

Presence and Characterization of Sensitization to Staphylococcal Enterotoxins in Patients with Allergic Rhinitis

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ABSTRACT

Exotoxins derived from Staphylococcus aureus appear to be involved in the pathogenesis of allergic diseases, especially atopic dermatitis (AD). However, little is known about sensitization to enterotoxins in cases of respiratory allergies. Because the nasal cavity is a primary site of colonization by S. aureus, we sought to determine the prevalence and role of serum immunoglobulin E (IgE) antibodies against staphylococcal enterotoxin A (SEA) and SEB in patients with allergic rhinitis (AR). The presence of SEA- and SEB-specific IgE was determined in 40 patients with AR and 16 healthy control subjects. In the rhinitis group, nasal symptom score, total serum IgE, sensitization to other inhaled allergens and the presence of sinusitis were determined. Twenty-five percent of patients with AR were sensitized to SEA/SEB whereas 6.3% of controls were sensitized to the toxins. Sensitization to SEB was predominant relative to SEA. There were no significant differences in the severity of nasal symptoms and complications of other allergic diseases including AD and bronchial asthma, between patients with or without sensitization to SEA/SEB. However, patients sensitized to these toxins were likely to show

increased total serum IgE and polyvalent sensitization, suggesting that exposure and subsequent sensitization to SEA/SEB may be involved in polyvalent sensitization. (American Journal of Rhinology 15, 417–421, 2001)

Superantigenic exotoxins produced by *Staphylococcus aureus*, such as staphylococcal enterotoxins (SE), exfoliative toxins, and toxic shock syndrome toxin 1 (TSST-1) appear to be involved in the pathogenesis of atopic diseases, especially atopic dermatitis (AD). These toxins, as well as toxin-producing *S. aureus*, preferentially occur in skin lesions of patients with AD in comparison to those with other skin diseases or healthy individuals.^{1–7} These superantigenic toxins elicit T-cell activation, which leads to the production of interleukin-4 (IL-4) and IL-5.^{8–11} Direct application of these toxins to the skin induces an inflammatory response *in vivo* in both humans and mice.^{12,13} In addition, it has been established that TSST-1 enhanced pollen-specific immunoglobulin E (IgE) production by peripheral blood mononuclear cells (PBMC) from patients with pollinosis during the pollen season.¹³ Recently, it was revealed that airway exposure to SEB induces airway inflammation and hyperresponsiveness.¹¹ On the other hand, *S. aureus*-derived toxins act as allergens, which induce specific IgE production against the toxins themselves.^{3,6,7,14} Histamine release from basophils was observed in AD patients sensitized to SE only on exposure to the relevant toxins. In contrast, no histamine release was observed in AD patients exhibiting no IgE antitoxin or in normal controls.³

These findings prompted us to investigate the involve-

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ment of toxins produced by *S. aureus* and IgE specific for these toxins in the pathogenesis of allergic rhinitis (AR). *S. aureus*, colonization of which is found often in the nostrils and nasal cavity, is one of the common pathogens responsible for acute infectious rhinosinusitis.^{15,16} Recently, Shiomori *et al.* showed that patients with perennial AR showed a higher rate of nasal carriage of both *S. aureus* and toxin-producing *S. aureus* compared with control subjects. In addition, PBMC from AR produced higher levels of IL-4 and IL-5 and decreased levels of interferon γ (IFN- γ) in response to the toxins.¹⁰ However, to our knowledge, little data can be found regarding IgE antibodies against these toxins in patients with AR.

In this study, we examined the prevalence of IgE-specific antibodies to SEs (SEA and SEB) in serum from patients with AR. Furthermore, we investigated clinical characteristics such as type of AR, total IgE levels, sensitization to other allergens, and disease severity with respect to age and sex in patients sensitized to SEA/SEB.

MATERIALS AND METHODS

Study Populations

Forty patients presenting with AR (mean age, 30.75 ± 14.58 years; range, 3–65 years; 19 men and 21 women) and 16 healthy volunteers (mean age, 37.40 ± 7.78 years; range, 25–50 years; 12 men and 4 women) were enrolled in this study. Twenty-six patients exhibited perennial rhinitis caused by house-dust mites. The remaining 14 patients exhibited seasonal pollenosis caused by Japanese cedar. Diagnosis of AR was based on the international consensus report.¹⁷ Sensitization to these allergens was confirmed by the presence of IgE-specific antibodies determined by capsulated hydrolytic carrier polymer (CAP)-RAST (Pharmacia, Uppsala, Sweden). Control subjects showed no history of atopic disorders such as AR and showed negative results for Phadiatop test (Pharmacia). The study was approved by the Human Research Committee of Okayama University Medical School. None of the patients had received immunotherapy or used immunosuppressive drugs, including topical steroids, before enrollment.

Assay of IgE Specific to SEs and Other Major Allergens

Serum samples were obtained from all patients and controls. Samples were analyzed for their IgE specificity to SEA and SEB using the CAP system.¹⁸ IgE specific to house-dust mites (*Dermatophagoides farinae*), Japanese red cedar, Hinoki cypress, orchard grass, short ragweed, mugwort, *Candida*, *Alternaria*, and trichophyton also were determined. Titers were defined as positive when the observed kilo Unit Allergen per liter (kUA/L) was ≥ 0.7 .

Clinical Characterization

The severity of nasal symptoms was determined by nasal symptom scores according to the criteria of Okuda *et al.*¹⁹ From a patient interview, this method involved determination of an average obtained from daily symptoms of sneeze, rhinorrhea, and nasal blockade during the 2 weeks before the subject's visit to Okayama University Hospital. In brief, the number of sneezing attacks, the number of nose blows, and the degree of mouth/nose breathing were graded on a scale of 0–3 according to the severity. The sum of the scores from the three nasal symptoms represents the nasal symptom score. Patients also were questioned regarding the age of onset of rhinitis, seasonal exacerbation of the disease, and complications of other atopic disorders including bronchial asthma, AD, eczema, urticaria, drug hypersensitivity, and allergic conjunctivitis. Patients were examined also for the presence of paranasal sinusitis by roentgenogram.

Statistical Analysis

Data are expressed as the mean $\pm$ SD. Statistical analyses were performed with a nonparametric Mann-Whitney *U* test and Fisher's exact probability test. Significant differences were accepted when the value of $p < 0.05$.

RESULTS

Prevalence of Sensitization to SEA and/or SEB in Patients with AR

Using the CAP system, titers of IgE specific for SEA and SEB were determined in 40 patients with AR and 16 healthy control subjects. Sensitization was defined as specific IgE concentrations of >0.70 kUA/L. In the control group, one individual (6.3%) showed sensitization to SEB. No control subjects showed sensitization to SEA. In contrast, in patients with AR, 5 (12.5%) patients showed positive sensitization to SEA and 9 (22.5%) patients exhibited positive sensitization to SEB. The titers of positive IgE to SEA and SEB ranged from 0.82 to 1.83 kUA/L and 0.79 to 12.90 kUA/L, respectively. The overall prevalence of sensitization to the enterotoxins was 25.0% (10/40 patients) in the allergic group, showing higher prevalence in comparison to healthy controls (6.3%) while the difference was not statistically significant ($p = 0.15$, Fisher's exact test; Fig. 1). However, of the 10 patients showing sensitization to the enterotoxins, 5 (50%) patients were sensitized to SEB alone, 4 (40%) patients were sensitized to both SEA and SEB, and 1 (10%) patient was sensitized to SEA alone. This result indicated that sensitization to SEB was predominant.

Clinical Characterization of Patients Sensitized to SEA/SEB

There were no significant differences regarding age, sex, type of rhinitis, sinusitis evaluated by roentgenogram, and allergic history, including AD, between SEA/SEB-

TABLE I

Characterization of Patients with AR with and without IgE Antibodies to SEA/SEB

	<i>n</i>	SEA/SEB-IgE−	SEA/SEB IgE+	<i>p</i> Value
Sex (M:F)	40	13:17	6:4	0.2918*
Age (years)				
At visit	40	33.93 + 2.78	21.20 + 1.95	0.0130#
At onset	40	28.17 + 15.41	10.50 + 8.72	0.0063#
Type of rhinitis	40			
Perennial	26	18	8	
Seasonal	14	12	2	0.225*
Symptom score	34	4.15 + 0.49	3.90 + 0.57	0.985#
Sinusitis	20	9/14 (64.29%)	5/6 (83.33%)	0.387*
Allergic history	31	6/21 (28.57%)	6/10 (60.00%)	0.100*
Serum total IgE (IU/mL)	40	137.90 + 34.93	1304.18 + 512.57	0.075#
Sensitization to other allergens				
House dust	39	12/29 (41.38%)	9/10 (90.00%)	0.0018*
Mite (<i>D. farinae</i>)	36	13/27 (48.15%)	8/9 (88.89%)	0.0355*
Tree pollen (Japanese red cedar and Hinoki cypress)	38	16/29 (55.17%)	7/10 (70.00%)	0.3309*
Grass pollen (orchard grass)	37	9/28 (32.14%)	5/9 (55.56%)	0.192*
Weed pollen (short ragweed and mugwort)	38	4/28 (14.29%)	5/10 (50.00%)	0.0362*
Mould (<i>Candida</i> , <i>Alternaria</i> , and <i>Trichophyton</i>)	36	3/28 (10.71%)	2/8 (25.0%)	0.3048*
Blood eosinophil (counts/mm ³)	22	259.73 + 249.37	522.43 + 391.28	0.0445#

*Fisher's exact test.

#Mann-Whitney's U test.

derived effects may lead to elevated total serum IgE levels seen in patients with AR. This observation may explain why SEA/SEB-sensitized patients are likely to exhibit polyvalent sensitization to other inhalant allergens. In addition, the presence of IgE against SEA/SEB was not caused by simply the high levels of serum IgE or polyclonal B cell activation. Consequently, 4 of 8 patients showing positive IgE titers to SEA/SEB (25.0%) showed low levels of total serum IgE (<70 IU/ml) and 4 of 22 patients (18.2%) with negative sensitization to SEA/SEB showed higher levels of total serum IgE (>250 IU/ml). Leung *et al.* showed the *S. aureus* toxin-induced histamine release by basophils from patients sensitized to exotoxins.³

Correlation between the presence of SEA/SEB-specific IgE and disease severity was not detected in this study. This finding was consistent with the report by Tada *et al.*, which noted no correlation between the prevalence of sensitization to the enterotoxins and disease severity in adult AD cases.¹⁴ On the other hand, Bunikowski *et al.* and Nomura *et al.* recently showed that the levels of SEB-specific IgE correlated with disease severity in childhood AD.^{6,7} More recently, Shiomori *et al.* reported that there was no significant correlation between the nasal carriage of enterotoxins and nasal symptom scores in patients with perennial rhinitis exhibiting allergy to house-dust mites despite the significantly higher scores of the *S. aureus*-positive patients in

comparison with those scores of the *S. aureus*-negative patients.¹⁰ These results suggest that both *S. aureus*-derived SEA/SEB and enterotoxin-specific IgE are not necessarily involved in the severity of AR.

In conclusion, we have shown that the prevalence of IgE specific for SEA/SEB was significantly higher in patients with AR in comparison with healthy control subjects. Although the presence of SEA/SEB-specific IgE did not correlate with disease severity, it appears to be involved in polyvalent sensitization and elevation of serum IgE levels. Although roles of other exotoxins or products derived from *S. aureus* and specific IgE against these toxins/products in the pathogenesis of AR should be investigated, both SEA and SEB are potentially new allergens in AR.

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REFERENCES

1. Leyden JJ, Marples RR, and Kligman AM. *Staphylococcus aureus* in the lesion of atopic dermatitis. Br J Dermatol 90:525–530, 1974.
2. Williams REA, Gibson AG, Aitchinson TC, et al. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis. Br J Dermatol 123:493–501, 1990.

3. Leung DYM, Harbeck R, Bina P, et al. Presence of IgE antibodies to Staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest* 92:1374–1380, 1993.
4. Mcfadden JP, Noble WC, and Camp RDR. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 128:631–632, 1993.
5. Akiyama H, Toi Y, Kanzaki H, et al. Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strains isolated from atopic dermatitis lesions. *Arch Dermatol Res* 288:418–420, 1996.
6. Bunikowski R, Mielke M, Skarabis H, 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* 103:119–124, 1999.
7. Nomura I, Tanaka K, Tomita H, et al. Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 104:441–446, 1999.
8. Marrack P, and Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 248:705–711, 1990.
9. Neuber K, Steinrucke K, and Ring J. Staphylococcal enterotoxin B affects in vitro IgE synthesis, interleukin-4 a, interleukin-4 a, interleukin-4 a, interleukin-4 and interleukin-5 production in atopic eczema. *Int Arch Allergy Immunol* 107:179–182, 1995.
10. Shiomori T, Yoshida S, Miyamoto H, et al. Relationship of nasal carriage of *Staphylococcal aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol* 105:449–454, 2000.
11. Herz U, Ruckert R, Wollenhaupt K, et al. Airway exposure to bacterial superantigen (SEB) induced lymphocyte-dependent airway inflammation associated with increased airway responsiveness—a model for non-allergic asthma. *Eur J Immunol* 29:1021–1031, 1999.
12. Strange P, Skov L, Lisby S, et al. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 132:27–33, 1996.
13. Saloga J, Leung DY, Reardon C, et al. Cutaneous exposure to the superantigen staphylococcal enterotoxin B elicits a T-cell-dependent inflammatory response. *J Invest Dermatol* 106:982–988, 1996.
14. Tada J, Toi Y, Akiyama H, et al. Presence of specific IgE antibodies to staphylococcal enterotoxins in patients with atopic dermatitis. *Eur J Dermatol* 6:552–554, 1996.
15. Kluytmans J, van Belkum A, and Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidermiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 10:505–520, 1997.
16. Suzuki K, Nishiyama Y, Sugiyama K, et al. Recent trends in clinical isolates from paranasal sinusitis. *Acta Otolaryngol Suppl (Stockh)* 525:51–55, 1996.
17. International Rhinitis Management Working Group. International consensus report on the diagnosis and treatment of allergic rhinitis. *Allergy* 49(suppl):5–34, 1994.
18. Pastorello EA, Incorvaia C, Pravettoni V, et al. A multicentric study on sensitivity and specificity of a new in vitro test for measurement of IgE antibodies. *Ann Allergy* 67:365–370, 1991.
19. Okuda M, Okamoto M, Nomura Y, et al. Clinical study on beclomethasone dipropionate powder preparation (TL-102) in perennial nasal allergy. *Rhinology* 24:113–123, 1986.
20. Blanco C, Carrillo T, Castillo R, et al. Avocado hypertrophy. *Allergy* 49:454–459, 1994.
21. Hoffmann-Sommergruber K, Dermoly P, Cramer R, et al. IgE reactivity to Api g1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v1. *J Allergy Clin Immunol* 104:478–484, 1999.
22. Hofer MF, Harbeck RJ, Schlievert PM, et al. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 112:171–176, 1999. □

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