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BRIEF COMMUNICATIONS

Evaluation of Staphylococcal Enterotoxin-Specific IgE Antibody in Tears in Allergic Keratoconjunctival Disorders

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Purpose: To investigate the presence of staphylococcal enterotoxin A (SEA) and B (SEB)-specific IgE antibodies in tears from patients with allergic conjunctival disorders.

Methods: The study included 8 eyes of 4 patients with perennial allergic conjunctivitis (PAC), 14 eyes of 7 patients with vernal keratoconjunctivitis (VKC), 12 eyes of 6 patients with atopic keratoconjunctivitis (AKC), and 10 eyes of 10 healthy volunteers as controls. Tears were sampled by the method of the Schirmer test I. Sampled tears were eluted and SEA- and SEB-specific IgE antibodies were analyzed by the AlaSTAT-IMMULIZE method.

Results: SEA-specific IgE antibodies in tears were positive in 9 of 14 eyes in VKC patients and in 1 of 12 eyes in AKC patients. SEB-specific IgE antibodies in tears were positive in 7 of 14 eyes in VKC patients and in 2 of 12 eyes in AKC patients. Values for antibodies were higher in patients with severe clinical findings. However, all the cases in the normal control and the PAC groups were negative for both antibodies.

Conclusion: Our data strongly suggested that staphylococcal enterotoxin may cause type I allergy, and may be an exacerbating factor for vernal keratoconjunctivitis and atopic keratoconjunctivitis. **Jpn J Ophthalmol 2003;47:609–611**

Key Words: Allergic conjunctivitis, atopic keratoconjunctivitis, specific IgE antibody, staphylococcal enterotoxin, vernal keratoconjunctivitis.

Introduction

Staphylococcus aureus (*S. aureus*) is the most frequent microorganism isolated from cutaneous lesions in patients with atopic dermatitis (AD). *S. aureus* secretes a variety of exotoxins such as staphylococcal enterotoxin (SE), toxic shock syndrome toxin-1 (TSST-1), and exfoliative toxin. However, these toxins are strain variable. About half of the staphylococcal strains isolated from cutaneous lesions of AD patients secrete exotoxins such as SE and TSST-1 that act as a superantigen. They are thought to be an exacerbating factor for the clinical findings in AD.[1]

S. aureus can secrete eight kinds of SE, such as staphylococcal enterotoxin A (SEA), SEB, SEC_1-C_3, SED, SEE, SEH. In 1993, Leung et al[2] reported that specific IgE antibodies to SEA, SEB, and/or TSST-1 were detected in 57% of the serum of patients with AD. Tada et al[3] reported that SEA- and SEB-specific IgE antibodies in adult patients with AD were positive in 77 of 96 patients (80%). In the infantile cases, it was reported that the levels and positive rates of SEA- or SEB-specific IgE antibodies in serum of patients with AD are significantly higher than those in non-atopic children, and levels of SEA- and SEB-specific IgE antibodies increased accordingly not only with the level of total IgE and specific IgE antibodies to food and inhalant allergens but also with the severity of the clinical score.[4,5] However, there have not been any studies regarding the relationship between specific IgE antibodies to the SEA and SEB in tears and the pathophysiology of allergic keratoconjunctival disorders.

In this study, we measured SEA- and SEB-specific IgE antibodies in tears to study the relationship between allergic keratoconjunctival disorders and the IgE antibodies against SEA and SEB.

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Materials and Methods

Subjects

This study was approved by the Ethics Committee for Clinical Study of the Nihon University School of Medicine. Demographic data are shown in Table 1. The study included 8 eyes of 4 patients with perennial allergic conjunctivitis (PAC), 14 eyes of 7 patients with vernal keratoconjunctivitis (VKC), and 12 eyes of 6 patients with atopic keratoconjunctivitis (AKC). The diagnosis of allergic conjunctival disorders, including PAC, VKC, and AKC, was based on the physical findings of an ophthalmological examination, such as keratoconjunctival findings by slit-lamp microscopy and serum antigen-specific IgE antibodies. Tears were collected after informed consent was obtained. The right eyes of 10 healthy normal volunteers who had neither allergic diathesis nor wore contact lens were used as controls.

Methods

Tears were collected based on the Schirmer method I using a filter paper (Schirmer Tear Production Measuring Strips; Showa Yakuhin Kako, Tokyo). Schirmer strips were cut at 5 mm length from the end inserted in the fornix, and stored in a microtube (Assist Tube; Assist Trading, Tokyo) and stored at −20ºC. The amount of sampled tears was determined by the wet-length of the Schirmer strip, ie, 1 uL per 1 mm. The cryopreserved Schirmer strips were thawed and eluted with 0.05 M phosphate buffered saline (PBS, pH 7.2). Values for SEA- and SEB-specific IgE antibodies in the elution solution were determined by the AlaSTAT-IMMULIZE (Iatoron, Tokyo) method. The IMMULIZE system is a fully automated continuous random access analyzer, which uses enzyme-amplified chemiluminescence as the detection system.[6] According to the manufacturer's recommendations on the use of the AlaSTAT-IMMULIZE method, the optimal cutoff levels as the definitions of tear SEA- and SEB-specific IgE positive antibodies were both 0.35 kU/L. Values less than this cutoff level were judged to indicate negative antibodies.

Results

The results of SEA- or SEB-specific IgE antibody examination are shown in Table 1 and Figure 1. SEA-specific IgE antibodies in tears were positive in 9 of 14 VKC patients and 1 of 12 AKC patients, but negative in 8 of 8 PAC patients. The calculated value for SEA-specific IgE antibodies, excluding the data for negative patients, was 7.95 ± 7.95 (mean ± SD) in VKC and 4.09 in AKC. In terms of SEB-specific IgE antibodies in tears, 7 of 14 VKC patients and 2 of 12 AKC patients were positive, but 8 of 8 PAC patients were negative. The calculated value for SEB-specific IgE antibodies, excluding the data for negative patients, was 1.84 ± 1.22 in VKC and 1.17 ± 0.59 in AKC. Both SEA-specific IgE antibodies and SEB-specific IgE antibodies were negative in all of the normal controls.

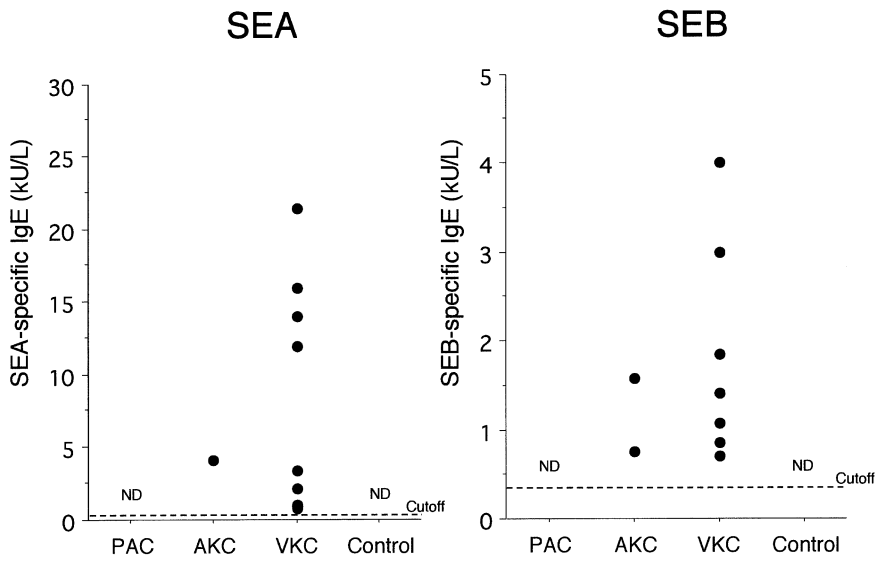
Discussion

In atopic dermatitis, *S. aureus* is thought to be an exacerbating factor for the allergic reaction because the bacterial cell component or exotoxin secreted by *S. aureus* plays a role as allergen, and also because this exotoxin is a superantigen.[1] In this study, SEA- and SEB-specific IgE antibodies in tears were studied. As a result, we found there was a high incidence of SEA- and SEB-specific IgE antibodies in VKC patients and a low incidence in AKC patients. The patients with severe clinical findings, such as VKC, showed higher values for the SEA-/SEB-specific IgE antibodies. On the other hand, all the normal control subjects and patients with PAC were negative for SEA- and SEB-specific antibodies. These results suggest that the incidence of SEA- and SEB-specific IgE antibodies in tears is high in patients with atopic dermatitis.

Table 1. Positive Rates and Levels of Tear Staphylococcal Enterotoxin A (SEA) and B (SEB)-specific IgE Antibodies in Studied Patients

	PAC* (n = 8)	VKC† (n = 14)	AKC‡ (n = 12)	Control (n = 10)
Sex (male:female)	2 : 6	10 : 4	4 : 8	2 : 8
Age (mean ± SD)	35.0 ± 20.4	20.3 ± 6.1	40.3 ± 14.5	30 ± 7.93
SEA-IgE antibody-positive cases (mean ± SD)§ (kU/L)	0 / 8 eyes	9 / 14 eyes (7.95 ± 7.95§)	1 / 12 (4.09§)	0 / 10
SEB-IgE antibody-positive cases (mean ± SD)§ (kU/L)	0 / 8 eyes	7 / 14 eyes (1.84 ± 1.22§)	2 / 12 (1.17 ± 0.59§)	0 / 10

*PAC: perennial allergic conjunctivitis.
†VKC: vernal keratoconjunctivitis.
‡AKC: atopic keratoconjunctivitis.
§This calculated value excluded values lower than the assay detection limit.



	SEA				SEB			
	PAC	AKC	VKC	Control	PAC	AKC	VKC	Control
No. of negative eyes	8	11	5	10	8	2	7	10
No. of positive eyes	0	1	9	0	0	10	7	0
Total	8	12	14	10	8	12	14	10

Figure 1. Distribution of staphylococcal enterotoxin A (SEA) and B (SEB)-specific IgE levels in the studied allergic keratoconjunctival disorders. Optimal cutoff levels as definition of positive tear SEA- and SEB-specific IgE positivity were 0. 35 kU/L. PAC: perennial allergic conjunctivitis, AKC: atopic keratoconjunctivitis, VKC: vernal keratoconjunctivitis, ND: not detected.

Moreover, it can be considered that the clinical significance is similar between antibody positivity in the tears of patients with allergic keratoconjunctival disorders and antibody positivity in the serum of patients with AD. The levels of SEA- and SEB-specific IgE antibodies have been reported not to correlate with AD severity; whereas it was reported that the severity correlates with the presence of SEA- and SEB-specific IgE antibodies.[5] It is speculated that the presence of SEA- and SEB-specific IgE antibodies exacerbates VKC and AKC by means of type I allergy. In patients with VKC and AKC, it is necessary to elucidate the correlation between the severity and the levels of SEA- and SEB-specific IgE antibodies by another study.

S. aureus is found to play a role in chronic allergic keratoconjunctivitis[7] also. Marginal keratitis, which is classified as a type III allergic disorder, is thought to have a relationship with staphylococcal exotoxin, and phlyctenular conjunctivitis is thought to show delayed hypersensitivity for the cellular proteins of *S. aureus*. In addition, *S. aureus* is thought to be a pathogenic factor for the blepharitis that accompanies atopic keratoconjunctivitis. Our results proved that there were many VKC and AKC patients in whom SEA- and SEB-specific IgE antibodies in tears were positive. Our data strongly suggested that staphylococcal enterotoxin may cause type I allergy on the ocular surface, and be an exacerbating factor for vernal conjunctivitis and atopic keratoconjunctivitis.

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