

Original Article

Staphylococcal enterotoxin-specific IgE antibodies in atopic dermatitis

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Abstract

Background: The authors clarified the clinical significance of the measurement of serum concentrations of specific IgE antibodies to staphylococcal enterotoxin (SE) A- and SEB in atopic dermatitis (AD).

Methods: The serum concentrations of SEA- and SEB-specific IgE antibodies in 140 pediatric patients with AD were measured with an immuno CAP –radioallergosorbent test system (RAST). To check the cross-reaction of specific IgE antibodies to SEA/SEB and other allergens, the CAP RAST fluorescent enzyme immunoassay inhibition test was performed.

Results: Forty-seven patients (33.6%) tested positive for either SEA- or SEB-specific IgE antibodies. School children showed higher positive rates of SEA/SEB-specific IgE antibodies than infants or young children. The patients with severe AD and those with exacerbation of symptoms in summer, had higher positive rates of SEA/SEB-specific IgE antibodies than patients with mild AD or those with exacerbation in winter. In addition, the positive rates of specific IgE antibodies to both dog-dander and cat-dander were higher in patients with positive SEA/SEB-specific IgE antibodies than in patients with negative ones. No cross-reactions occurred among specific IgE antibodies to SEA/SEB and dog/cat dander with one patient's serum, which had positive IgE-specific antibodies against cat/dog dander and SEA/SEB. The positive rate of SEA/SEB-specific IgE antibodies in the patients with dogs and/or cats as pets was 48.4%, which was higher than in those with no pets.

Conclusions: Atopic dermatitis patients who exhibit high positive rates of SEA/SEB-specific IgE antibodies were found to be school children, severe cases, cases with high serum concentrations of total IgE, cases with exacerbation in summer, and cases with dogs and/or cats as pets. The measurement of serum concentrations of specific IgE antibodies to SEA and SEB, thus has some value for evaluating AD patients.

Key words

atopic dermatitis, cat, dog, RAST, SEA-specific IgE antibodies, SEB-specific IgE antibodies.

Atopic dermatitis (AD) is defined as a chronically relapsing inflammatory skin disease often associated with a family history of atopy, and diagnosed on the basis of a combination of characteristic clinical findings.^{1,2} Its pathology comprises the destruction of skin barrier mechanisms and the impaired ability to retain moisture in the skin. Some bacteria, such as *Staphylococcus aureus* (*S. aureus*), have been isolated from weeping lesions at high frequency, and *S. aureus* is well known to exacerbate AD.^{3–5} It has been reported that the serum concentrations of specific IgE antibodies to staphylococcal enterotoxin (SE) A and SEB are increased in adult and

pediatric patients with AD.^{6–10} The prevalence of positive rates of SEA/SEB-specific IgE antibodies in children with AD ranged from 35.1% to 57% in those reports. However, the clinical significance of the measurement of SEA and SEB in pediatric AD patients remained unclear.

We investigated the serum concentrations of SEA/SEB-specific IgE antibodies in pediatric patients aged 15 years or less to clarify their clinical significance. Also, we focused on the association between SEA/SEB-specific IgE antibodies and exposure to pets.

Methods

Subjects

The subjects were 140 pediatric patients with AD, comprising 92 boys and 48 girls aged from 3 months to 15 years

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Table 1 Prevalence of positive rates of staphylococcal enterotoxin (SE)-A- and SEB-specific IgE antibodies in atopic dermatitis and bronchial asthma patients

	No.	Patients with positive specific IgE antibodies		
		SEA (%)	SEB (%)	SEA/SEB (%)
Atopic dermatitis				
Total	140	25 (18)	41 (29)	47 (34)
Severe	13	7 (54)	10 (77)	10 (77)
Moderate	50	11 (22)	16 (32)	21 (42)
Mild	77	7 (9)	15 (19)	16 (21)
Bronchial asthma	108	4 (4)	8 (7)	10 (9)

(mean, 4.4 years) who visited Yamaguchi University Hospital, Yamaguchi, Japan or Doai Memorial Hospital, Tokyo, Japan, between April 2000 and September 2001, with the chief complaint of skin lesions. The AD patients were divided into three groups according to their age: 40 infants (<2 years), 52 young children (2–6 years), and 48 school children (≥ 6 years). The severity of AD was evaluated according to the 1999 Japanese Therapeutic Guidelines for Atopic Dermatitis, and the subjects were classified into one of the following groups: mild ($n = 77$), moderate ($n = 50$), or severe ($n = 13$). All patients were treated according to the guidelines and followed up for over a year. Fifty-eight patients complained of aggravation of the dermatological symptoms in summer or winter. Twenty-three out of these 58 AD patients complained of aggravation of the dermatological symptoms in summer, the other 35 complaining of aggravation in winter. A total of 108 patients with bronchial asthma (BA) without the complication of AD, comprising 74 boys and 34 girls aged from 3 months to 15 years (mean, 5.0 years), were also examined as an allergic disease control with high serum concentrations of total IgE.

Measurement of serum concentrations of total IgE and antigen-specific IgE antibodies

Measurement of the serum concentrations of total IgE was conducted by means of a radioimmunosorbent test. The titers of specific IgE antibodies to SEA, SEB and other allergens, such as hen's eggs, milk, soybeans, house dust, mites, cedar pollen, cat dander and dog dander, were measured with an immuno CAP-radioallergosorbent test (RAST) system (Pharmacia & Upjohn, Uppsala, Sweden), as reported previously.^{8,11} Specific IgE antibody titers exceeding 0.7 U/mL (Class 2) were regarded as positive.

Culture of *Staphylococcus aureus* from the skin of patients

When AD patients had the complication of moist skin lesions, we tried to isolate *S. aureus* from the lesions. Samples were collected by directly scrubbing eczematous lesions on the wrists and elbows with sterile saline-soaked

swabs. The samples were cultured in 5% sheep blood agar medium at 35°C for 20 h. Coagulase activity and catalase activity were examined to identify *S. aureus*.

CAP RAST fluorescent enzyme immunoassay inhibition test

The CAP RAST fluorescent enzyme immunoassay (FEIA) inhibition test was performed to investigate the cross-reaction of specific IgE antibodies to SEA and SEB, and cross-reactions to dog dander and cat dander.^{12,13} The serum obtained from a 5-year-old girl who tested positive for specific IgE antibodies to SEA (Class 3), SEB (Class 3), dog dander (Class 5), and cat dander (Class 4) was used in this assay. Briefly, each allergen (i.e. SEA, SEB, and dog and cat allergen extracts (Pharmacia & Upjohn)), was serially diluted ($1-10^{-2}$) with 0.1 mol phosphate buffer (pH 7.4), and then 30 μ L of each dilution was mixed with 20 μ L of patient serum and preincubated at room temperature with continuous shaking for 2 h. As a control, 30 μ L of the same buffer was used instead of the allergen. Then the residual specific IgE antibodies in the mixture were measured with the CAP RAST system to calculate the rate of inhibition of specific IgE antibodies to SEA or SEB.¹³

Statistical analyses

Statistical analyses were performed using the χ^2 test, Fisher's exact test and Wilcoxon rank sum test.

Results

Prevalence of specific IgE antibodies to SEA and SEB

Twenty-five out of the 140 AD patients (17.9%) were positive for SEA-specific IgE antibodies and 41 (29.3%) were positive for SEB-specific IgE antibodies (Table 1). A total of 47 AD patients (33.6%) tested positive for either SEA-specific or SEB-specific IgE antibodies, while the positive rate of SEA/SEB-specific IgE antibodies was low in

Table 2 Association of specific IgE antibodies to staphylococcal enterotoxin (SE)-A/SEB with ones to dog or cat dander

Specific IgE antibodies to SEA/SEB	Specific IgE antibodies to dog dander		Specific IgE antibodies to cat dander	
	Positive	Negative	Positive	Negative
Positive ($n = 39$)	32*	7	28*	11
Negative ($n = 85$)	22	63	18	67
Total	54	70	46	78

*The patients with positive SEA/SEB-specific IgE antibodies have higher positive rates of specific IgE antibodies to dog dander or cat dander. $P < 0.01$, χ^2 test. Each value shows the number of patients.

the patients with BA. In addition, the positive rate of SEA/SEB-specific IgE antibodies in the severe group was 77%, which was higher than those in the moderate and mild groups of AD (Table 1). The positive rate of SEA/SEB-specific IgE antibodies was 54.2% in school children, which was higher than those in infants and young children (5% and 36.5%, respectively). The serum concentrations of total IgE in young children and school children with positive SEA/SEB-specific IgE antibodies were 2948 ± 4755 U/mL and 2952 ± 3255 U/mL. These concentrations were higher than those in young children and school children with negative SEA/SEB-specific IgE antibodies (566 ± 675 U/mL, $P < 0.05$, and 908 ± 743 U/mL, $P < 0.01$, respectively). Because two patients were positive for SEA/SEB-specific IgE antibodies and 38 were negative in the infant group, we could not compare the serum concentrations of total IgE in this group.

The serum concentrations of SEA/SEB-specific IgE antibodies in 58 patients with AD were measured twice in summer and winter. The serum concentrations of SEA/SEB-specific IgE antibodies showed no change in winter or in summer in each patient. The AD patients with exacerbation in summer showed higher positive rates of SEA/SEB-specific IgE antibodies than those with exacerbation in winter (73.9% vs 20.0%; $P < 0.001$).

Association between isolation of *Staphylococcus aureus* and a positive rate of SEA/SEB-specific IgE antibodies

Bacterial cultures of the skin lesions of 53 AD patients were performed. *S. aureus* was isolated from the skin lesions of 44 (83%) of these AD patients. *S. aureus* was isolated from all patients with positive SEA/SEB-specific IgE antibodies ($n = 20$), but from only 24 (72.7%) of those with negative SEA/SEB-specific IgE antibodies ($n = 33$). *S. aureus* was isolated more frequently from patients with positive SEA/SEB-specific IgE antibodies than ones with negative SEA/SEB-specific IgE antibodies ($P < 0.05$). One-third of the samples included methicilline-resistant *S. aureus* (MRSA), the other samples showing methicilline-susceptible *S. aureus*. There was no difference in the incidence of MRSA between patients with positive SEA/SEB-specific IgE antibodies and those with negative ones.

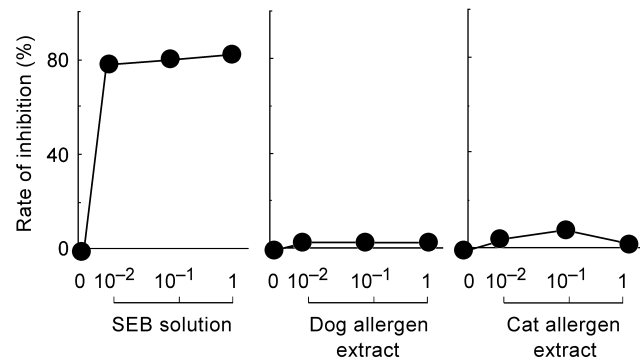


Fig. 1 Rate of inhibition by staphylococcal enterotoxin (SE) B-specific IgE antibodies measured by the CAP RAST fluorescent enzyme immunoassay inhibition test. The x-axis shows the dilution of the SEB solution, dog allergen (dander) extract or cat allergen (dander) extract in phosphate buffer. The y-axis shows the rate of inhibition by SEB-specific IgE antibodies. A serum specimen collected from a 5-year-old girl who tested positive for specific IgE antibodies to SEA (Class 3), SEB (Class 3), dog dander (Class 5), and cat dander (Class 4) was used in this test. The SEB solution, even when diluted 1 : 100, but not the dog allergen extract nor cat allergen extract, caused almost 80% inhibition of the reaction. No cross-reaction occurred between SEB, and dog or cat dander-specific IgE antibodies.

SEA/SEB-specific IgE antibodies in patients with dogs and cats as pets

The patients with positive SEA/SEB-specific IgE antibodies had higher positive rates of specific IgE antibodies to dog and cat dander ($P < 0.01$, Table 2). The CAP RAST FEIA inhibition test showed the absence of cross-reaction between dog/cat dander and SEB-specific IgE antibodies with one patient's serum (Fig. 1). The results obtained in the SEA-specific IgE antibody inhibition test were the same (data not shown). Also, the patients with positive SEA/SEB-specific IgE antibodies had higher positive rates of specific IgE antibodies to house dust and mites ($P < 0.01$), but not to other allergens (i.e. hen's eggs, cow's milk, soybeans, and cedar pollen).

We obtained information about the keeping of dogs and cats as pets from 113 patients with AD. The positive rate of

Table 3 The positive rates of specific IgE antibodies to allergens in patients with dogs and/or cats as pets and without pets

Specific IgE antibodies	Patients keeping dogs and/or cats (<i>n</i> = 31)	Patients without dogs or cats (<i>n</i> = 82)
Hen's eggs	25.8	29.6
Cow's milk	25.8	14.8
Soybeans	32.2	23.4
House dust	83.9*	63
Mites	83.9*	63
Cedar pollen	48.4	40.7
Cat dander	64.5**	30.9
Dog dander	61.3*	35.8
SEA	19.3	12.3
SEB	38.7*	21
SEA/SEB	48.4**	23.4

*Significant at $P < 0.05$ vs patient group without dogs or cats. **Significant at $P < 0.01$ vs patient group without dogs or cats.

SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B. Values are the mean percentages of patients who showed positive specific IgE antibodies to each allergen.

SEA/SEB-specific IgE antibodies in the patients who kept dogs and/or cats was 48.4%, which was higher than in those who did not have pets (Table 3). There were no significant differences of the severity of AD in the patients keeping pets and those not keeping pets. Regarding other allergens, the patients keeping pets showed higher positive rates of specific IgE antibodies to house dust and mites, and cat/dog dander.

Discussion

In this study, we found that the prevalence of positive rates of SEA/SEB-specific antibodies was 33.6% in all patients with AD. It is likely that the positive rate of SEB-specific IgE antibodies was higher than that of SEA-specific IgE antibodies because SEB is associated with local infections, while SEA is often encountered in patients with toxic shock syndrome.¹⁴ The positive rate of SEA/SEB-specific antibodies in the patients suffering from severe AD was higher than in those with mild AD. Moreover, the positive rate of SEA/SEB-specific antibodies in school children was higher than that in young children. The serum concentrations of total IgE in the patients with positive SEA/SEB-specific IgE antibodies were higher than in those with negative SEA/SEB-specific IgE antibodies. These findings support previous reports.^{6–10}

Compared with the patients with exacerbation in winter, those with exacerbation in summer showed a higher positive rate of SEA/SEB-specific IgE antibodies. It can be hot and humid in the summer and dry in the winter on the Pacific Ocean coast of Japan. Because such weather promotes bacterial proliferation, especially *S. aureus* on the skin in summer, there was a greater chance of being colonized with Staphylococcus-producing SEA/SEB. This effect may result in vulnerability of those who test positive for SEA- and SEB-specific IgE antibodies to aggravation of dermatological

symptoms. As previously reported, *S. aureus* was isolated from skin lesions from 83% of the AD patients.¹⁵ It has been reported that the incidence of MRSA accounting for *S. aureus* was 31.1% in the skin lesions of adult patients with AD.¹⁶ In this study, we proved that the incidence of MRSA was 33% in the skin lesions of pediatric patients with AD. The detection rate of MRSA in pediatric patients with AD was similar to that in adult AD patients in Japan. There was no difference in the incidence of MRSA between the patients with positive SEA/SEB-specific IgE antibodies and those with negative ones.

In this study, the patients with positive SEA/SEB-specific IgE antibodies had higher positive rates of specific IgE antibodies to dog/cat dander, house dust and mites. We focused on the association between SEA/SEB-specific IgE antibodies and exposure to pets. The presence of SEA/SEB-specific IgE antibodies was associated with the presence of dog and cat dander-specific IgE antibodies. The FEIA inhibition test proved these IgE antibodies exhibited no cross-reaction with one patient's serum. In practice, the positive rates of SEA- and SEB-specific IgE antibodies in the patients with dogs and/or cats as pets were higher than those without pets. Also, the patients with dogs and/or cats as pets showed higher positive rates of specific IgE antibodies to not only cat and dog dander, but also house dust and mites, than those without pets. Because there were no differences in the severity of AD between the patients keeping pets and those without pets, it is likely that the keeping of dogs/cats as pets might cause increases in the serum concentrations of specific IgE antibodies to not only cat and dog dander, but also SEA/SEB, house dust and mites. These allergens are related to exposure to pets.

There are two possible explanations for the presence of SEA/SEB-specific IgE antibodies in AD patients with dogs and/or cats as pets. One is that *S. aureus* infection results from heavier contamination by oral zoonotic bacteria,

especially with deep puncture wounds, since it is well known that dogs and cats have SE-producing *S. aureus* as a resident floral component.¹⁷ Another possibility is that skin injury may facilitate the transfer of SEA or SEB produced by *S. aureus* existing on the surface, to deep layers of AD patients' skin when the patients are bitten, scratched or touched by dogs and cats. It remains unknown whether sensitization to pets is a risk factor for allergic diseases or not.¹⁸ However, it is better to consider dogs and cats as one of the possible sources of *S. aureus*, which causes exacerbation in AD patients.

In summary, the AD patients who have high positive rates of SEA/SEB-specific IgE antibodies were found to be school children, severe cases, cases with high serum concentrations of total IgE, cases with exacerbation in summer, and cases exposed to pets (dogs and/or cats). The measurement of specific IgE antibodies to SEA and SEB in serum thus has some value for evaluating AD patients.

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