

## Review article

# Bacterial infections and atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease which often begins in infancy and runs a course of remission and exacerbation. The acute eczematous lesions of AD are characterized by erythema, oozing, and crusting, whereas the chronic lesions show thickened skin and papules. Furthermore, pruritus and sleeplessness are characteristics of AD (1).

There is increasing evidence that environmental allergens and food allergens are major provocation factors for the flare-ups of AD (2). Specific IgE antibodies to food or environmental allergens can be detected in the serum of 80% of patients with AD. A subgroup of patients with specific IgE and positive skin prick tests to inhalant or food allergens exhibit positive cutaneous late-phase reactions after epicutaneous application of allergens (3–5). The mononuclear infiltrate in the eczematous reactions to patch testing and in the lesional skin of AD is similar to that of allergic contact dermatitis when stained with immunohistochemical techniques. Mononuclear cells and eosinophil granulocytes can be found mainly in the dermis (4, 6, 7), and Langerhans cells with specific IgE bound to FcεRI on their surface in the epidermis (8, 9). CD4-positive T-helper cells predominate in the cellular infiltrate.

### Allergen-specific T cells in AD

The incubation of blood lymphocytes of AD patients with inhalant and food allergens leads to a proliferative response, indicating the presence of a specific T-cell-mediated hypersensitivity in AD (5). House-dust-mite-

specific T cells could be generated from biopsies of patch test lesions (10–12). Furthermore, food-specific T-cell clones were obtained from blood and skin lesions after oral food challenges (13–15).

The allergen-specific response was observed in the subset of circulating lymphocytes expressing the skin-homing molecule CLA. This observation points to a mechanism which targets allergen-specific T cells to the skin. Numerous studies at the mRNA level, as well as at the protein level, performed in recent years have demonstrated that the TH2-like cytokine IL-4 plays a role in the initial phase of cutaneous inflammation, whereas the TH1-like cytokine IFN-γ predominates in spontaneous lesions or older patch test lesions in AD (17, 18).

By the use of limiting dilution cultures, inhalant allergen-specific T cells have been shown to represent only a minority (1–5%) of the infiltrating T cells in lesional skin (10, 12). Therefore, other factors which lead to the activation of T cells at the site of inflammation are probably involved in the pathogenesis of AD. Staphylococcal exotoxins, which exhibit superantigenic properties, are potential candidates for polyclonal T-cell activation.

### Bacterial superantigens

Superantigens are high-molecular-weight proteins comprising a group of molecules produced by various microorganisms, such as bacteria (staphylococci, streptococci, *Yersinia*, and *Mycoplasma*) and viruses. They are involved in the pathogenesis of several human

diseases including food poisoning, septic shock, Kawasaki syndrome, and psoriasis (19). Bacterial superantigens can activate T cells in an MHC-unrestricted, unspecific way by interacting directly with the TCR-V $\beta$  chain and the MHC class II molecule outside the peptide-binding groove area (20–22). The toxins have significant binding affinities for certain defined constant parts of the TCR-V $\beta$ -chain (about 2–6 per toxin). Both autologous and allogeneic, and even xenogeneic, antigen-presenting cells (APC) are able to stimulate up to 25% of the T-cell pool in the presence of superantigens, which are strong mitogens even in a concentration of  $10^{-10}$  M (23). On the other hand, a “conventional” allergen which undergoes classical antigen presentation may stimulate about 0.1% of allergen-specific T cells in a MHC-restricted fashion via their TCR-V $\alpha$  and - $\beta$  chain (Fig. 1). The group of the *Staphylococcus aureus*-derived superantigens mainly comprises the enterotoxins A–D (SEA–D) and the toxic shock syndrome toxin-1 (TSST-1).

The influence of bacterial superantigens on the immune system has been well investigated particularly in animal models. After intravenous application of superantigen (1  $\mu$ g of SEB) in mice, almost all V $\beta$ 8+ T cells become activated, leading to a substantial production of cytokines and a loss of L-selectin on the cell surface. In the initial phase of T-cell activation 1–4 h after application of superantigens, TNF- $\alpha$ , IL-2, IL-4, IL-6, and IFN- $\gamma$  are traceable in the murine serum. This initial phase is followed by a (partial) deletion and anergy of SEB-reactive cells (24). T cells activated by superantigens undergo apoptosis after a distinct number of cell divisions (25).

### *S. aureus* and AD – colonizing features

Of patients with AD, 80–100% are colonized with *S. aureus* (26–29). In contrast, *S. aureus* can be found on the skin of only 5–30% of normal individuals, mainly in intertriginous areas. Persistent nasal carriage is present in 20% of normal adults. *S. aureus* can be isolated from clinically affected and unaffected skin, and both acute and chronic AD lesions are colonized. Staphylococcal colonization density is significantly lower in healthy individuals than in patients with AD, and bacterial counts on unaffected skin are lower than on affected skin (30).

*S. aureus* often constitutes up to 80% of the normal flora in AD and large numbers of this microorganism seem to eliminate the lipophilic coryneform bacteria from the skin (31). The colonization density can reach up to  $10^7$  colony-forming units/cm<sup>2</sup> without clinical signs of infection (29). The susceptibility of the atopic skin to colonization with *S. aureus* may have several causes. *S. aureus* cell walls contain receptors, the so-called adhesins, for epidermal and dermal laminin and

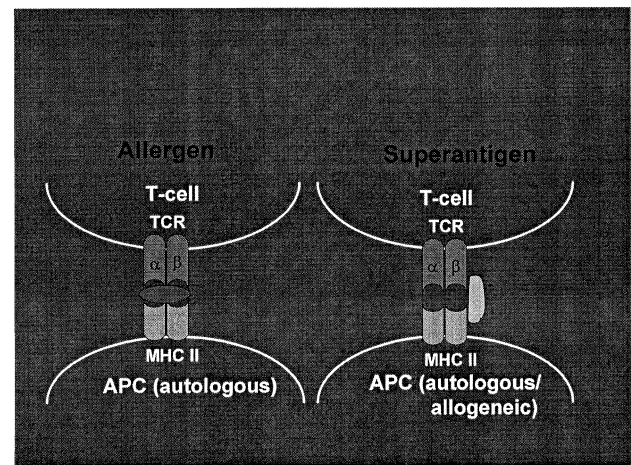


Figure 1. Activation of T cells by antigens and superantigens. In contrast to antigens, superantigens are not processed and can bind outside antigen-binding groove of MHC molecule. Superantigens interact with certain defined constant parts of TCR V $\beta$  chain, thus activating up to 25% of T-cell pool.

fibronectin. Since the skin of patients with AD lacks an intact stratum corneum, dermal fibronectin receptors might be uncovered and increase the adherence of *S. aureus* (32, 33). Fibrillar and amorphous structures have been traced between *S. aureus* cells and corneocytes, and may result in a bacterial biofilm that contributes to the adherence of *S. aureus* (34). Skin-surface lipids, such as free fatty acids and polar lipids, have been shown to exhibit antibacterial activity (35). The observation that *S. aureus* penetrates into the intracellular spaces of the epidermis suggests that skin-surface lipids are deteriorated in patients with AD (34). Strikingly significant increases in the carriage of *S. aureus* were found in the anterior nares and hands of caregivers of children with AD compared to caregivers of healthy children, a finding which suggests transmission (36).

The density of *S. aureus* on AD lesions has been shown to correlate with cutaneous inflammation (31, 37). In contrast, Akiyama et al. could not find such a correlation (38).

The staphylococcal enterotoxins A–D (SEA–D) and TSST-1 have been found to be secreted by *S. aureus* strains isolated from the skin of up to 65% of AD patients who are colonized with this microorganism (28, 37–41). Superantigens secreted by *S. aureus* have been shown to penetrate the epidermis and dermis, where they interact with different cells of the cutaneous immune system. In a previous study, the application of SEB on the skin of normal individuals and patients with AD resulted in an inflammatory reaction at the application site (42), and it could be shown in a mouse model that this inflammation is T-cell dependent (43, 44). Various studies suggest that at least two different pathways are involved in the exacerbation of cutaneous inflammation by staphylococcal exotoxins. Firstly, they can act as superantigens, leading to polyclonal activation of T cells;

secondly, they may induce the generation of exotoxin-specific T cells able to promote the generation of exotoxin-specific IgE antibodies in their function as "conventional" allergens. Furthermore, staphylococcal exotoxins may influence cutaneous professional and nonprofessional APC directly via binding to MHC class II molecules.

However, it is still not clear which of these mechanisms predominates in individual patients.

### Impact on T cells

Different pathways of T-cell activation may be involved after stimulation with different concentrations of exotoxins in patients with AD. The influence of exotoxins on T-cell proliferation and production of lymphokines seems to be dependent on their concentration. In the nanogram range and above, TSST-1 induces a strong proliferation of PBMC with an increase in IFN- $\gamma$  secretion, and consequently causes an inhibition of the synthesis of total IgE. At picogram and femtogram concentrations, TSST-1 leads to a lower IFN- $\gamma$  production and promotes a polyclonal B-cell activation by bridging the TCR and the MHC class II on B cells with an increase of total IgE (45). Similarly, in a humanized SCID mouse model, where hu-PBMC were administered intraperitoneally followed by an intradermal injection of autologous PBMC, the epidermal application of both SEB (50 ng) and mite allergens together elicited a profound dermal and epidermal inflammation, whereas the application of SEB alone resulted in a weaker inflammation. Mite allergens alone had no effect, although the donor was sensitized to mites. Intraperitoneal application of SEB and mite allergen together resulted in an IFN- $\gamma$ - and IL-5-induced inhibition of total and specific IgE synthesis of peritoneal PBMC (46).

These data indicate that exotoxins at higher concentrations induce a strong proliferation of T cells and favor a TH1-like cytokine profile. Several groups have shown that exotoxins can exacerbate cutaneous lesions of AD by acting as "superantigens". The T cells of patients with AD expressing certain TCR-V $\beta$ -chains are activated upon incubation with staphylococcal superantigens in the skin. Strickland et al. demonstrated that the colonization with toxigenic *S. aureus* strains is associated with an expansion of T cells expressing the appropriate TCR-V $\beta$  chains among the cutaneous lymphocyte antigen (CLA)-positive T-cell subset in the peripheral blood (47). Similarly, patients with active AD had a higher percentage of cells positive for the TCR V $\beta$ 2 and V $\beta$ 5.1 in the CLA<sup>+</sup>, but not in the CLA<sup>-</sup>, subset. Furthermore, the authors found an increased proportion of HLA-DR<sup>+</sup> cells in the compartment

expressing CLA and TCR V $\beta$ 5.1 in patients with active AD (48). Leung et al. demonstrated that staphylococcal exotoxins can upregulate the expression of the cutaneous homing receptor CLA on normal PBMC by induction of IL-12. Furthermore, selective TCR V $\beta$ -chains were induced (49). These findings indicate that exotoxins may contribute to the pathogenesis of AD by increasing the frequency of superantigen-reactive T cells able to migrate to AD lesions.

In some patients with AD, preferential expression of certain TCR V $\beta$ -chains was also found in lesional skin. The pattern of TCR V $\beta$  expression was heterogeneous among the patients and was related to different exotoxins in one patient (50). Neuber et al. (51) found that most of the T cells detected in lesional skin were TCR V $\beta$ 3<sup>+</sup>, and that PBMC from patients with AD showed a significantly higher proliferation and IL-5 secretion than healthy individuals after stimulation with monoclonal antibodies against TCR V $\beta$ 3 and TCR V $\beta$ 8.

A shift in the intradermal TCR-V $\beta$  repertoire corresponding to the respective superantigen was found in the lesional skin of children with AD by immunohistochemical staining (37), and skin biopsy specimens obtained from SEB-treated areas demonstrated a selective accumulation of T cells expressing SEB-reactive TCR-V $\beta$  elements in patients with AD and healthy individuals (52).

Different groups have shown that the disease activity in children colonized with toxigenic *S. aureus* strains is higher than in patients colonized with nontoxigenic strains, a finding which underlines the clinical importance of these data (37, 53). In addition, superantigens might enhance the specific T-cell response to aeroallergens in patients with AD by the recruitment into the skin of superantigen-reactive T cells bearing the appropriate TCR-V $\beta$  elements that are, in parallel, specific to aeroallergens.

Leung et al. (28) first proposed that superantigens may exacerbate AD by acting as a new group of allergens, since specific IgE to SEA, SEB, and TSST-1 could be detected in the sera from 57% of AD patients, most of whom were identified as carriers of toxigenic *S. aureus* strains. The basophils of these patients were found to release histamine upon incubation with the respective toxin, indicating a functional role of these antibodies in patients with AD.

Recently, we were able to show that adults suffering from AD who are sensitized to SEB have a higher disease activity than nonsensitized patients when assessed clinically and by determination of eosinophil protein mediators in serum and urine as *in vitro* parameters of inflammation (54). Our results are concordant with the findings of Nomura et al., who demonstrated a correlation between IgE anti-SEB

levels and severity of AD in children and adolescents (40). In addition, Bunikowski et al. found a relationship between a sensitization to SEA and SEB, which could be found in 34% of children with AD, and the severity of AD (41). One can speculate that staphylococcal exotoxins may exacerbate skin lesions in AD through the same mechanism that is now widely accepted for inhalant allergens. The presence of exotoxins on the skin could lead to a release of proinflammatory mediators from cutaneous mast cells and a subsequent pruritus and scratching. Furthermore, the toxins may bind to specific IgE on the surface of Langerhans cells, thus leading to a facilitated allergen presentation and to the activation of specific T cells.

In a recent publication, Campbell & Kemp showed that children under 7 years of age with AD more frequently have IgG antibodies to SEB than normal controls. In addition, atopic children have higher levels of SEB-specific IgG than controls. The authors assume that the increased titers of SEB-specific IgG are caused by increased superantigen exposure; i.e., colonization with SEB-producing strains (55).

To investigate to what extent staphylococcal exotoxins activate T cells as superantigens or as allergens, we generated exotoxin-reactive TCL from the lesional skin and blood of three adult patients suffering from long-standing severe AD who were colonized with exotoxin-producing *S. aureus* strains (unpublished data). We found not only a superantigen-mediated T-cell response, but also an allergen-specific T-cell response to staphylococcal exotoxins in the lesional skin and blood of patients with AD who were colonized with toxigenic *S. aureus* strains. Both superantigen-specific TCL, which were activated by exotoxins only in the presence of autologous APC, and superantigen-reactive TCL, which were activated by both autologous and allogeneic APC, could be generated from the lesional skin and blood of these patients. In two of our patients, about 70% (75%/69.2%) and in one patient 42.9% of all cutaneous exotoxin-reactive TCL were superantigen-reactive. The stimulation indices of the superantigen-reactive TCL were significantly higher than the stimulation indices of the superantigen-specific TCL when stimulated in the presence of autologous APC, a finding which points to a higher degree of activation. We investigated, for the expression of TCR-V $\beta$  elements, superantigen-reactive and superantigen-specific TCL isolated from the skin and peripheral blood of one patient who was colonized by SEC-producing strains. Superantigen-reactive TCL were found to express the SEC-reactive TCR-V $\beta$ -chains 13 and 17 on their surface, confirming the immunohistologic features found in lesional skin by Bunikowski et al. (37), and Skov et al. (52) on a clonal level. In contrast, superantigen-specific TCL did not express any SEC-reactive TCR-V $\beta$  elements.

Interestingly, a relatively high proportion of superantigen-specific TCL could be isolated from the skin of one patient who had no specific IgE to the respective toxin, compared to patients who were also sensitized to the cutaneous exotoxins by IgE. Therefore, T-cell-dependent mechanisms that do not involve the production of specific IgE antibodies are likely to contribute to the cutaneous inflammation. Recently, Akdis et al. (56) showed that SEB induced, in a dose dependent manner, a significant proliferation of T cells derived from the lesional skin of patients with both the extrinsic and the intrinsic forms of AD. In contrast to T cells derived from the skin of patients with the extrinsic form of AD, cells from patients with intrinsic AD failed to produce significant amounts of IL-13 upon incubation with SEB, which may, therefore, be unable to help B cells to produce specific IgE in these patients (56).

Further studies are needed to characterize the T-cell response to staphylococcal exotoxins specifically in the IgE-independent (intrinsic) form of AD.

#### Influence of superantigens on APC and on keratinocytes

Both epidermal Langerhans cells and IFN- $\gamma$ -pre-treated keratinocytes can serve as accessory cells for the polyclonal T-cell response to bacterial superantigens, and TNF- $\alpha$  could be identified as one major factor responsible for T-cell activation (57, 58).

There is a large body of evidence that staphylococcal exotoxins have an influence not only on T cells, through bridging of MHC class II molecules and TCR-V $\beta$  chains, but also on professional and nonprofessional APC, which can be stimulated directly to express adhesion molecules and inflammatory cytokines through engagement of MHC class II molecules by the toxins.

SEB has been shown to induce calcium influx and upregulation of ICAM-1 on IFN- $\gamma$ -preactivated keratinocytes through binding to MHC class II molecules (59). Similarly, Matsunaga et al. found not only a strong induction of ICAM-1 at both the protein and mRNA level but also increased expression of VCAM-1 at the mRNA level upon incubation of cultured human keratinocytes with staphylococcal exotoxins. Furthermore, IL-1 and TNF- $\alpha$  were induced (60). Similar results were obtained by another group, who added SEA and SEB to keratinocytes and detected the secretion of TNF- $\alpha$  after 6–12 h of incubation (61). After application of SEB to the skin of healthy individuals, IL-1 $\beta$  could be detected in blister roofs, but not in the blister fluid, a finding which points to the role of epidermal cells in the synthesis of this cytokine (62).

Recently, TSST-1 has been shown to inhibit monocyte apoptosis in patients with AD. This effect could be inhibited by neutralizing antibodies to GM-

CSF, a finding which points to a mechanism likely to contribute to the inflammation in AD (63). Furthermore, recent data suggest that *S. aureus* exotoxins also have profound effects on human eosinophils, since staphylococcal enterotoxins and TSST-1 were found to inhibit eosinophil apoptosis and modulate the expression of cell-surface antigens (63a).

### Other pathogenic factors of *S. aureus*

#### Other toxins

*S. aureus* strains have been shown to release not only toxins with superantigenic properties but also thermostable toxins such as  $\alpha$ -toxin, which is known as a cytotoxin.  $\alpha$ -Toxin was found to be produced by *S. aureus* strains isolated from patients with AD and psoriasis. It causes cytotoxic damage in keratinocyte cell lines and stimulates the release of TNF- $\alpha$  from keratinocytes *in vitro*. These cytotoxic effects exhibit the characteristics of necrosis, indicating that staphylococcal products may not only stimulate inflammatory cells but also cause direct damage to epidermal cells (61).

#### Cell walls

Specific IgE to *S. aureus* cell-wall components has been detected in up to 25% of patients with AD. Some authors found sensitization to staphylococcal cell walls to be associated with the severity of the disease (64); others could not establish such a correlation (65, 66). Taken together, these conflicting results might be caused by the different methods and reagents (complete bacteria as compared with cell-wall components) used in these studies.

Bacterial cell-wall components may also influence B cells directly, since costimulation of PBMC from patients with AD with IL-4 and teichoic acid or peptidoglycans led to a pronounced increase of IgE synthesis and CD23 expression *in vitro* (67). Moreover, an increased stimulation index could be measured in patients with AD compared to normal individuals, upon incubation of PBMC with *S. aureus* strain Wood 46 and purified *S. aureus* cell walls, indicating an increased cell-mediated immune response (68).

Recently, Jahreis et al. demonstrated that a subgroup of patients with AD showed a significantly diminished IFN- $\gamma$  production and enhanced IL-4 synthesis of their PBMC after incubation with the staphylococcal surface proteins p70 and NP-tase,

which are typically presented allergens. The authors concluded that such patients have impaired ability to clear *S. aureus* colonization (69).

### Treatment of AD with antimicrobial substances

If colonization with *S. aureus* contributes to the cutaneous inflammation in patients with AD, one would expect a therapeutic effect from an antimicrobial treatment. Several studies have investigated the effect of topically or systemically applied antibiotics on colonization by *S. aureus* and the severity of inflammation, and the results of these studies have been conflicting.

In a double-blind, placebo-controlled study, mupirocin plus corticosteroid applied topically on the skin of children with AD who showed no signs of superinfection led to a significant reduction of skin colonization and to a sustained improvement of the skin condition for an observation period of 6 weeks. Furthermore, when topical steroids were applied together with antibiotics, the therapeutic effect was better than with corticosteroids alone, suggesting a steroid-saving effect of the antibiotic treatment (70). However, recolonization appeared after 2–4 weeks. *S. aureus* can be eliminated from the skin of patients with AD by a treatment with very potent corticosteroids over a period of 2 weeks, thus suggesting the contribution of immunologic factors that support colonization (71). The stimulation of T cells by superantigens may be reduced by antibiotics, leading to a steroid-saving effect. In another study, the application of gentian violet reduced not only the bacterial density but also the severity of the eczema (72).

In contrast, the treatment of children with oral flucloxacillin, especially Ceftin, in double-blind, placebo-controlled studies resulted in a significant reduction of *S. aureus* colonization, but no significant improvement of AD. As in the study of Lever et al. (70), recolonization appeared soon after the completion of the treatment (73, 74). One could argue that these studies did not take into account the nasal carriage of *S. aureus* and the possible carriage and transmission of *S. aureus* between the patients and the contact persons. A consequent eradication of *S. aureus* from the patients' skin and nares together with topical treatment of their contact persons might result in a sustained eradication of *S. aureus* and consequently improve the skin condition. However, this has been shown only in open therapeutic trials so far.

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