

Original article

Comparison of serum specific IgE antibodies to staphylococcal enterotoxins between atopic children with and without atopic dermatitis

Background: The skin of patients with atopic dermatitis (AD) exhibits a striking susceptibility to colonization and infection by *Staphylococcus aureus*. The exotoxins secreted by *S. aureus* can act as superantigens and classic allergens, inducing the production of functionally relevant specific IgE antibodies. The aim of this study was to compare the levels and positive rates of serum staphylococcal enterotoxin A (SEA)- and staphylococcal enterotoxin B (SEB)-specific IgE between atopic children with and without AD.

Methods: Sixty children with AD, 55 children with respiratory allergy without AD, and 24 nonatopic healthy children were studied. The levels and positive rates of serum SEA- and SEB-specific IgE were compared among three groups. The correlation between the levels or positive rates of serum SEA/SEB-specific IgE and the severity of AD or the presence of previous skin infections was studied.

Results: The children with AD had significantly higher levels and positive rates of serum SEA- and SEB-specific IgE than the atopic children without AD ($P < 0.001$) and the nonatopic children ($P < 0.001$). There was no significant difference in the levels and positive rates of serum SEA- and SEB-specific IgE between the atopic children without AD and the nonatopic children. With or without adjustment for the potential confounding effect of total serum IgE levels, the levels and positive rates of serum SEA- and SEB-specific IgE were significantly correlated with severity of AD ($P < 0.005$), but they were not significantly different between AD children with and without previous skin infections.

Conclusions: SEA and SEB may contribute to chronic inflammation and exacerbation of AD through the IgE-mediated immune response.

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Atopic dermatitis (AD) is a chronically relapsing pruritic inflammatory skin disorder often associated with elevated total serum IgE levels and other atopic diseases such as asthma or allergic rhinitis (1, 2). It is the most common chronic skin disease in young children. Unfortunately, patients with AD usually require chronic use of topical corticosteroids without satisfactory therapeutic effects. Further understanding of the immunopathogenesis of AD may provide the rationale for developing specific therapy and point the way toward prevention of this disease.

The skin of patients with AD exhibits a striking susceptibility to colonization and infection by *Staphylococcus aureus*, which can secrete various exotoxins, including staphylococcal enterotoxins A, B, and C (SEA, SEB, and SEC, respectively) and toxic

shock syndrome toxin-1 (TSST-1) (3–7). These exotoxins may penetrate the skin and contribute to persistent inflammation and exacerbation of AD (8). They can act as superantigens and directly interact with several immunocompetent cell populations (9–13). In addition, they may also act as classic allergens, inducing the production of functionally relevant specific IgE antibodies and triggering the release of mediators from sensitized basophils (6, 7, 14).

The aim of this study was to compare the levels and positive rates of serum SEA- and SEB-specific IgE between atopic children with and without AD. Furthermore, the correlation between the levels or positive rates of serum SEA/SEB-specific IgE and severity of AD or the presence of previous skin infections was studied.

Material and methods

Study populations

From July 1998 to June 1999, 60 children with AD, fulfilling the diagnostic criteria of Hanifin & Rajka (1), were enrolled in this cross-sectional study as group I. It included mild AD in 19, moderate AD in 18, and severe AD in 23 children, the severity of AD being determined by the criteria of Rajka (2). Fifty-five of them also suffered from respiratory allergy (asthma and/or allergic rhinitis). A history of previous superficial skin infections characterized by small pustules, pus, folliculitis, furuncles, impetigo, or crusting was noted in 17 with severe AD, four with moderate AD, and three children with mild AD. Only one child, who had mild AD, had a history of previous invasive *S. aureus* skin infection presenting with lymphadenitis. All of the children with previous skin infections had received systemic and/or topical antibiotic therapy. Two control groups were studied: 55 children with respiratory allergy (asthma and/or allergic rhinitis) without AD as group II; 24 nonatopic healthy children as group III. The ages and sexes of the children in groups I, II, and III are listed in Table 1.

Determination of total serum IgE and serum allergen-specific IgE levels

In all studied children, the levels of total serum IgE and serum specific IgE to eight inhalant and food allergens, including mite, egg white, egg yolk, cow's milk, peanut, soybean, shrimp, and *Candida albicans*, were measured by the Pharmacia CAP assay (Pharmacia and Upjohn, Uppsala, Sweden) according to the manufacturer's instructions.

Determination of serum SEA- and SEB-specific IgE levels

In all studied children, serum SEA/SEB-specific IgE levels were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, flat-bottomed Costar EIA/RIA microtiter ELISA plates (Costar Corporation, Cambridge, MA, USA) were coated with 100 µl/well of purified mouse monoclonal anti-human IgE (PharMingen, San Diego, CA, USA) at 5 µg/ml in some wells for establishment of standard curve, and they were coated with 100 µl/well of highly purified SEA or SEB (Sigma Chemical Co., St Louis, MO, USA) at 2 µg/ml in other wells in carbonate buffer (0.035 M NaHCO₃ and 0.015 M Na₂CO₃, pH 9.6) overnight at 4°C. Each well was aspirated and washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween). Each well was blocked with 200 µl of blocking solution (1% bovine serum albumin and 0.05% NaN₃ in 0.1 M Tris buffer, pH 8.0–8.2) for 2 h at room temperature. Thereafter, each well was aspirated and washed with PBS-Tween; then 100-µl serum samples or serial dilutions of purified human IgE (Chemicon, Temecula, CA, USA) standards in diluent (1% bovine serum albumin, 0.05% NaN₃, and 0.05% Tween 20 in 0.1 M Tris buffer, pH 8.0–8.2) were added and incubated overnight at 4°C. After being aspirated and washed with PBS-Tween, each well was filled with 200 µl of biotin-conjugated mouse monoclonal anti-human IgE (PharMingen, San Diego, CA, USA) at 500 ng/ml in diluent and incubated for 2 h at room temperature. After repeating the aspiration and wash as above, 100 µl/well of horseradish peroxidase-conjugated avidin (Pierce, Rockford, IL, USA) at 2 µg/ml in PBS-Tween was added and incubated for 2 h at room temperature, and then each well was washed as above. Thereafter, 100 µl of substrate solution consisting of 3% 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

Table 1. Levels and positive rates of total serum IgE and serum SEA/SEB-specific IgE antibodies in studied children

	Group I (n=60)	Group II (n=55)	Group III (n=24)	P value†		
				I vs II	I vs III	II vs III
Sex (M:F)	40:20	34:21	10:14			
Age (years)*	7.2 (3.6)	8.2 (3.8)	8.4 (3.8)			
	6.9 (1.3, 14.8)	7.3 (2.6, 16)	8.5 (1, 14)	0.237	0.203	0.522
Total serum IgE (kU/l)*	17191 (109 094)	860 (1025)	50 (45)			
	982 (36, 846 000)	467 (35, 5610)	37 (4, 169)	<0.001	<0.001	<0.001
SEA-IgE (kU/l)*	6.92 (40.1)	0.43 (1.1)	0.12 (0.2)			
	0.80 (0, 312)	0.00 (0, 6.5)	0.00 (0, 0.6)	<0.001	<0.001	0.645
SEB-IgE (kU/l)*	119.6 (895)	1.03 (1.6)	0.38 (0.7)			
	2.40 (0, 6934)	0.14 (0, 6.7)	0.00 (0, 3.2)	<0.001	<0.001	0.076
a. SEA-IgE >0.16 kU/l#	42 (70%)	19 (35%)	6 (25%)	<0.001	<0.001	0.402
b. SEB-IgE >0.7 kU/l#	42 (70%)	19 (35%)	4 (17%)	<0.001	<0.001	0.177
a. or b.#	53 (88%)	25 (45%)	8 (33%)	<0.001	<0.001	0.336

SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B; group I: children with atopic dermatitis; group II: children with respiratory allergy without atopic dermatitis; group III: nonatopic healthy children.

* Mean (SD)/median (minimum, maximum).

Number (positive rate).

† P value of Mann-Whitney test and Fisher's exact test.

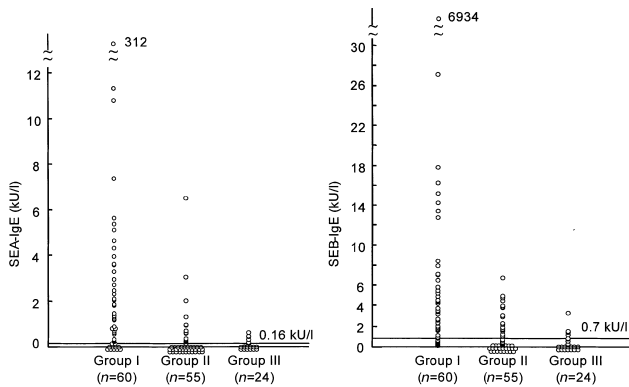


Figure 1. Distributions of serum SEA/SEB-specific IgE levels in studied children. SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B; group I: children with atopic dermatitis; group II: children with respiratory allergy without atopic dermatitis; group III: nonatopic healthy children. Optimal cutoff levels as definitions of serum SEA- and SEB-specific IgE positivity were 0.16 and 0.7 kU/l, respectively.

(ABTS; Sigma Chemical Co., St Louis, MO, USA) and 0.001% H_2O_2 in 0.05 M citrate buffer, pH 4.0, was added to each well. After 10 min at room temperature, the absorbance was determined at 420 nm on an ELISA reader (Anthos 2010, Anthos Labtec Instruments, GmbH, Australia). Serial dilutions of purified human IgE standards were included in each assay plate, and the standard curve was established from this. Serum SEA/SEB-specific IgE levels were determined by reference to the standard curve. The specificity of these IgE antibodies was demonstrated by the inhibition of binding of serum IgE to the wells coated with SEA or SEB after preincubation of positive sera with the corresponding toxin at 1 $\mu\text{g}/\text{ml}$ overnight at 4°C.

Statistical analyses

Serum SEA/SEB-specific IgE positivity was considered to exist when the serum levels were higher than the optimal cutoff values determined by the receiver operator characteristic (ROC) analysis for discrimination between AD and non-AD children (15, 16). The levels of total serum IgE and serum SEA/SEB-specific IgE among different groups were compared by nonparametric methods including the Mann–Whitney test for the difference between two groups and the Kruskal–Wallis test for the differences among three groups. The positive rates of serum SEA/SEB-specific IgE among the different groups were compared by Fisher's exact test. The correlation between total serum IgE and serum SEA/SEB-specific IgE levels was determined by Spearman's rank correlation. Considering the correlation between total serum IgE and SEA/SEB-specific IgE levels, and in order to clarify the correlation between AD or severity of AD and the levels or positive rates of serum SEA/SEB-specific IgE in the presence of concomitant variation of total serum IgE levels, we used analysis of covariance (ANCOVA) and multiple logistic regression analysis to

control the potential confounding effect of total serum IgE levels. All tests were two-tailed, and $P < 0.05$ was considered statistically significant. The SPSS 8.0 statistical software for Windows (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Total serum IgE and serum allergen-specific IgE levels

Total serum IgE levels were significantly different among groups I, II, and III ($P < 0.001$) (Table 1). All children in groups I and II had serum specific IgE more than 1 kU/l to at least one of the eight inhalant and food allergens. No children in group III had detectable serum allergen-specific IgE antibodies.

Serum SEA- and SEB-specific IgE levels

The distributions of serum SEA/SEB-specific IgE levels in groups I, II, and III are shown in Fig. 1. The children with AD had significantly higher serum SEA/SEB-specific IgE levels than the atopic children without AD ($P < 0.001$) and the nonatopic children ($P < 0.001$) (Table 1). There was no significant difference in serum SEA/SEB-specific IgE levels between the atopic children without AD and the nonatopic children (Table 1). There was a significant correlation between total serum IgE and serum SEA/SEB-specific IgE levels with the respective correlation coefficients of 0.31 ($P < 0.001$) and 0.34 ($P < 0.001$) (Fig. 2). After adjustment by ANCOVA for the potential confounding effect of total serum IgE levels, serum SEA/SEB-specific IgE levels were still significantly correlated with AD ($P = 0.003$ and < 0.001 , respectively). In addition, there was a significant correlation between serum SEA-specific IgE levels and SEB-specific IgE levels in the atopic children with or without AD ($P < 0.001$). Serum SEA/SEB-specific IgE levels were significantly different among the three groups of children with

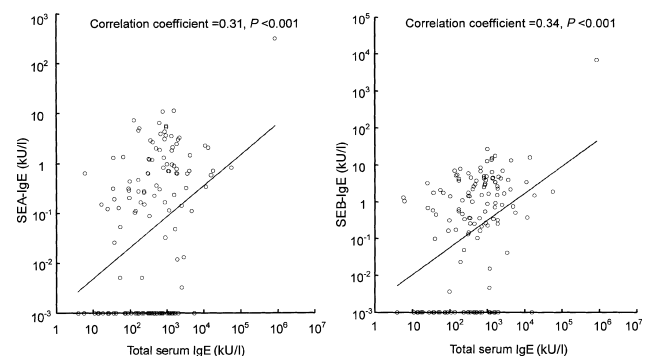


Figure 2. Scatter plot depicting correlation between total serum IgE and serum SEA/SEB-specific IgE levels in studied children. SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B. Correlation coefficient was determined by Spearman's rank correlation.

different severity of AD ($P < 0.001$). After adjustment by ANCOVA for the potential confounding effect of total serum IgE levels, serum SEA/SEB-specific IgE levels were still significantly correlated with severity of AD ($P = 0.002$ and 0.003 , respectively) (Table 2). The distributions of serum SEA/SEB-specific IgE levels in the AD children with and without previous skin infections are demonstrated in Fig. 3. There was no significant difference in serum SEA/SEB-specific IgE levels between the AD children with and without previous skin infections (Table 3).

Positive rates of serum SEA- and SEB-specific IgE antibodies

According to the ROC analysis, the optimal cutoff levels as the definitions of serum SEA- and SEB-specific IgE positive were 0.16 and 0.7 kU/l, respectively. With these selected cutoff levels, the tests of serum SEA- and SEB-specific IgE had the highest diagnostic sensitivity (70% and 70%, respectively) and specificity (68% and 71%, respectively) on AD. According to the above definitions, serum SEA- or SEB-specific IgE was positive in the majority (88%) of children with AD. The children with AD had significantly higher positive rates of serum SEA/SEB-specific IgE (70% and 70%) than the atopic children without AD (35% and 35%) ($P < 0.001$) and the nonatopic children (25% and 17%) ($P < 0.001$) (Table 1). There was no significant difference in the positive rates of serum SEA/SEB-specific IgE between the atopic children without AD and the nonatopic children (Table 1). The positive rates of serum SEA/SEB-specific IgE were significantly different among the three groups of children with different severity of AD ($P < 0.001$). After adjustment by multiple logistic regression analysis for the potential confounding effect of total serum IgE levels, the positive rates of serum SEA/SEB-specific IgE were still significantly correlated with severity of AD ($P < 0.001$) (Table 2). However, there was no significant

difference in the positive rates of serum SEA/SEB-specific IgE between the AD children with and without previous skin infections (Table 3).

A 13-year-old boy who had suffered from severe AD from the age of 10 years, and who had a history of superficial skin infections, had extremely high levels of total serum IgE and serum SEA- and SEB-specific IgE up to 846 000, 312, and 6934 kU/l, respectively. No history of recurrent invasive infections, eosinophilia, or respiratory allergy was noted in this child. Thus, he did not fulfill the criteria for the diagnosis of hyper-IgE syndrome. If the extreme data of this patient are excluded, the means $\pm$ standard deviations of total serum IgE levels, and serum SEA- and SEB-specific IgE levels in the remaining 59 children with AD were 3143 ± 7906 , 1.75 ± 2.47 , and 4.14 ± 5.38 kU/l, respectively. However, the extreme data would not bias the results of the comparison of serum SEA/SEB-specific IgE levels among the different groups because non-parametric methods were used for statistical analyses.

Discussion

Leung et al. detected serum specific IgE antibodies to SEA, SEB, or TSST-1 in 32 (57%) of 56 patients with AD (6). Tada et al. found that serum SEA- or SEB-specific IgE levels were higher than 0.35 kU/l in 77 (80%) of 96 adults with AD (14). Bunikowski et al. reported that serum SEA- or SEB-specific IgE levels were higher than 0.7 kU/l in 20 (34%) of 58 children with AD (7). However, serum SEA- and SEB-specific IgE positivity was not defined. In fact, the results of our study, as well as previous studies, showed that the distributions of serum SEA- and SEB-specific IgE levels were not the same. It would not be appropriate to use the same cutoff levels as the definitions of serum SEA- and SEB-specific IgE positivity. According to the ROC analysis, our study determined serum levels higher than 0.16 and 0.7 kU/l as the respective optimal definitions of serum SEA- and SEB-specific IgE positivity, a finding which could maximize the discrimination between AD and non-AD children. This would reduce the possibility of misclassification of test results and help to clarify the correlation between positive rates of serum SEA/SEB-specific IgE and other factors (15, 16). According to the above definitions, serum SEA- or SEB-specific IgE was positive in the majority (88%) of children with AD in our study.

Previous studies showed that specific IgE antibodies to staphylococcal exotoxins were extremely rare in nonatopic individuals, including patients with nonatopic eczematous skin diseases such as psoriasis or contact dermatitis, although chronic colonization of superantigen-secreting *S. aureus* was noted on their skin (6, 7, 14). However, the levels of serum specific IgE to staphylococcal exotoxins had never been determined in

Table 2. Levels and positive rates of serum SEA/SEB-specific IgE antibodies in children with different severity of AD

	Severe AD (n=23)	Moderate AD (n=18)	Mild AD (n=19)	P value†
SEA-IgE (kU/l)*	14.95 (64.8)	2.95 (3.6)	0.97 (1.4)	0.002
	1.84 (0, 312)	0.83 (0, 11.3)	0.11 (0, 4.6)	
SEB-IgE (kU/l)*	306.4 (1445)	4.84 (4.2)	2.36 (3.4)	0.003
	4.50 (0, 6934)	1.91 (0.2, 17.8)	1.52 (0, 14.2)	
a. SEA-IgE > 0.16 kU/l#	19 (83%)	14 (78%)	9 (47%)	<0.001
b. SEB-IgE > 0.7 kU/l#	21 (91%)	16 (89%)	11 (58%)	<0.001
a. or b.#	22 (96%)	17 (94%)	15 (79%)	<0.001

AD: atopic dermatitis; SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B.

* Mean (SD)/median (minimum, maximum).

Number (positive rate).

† P value of analysis of covariance (ANCOVA) and multiple logistic regression analysis with adjustment for potential confounding effect of total serum IgE levels to test differences among three groups of children with different severity of AD.

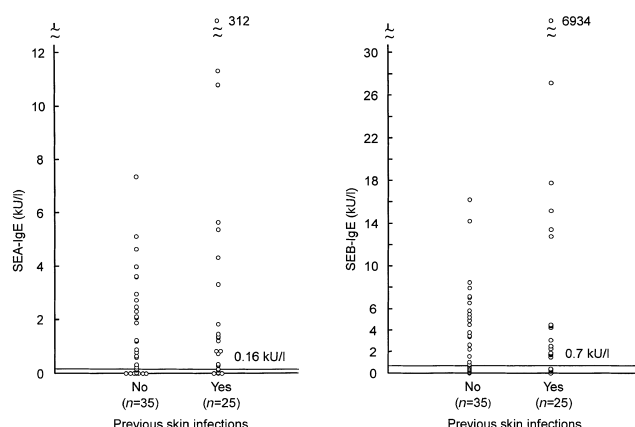


Figure 3. Distributions of serum SEA/SEB-specific IgE levels in children with atopic dermatitis with and without previous skin infections. SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B. Optimal cutoff levels as definitions of serum SEA- and SEB-specific IgE positivity were 0.16 and 0.7 kU/l, respectively.

atopic children without AD. Our study showed that the children with AD had significantly higher levels and positive rates of serum SEA/SEB-specific IgE than the atopic children without AD and the nonatopic children. However, there was no significant difference in the levels and positive rates of serum SEA/SEB-specific IgE between the atopic children without AD and the nonatopic children. These findings suggested that serum SEA/SEB-specific IgE antibodies may be specific to AD and play an important role in the majority of patients with this disease.

Tada et al. showed that there was no significant correlation between severity of AD and the prevalence of serum SEA/SEB-specific IgE antibodies (14). However, Bunikowski et al. reported that severe AD was significantly more often observed in SEA/SEB-sensitized children (7). In our study, after adjustment for the potential confounding effect of total serum IgE levels, the levels and positive rates of serum SEA/SEB-specific IgE were still significantly correlated with severity of AD. These findings suggested that SEA and SEB may contribute to persistent inflammation and exacerbation of AD through the IgE-mediated immune response.

Bunikowski et al. showed that SEA/SEB-sensitized AD children had a higher incidence of *S. aureus* skin infections requiring antimicrobial therapy than did nonsensitized AD children (7). In our study, however, there was no significant difference in the levels and

Table 3. Levels and positive rates of serum SEA/SEB-specific IgE antibodies in AD children with and without previous skin infections

	Previous skin infections		P value†
	No (n=35)	Yes (n=25)	
SEA-IgE (kU/l)*	1.54 (1.8)	14.50 (62.1)	0.408
	0.83 (3.14)	0.64 (2.32)	
SEB-IgE (kU/l)*	3.54 (3.9)	282.0 (1386)	0.697
	2.29 (4.11)	2.60 (4.92)	
a. SEA-IgE > 0.16 kU/l#	23 (66%)	19 (76%)	0.286
b. SEB-IgE > 0.7 kU/l#	24 (69%)	18 (72%)	0.503
a. or b.#	31 (89%)	22 (88%)	0.625

AD: atopic dermatitis; SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B.

* Mean (SD)/median (IQR).

Number (positive rate).

† P value of Mann-Whitney test and Fisher's exact test.

positive rates of serum SEA/SEB-specific IgE between the AD children with and without previous skin infections. Clinically, patients with AD, regardless of whether there is a skin infection, respond better to combined treatment with topical corticosteroids and antistaphylococcal antibiotics than to topical steroid therapy alone (17). These findings suggested that *S. aureus* induced the production of specific IgE to the superantigens, not by infection of this organism, but by penetration of the exotoxins from the colonized skin.

The results of this study suggest that SEA and SEB may contribute to chronic inflammation and exacerbation of AD through the IgE-mediated immune response. Consequently, the determination of serum SEA/SEB-specific IgE antibodies may provide important information in the follow-up of patients with AD. In addition, it is clear that antibiotics can transiently reduce the amount of *S. aureus* on the inflammatory skin, but cannot eradicate the organism. New strategies for the treatment of AD may include ones that interfere in the production of exotoxins by *S. aureus*. Further studies are required to elucidate the role of bacterial superantigens in the initiation, maintenance, and exacerbation of skin inflammation in AD.

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