

Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis

Ichiro Nomura, MD, PhD,^a Kazuko Tanaka, MSc,^b Hisashi Tomita, BSc,^b Toshio Katsunuma, MD, PhD,^a Yukihiro Ohya, MD, PhD,^a Norikazu Ikeda, PhD,^c Tae Takeda, MD, PhD,^c Hirohisa Saito, MD, PhD,^b and Akira Akasawa, MD, PhD^a Tokyo, Japan

Background: Superantigenic exotoxins produced by *Staphylococcus aureus* and their specific IgE antibodies are thought to be important precipitating factors of atopic dermatitis (AD), but there are few reports evaluating these 2 factors at the same time.

Objective: We examined whether the presence of the exotoxins sampled from the skin of patients with AD and the levels of anti-exotoxin IgE antibodies in their sera correlated with their severity of AD.

Methods: Patients with mild-to-severe AD, 1 to 22 years of age, were evaluated by using Leicester's scoring system. Specific IgE antibodies against these exotoxins were determined by using ELISA. *S aureus* was isolated from 3 different areas of the skin. We examined whether the exotoxin (staphylococcal enterotoxin [SE]A, SEB, SEC, SED, and toxic shock syndrome toxin-1) could be detected.

Results: The levels of SEB-specific IgE were correlated with the severity of AD. Five of 6 patients having very high SEB-specific IgE antibody titers were under 6 years of age, and SEB was most frequently isolated (41%). There was no difference in severity between patients with or without exotoxin-producing *S aureus*. The severity of 9 patients who had both exotoxin-producing *S aureus* on the skin and specific IgE antibody against the same exotoxin in sera was significantly higher than that of the other patients.

Conclusions: Anti-SEB IgE titers correlate well with the severity of AD. The presence of exotoxin-producing *S aureus* may precipitate AD through its specific IgE antibody. (J Allergy Clin Immunol 1999;104:441-6.)

Key words: Atopic dermatitis, staphylococcal exotoxin, specific IgE, Leicester's disease activity score

Staphylococcus aureus frequently colonizes (over 95%) the eczematous skin of patients with atopic dermatitis (AD) and is thought to be an important precipitating factor of AD.¹ The mechanisms of worsening AD by *S aureus* are thought to be that (1) α -toxin and other cytolytic toxins directly destroy the skin tissue, (2) cellular proteins of *S aureus* act as allergens,² and (3)

Abbreviations used

AD: Atopic dermatitis

SE: Staphylococcal enterotoxin

TSST-1: Toxic shock syndrome toxin-1

superantigenic exotoxins act as superantigen and allergen.

S aureus can produce superantigenic exotoxins (Fig 1), such as staphylococcal enterotoxin [SE]A, SEB, SEC, SED, SEE, toxic shock syndrome toxin (TSST)-1, and exfoliative toxin-A and -B. Superantigens activate T cells in a nonspecific manner against antigens. Several studies have reported evidence concerning the action of exotoxins as superantigens. When PBMCs from donors with AD are treated with staphylococcal superantigens, the amount of messenger RNA of IL-4, IL-5,⁴ and IgE^{5,6} are increased. SEB and TSST-1 induced the production of cutaneous lymphocyte antigens of T cells from healthy donors.⁷ Intracutaneous injection of SEB induces a T cell-dependent inflammatory response in mice.⁸ On the contrary, Akiyama et al⁹ have reported that the existence of the exotoxins is not reflective of AD severity.

Leung et al¹⁰ have reported that specific IgE antibodies to staphylococcal exotoxins (ie, SEA, SEB, and/or TSST-1) are found in 57% of sera from patients with AD. Tada et al¹¹ and Nissen et al¹² also found similar results. Specific IgE antibodies against staphylococcal exotoxins are also thought to precipitate AD by mediating type I allergy. The levels of anti-exotoxin IgE have been reported not to correlate with AD severity,¹¹ whereas Bunikowski et al¹³ have recently reported that the severity correlates with the presence of SEA/SEB-specific IgE antibodies.

It is speculated that staphylococcal exotoxins worsen AD by acting as allergens by means of type I allergy or by acting as superantigens by means of nonspecific activation of T cells (Fig 1). Here we focused on the relationship between AD severity and levels of serum anti-exotoxin IgE and skin exotoxin-producing *S aureus* at the same time.

METHODS

Patients

Ninety-four patients with mild-to-severe AD, fulfilling the criteria of Hannifin and Rajka,¹⁴ took part in this study. The patients were 56 males and 38 females (age range, 1 to 22 years; mean, 7.78

From ^athe Department of Allergy, National Children's Hospital, Tokyo; and ^bthe Department of Allergy and ^cthe Department of Infectious disease, National Children's Medical Research Center, Tokyo.

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Reprint requests: Akira Akasawa, MD, PhD, Department of Allergy, National Children's Hospital, 3-35-31, Taishido, Setagayaku, Tokyo, Japan.

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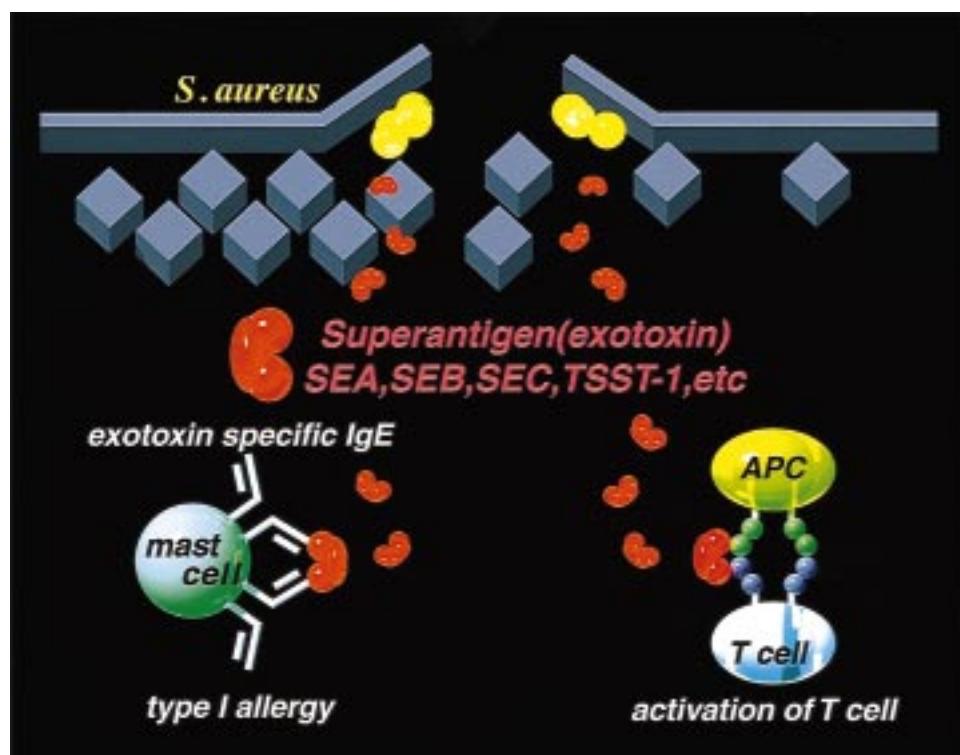


FIG 1. Schematic model of the pathogenesis of AD induced by staphylococcal exotoxins. The three-dimensional structure of exotoxin has been reported.¹⁶

TABLE I. Patient profiles of asthmatic control subjects and patients with AD

	Asthmatic control subjects (n = 25)	Patients with AD (n = 94)	Patients with AD* (n = 39)
Sex (F/M)	8:17	38:56	15:24
Age (y)	4.7 ± 2.3	7.8 ± 4.9	8.8 ± 4.9
Leicester's disease activity score	0	35.4 ± 22.2	44.9 ± 16.1
Total IgE (IU/mL)	251 ± 440	5609 ± 10,020	7323 ± 8011
SEA-specific IgE (U)	0.15 ± 0.46	9.1 ± 19.5	7.8 ± 18.3
SEB-specific IgE (U)	4.2 ± 4.1	14.7 ± 21.8	16.2 ± 26.8
SEC-specific IgE (U)	2.0 ± 2.9	8.8 ± 15.5	11.5 ± 17.3
TSST-1-specific IgE (U)	1.7 ± 3.3	8.76 ± 22.5	7.7 ± 22.2

*Patients with AD who were examined for exotoxin production by *S aureus*. They were included in the 94 patients with AD.

± 4.91 years; Table I). The severity of AD was scored on the basis of the modified Leicester system.¹⁵ Five clinical features (erythema, papule, excoriation, oozing, and lichenification) were graded at 6 body sites (head, trunk, elbow, hand, knee, and foot) on a scale of 0 (none) to 3 (severe). This gave a disease activity score with a maximum of 90 points. We defined 0 to 19 points as mild AD, 20 to 49 points as moderate AD, and 50 to 90 points as severe AD. The mean score of disease activity was 35.4 ± 22.2. None of these patients had a history of deep-seated skin infection by *S aureus*. Serum samples obtained from all the patients were analyzed for their specific IgE to SEA, SEB, SEC, and TSST-1. Specific IgE to house dust mite, cedar pollen, and egg white, as well as total serum IgE, were also determined. Control serum sam-

TABLE II. Identification of exotoxin-producing *S aureus* from the skin of 39 patients with AD

Exotoxins	No. of patients (%)
SEA only	2 (5.1)
SEB only	13 (33.3)
SEC only	1 (2.6)
SED only	1 (2.6)
TSST-1 only	1 (2.6)
SEA + SEB	1 (2.6)
SEB + SEC	1 (2.6)
SEB + TSST-1	1 (2.6)
Exotoxin (–) <i>S aureus</i>	16 (41.0)
No growth	2 (5.1)

Thirty-seven of 39 patients (95%) had *S aureus* on the skin. Sixteen patients (41%) had SEB-producing *S aureus* on the skin; 13 patients had SEB-producing *S aureus* only, and 3 patients had SEB-producing *S aureus* and other exotoxin-producing *S aureus*.

ples were obtained from 25 patients with bronchial asthma who had no history of AD (average age, 4.7 ± 2.3 years).

Assay of specific IgE to staphylococcal exotoxins

Flat-bottomed, EIA/RIA plates (Costar Corp, Cambridge, Mass) were filled with 100 µL of a 5 mg/mL (SEA) and a 2.5 mg/mL (SEB and TSST-1) solution of highly purified staphylococcal exotoxin (Toxin Technology, Sarasota, Fla) in 0.01 mol/L Na₂CO₃ buffer (pH 9.1). The plates were covered and placed overnight at 4°C. The following day, the plates were washed 3

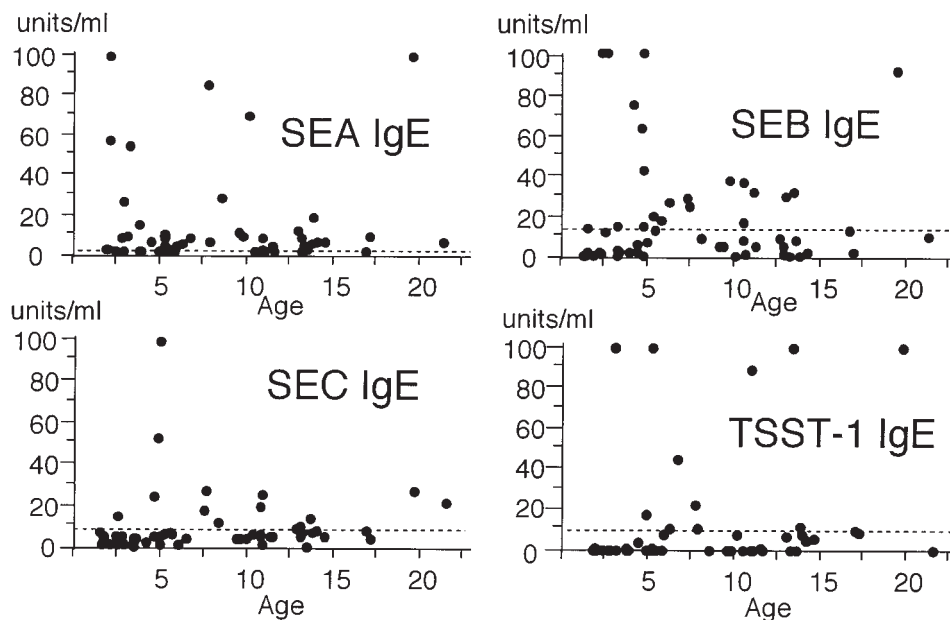


FIG 2. Age distribution of the patients with AD ($n = 94$) for specific IgE of staphylococcal exotoxins determined by using ELISA. Anti-exotoxin IgE of asthma control sera were as follows: SEA, 0.15 ± 0.46 U (mean $\pm$ SD); SEB, 4.2 ± 4.1 U; SEC, 2.0 ± 2.9 U; and TSST-1, 1.7 ± 3.3 U. When cut-off points were defined as control mean $+ 2$ SD, the positive ratio of anti-SEA IgE was 78.7%, that of SEB was 35.1%, that of SEC was 21.3%, and that of TSST-1 was 18.1%. The cut-off points were drawn as broken lines. Five of 6 patients with very high SEB-specific IgE titers were under 6 years of age.

times with PBS with 0.1% Brij 35 (Sigma Diagnostics, St Louis, MO) and then filled with 200 μ L of 0.1% human serum albumin (Sigma Chemical Co, St Louis, Mo). The plates were incubated at room temperature for 2 hours and then washed 3 times with PBS-Brij. Serum to be tested was diluted 1:16 in PBS-Brij, and 100 μ L was placed in the wells. The plates were incubated at room temperature for 2 hours and then washed 6 times with PBS-Brij. One hundred microliters of a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-human IgE (KPL, Gaithersburg, Md) was placed in the wells. The plates were incubated at room temperature for 1 hour and then washed 6 times with PBS-Brij. One hundred microliters of equal volume mixture of tetramethyl benzidine peroxidase substrate and peroxidase solution (KPL) were placed in the wells. After 20 minutes, 100 μ L of 1 mol/L phosphate solution was added in each well to stop the reaction, and results were read at 450 nm. The background without serum samples was under 0.1 and was subtracted from the values obtained with the test samples. Serum containing high antitoxin titer was defined as 100 U for calibrating the other serum samples.

Skin bacterial culture and exotoxin productivity

Thirty-nine patients who were first seen by Dr Nomura could be examined. They were included in the total 94 patients with AD. Three different lesions (ie, exudative dermatitis, dry dermatitis, and normal skin) were tested by rubbing 2×2 cm of skin surface with a swab, and the swabs were then spread over the surface of mannitol salt agar plates. Agar plates were then incubated for 2 days at 37°C. Five coagulase-positive colonies per plate were randomly selected to incubate for 18 hours in trypticase soy broth with shaking, and then culture broth was centrifuged at 3000 rpm for 20 minutes. Supernatants were diluted to 1:10, 1:100, 1:1000, and 1:10,000. They were mixed with specific rabbit IgG against exotox-

ins (SEA, SEB, SEC, SED, and TSST-1; purchased from Denka Seiken, Tokyo, Japan). After 20 hours of incubation at room temperature, the wells were checked for agglutination and identified as follows: (-), ($\pm$), (+), (++) , (+++). Those identified as (+) or greater were considered positive.

Statistical analysis

Data collected were the mean of duplicated determinations. Statistical analysis was first performed with a one-factor ANOVA test. When a significant deviation was observed, the Scheffe F test was performed as a post hoc test by using the STATVIEW IV program. A P value of less than .05 was accepted as significant.

RESULTS

Serum exotoxin-specific IgE

Serum IgE antibodies from 94 patients with AD against SEA, SEB, SEC, and TSST-1 were determined by using ELISA (Table I). When cut-off points were determined as control mean $+ 2$ SD, the number of patients having exotoxin-specific IgE was as follows: SEA-specific IgE, 74 (78.7%); SEB-specific IgE, 33 (35.1%); SEC-specific IgE, 20 (21.3%); and TSST-1-specific IgE, 17 (18.1%). Five of 6 patients having high SEB-specific antibody titer (>50 U) were less than 6 years of age (Fig 2).

Patients with AD were divided into 3 groups on the basis of their Leicester's disease activity scores. The difference of each antitoxin IgE titer among the 3 severity groups were examined (Fig 3). Anti-SEB IgE titers showed statistically significant differences between patients with mild and severe AD and between patients

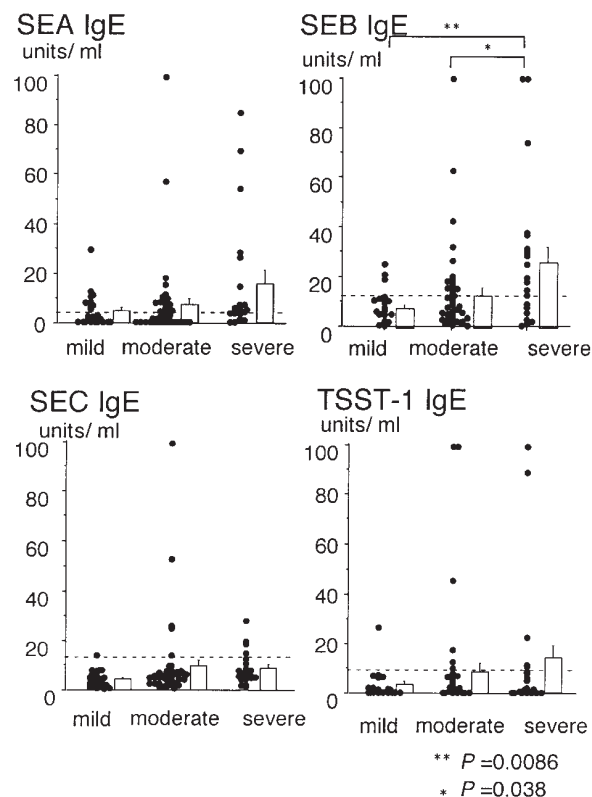


FIG 3. Exotoxin-specific IgE and severity of AD. Patients with AD were divided into 3 groups on the basis of their Leicester's disease activity scores (ie, 1 to 19 points was defined as mild, 20 to 49 points as moderate, and 50 to 90 points as severe). Difference of each anti-exotoxin IgE titer between the 3 groups were then examined. Anti-SEB IgE titers of the group of patients with severe AD showed statistically significant differences from the mild and moderate groups, as determined by the Scheffe F test. Each closed circle represents a patient with AD. Each column and bar represents the mean $\pm$ SEM of the group. The cut-off points are indicated as broken lines.

with moderate and severe AD by means of the Scheffe F test. This result was different from that reported by Tada et al,¹¹ which showed that anti-exotoxin IgE titers were not different among the 3 groups of AD severity. This discrepancy might be due to the average age of the patients being different. The average age of our patients was 7.8 ± 4.9 years, whereas the patients in the study by Tada et al had an average age of 20.2 ± 9.8 years. Thus we divided the patient group at the age of 7 years. There were statistically significant differences of anti-SEB IgE titers among the 3 groups of AD severity in the subgroup of patients less than 7 years of age ($P = .0137$, as determined by one-factor ANOVA). On the other hand, there was not a significant difference in the levels among the 3 groups of severity in patients older than 7 years ($P = .141$).

For determination of other allergen-specific IgE titers (eg, house dust mite, cat, cedar pollen, and egg white) and total IgE titer, the same procedures as in Fig 3 were performed. Only specific IgE titers to house dust mite ($P < .01$) and total IgE titers ($P < 0.01$) showed statistically

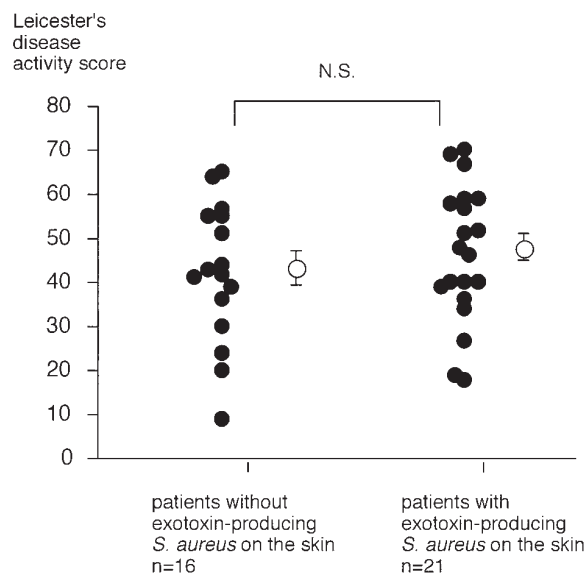


FIG 4. Disease activity scores of the patients with or without exotoxin-producing *S aureus*. Disease activity scores (Leicester's score) and presence of skin exotoxin-producing *S aureus* of the patients with AD were evaluated. The patients were divided into 2 groups, one with exotoxin-producing *S aureus* on their skin and one without. The difference of disease activity scores between the 2 groups was not statistically significant.

significant differences in the levels among the 3 groups of severity.

Exotoxins secreted by *S aureus* grown from AD skin lesions

S aureus was isolated from 37 of 39 patients with AD (Table II). *S aureus* isolated from 21 patients was found to secrete exotoxins. SEB was identified from 16 patients (41%) and was the most frequently detected. These data were in agreement with previous reports.^{9,10} There was no statistically significant difference in disease activity scores between patients with or without exotoxin-producing *S aureus* (Fig 4).

Because anti-SEB IgE titers were related to disease severity, the relationship between the presence of SEB-producing *S aureus* and SEB-specific IgE titers were compared. However, SEB-specific IgE titers in patients who have SEB-producing *S aureus* and those in patients having no SEB were, respectively, 8.1 ± 9.0 and 20.7 ± 6.4 , and were not significantly different.

Pairing of skin exotoxin producing *S aureus* and serum IgE antibody specific to the same exotoxin

Pairing (+) means that a patient has both exotoxin-producing *S aureus* on the skin and serum IgE specific to the same exotoxin (over cut-off point: asthma control + 2 SD) at the same time. There were 9 patients with pairing. Their disease severity was significantly higher than that of the other patients (Fig 5). Total IgE titers of these 9 patients and the other patients were not significantly dif-

ferent. The disease activity scores (means $\pm$ SEM) obtained in the patients who had (1) high levels of anti-exotoxin IgE in sera and the other exotoxins detected on their skin, (2) high levels of anti-exotoxin IgE in sera without detectable exotoxins on their skin, (3) low levels of anti-exotoxin IgE in sera and exotoxins detected on their skin, and (4) low levels of anti-exotoxin IgE in sera without detectable exotoxins on their skin were, respectively, 41.7 ± 5.4 , 47.1 ± 4.7 , 41.8 ± 5.8 , and 36.0 ± 8.2 . Each of the above scores was not significantly different from the scores obtained from the rest of the patients.

DISCUSSION

SEB was most frequently (41% of the patients) detected on the AD-affected skin of patients in this study, as has been reported. There was no statistically significant difference in the AD disease activity score between the patients with or without exotoxin-producing *S aureus*. Although these exotoxins are superantigens, they might be neutralized by IgG or some other mechanisms, as has been reported.

We have focused on specific IgE antibodies against these exotoxins in this study. Eighteen percent to 78.7% of the patients were judged to be positive, as has been reported by others.

Next we examined the relationship between age distribution and anti-exotoxin IgE of patients with AD. IgE antibodies against these exotoxins were inclined to be found in younger children, also suggesting that these exotoxins might be neutralized in the older patients.

In our study anti-SEB IgE antibodies were more frequently detected in patients with severe AD than in patients with mild and moderate AD who were an average age of 7.8 ± 4.91 years. When we were preparing this manuscript, Bunikowski et al¹³ had just reported results similar to those obtained in our study; that is, disease severity of the patients (median age, 2.5 years) correlated with the presence of SEA/SEB-specific IgE. On the other hand, Tada et al¹¹ reported that in patients with AD aged 20.2 ± 9.8 years, anti-exotoxin IgE titers were not different among the 3 groups of AD severity. This discrepancy might be due to differences in the average ages of the patients. We found that the mean anti-SEB IgE titer in the group of patients with severe AD was significantly higher than that in children younger than 7 years of age, whereas these levels were not different in the 3 groups of patients older than 7 years.

With regard to other allergen-specific IgE titers (eg, house dust mite, cat, cedar pollen, and egg white) and total IgE titer, only specific IgE titers to house dust mite and total IgE titers showed statistically significant difference in the levels among the 3 groups of AD severity. Anti-SEB IgE titers are thought to be as important as house dust mite-specific IgE or total IgE in the evaluation of AD severity.

The serum levels of anti-SEB IgE from the patients with SEB-producing *S aureus* did not have significantly higher titers than those obtained from the patients with-

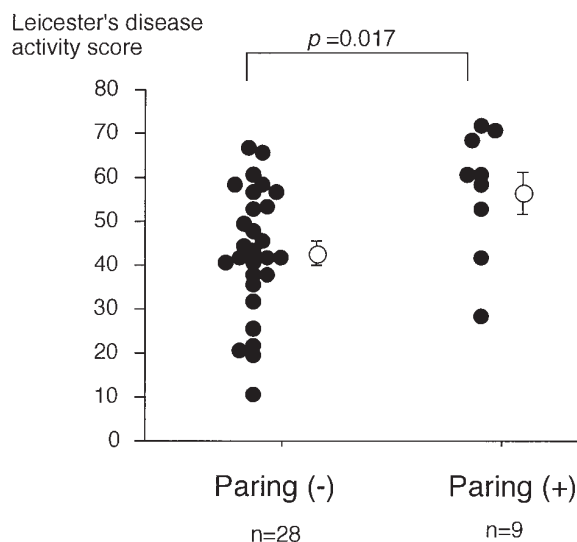


FIG 5. There were 9 patients who had detectable exotoxin-producing *S aureus* on the skin and positive serum-specific IgE to the same exotoxin (over the cut-off point). Their disease activity scores of AD were significantly higher than those of the other patients. Four patients had SEB-producing *S aureus* on the skin and serum SEB-specific IgE (SEB paired). Three patients were SEA paired, one was SEC paired, and one was TSST-1 paired.

out these bacteria. This result again supports the idea of some neutralizing substance in the skin of these patients.

Finally, we intended to determine the relationship between AD severity and the levels of anti-exotoxin IgE, especially when the exotoxins were detected from the skin. The severity of 9 patients who had both exotoxin-producing *S aureus* on the skin and its specific IgE in sera was significantly higher than that of the other patients. Therefore it is suggested that exotoxins may worsen AD through type I allergy. Exotoxin-specific IgE antibodies and exotoxin-producing *S aureus* should be measured at the same time to evaluate the effect of exotoxins on AD severity.

In summary, we found that (1) SEB-producing *S aureus* was most frequently detected on the skin of patients with AD, (2) severity of AD was not related to the presence of exotoxin-producing *S aureus*, (3) 5 of 6 patients with high SEB-specific IgE titers were younger than 6 years of age, (4) anti-SEB IgE levels correlate well with the severity scores of patients with AD and are thought to be as important as house dust mite-specific IgE or total IgE in the evaluation of AD severity, and (5) the simultaneous presence of exotoxin-producing *S aureus* and the corresponding exotoxin-specific IgE antibody was often found in patients with severe AD. The presence of exotoxin-producing *S aureus* may precipitate AD through its specific IgE antibodies.

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REFERENCES

1. Leung DY. Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 1995;96:302-18.
2. Motala C, Potter PC, Weinberg EG, Malherbe D, Hughes J. Anti-*Staphylococcus aureus*-specific IgE in atopic dermatitis. *J Allergy Clin Immunol* 1986;78:583-9.
3. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990;248:705-1.
4. Neuber K, Steinrucke K, Ring J. Staphylococcal enterotoxin B affects in vitro IgE synthesis, interferon- γ , interleukin-4 and interleukin-5 production in atopic eczema. *Int Arch Allergy Immunol* 1995;107:179-82.
5. Hofer MF, Lester MR, Schlievert PM, Leung DY. Upregulation of IgE synthesis by staphylococcal toxic shock syndrome toxin-1 in peripheral blood mononuclear cells from patients with atopic dermatitis. *Clin Exp Allergy* 1995;25:1218-27.
6. Jabara HH, Geha RS. The superantigen toxic shock syndrome toxin-1 induces CD40 ligand expression and modulates IgE isotype switching. *Int Immunol* 1996;8:1503-10.
7. Leung DY, Gately M, Trumble A, Ferguson DB, Schlievert PM, Picker LJ. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. *J Exp Med* 1995;181:747-53.
8. Saloga J, Leung DY, Reardon C, Giorno RC, Born W, Gelfand EW. Cutaneous exposure to the superantigen staphylococcal enterotoxin B elicits a T-cell-dependent inflammatory response. *J Invest Dermatol* 1996;106:982-8.
9. Akiyama H, Toi Y, Kanzaki H, Tada J, Arata J. Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strains isolated from atopic dermatitis lesions. *Arch Dermatol Res* 1996;288:418-20.
10. Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993;92:1374-80.
11. Tada J, Toi Y, Akiyama H, Arata J, Kato H. Presence of specific IgE antibodies to staphylococcal enterotoxins in patients with atopic dermatitis. *Eur J Dermatol* 1996;6:552-4.
12. Nissen D, Pedersen LJ, Skov PS, Vejlsgaard GL, Poulsen LK, Jarlov JO, et al. IgE-binding components of staphylococcal enterotoxins in patients with atopic dermatitis. *Ann Allergy Asthma Immunol* 1997;79:403-8.
13. Bunikowski R, Mielke M, Skarabis H, Herz U, Bergmann RL, Wahn U, et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 1999;103:119-24.
14. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;9:44-7.
15. Sowden JM, Berth JJ, Ross JS, Motley RJ, Marks R, Finlay AY, et al. Double-blind, controlled, crossover study of cyclosporin in adults with severe refractory atopic dermatitis. *Lancet* 1991;338:137-40.