

Short communication

Severe atopic dermatitis is associated with sensitization to staphylococcal enterotoxin B (SEB)

Background: *Staphylococcus aureus* has been identified as a possible trigger factor in atopic dermatitis (AD). Some 30–60% of *S. aureus* strains isolated from patients with AD are able to produce exotoxins with superantigenic properties, mostly staphylococcal enterotoxins A, B, C, and D (SEA–D) and toxic shock syndrome toxin-1 (TSST-1). Recently, it was demonstrated that the presence of IgE antibodies to SEA and SEB is correlated with the severity of skin lesions in children with AD. To determine the relevance of staphylococcal enterotoxins in adult patients with AD, we investigated the relationship between the severity of skin lesions and sensitization to SEA and SEB.

Methods: Clinical severity was determined by the SCORAD index. Circulating IgE antibodies to SEA and SEB, serum eosinophil cationic protein (ECP) levels, and urine eosinophil protein X (EPX) levels were measured.

Results: The skin condition was significantly worse in patients sensitized to SEB than in unsensitized patients. Serum ECP and urine EPX levels were found to be significantly higher in SEB-sensitized patients, confirming the higher degree of cutaneous inflammation.

Conclusions: Our results demonstrate a relationship between severity of skin lesions and sensitization to SEB in adult patients with AD, but a relationship between disease activity and sensitization to SEA could not be shown.

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Key words: atopic dermatitis; eczema; eosinophil granule proteins; exotoxins; specific IgE; *Staphylococcus aureus*; superantigens; T cells.

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Accepted for publication 3 January 2000

Atopic dermatitis (AD) is a chronic inflammatory skin disease which often begins in infancy and runs a course of remission and exacerbation. In recent years, the incidence of AD has increased worldwide. Acute eczematous lesions are characterized by erythema, oozing, and crusting, whereas chronic lesions show thickened skin and papules. Furthermore, intense pruritus and, consequently, sleeplessness are hallmarks of AD (1).

Although AD is clinically well characterized, the pathogenesis is not fully understood. Antigen-specific T cells play a crucial role in the pathogenesis of AD at the cellular level (2). Proliferative responses can be elicited in blood lymphocytes by inhalant allergens, including house-dust-mite and pollen or food antigens, a fact which indicates the presence of specific T-cell-mediated hypersensitivity in AD (3, 4). Allergen-specific T cells from lesional skin were found to produce different cytokines (5, 6). IL-5 is able to activate eosinophils, which may play an important effector

function in the lesional skin of AD (7) since they are able to release cytotoxic mediators such as eosinophil cationic protein (ECP) and eosinophil protein X (EPX). The level of serum ECP has been found to increase when the clinical skin condition in AD worsens (8). IL-4 is able to induce the production of allergen-specific IgE antibodies, which are found in 60–80% of all AD patients (9). Specific IgE antibodies are thought to play a role in antigen presentation via the Fc receptor for IgE (Fc ϵ R-I) on epidermal Langerhans cells (10). Degranulation of dermal mast cells by allergen-induced cross-linking of IgE antibodies bound to Fc ϵ R on the cell surface may also contribute to the allergic inflammation in AD.

Staphylococcus aureus, a Gram-positive bacterium, has been identified as a possible trigger factor in AD. About 80–100% of AD patients are colonized with *S. aureus* (11–13). On the other hand, only 5–10% of healthy individuals are carriers of this microorganism. *S. aureus* could be isolated from acute and chronic skin lesions in AD (14), and it was demonstrated that the colonization density can reach 10⁷ colony-forming units/cm² (15). The anterior nares have also been identified as a reservoir of *S. aureus*.

Abbreviations: AD: atopic dermatitis; SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B; TSST-1: toxic shock syndrome-1; ECP: eosinophil cationic protein; EPX: eosinophil protein X.

Williams et al. could show that the density of *S. aureus* on AD lesions exhibits a strong correlation with cutaneous inflammation (13), and *S. aureus* was found to have an unusual ability to adhere to atopic corneocytes when compared to corneocytes obtained from patients with other cutaneous diseases (16). Previous studies have shown that 30–60% of *S. aureus* strains isolated from patients with AD secrete exotoxins with superantigenic properties; e.g., staphylococcal enterotoxins A, B, C, and D (SEA–D) and toxic shock syndrome toxin-1 (TSST-1) (17–19). In a recent study, the application of SEB on the skin of normal individuals and patients with AD resulted in an inflammatory reaction at the application site (20). Furthermore, a correlation between the severity of skin lesions and sensitization to staphylococcal exotoxins could be demonstrated in children with AD (17). These findings point to the importance of staphylococcal exotoxins as a new group of allergens.

To date, there are no studies on the role of superantigen-specific IgE in adult patients with AD. Therefore, we investigated the relationship between the presence of circulating IgE antibodies to SEA and SEB and the clinical severity of skin lesions. Moreover, serum ECP and urine EPX concentrations as objective parameters of allergic inflammation were correlated to the presence of exotoxin-specific IgE.

Material and methods

Patients

Seventy-one patients with mild to severe AD were enrolled in the study (29 male, 42 female patients; median age 32 years, range 18–65 years). Patients were recruited from the Department of Dermatology and Allergology of the Hannover Medical University (Germany) on their first visit. All investigations were performed before treatment. AD was diagnosed by the criteria of Hanifin & Rajka (21), which include pruritus, typical morphology and distribution of eczematous lesions, chronicity of the disease, and personal or family history of atopy. The clinical severity of AD was determined by the SCORAD index, which is a scoring system combining extent, severity, and subjective symptoms of AD (22).

Determination of total and superantigen-specific IgE

Levels of total IgE were determined in the sera from AD patients by CAP RAST, according to the manufacturer's instructions (Pharmacia, Freiburg, Germany). Specific IgE to SEA and SEB were measured by the AlaSTAT test, which is a liquid-phase enzyme immunoassay (DPC Biermann, Bad Nauheim, Germany).

Determination of eosinophil-derived proteins

Blood samples were coagulated for 60 min at room temperature, followed by 30-min incubation at 4°C. Samples were centrifuged for 10 min (4°C, 850 g). Sera were frozen immediately and stored at –20°C. ECP was determined by the CAP System, ECP FEIA (Pharmacia, Freiburg, Germany), and in parallel by the AlaSTAT system (DPC Biermann) after the completion of the study.

Urine samples from 35 of 71 patients were frozen immediately and stored at –20°C. EPX was measured with a competitive RIA, according to the manufacturer's instructions (Pharmacia, Freiburg, Germany).

Both ECP and EPX concentrations were batch assayed after the completion of the study.

Statistical analysis

The comparison of the medians of nonparametric distributed results of different patient groups was performed with the Mann–Whitney rank sum test. The correlation coefficients between different variables were calculated with the Pearson product moment correlation.

Results

Sensitization to SEA/SEB in AD patients

In 71 adult patients with AD, serum IgE antibodies to the staphylococcal exotoxins SEA and SEB were measured. Specific IgE concentrations of more than 0.35 kU/l were judged to be positive, as samples of normal blood donors are below this value according to the information given by the manufacturer. Forty patients (56%) were found to be sensitized to staphylococcal exotoxins (SEA and/or SEB). Nineteen of these patients (47.5%) were sensitized to both SEA and SEB; in 12 patients (30%), only IgE antibodies to SEA and in nine patients (22.5%) only IgE antibodies to SEB were detected.

Patients sensitized to SEB had median serum-SEB-IgE levels of 2.06 kU/l (range 0.47–30.54 kU/l), whereas SEA-sensitized patients had median serum-SEA-IgE levels of 1.80 kU/l (range 0.37–80.46 kU/l). The median levels of the patients who were not sensitized to SEA or SEB were 0.00 kU/l (ranges 0.00–0.28 kU and 0.00–0.26 kU/l, respectively).

We found higher levels of total IgE in patients sensitized to staphylococcal exotoxins than in unsensitized patients. On the other hand, IgE antibodies specific to SEA or SEB were not detectable in patients with high amounts of total IgE and low SCORAD score, an observation which points to the specificity of the antibody detection system (Table 1).

Interdependence of sensitization to staphylococcal exotoxins and SCORAD index

The clinical severity of AD was determined by the SCORAD index, which ranged from 14 to 103 points. The median score of the sensitized patients was compared to the median score of the unsensitized patients with the Mann–Whitney rank sum test. There was a statistically significant difference in clinical severity when SEB-IgE-positive patients were compared with SEB-IgE-negative patients (median values 50.4 and 33.3 points, respectively, $P < 0.0001$) (Fig. 1). There was no significant difference when patients who were sensitized to SEA and patients who were not sensitized to SEA were compared ($P = 0.156$). The SCORAD scores were not correlated with the absolute concentrations of SEA or SEB (Pearson product moment correlation).

Interdependence of sensitization to staphylococcal exotoxins and ECP/EPX concentrations

Eosinophil granule proteins are released by activated eosinophils and have been shown to be toxic to epithelial cells and nerves. They may thus contribute to tissue damage in inflammatory processes. Since eosinophil granule proteins are known to be objective parameters of atopic inflammation, we determined the concentrations of serum ECP and urine EPX. ECP concentration was analyzed in the sera of all patients (median value 13.8 $\mu\text{g/l}$, range 2.09–114.0 $\mu\text{g/l}$). ECP was assayed with two systems which showed a high correlation of the results ($R = 0.96$, $P < 0.0001$). The median ECP level of the sensitized patients was compared to the ECP levels of the unsensitized patients with the Mann–Whitney rank sum test. As shown in Fig. 2, serum ECP levels were significantly higher in patients sensitized to SEB than in unsensitized patients (median values 22.7 and 12.9 $\mu\text{g/l}$, $P = 0.018$). There was also a significant difference when SEA-sensitized and unsensitized patients were compared (median values 22.7 and 12.0 $\mu\text{g/l}$, $P = 0.0038$).

Urine samples were obtained for analysis of EPX concentration from 35 of 71 patients (median value 103.1 $\mu\text{g}/\text{mmol}$ creatinine, range 26.3–390 $\mu\text{g}/\text{mmol}$ creatinine). Again, the median EPX levels of the

sensitized patients were compared with the levels of the unsensitized patients with the Mann–Whitney rank sum test. Urine EPX levels were significantly higher in patients sensitized to SEB than in unsensitized patients (median values 176.3 and 73.3 $\mu\text{g}/\text{mmol}$ creatinine, $P = 0.0096$) (Fig. 3). A significant difference could not be shown when SEA-IgE-positive/-negative patients were compared ($P = 0.2338$). We found no significant correlation between the SCORAD score and ECP/EPX concentration (Pearson product moment correlation).

Discussion

There is increasing evidence that cutaneous and nasal colonization with *S. aureus* contributes to the pathogenesis of AD. In our study, we could demonstrate that severe skin lesions are associated with sensitization to SEB.

Leung et al. (11) 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. Basophils of these patients were found to release histamine upon incubation with the respective toxin. Thus, colonization with superantigen-producing *S. aureus* strains could lead to a permanent release of proinflammatory mediators from cutaneous mast cells, in this way acting as a trigger factor in AD. However, the level of superantigen-specific IgE was not compared with the severity of skin lesions in this study.

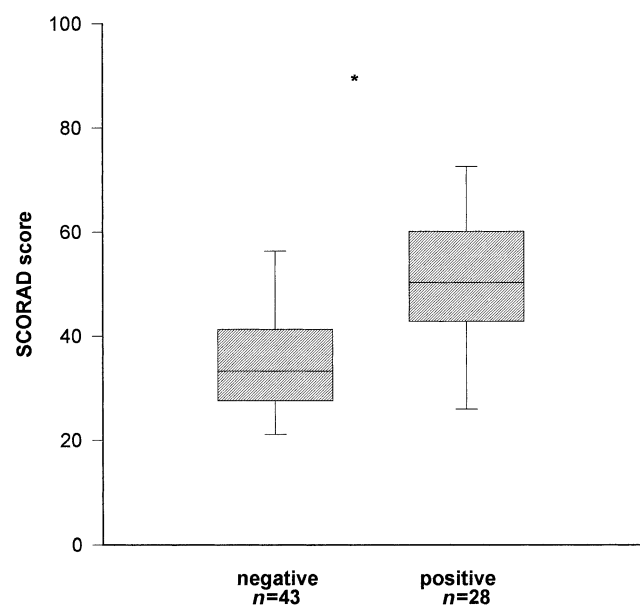


Figure 1. Clinical severity of AD assessed by SCORAD score in patients sensitized to SEB compared with patients not sensitized to SEB. Horizontal lines indicate median values of SCORAD points; boxes show 25th and 75th percentiles. *Mann–Whitney rank sum test, $P < 0.0001$.

Table 1. Patients with high levels of total IgE and low SCORAD score not sensitized to SEA or SEB

Patient	SCORAD score (points)	ECP ($\mu\text{g/l}$)	Total IgE (kU/l)	Anti-SEA-IgE (kU/l)	Anti-SEB-IgE (kU/l)
1	28.3	6.47	3340.0	<0.35	<0.35
2	29.1	15.2	5160.0	<0.35	<0.35
3	21.5	13.8	4060.0	<0.35	<0.35
4	20.7	7.64	6550.0	<0.35	<0.35
5	36.8	5.94	1091.0	<0.35	<0.35
Mean	27.3	9.81	4040.2	<0.35	<0.35

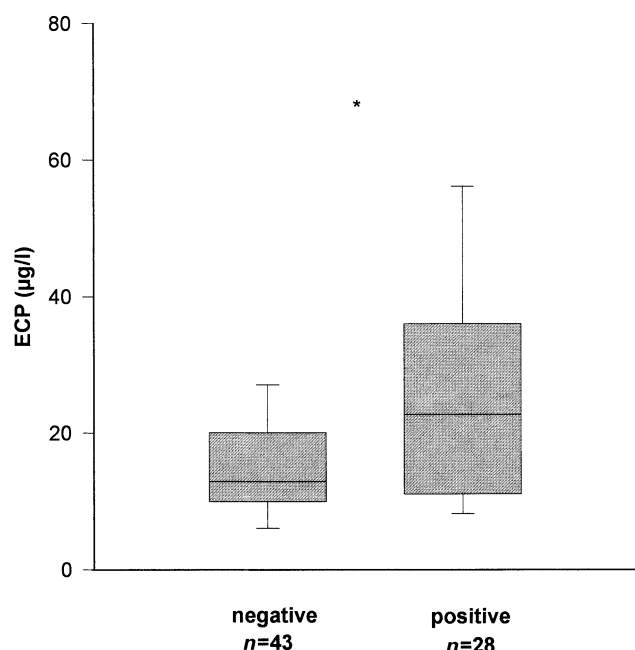


Figure 2. Serum ECP levels of patients sensitized to SEB in comparison with patients not sensitized to SEB. Horizontal lines represent median ECP levels; boxes contain 25th and 75th percentiles. *Mann-Whitney rank sum test, $P=0.018$.

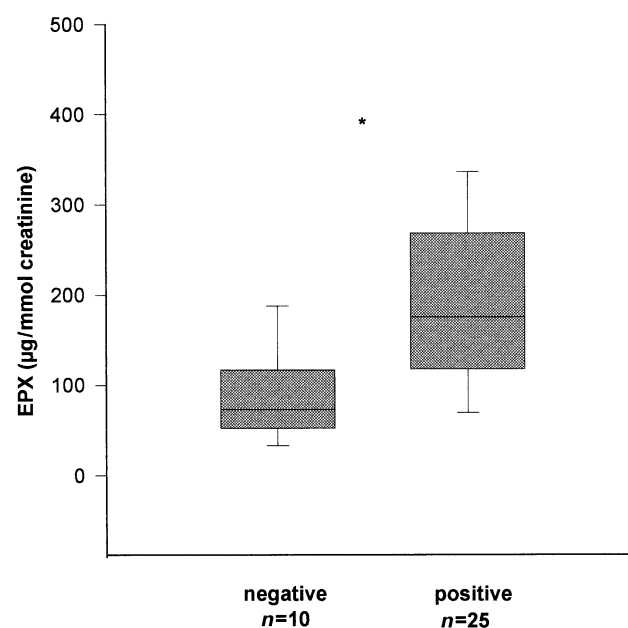


Figure 3. Urine EPX levels of patients sensitized to SEB compared with patients not sensitized to SEB. Horizontal lines indicate median EPX concentrations; boxes represent 25th and 75th percentiles. *Mann-Whitney rank sum test, $P=0.0038$.

We could show here that the presence of IgE antibodies to SEB is associated with severe skin lesions quantified by the SCORAD score in adults.

In addition to the SCORAD index, serum ECP and urine EPX concentrations, as *in vitro* parameters of allergic inflammation, were elevated in patients sensitized to SEB.

The serum concentration of ECP has been shown to be a sensitive measure of disease activity in AD (8). A clear relationship could also be demonstrated between urine concentration of EPX and disease activity in asthma (23), but studies on AD are missing.

In a recent study on children with AD, the presence of IgE antibodies to both SEA and SEB, which were found in 34% of the subjects, was correlated with the severity of skin lesions (17). We demonstrate here that sensitization to SEB is correlated with severity of cutaneous inflammation as determined by the SCORAD score, and ECP and EPX levels. For anti-SEA IgE, we could demonstrate an association only with serum ECP concentrations, and not with EPX concentrations as with the SCORAD scores. In keeping with our results, Nomura et al. were able to show that the severity of AD is related to the level of SEB-specific IgE, and not to the level of SEA- or TSST-1-specific IgE in children and adolescents with AD (24).

These findings point to a higher immunogenicity of SEB than SEA in patients with AD. In amino-acid sequence, staphylococcal enterotoxins fall into two groups. SEA and SED are similar, as are SEB and SEC

(25). Therefore, SEB (and SEC) might be more immunogenic due to its molecular structure. Alternatively, SEB-producing staphylococci could colonize the skin more persistently, leading to a permanent release of inflammatory mediators from cutaneous mast cells with subsequent pruritus and scratching. Moreover, SEB may bind to specific IgE on the surface of Langerhans cells, thus leading to the activation of specific T cells.

Bunikowski et al. demonstrated that the density of colonization with SEA-/SEB-producing *S. aureus* strains is higher in children sensitized to these superantigens than in unsensitized children (17). In a recent publication, Campbell et al. (26) have demonstrated that children under 7 years with AD more frequently have IgG antibodies to SEB than normal controls, but not antibodies to SEA and TSST-1. Furthermore, atopic children have higher levels of SEB-specific IgG than the 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.

Like Campbell et al., we did not investigate the cutaneous colonization with toxigenic strains, and future studies should address this aspect.

In summary, staphylococcal exotoxins, especially SEB, may exacerbate skin lesions in AD not only in their function as superantigens, but also as a new group of allergens. Further studies have to elucidate these functions at the cellular level.

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