for either immediate hypersensitivity (SPT and IgE) or delayed-type hypersensitivity (APT). Fischer *et al.* [9] performed SPT and APT with different recombinant allergens in AD patients, and showed that there is a preferential sensitization to rMal s5, rMal s6 and rMal s9.

However, the mechanisms of allergic response are still not fully understood. Only recently, interactions between dendritic cells and *Malassezia* have been investigated in detail [35–38]. Using different receptors (mannose receptors, etc.) or pinocytosis, *Malassezia* allergens are taken up by APCs. Among these, Langerhans cells, usually increased in number in atopic skin and expressing MHC class II on their cell surface, and AD-specific inflammatory dendritic epidermal cells play a key role in the first sensitization phase. This process is associated with a release of proinflammatory and immunoregulatory cytokines from activated APCs (TNF- α , IL-1 β , IL-16 and IL-18) and surrounding keratinocytes (human thymic stromal lymphopoietin), which act as migratory signals for T helper type 2 cells and therefore T helper type 2 immune response.

Another important linking element between *Malassezia* colonization and AD is the decreased expression of β -

defensin-2 in keratinocytes (see preceding paragraph). Usually, β -defensin 2 expression is upregulated after *Malassezia* uptake in human keratinocytes, which limits the uptake of further *Malassezia* cells from the extracellular environment and reflects the regulated innate host defence [39]. This first-line immune defence is deficient in AD and permits the uncontrolled penetration of *Malassezia* spp. into keratinocytes (see Table 1).

Other fungal agents in atopic dermatitis

Not only *Malassezia* spp. can be identified in higher densities on atopic skin. Basidiomycetous yeasts such as *Cryptococcus diffluens* and *Cryptococcus liquefaciens* have been identified in more than 40% of AD patients, and from 20% of healthy subjects [40]. In such studies there was no support for these findings by means of positive proof for specific IgE antibodies. In addition to these yeasts, Fischer *et al.* [41] showed that *Coprinus comatus* can induce eczematous skin lesions in AD patients. The authors demonstrated that 12 out of 38 (32%) had positive APT reactions, 20 out of 38 (57%) also having a positive SPT reaction to *C. comatus* extract.

Intestinal colonization with *Candida albicans*

Conflicting results have been reported on the role of intestinal or skin colonization with *Candida albicans* in AD. *Candida* species, especially *C. albicans* have been cultured more frequently from patients with AD, in particular from the gastrointestinal tract, than from healthy donors. However, as of now there is no evidence for any correlation between disease severity and fungal colonization. There is a strong variation in the prevalence of patients with AD and positive SPT to *C. albicans* extract and specific IgE. In-vitro *C. albicans* induces a high secretion of IL-4 and macrophage-derived chemokines and only low levels of IFN- γ , which indicates a T helper type 2 immune response and a possible sensitization to this yeast [42].

Antimycotic therapy with ketoconazole may be efficacious in head/neck type dermatitis when taken over a long period of time (>3 months) [43]. There is currently no recommendation for this therapy because recent studies have failed to show a rapid improvement of AD within one month in contrast to treatment with topical steroids.

Atopy, parasitic colonization and the hygiene hypothesis

For many years the 'hygiene hypothesis' has been gaining support from the finding that there is an increased prevalence of atopy among individuals with a higher social status, improved household hygiene and fewer childhood infections [44]. It has been debated whether microbial antigens and bacterial endotoxins during childhood direct the immune system towards a T

helper type 1 cell immune response, which counterbalances proallergic responses of T helper type 2 cells. This hypothesis was disproved only recently, when a large case-control study of 307 children with AD and 297 healthy controls showed that there is no evidence that exposure to infection in early childhood contributes to a protective effect [45**], whereas there is an inverse association with the development of asthma [46].

Moreover, it has been postulated that endoparasitic infestation provides a protective immunomodulatory network against allergic diseases and atopy by parasite-induced diversion of the immune system away from a T helper type 2-like response. Recent studies have shown a consistent inverse relationship between infections (schistosomiasis and intestinal helminths) and inhalant allergies caused by a greater production of IL-10, an inflammatory T helper type 2-dependent and regulatory T-cell-dependent cytokine, and transforming growth factor beta, in response to endoparasitic infection [47,48*]. Taken together, endoparasites seem to influence the outcome of inhalant allergies, but not, or only to a very limited extent, AD.

Conclusion

In the past 2 years the new findings on the relationship between AD and microbial colonization have offered important insights into the role of microorganisms in the course of the disease. The current understanding of *S. aureus* and its superantigens, playing a key role in initiating and modulating the immune response, may lead to more beneficial treatments, e.g. the topical application of ceramide-mimicking substances. In contrast, the specific function of *Malassezia* in the immune cascades leading to AD is still unclear. The use of recombinant allergens will contribute to the elucidation of the mode of action of sensitization to *Malassezia*, and in the future may hopefully provide patients with new therapeutic options in terms of immunotherapy with recombinant fungal allergens.

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