

Possible influences of *Staphylococcus aureus* on atopic dermatitis — the colonizing features and the effects of staphylococcal enterotoxins

Y. MORISHITA, J. TADA, A. SATO, Y. TOI, H. KANZAKI, H. AKIYAMA and J. ARATA

Department of Dermatology, Okayama University Medical School, Okayama, Japan

Summary

Background Heavy colonization of atopic dermatitis (AD) with *Staphylococcus aureus* is well documented. This phenomenon suggests that *S. aureus* in AD lesions influences the disease processes of AD.

Objective We describe the importance of the presence of *S. aureus* and staphylococcal enterotoxins A and B (SEA, SEB) in AD lesions.

Methods We investigated the colonizing features of *S. aureus* in AD lesions using electron microscopy, the distribution of SEB in the eczematous skin of AD using immunofluorescence, the effects of SEA and SEB on normal human epidermal keratinocytes in organ culture, and the presence of specific IgE antibodies to SEA and/or SEB in serum of AD patients by enzyme immunoassay.

Results *S. aureus* in AD lesions colonized on and in the horny layers of the eczematous skin. SEB produced by *S. aureus* was distributed mainly on the dermal-infiltrated cells, especially on eosinophils. SEA and SEB stimulated expression of ICAM-1 and HLA-DR in normal human keratinocytes. More than half of the AD patients in the present study had specific IgE antibodies to SEA and/or SEB in their serum.

Conclusion *S. aureus* and SEs have important roles in the exacerbation and prolongation of AD.

Keywords: atopic dermatitis, eosinophil, IgE, staphylococcal enterotoxin, *Staphylococcus aureus*

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease with a tendency for exacerbation and remission over time. The clinical course of AD is influenced by a number of environmental factors including colonization of the skin with *Staphylococcus aureus*. Heavy colonization with *S. aureus* of AD lesions is well documented [1–5]. *S. aureus* strains isolated from AD subjects release superantigens such as staphylococcal enterotoxin A and B (SEA, SEB), and toxic shock syndrome toxin-1 (TSST-1) [6–8].

S. aureus and staphylococcal superantigens can contribute to the inflammation of AD by causing continued T-cell activation and release of pro-inflammatory mediators [9,10]. SEB induces inflammatory reactions following application to intact skin of normal and atopic subjects [11]. Although *S. aureus* colonization on AD lesions can be eliminated to some degree after treatment with a combination of topical corticosteroid and antibiotic therapies or a very potent corticosteroid alone [12,13], it is very difficult to completely remove *S. aureus* from AD skin lesions using any therapeutic method. This phenomenon suggests that *S. aureus* in AD lesions may colonize not only on the surfaces of eczematous lesions but in the intercellular spaces of the epidermal horny layers, often forming biofilm [14] and,

Correspondence: J. Tada, Department of Dermatology, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700-8558, Japan.

furthermore, that *S. aureus* and staphylococcal toxins have an important role in exacerbation and prolongation of AD.

The present study investigated the colonizing features of *S. aureus* on and in eczematous lesions, the distribution of SEB in the eczematous skin of AD patients, and the effects of SEA and SEB on the epidermal keratinocytes; furthermore, SEA- and SEB-specific IgE antibodies were detected in the serum of AD patients.

Materials and methods

Patients

AD was diagnosed according to the criteria of Hanifin and Rajka [15] and of the Japanese Dermatological Association [16]. Patients with mild to severe AD were selected in this study ($n=149$). The patients included 66 males and 83 females (mean age 21.4 ± 9.7 years, range: 1–73). The severity of dermatitis over the whole body was assessed according to the criteria of Rajka [17] (36 patients with mild AD, 64 with moderate AD and 40 with severe AD).

Serum samples were obtained from all patients for analysis of specific IgE against SEA, SEB, and α -toxin, and from 134 out of 149 patients for analysis of total serum IgE. None of these patients had a history of deep-seated *S. aureus* skin infections. Control serum samples were obtained from skin-diseased patients (control 2; five males and 12 females, mean age 36.8 ± 14.8 years) with eczematous lesions without atopic disorders. Furthermore, as a normal control group (control 1), 11 serum samples were obtained from healthy subjects (three males and eight females, mean age 26.9 ± 7.3 years).

Specific IgE antibodies against SEA, SEB, and α -toxin were measured using the AlaSTAT kit (Nippon DPC Corporation, Chiba, Japan), a liquid-phase enzyme immunoassay for allergen-specific IgE antibody determination [18]. The specific IgE is considered to be positive when the level is greater than 0.35 IU/mL.

Statistical analysis was performed using the unpaired *t*-test.

Samples

After informed consent was obtained from the patients, biopsy specimens were taken from chronic eczematous lesions of five AD patients. The specimens were divided into two pieces for immunofluorescence and electron microscopy, and one part of the former was snap-frozen in liquid nitrogen and stored at -70°C until use.

Immunofluorescence

To confirm the distribution of SEB in the skin, double

immunofluorescence staining was performed with an anti-eosinophil major basic protein (MBP) monoclonal antibody (mAb) (Chemicon International Inc., Temecula, CA) or an anti-CD1 mAb (DAKO, Glostrup, Denmark) and a polyclonal rabbit anti-SEB antibody (Ab) (Toxin Technology Inc., Sarasota, FL, USA). Five-micrometre cryosections were prepared and blocked with 10% control goat serum in PBS for 1 h at room temperature (RT). After reaction with the anti-MBP (1:1000) or the anti-CD1 mAb (1:20) overnight at 4°C , the samples were incubated with FITC-conjugated antimouse IgG Ab (1:50, DAKO) for 1 h at RT, with anti-SEB Ab (1:20) for 1 h RT and rhodamine-conjugated antirabbit IgG Ab (1:40, DAKO) for 1 h at RT, successively. During each step, the samples were rinsed with three changes of PBS for 10 min each. For control studies, nonimmunized mouse or rabbit serum was used instead of the first antibody.

For double staining of IgE and SEB, a FITC-conjugated anti-IgE Ab (1:50, ICN ImmunoBiologicals, Lisle, IL, USA) was incubated as a first antibody, followed by SEB staining as described above. As a control for the IgE staining, a FITC-conjugated antimouse Ab was used.

Electron microscopy

Appropriate small tissues mentioned above were fixed with 2.5% glutaraldehyde for 2 h at 4°C and post-fixed with 1% osmium tetroxide for 3 h at 4°C . For some specimens, ruthenium red staining (0.05%) was performed to detect glycocalyx produced by *S. aureus*. After dehydration in ethanol series, they were embedded in Epon 812. Ultra-thin sections were double-stained with uranyl acetate and lead citrate, then examined with a JEM 100-SX electron microscope.

Organ culture and immunohistochemistry

Normal human skin (0.3–0.5 mm thick) obtained from plastic surgery was cut into small pieces of 5×5 mm. These tissues were placed with their epidermal sides upward on lens-papers floating on MEM (Gibco, Gland Island, NY, USA) containing enterotoxins (SEA or SEB; Toxin Technology) at a concentration of 10 ng/mL. These were incubated at 37°C for 2–24 h. After appropriate incubation time, the tissues were snap-frozen in liquid nitrogen, and then cryosections ($5 \mu\text{m}$) were prepared. The sections were air-dried, washed in PBS three times for 10 min each, and blocked for endogenous peroxidase activity with hydrogen peroxide (0.3%) for 20 min. They were incubated with 5% bovine serum albumin in PBS (BSA/PBS) for 10 min to block non-specific labelling. After these pretreatments they were incubated with primary antibodies, anti-HLA-DR mAb (1:200, Becton Dickinson, San Jose, CA) or anti-ICAM-1 mAb (1:20, Becton Dickinson) for 1 h

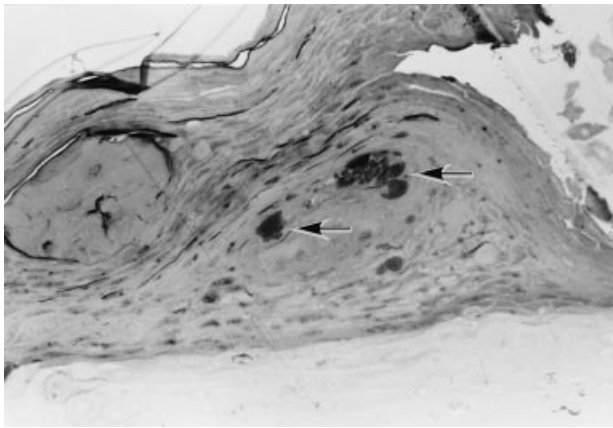


Fig. 1. Semithin section embedded in Epon 812 stained with toluidine blue. Clustered *S. aureus* cells forming colonies (arrows) are present in crust-like horny layers.

at RT. The sections were washed in PBS three times for 5 min, followed by 10-min incubation with biotinylated antimouse immunoglobulin (DAKO Japan, Kyoto, Japan), and further incubated with streptavidin conjugated to horse-radish peroxidase (1:100) for 30 min. After washing in PBS, the immune aggregates were visualized with a 0.02% diaminobenzidine solution. Incubation with BSA/PBS instead of the primary antibodies was carried out as a negative control. For another control, the tissue samples were incubated without the enterotoxins.

Keratinocyte culture and detection of *RANTES* mRNA

Normal human epidermal keratinocytes were obtained as primary culture from Biowhittaker (Walkerville, MD, USA) and maintained in serum-free keratinocyte growth medium (KGMTM; Biowhittaker). The cells were grown in 6-well flat-bottomed culture plates to 70–80% confluency, then were stimulated with SEB (500 ng/mL), IFN- γ (25 U/mL), or tumour necrosis factor- α (TNF- α ; 10 mg/mL) for 18 h with 5% CO₂ in air at 37°C. Next, normal human epidermal keratinocytes were incubated with 25 U/mL of IFN- γ for 48 h. The cells were washed with PBS, then medium was replaced with fresh KGM containing either no stimulants or SEB (500 ng/mL) for 18 h. The cells after stimulation were placed in a solution containing phenol and guanidium isothiocyanate (TRIzolTM Reagent; Life Technologies, Gaithersburg, MD, USA). After addition of chloroform, the mixture was homogenized, centrifuged, and the aqueous phase was recovered. RNA was precipitated by adding isopropyl alcohol, then the precipitate was vacuum-dried. After dissolving in H₂O, 1 μ g of total RNA was denatured at 95°C for 5 min, and single-strand DNA was synthesized by 1-h incubation with You-Prime First-Strand Beads (Ready to goTM; Pharmacia Biotech, Grand Island, NY, USA) and

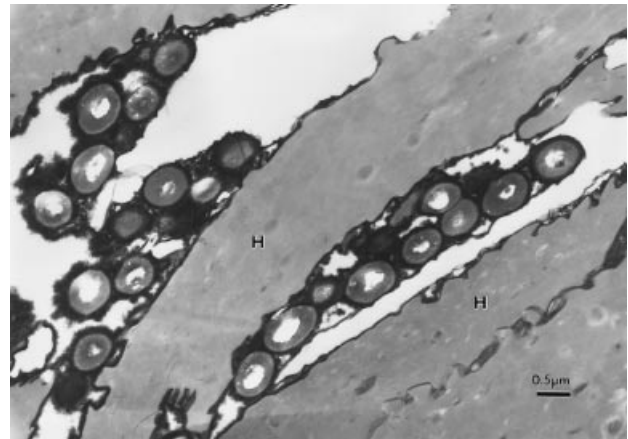


Fig. 2. *S. aureus* cells multiplying among corneocytes. Amorphous or fine materials stained positive for ruthenium red, suggesting the abundant production of glycocalyx by *S. aureus*, surround almost all *S. aureus* cells. H; corneocyte.

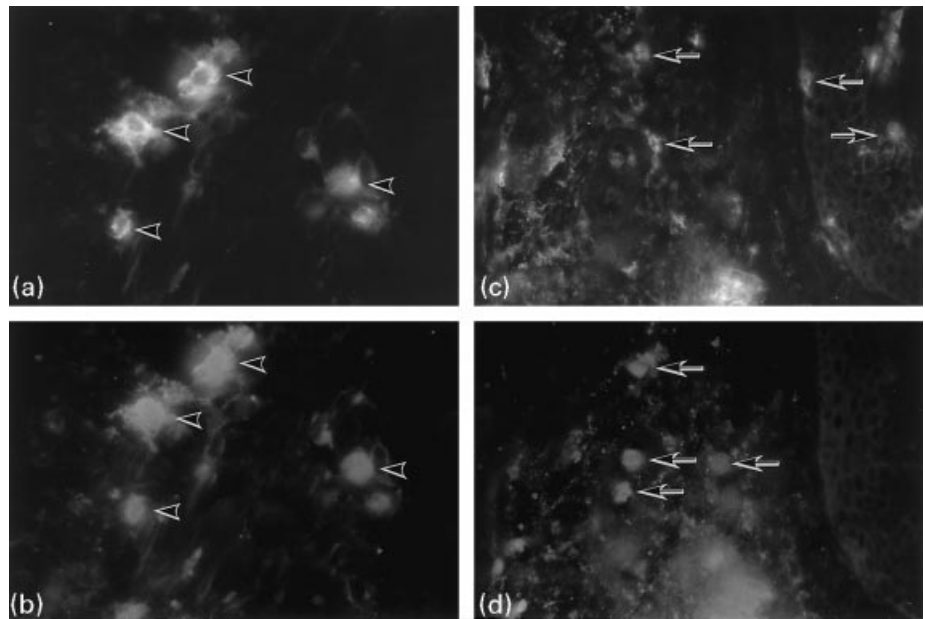
synthesized oligo-dT. Aliquots of cDNA samples were amplified with Taq polymerase (Gibco, Grand Island, NY, USA) and primers specific for *RANTES* and *G3PDH* (Clontech, Palo Alto, CA, USA) for 30 cycles in a DNA thermal cycler (PC-700; ASTEC, Fukuoka, Japan); PCR conditions consisted of denaturing at 94°C for 45 s, annealing at 58°C for 45 s, and extending at 70°C for 2 min. The cDNA samples were normalized to yield comparable amounts of *G3PDH* cDNA. PCR products were electrophoresed on 2% agarose gels, and visualized with ethidium bromide. The results were quantified by analysis of Polaroid negative using the NIH image programme.

Results

Light and electron microscopy

Examining semithin sections stained with toluidine blue from Epon-embedded tissues of AD lesions, the number of *S. aureus* cells found in the sections varied in each sample although their adhesive characteristics were similar. Scattered or clustered *S. aureus* cells attached to uppermost horny cells or were present in crust-like horny layers forming colonies (Fig. 1). On electron microscopy, their localization patterns were similar to those in light microscopy. They multiplied, attaching to uppermost horny cells and among corneocytes, and some *S. aureus* cells were found arranged linearly along desquamating corneocytes. Amorphous or fine materials stained positive for ruthenium red and surrounded almost all *S. aureus* cells, suggesting an abundant production of glycocalyx by *S. aureus*. In addition, *S. aureus* cells appeared to actively multiply based on the observation of many splitting *S. aureus* cells (Fig. 2).

Fig. 3. Double immunofluorescence staining of MBP (a, fluorescein) and SEB (b, rhodamine), and CD1 (c, fluorescein) and SEB (d, rhodamine). Almost all SEB⁺ cells in the dermis (b, arrowheads) are positively stained with anti-MBP Ab (a, arrowheads). In contrast, the distribution of SEB⁺ cells (d, arrows) is not always consistent with that of CD1⁺ cells (c, arrows). The staining pattern with anti-SEB Ab showed diffuse immunofluorescence in the cytoplasm of the corresponding cells (b, d). In contrast, that with anti-MBP Ab was much clearer in the periphery of the corresponding cells (a).



Fibrillar structures were occasionally observed between *S. aureus* cells that were in contact with each other and between *S. aureus* cells and corneocytes.

Distribution of staphylococcal enterotoxin B

In AD lesions, many SEB⁺ cells were observed in the dermis (Fig. 3) and less often in the epidermis. Micro-organisms colonizing on uppermost horny layers and among the horny cells, which were considered to be *S. aureus* cells, were also positively stained with anti-SEB Ab. With double staining, almost all SEB⁺ cells in the dermis (Fig. 3b) were positively stained with anti-MBP Ab (Fig. 3a). Many IgE⁺ cells were observed in the epidermis as well as in the dermis, although with double staining of IgE and SEB some IgE⁺ cells showed negative staining with anti-SEB Ab in the skin. With double staining of CD1 and SEB, the distribution of SEB⁺ cells (Fig. 3d) was not always consistent with that of CD1⁺ cells (Fig. 3c). The staining pattern of anti-SEB Ab showed diffuse immunofluorescence in the cytoplasm of the corresponding cells (Fig. 3b,d). In contrast, the staining pattern of anti-MBP Ab was much clearer in the periphery of the corresponding cells (Fig. 3a).

HLA-DR and ICAM-1 expression in the normal organ-cultured skin

The expression of HLA-DR and ICAM-1 was similarly detected in the epidermis, mainly at basal and suprabasal cell layers, after 8 h incubation with either SEA or SEB, and continuously observed throughout 24 h incubation (Fig. 4a). Furthermore, ICAM-1 expression was observed

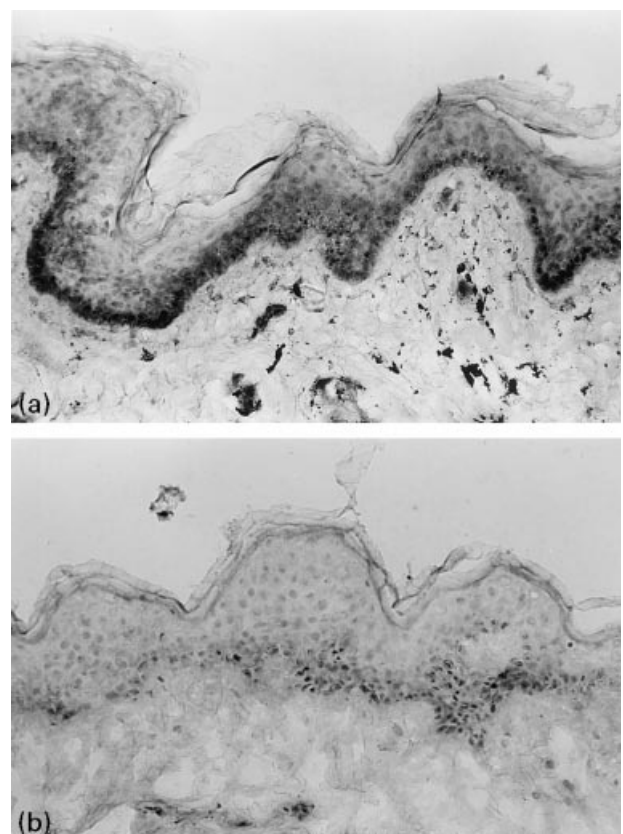


Fig. 4. Normal human skin incubated with SEB for 8 h (a). Immunohistochemically, the expression of ICAM-1 is detected on the basal and suprabasal keratinocytes in the epidermis. No expression of ICAM-1 is observed in the control skin incubated without enterotoxins (b).

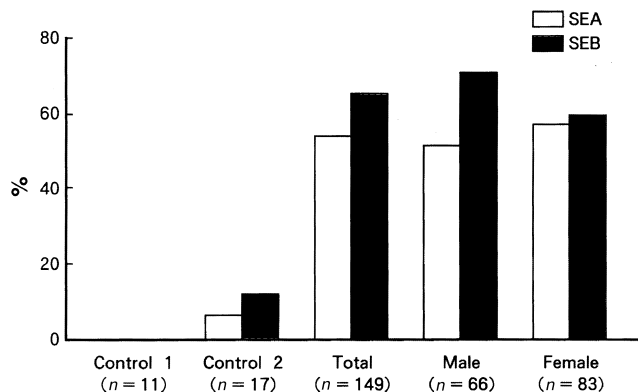


Fig. 5. Prevalence of specific IgE antibodies against SEA or SEB. In two control groups, the prevalence is significantly lower than that in AD. There is no significant difference in the prevalence between males and females. Control 1; 11 healthy subjects, and Control 2; 17 skin diseased patients without atopic disorders.

on endothelial cells of dermal vessels. No expression of HLA-DR or ICAM-1 was found in the control skin incubated without enterotoxins (Fig. 4b).

Specific IgE antibodies to staphylococcal toxins (SEA, SEB, and α -toxin)

The prevalence of positive IgE antibodies against SEA or SEB is shown in Fig. 5. Of 149 AD serum samples tested, 107 (71.7%) had specific IgE antibodies against either SEA or SEB or both. There were 81 (54.4%) AD serum samples that reacted with anti-SEA Ab, 97 (65.1%) that reacted with anti-SEB Ab, and 71 (47.7%) that reacted with both SEA and SEB Abs. The titres of SEA-specific IgE ranged from 0.38 to >30 IU/mL and those to SEB-specific IgE ranged from 0.36 to >50 IU/mL. Of the patients with both SEA- and SEB-specific IgE, those with high titres of one did not always show high titres of the other. We examined the prevalence of specific IgE-positive patients in terms of gender, age, AD severity, and total serum IgE levels. There was no significant correlation between the prevalence and sex, age, or AD severity (Fig. 5). The higher incidence of IgE antibodies specific for SEA and/or SEB was found in the AD patients with higher levels (more than 500 IU/mL) of total serum IgE levels. There was a statistically significant difference ($P < 0.01$) between the patients with high total serum IgE levels (more than 500 IU/mL) and those with normal total serum IgE levels. No specific IgE antibodies to staphylococcal α -toxin were detected in these AD patients.

Control sera from 17 patients with nonatopic skin diseases and normal control sera from 11 healthy subjects were also tested for SEA-, SEB- and α -toxin-specific IgE, and for total serum IgE levels. Only three of these serum samples

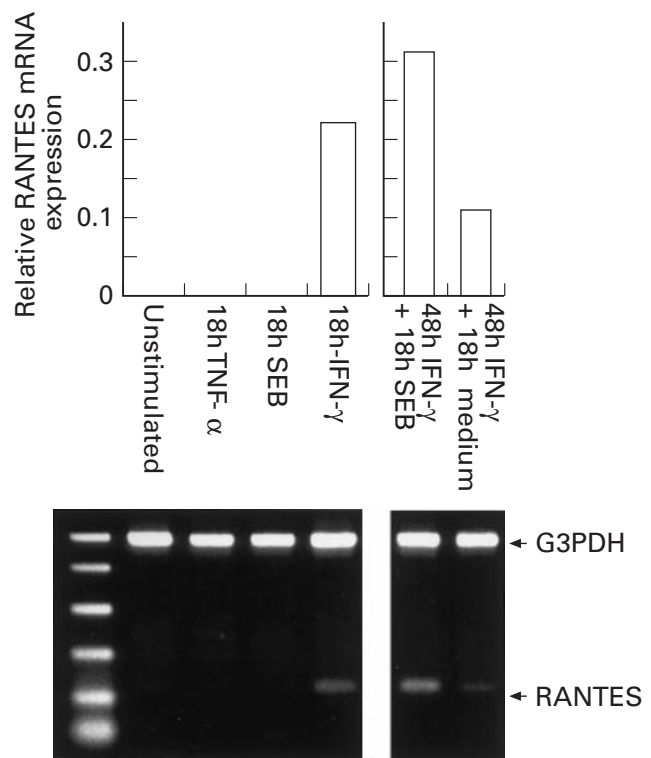


Fig. 6. RT-PCR analysis of the kinetics of RANTES mRNA expression relative to G3PDH in cultured human keratinocytes with or without stimulation. RT-PCR was performed starting from cDNA corresponding to 1 μ g of total RNA. Area of RANTES/G3PDH expression was calculated as described in Material and methods.

were positive to SEA or SEB in Control 2 and all of their total serum IgE levels were within normal limits. Compared with the prevalence of positive specific IgE antibodies in AD patients, that in control groups was significantly lower ($P < 0.001$).

RANTES mRNA expression on IFN- γ -treated keratinocytes

RANTES mRNA was expressed on cultured human keratinocytes incubated with IFN- γ for 18 h, although it was not detected in the incubation with SEB or TNF- α (Fig. 6). With the addition of 500 ng/mL SEB, RANTES mRNA expression on keratinocytes preincubated with 25 U/mL of IFN- γ for 48 h was upregulated or maintained for a long time, compared with the condition with no stimulants (Fig. 6).

Discussion

The bacterial flora of skin consists primarily of staphylococci, micrococci, corynebacteria, and propionibacteria

[19]. Among these skin bacteria, *S. aureus* is found only in 2–25% on the skin of normal healthy individuals [1,2,20–23]. In contrast, not only eczematous but normal-appearing skin in almost all patients with AD is colonized with *S. aureus* in higher prevalence and density than in normal skin of healthy subjects or other types of eczematous skin [1–5,20–23]. The increased colonization by *S. aureus* in patients with AD might be caused by enhanced adherence of *S. aureus* to skin as this micro-organism exhibited a statistically much greater degree of adherence to corneocytes of patients with AD, compared with corneocytes collected from normal controls or those with psoriatic lesions or other skin diseases [24]. These investigations, however, mainly reflect only quantitative or prevalence of colonizing *S. aureus* in AD lesions. Few examinations of the adhesive features of *S. aureus* colonizing on AD skin lesions have been reported [25]. The current study revealed that the bacterial cells tightly attached to the uppermost corneocytes and could penetrate the epidermis via the intercellular spaces. Fibrillar and amorphous structures, which were positive for ruthenium red stain and might be derived from products of *S. aureus* cells, plasma components, or a mixture of both, were found between the *S. aureus* cells in contact with each other and between *S. aureus* cells and corneocytes. Similar materials, glycocalyx, were also demonstrated in an *in vivo* experiment of infection with *S. aureus* in mice [26], and they seem to contribute to the strong adherence among *S. aureus* cells and between *S. aureus* cells and corneocytes, resulting in bacterial biofilms [14]. The findings in this study, especially penetration of *S. aureus* into intercellular spaces of the epidermis, strongly suggest that skin surface lipids in AD patients are deteriorated. The antibacterial activity of skin surface lipids such as free fatty acids, polar lipids, and glycosphingolipids have been recognized for years [27].

Most strains of *S. aureus* isolated from AD often produce exotoxins such as SEA, SEB, and toxic shock syndrome toxin-1 (TSST-1) [6–8]. The staphylococcal toxins secreted on the skin surfaces can act as superantigens which, with the help of HLA-DR on Langerhans cells or macrophages might stimulate them to produce proinflammatory cytokines such as interleukin-1 (IL-1) and TNF- α [28]. IL-1 and TNF- α then may contribute to skin inflammation by induction of vascular endothelial leucocyte adhesion molecules such as ICAM-1 and ELAM-1 [29,30]. Furthermore, the expression of MHC class II molecules on keratinocytes is induced during inflammation and potently activates T cells in the presence of superantigens [31,32]. The T-cell activation by class II-bearing keratinocytes will lead to further inflammation and cytokine release.

The present investigation of organ culture incubated with

SEA or SEB demonstrated the expression of HLA-DR and ICAM-1 on normal keratinocytes. Though it is not clear whether the effects are due to direct effect of SEA or SEB on keratinocytes, or through cytokines released from lymphocytes present in the dermis, the findings clearly suggest that the staphylococcal enterotoxins provoke the inflammation [33,34]. Further studies and our previous report [35] revealed that 70–80% of all the patients tested had a significantly higher incidence of circulating IgE antibodies specific for SEA and/or SEB ($P < 0.001$) compared with those in two control groups. Because the staphylococcal enterotoxins seem to penetrate the injured skin barrier in patients with AD more easily than they do an intact skin barrier, these findings strongly support the possibility that in the majority of AD patients SEA and SEB have a role in exacerbation and prolongation, and/or act as a trigger of AD through IgE-dependent immunoreaction. Even in patients with mild AD, staphylococcal toxins might have similar effects on the lesions as those in patients with severe AD.

AD is closely associated with eosinophils [36,37], and RANTES (regulated on activation, normal T expressed and secreted) is a chemotactic and activating factor for human eosinophils [38]. The clinical severity of AD shows a positive correlation with the number of blood eosinophils and the concentration of serum eosinophil cationic protein (ECP) [39,40]. In AD lesions, the deposition of MBP [41] as also shown by immunofluorescence in this study and of ECP [42] has been demonstrated, suggesting the degranulation of eosinophils in inflammatory sites of AD. In the present study, SEB upregulated RANTES mRNA expression on cultured human keratinocytes preincubated with IFN- γ . With electron microscopy, abundant degranulated eosinophil-specific granules were observed in the inflamed dermis (data not shown). Our immunofluorescence study on the distribution of SEB demonstrated that it was almost always localized on dermal-infiltrated eosinophils, not always CD1⁺ or IgE-bearing cells. The findings indicate that at least SEB has an important interaction especially with eosinophils directly and through epidermal keratinocytes, as well as IgE-bearing cells through IgE-mediated responses, which suggests that SEB has a crucial role in eosinophilic inflammation of AD lesions.

Flare-ups of AD often correlate with heavy colonization of *S. aureus* on the skin surfaces [7]. The skin rash frequently resolves when *S. aureus* is reduced following antibiotic therapy, topical high potent corticosteroid application, or a combination of both [8,12,13], but *S. aureus* cannot be completely eliminated. The data we present here suggest that the enterotoxins continuously stimulate infiltrated corresponding cells such as lymphocytes, eosinophils, and monocytes/macrophages in AD lesions to produce IgE and to induce the relevant cells [43], resulting

in the exacerbation and prolongation of the inflammation in AD.

References

- Leyden JJ, Marples RR, Kligman AM. Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol* 1974; 90:525–30.
- Aly R, Maibach HI, Shinefield HR. Microbial flora of atopic dermatitis. *Arch Dermatol* 1977; 113:780–2.
- Svejgaard E. The role of microorganisms in atopic dermatitis. *Sem Dermatol* 1990; 9:255–61.
- Ring J, Abeck D, Neuber K. Atopic eczema: role of microorganisms on the skin surface. *Allergy* 1992; 47:265–9.
- Akiyama H, Ueda M, Toi Y, Kanzaki H, Tada J, Arata J. Comparison of the severity of atopic dermatitis lesions and the density of Staphylococcus aureus on the lesions after antistaphylococcal treatment. *J Infect Chemother* 1996; 2:70–4.
- McFadden JP, Noble WC, Camp RDR. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 1993; 128:631–2.
- Leung DY, Harbeck R, Bina P et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest* 1993; 92:1374–80.
- 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.
- Tokura Y, Furukawa F, Wakita H, Yagi H, Ushijima T, Takigawa M. T-cell proliferation to superantigen-releasing Staphylococcus aureus by MHC class II-bearing keratinocytes under protection from bacterial cytolysin. *J Invest Dermatol* 1997; 108:488–94.
- Campbell DE, Kemp AS. Proliferation and production of interferon-gamma (IFN- γ) and IL-4 in response to Staphylococcus aureus and staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997; 107:392–7.
- Strange P, Skov L, Lisby S, Nielsen PL, Baadsgaard O. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996; 132:27–33.
- Lever R, Hadley K, Downey D, Mackie R. Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. *Br J Dermatol* 1988; 119:189–98.
- Nilsson EJ, Hennings CG, Magnusson J. Topical corticosteroids and Staphylococcus aureus in atopic dermatitis. *J Am Acad Dermatol* 1992; 27:29–34.
- Akiyama H, Ueda M, Kanzaki H, Tada J, Arata J. Biofilm formation of Staphylococcus aureus strains isolated from impetigo and furuncle: role of fibrinogen and fibrin. *J Dermatol Sci* 1997; 16:2–10.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Suppl.)*(Stockh) 1980; 92:44–7.
- The Japanese Dermatological Association. Japanese Dermatological Association criteria for the diagnosis of atopic dermatitis. *J Dermatol (Tokyo)* 1996; 23:66–7.
- Rajka G. Natural history and clinical manifestations of atopic dermatitis. *Clin Rev Allergy* 1986; 4:3–26.
- McSharry C, McKay IC, Lewis C. Methods for quantifying allergen-specific IgE antibodies: solid-phase and liquid-phase techniques. *J Clin Immunol* 1993; 16:153–8.
- Leyden JJ, McGinley KJ, Hoelzle E, Labows JN, Kligman AM. The microbiology of the human axilla and its relationship to axillary odor. *J Invest Dermatol* 1981; 77:413–6.
- Hoeger PH, Lenz W, Boutonnier A, Fournier JM. Staphylococcal skin colonization in children with atopic dermatitis: Prevalence, persistence, and transmission of toxigenic and nontoxigenic strains. *J Inf Dis* 1992; 165:1064–8.
- Williams REA, Gibson AG, Aitchinsons TC, Lever R, Mackie RM. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis. *Br J Dermatol* 1990; 123:493–501.
- White MI, Noble WC. Consequences of colonization and infection by Staphylococcus aureus in atopic dermatitis. *Clin Exp Dermatol* 1986; 11:34–40.
- Gloor M, Peters G, Stoika D. On the resident aerobic bacterial skin flora in unaffected skin of patients with atopic dermatitis and in healthy controls. *Dermatologica* 1982; 164:258–65.
- Cole GW, Silverberg NL. The adherence of Staphylococcus aureus to human corneocytes. *Arch Dermatol* 1986; 122: 166–9.
- Tada J, Toi Y, Arata J. Ultrastructural adhesive modes of Staphylococcus aureus cells colonizing on the skin surfaces in atopic dermatitis. In: Ishibashi Y, Nakagawa H, Suzuki H, eds. *Electron Microscopy in Dermatology — Basic and Clinical Research*. Amsterdam: Elsevier Science B.V., 1994: 75–80.
- Akiyama H, Kanzaki H, Tada J, Arata J. Staphylococcus aureus infection on cut wounds in the mouse skin: experimental staphylococcal botryomycosis. *J Dermatol Sci* 1996; 11: 234–8.
- Miller SJ, Aly R, Shinefeld HR, Elias PM. In vitro and in vivo antistaphylococcal activity of human stratum corneum lipids. *Arch Dermatol* 1988; 124:209–15.
- Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990; 248:705–11.
- Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991; 67:1033–6.
- Leung DYM, Cotran RS, Pober JS. Expression of an endothelial leukocyte adhesion molecule (ELAM-1) in elicited late phase allergic skin reaction. *J Clin Invest* 1991; 87:1805–10.
- Tokura Y, Yagi J, O'Malley M et al. Superantigenic staphylococcal exotoxins induce T cell proliferation in the presence of Langerhans cells or class II-bearing keratinocytes and stimulate keratinocytes to produce T cell-activating cytokines. *J Invest Dermatol* 1994; 102:31–8.
- Strange P, Skov L, Baadsgaard O. Interferon gamma-treated keratinocytes activate T cells in the presence of superantigens: involvement of major histocompatibility complex class II molecules. *J Invest Dermatol* 1994; 102:150–4.
- Wakita H, Tokura Y, Furukawa F, Takigawa M. Staphylococcal enterotoxin B upregulates expression of ICAM-1 molecules on IFN- γ -treated keratinocytes and keratinocyte cell line. *J Invest Dermatol* 1995; 105:536–42.

- 34 Matsunaga T, Katayama I, Yokozeki H, Nishikawa K. Superantigen-induced cytokine expression in organ-cultured human skin. *J Dermatol Sci* 1996; 11:104–10.
- 35 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.
- 36 Kapp A. The role of eosinophils in the pathogenesis of atopic dermatitis — eosinophil granule proteins as markers of disease activity. *Allergy* 1993; 48:1–5.
- 37 Desreumaux P, Capron M. Eosinophils in allergic reactions. *Curr Opin Immunol* 1996; 8:790–5.
- 38 Alam R, Stafford S, Forsythe P et al. RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* 1993; 150:3442–7.
- 39 Czech W, Krutmann J, Schuöpf E, Kapp A. Serum eosinophil cationic proteins (ECP) is a sensitive measure for disease activity in atopic dermatitis. *Br J Dermatol* 1992; 126:351–5.
- 40 Kägi MK, Joller-Jemelka H, Wüthrich B. Correlation of eosinophils, eosinophil cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. *Dermatology* 1992; 185:88–92.
- 41 Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis. *N Engl J Med* 1985; 313:282–5.
- 42 Maeda K, Yamamoto K, Tanaka Y, Anan S, Yoshida H. The relationship between eosinophils, OKT6-positive cells and house dust mite (HDM) antigens in naturally occurring lesions of atopic dermatitis. *J Dermatol Sci* 1992; 3:151–6.
- 43 Neuber K, Steinrücke 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.