

The Role of Immune Response to *Staphylococcus aureus* Superantigens and Disease Severity in Relation to the Sensitivity to Tacrolimus in Atopic Dermatitis

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Key Words

Atopic dermatitis • Sensitivity • Superantigens • Toxic shock syndrome toxin-1 • Tacrolimus

Abstract

Background: *Staphylococcus aureus*-producing superantigens (SAGs), such as staphylococcal enterotoxin B (SEB) or toxic shock syndrome toxin-1 (TSST-1), are frequently observed in atopic dermatitis (AD). However, little has been done to establish the association of immune responses to SAGs and the therapeutic response to immunosuppressive drugs in AD. Therefore, we investigated the prevalence and role of SAGs in the pathophysiology and immunosuppressive drug sensitivity in AD patients. **Methods:** We classified 29 patients into two groups on the basis of their clinical AD scores: a low-score group (n = 14) corresponding to mild to moderate patients and a high-score group (n = 15) corresponding to severe patients. We estimated the plasma anti-SEB or TSST-1 IgE of these patients and healthy subjects by ELISA. We also estimated individual drug sensitivity by determining drug concentrations that would give 50% inhibition (IC₅₀) of peripheral-blood mononuclear cell (PBMC) proliferation in vitro. **Results:** The levels of plasma anti-SEB or

TSST-1 IgE in the severe patients were significantly higher than those in the mild to moderate patients (p < 0.05 and p < 0.01, respectively). When stimulated with concanavalin A in vitro, PBMCs in the severe patients exhibited low sensitivity to the suppressive efficacy of tacrolimus (FK506) as compared to the mild to moderate patients (p < 0.01). Furthermore, there was a significant correlation between the IC₅₀s of FK506 and plasma anti-TSST-1 IgE levels (p < 0.01). **Conclusions:** We showed that PBMCs in severe AD patients exhibited lower sensitivity to FK506, and had higher plasma levels of anti-TSST-1 IgE as compared to the mild AD patients. SAGs appear to be one of the causes of decreased PBMC sensitivity to FK506, and therefore an alternative treatment would be useful based on the individual drug sensitivity data and anti-TSST-1 IgE levels. Copyright © 2006 S. Karger AG, Basel

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease frequently associated with increased IgE synthesis and skin colonization with *Staphylococcus aureus*-producing superantigens (SAGs) [1, 2]. SAGs from

S. aureus include staphylococcal enterotoxin A, B, C (SEA, SEB, SEC) and toxic shock syndrome toxin-1 (TSST-1) [3]. A role of skin colonization by SAg-producing *S. aureus* in the pathogenesis of AD has been suggested in recent reports [1, 4, 5]. Moreover, the degree of skin colonization was found to be associated with disease severity [1, 6, 7], and has been suggested to be implicated in the development of insensitivity to glucocorticoid (GC) treatment [8].

SAGs comprise a group of microbial products, which have powerful immunomodulatory properties possibly playing a key role in the pathogenesis of several human diseases [9]. Unlike conventional antigens, SAGs bind to certain regions of major histocompatibility complex class II molecules of antigen-presenting cells outside the classical antigen-binding groove [10]. SAGs concomitantly bind in their native form to T cells at specific motifs of the variable region of the β chain of T cell receptor [10]. The pathophysiology induced by SAG is related to the ability of SAG to interact with a high proportion of peripheral T cells that leads to the release of high amounts of various cytokines and other effectors by immune cells [11, 12]. These changes induced by SAGs are suggested to be implicated in the pathogenesis of toxic shock syndrome [13], Kawasaki disease [14], rheumatoid arthritis [15], bronchial asthma [16], persistent allergic rhinitis [17], psoriasis [18], and AD [1, 2].

Recent articles have reported that specific IgE antibodies against SAGs, SEA, SEB, and/or TSST-1, are found in serum of 57–80% of AD patients [1, 19]. Concurrently, a correlation between the amount of serum SAG-specific IgE and the severity of AD has also been found [20, 21]. This correlation is strongest when patients produce specific IgE against SEA and/or SEB originating from *S. aureus* on their skin surface [20, 21]. SAGs continuously secreted from *S. aureus* on the skin surface may be triggering mediator/cytokine release from IgE-bearing immune cells [20, 21].

The aim of this study was to examine the prevalence and role of SAGs on the pathophysiology and immunosuppressive drug sensitivity in patients with AD. For this purpose, the relationship between plasma titers for specific SAG IgE antibodies and the ability of peripheral blood mononuclear cells (PBMCs) to proliferate in response to SAGs, PBMC sensitivity to immunosuppressive drugs *in vitro*, or the severity of AD evaluated by clinical score were examined.

Methods

Subjects

The ethical committee of Tokyo Medical University approved this study, and written informed consent was obtained from subjects included in this study. Venous blood was taken from 29 patients with AD (18 males and 11 females, 34.8 ± 11.7 years of age) and 13 healthy volunteers (7 males and 6 females, 25.8 ± 3.2 years of age). None of the healthy subjects had taken immunosuppressive agents, including GCs, for at least 6 months prior to or during the study. For more than 6 months, 22 out of the 29 AD patients were treated with FK506 ointment therapy. Moreover, 25 out of the 29 patients also received topical steroid ointment, and/or moisturizing cream in combination with oral antihistamine and anti-mast cell drugs that block the release of chemical mediators. AD severity was assessed by the same observer using a clinical score as described by 'The Guidelines for the Therapy for Atopic Dermatitis 2004' published by the Japanese Dermatological Association in 2004 (see below) [22].

Preparation of PBMCs for Cell Cultures

PBMCs were isolated from healthy volunteers and AD patients as described previously [23]. In short, 10 ml of the heparinized blood were loaded on 3 ml of Ficoll Hypaque and centrifuged at 2,000 g for 20 min, and PBMCs including lymphocytes were separated. The cells were then washed and suspended in RPMI 1640 medium (Gibco, Grand Island, N.Y., USA) containing 10% heat-inactivated fetal bovine serum (Gibco), 100,000 IU/l penicillin (Meiji Seika, Tokyo, Japan), and 100 mg/l streptomycin (Meiji Seika) to a cell density of 1×10^6 cells/ml.

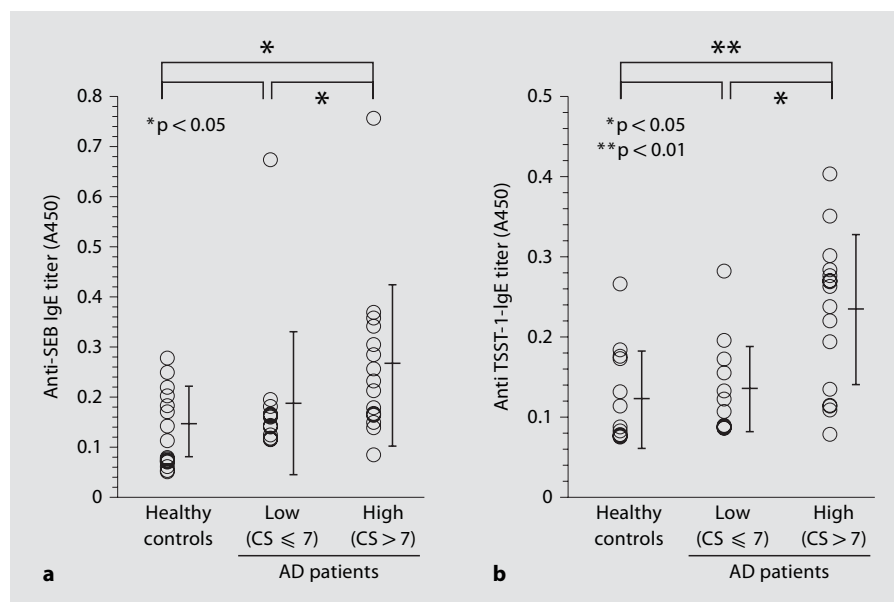
Cell Proliferation Assay

Two hundred microliters of cell suspension prepared as above were placed into each of the 96 flat-bottom wells of a microtiter plate. Concanavalin A (ConA; Seikagaku Kogyo, Tokyo, Japan), SEB, or TSST-1 (Sigma Chemical Co., St. Louis, Mo., USA) was added to each well to a final concentration of 5 μ g/ml for ConA or 1 ng/ml for SAGs, respectively. Subsequently, 4 μ l of an ethanol solution containing PSL (Sigma Chemical Co.) and FK506 (Astellas, Tokyo, Japan) was added. The plate was incubated for 92 h in 5% CO₂ air at 37°C. The cells were pulsed with ³H-thymidine (New England Nuclear, Boston, Mass., USA) for the last 20 h of incubation and then collected on a glass fiber filter paper with an automatic cell harvester. PBMC proliferation was assessed by ³H-thymidine incorporation as disintegrations per minute. The immunosuppressant concentration that would give 50% PBMC mitogenesis inhibition was determined from the dose-response curve. Stimulation index (SI) was calculated by a relative proliferation of PBMCs in response to mitogen or SAGs as compared to the proliferation of PBMCs in the absence of any stimuli.

Assay for Specific IgE Antibodies to SAGs

ELISA kits for measuring plasma antibody titers against SAGs were constructed as previously reported [24]. In short, purified SAGs were diluted in PBS at a concentration of 0.5 μ g/ml, and 0.1 ml volume of this solution was added to the wells of the ELISA plates. The plates were incubated overnight at 4°C. The unbound reagents were removed by aspiration and then treated with 0.4 ml of PBS containing 0.5% BSA for 2 h at 25°C. Before use, the plates were rinsed once with a washing buffer. Human plasma samples

Fig. 1. Plasma levels of anti-SEB (a) and TSST-1 IgE (b) in healthy subjects and AD patients. AD patients were divided into two groups according to their clinical scores by a median clinical score (CS) of 7 as a threshold. Low = Low clinical score group (n = 14); high = high clinical score group (n = 15).



were diluted 10-fold with dilution buffer C (PBS containing 0.25% BSA, 0.05% Tween 20), 0.1 ml of this solution was added to each of the test wells, and then the plates were incubated for 1 h at 25°C. The unbound reagents were rinsed 3 times with a washing buffer. Mouse monoclonal antibodies against human IgE were diluted in dilution buffer C to 0.25 µg/ml, and 0.1 ml of this solution was added to each of the test wells. Horseradish peroxidase-labeled rabbit monoclonal antibodies against mouse IgG were added to each of the test wells. The plates were incubated for 30 min at 25°C, then the unbound reagents were removed, and the wells were rinsed 3 times with a washing buffer. Substrate solution (0.1 ml) was then added, and the plates were incubated for 30 min at 25°C. Enzyme reaction was terminated with the addition of 0.1 ml of 0.5 M sulfuric acids, and then the ELISA plates were read spectrophotometrically at 450 nm. When the absorbance was greater than 2.0, the diluted plasma samples were further diluted 5-fold with dilution buffer C and applied to the ELISA.

Estimation of AD Clinical Scores

AD clinical scores were estimated from two independent indices, i.e., eruption severity and area. The eruption severity index was categorized into four intensities, i.e. severe, moderate, mild and slight, each of which scored as 3, 2, 1 and 0 points, respectively. Patients exhibiting the following skin symptoms, high swelling, edema, infiltration, lichenification, multiple papules, high flakiness, adhesion of crust, bullous, erosions, scratching mark, and/or prurigo nodularis, were given a score of 3. Patients exhibiting the following skin symptoms, moderate erythema, flakiness, slight papules, and/or lichenification, were given a score of 2. Patients exhibiting dryness, slight erythema, and/or flakiness were given a score of 1. Patients exhibiting only a slight dryness were given a score of 0. On the other hand, the eruption area index was categorized into the following four intensities: more than two thirds of the face or body area (score 3), one to two thirds of the face or body area (score 2), less than one third of the

face or body (score 1), and negligible (score 0). These two independent indices were evaluated by the same observer (physician), and clinical scores were estimated by a sum of the eruption severity and area scores for the face and body. Accordingly, the maximum clinical score amounts to 12 points, while the lowest one is zero.

Statistical Analysis

Data are expressed as the mean $\pm$ SD [for specific IgE and inhibitory concentration of 50% (IC₅₀) values] for each group as indicated. We analyzed the data using Kruskal-Wallis test and Dunn as a post hoc test for comparisons. The Spearman correlation coefficient by rank was used for correlation analysis. We performed multiple analyses of variance by MANOVA. These analyses were performed with Stat View and Graph Pad Prism. In each case, two-sided p values <0.05 were considered to be significant.

Results

Anti-SAg IgE Levels and AD Severity

Anti-SEB-specific IgE titers in the plasma of the 13 healthy subjects and 29 AD patients were determined by ELISA. The AD patients were divided into two groups according to their clinical scores, i.e., the low-score group (clinical score ≤ 7 ; n = 14) and the high-score group (clinical score > 7 ; n = 15). The high-score group had significantly higher levels of SEB-specific IgE titers than the healthy subjects and the low-score group (p < 0.05 and p < 0.05, respectively) (fig. 1a). The mean titer of anti-SEB IgE in the healthy subjects and two AD subgroups were

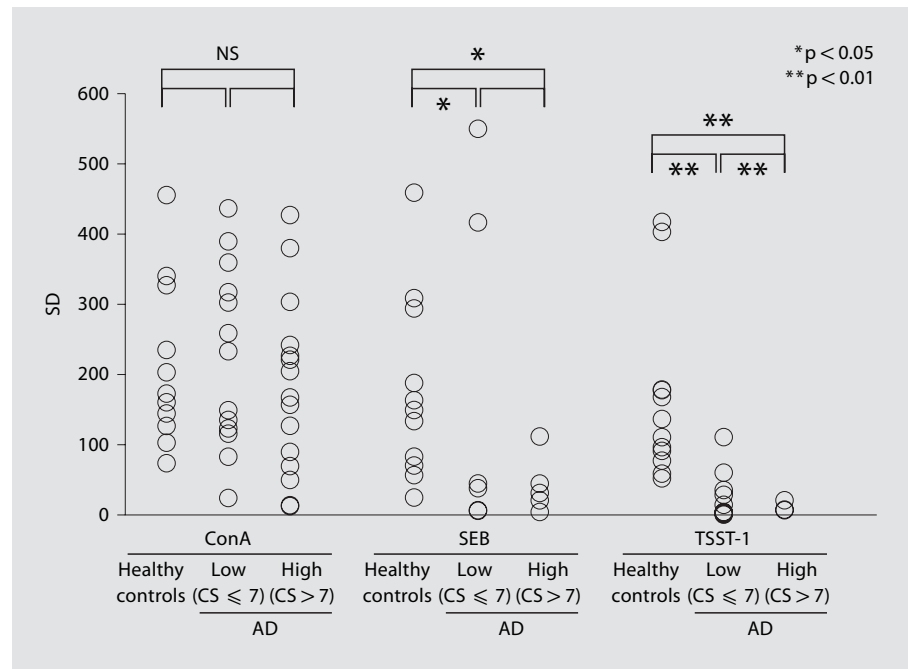


Fig. 2. Comparison of proliferation abilities of PBMCs between healthy subjects and AD patients with low and high clinical scores (CS) in response to ConA, SEB, and TSST-1 in vitro.

0.147 ± 0.075 , 0.188 ± 0.142 and 0.265 ± 0.161 , respectively. Moreover, the high-score AD group had significantly higher plasma levels of anti-TSST-1 IgE than the healthy subjects and the low-score group ($p < 0.01$ and $p < 0.05$, respectively) (fig. 1b). The mean levels of anti-TSST-1 IgE in the healthy subjects and the AD subgroups were 0.121 ± 0.059 , 0.134 ± 0.056 and 0.232 ± 0.093 , respectively. In addition, we found that the mean levels of total IgE concentrations in the high- and the low-score AD group were $2,707.8 \pm 3,088.3$ and $7,183.3 \pm 5,600.8$, respectively. The levels of anti-SEA and SEC IgE were also detected in the healthy and the AD subgroups. There were no significant differences in the levels between the healthy subjects and AD patients (data not shown). There were significantly higher plasma levels of the total IgE in the high-score AD group than the low-score AD group ($p < 0.05$; data not shown).

PBMC Responses to ConA and Sags in Relation to Clinical Scores and Anti-SAg Antibody Titers in AD Patients

PBMC ability to proliferate in response to ConA or Sags in vitro was estimated in the AD patients and healthy subjects, using a generalized mitogen assay procedure [23], as shown in figure 2. PBMCs from high-score patients showed significantly low proliferative responses to either of the stimuli, SEB and TSST-1, com-

pared to those from the healthy subjects ($p < 0.05$ and $p < 0.01$, respectively). A statistically significant difference in the proliferation ability of PBMCs in response to TSST-1 was also observed between the low- and high-score groups ($p < 0.01$; fig. 2). PBMC proliferation response to TSST-1 in vitro, as estimated with SI in the AD patients, showed a negative correlation with plasma IgE titers against TSST-1 as can be seen in figure 3 ($p < 0.01$). On the other hand, no such significant correlation was observed between the SI values in response to SEB and the plasma anti-SEB titers in those patients (data not shown).

PBMC Sensitivity to Immunosuppressive Drugs in AD in Relation to Clinical Scores and Anti-SAg IgE Titers

GCs and FK506 have been used topically for AD treatment. We examined PBMC sensitivity to these drugs in vitro in relation to the clinical scores of the AD patients. PSL and FK506 concentrations that induced 50% inhibition of ConA-stimulated PBMC proliferation were determined individually from their dose-response curves. Typical dose-response curves of PSL and FK506 on the proliferation of PBMCs from a patient with AD are shown in figure 4. As shown in figure 5, considerable individual variations in the IC_{50} values for these drugs were observed, especially in the AD patients. PSL IC_{50} values in the PBMCs of the healthy subjects ranged from 0.1 to

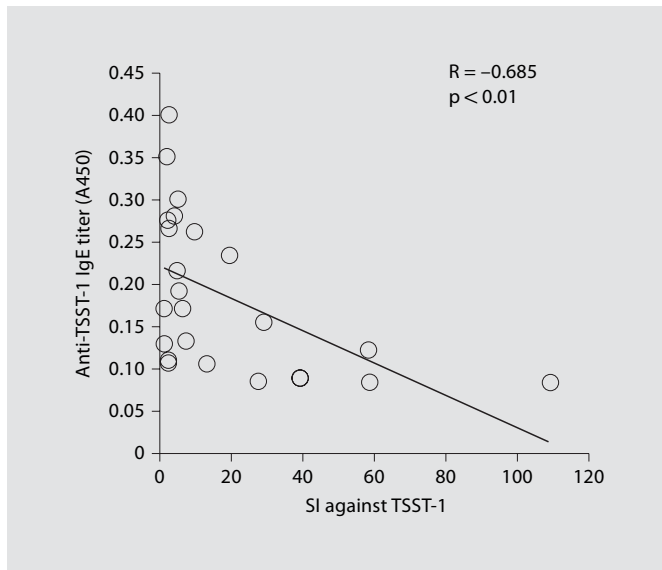


Fig. 3. Negative correlation between proliferation ability (SI) of PBMCs in response to TSST-1 in vitro and plasma anti-TSST-1 IgE titer.

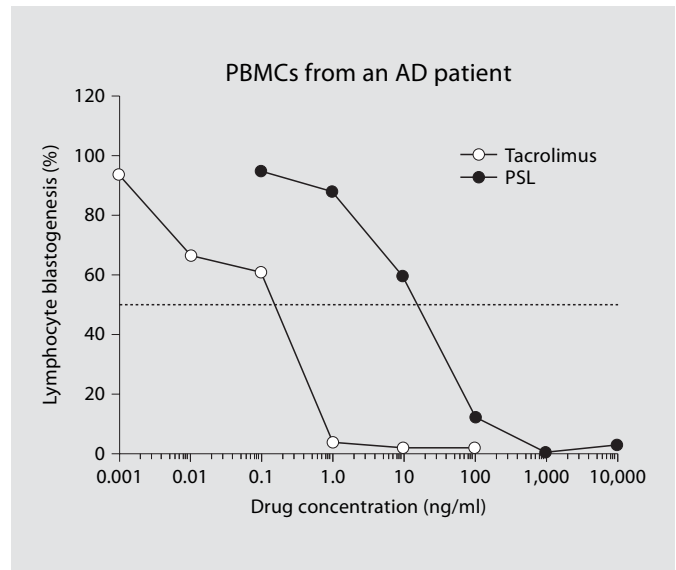
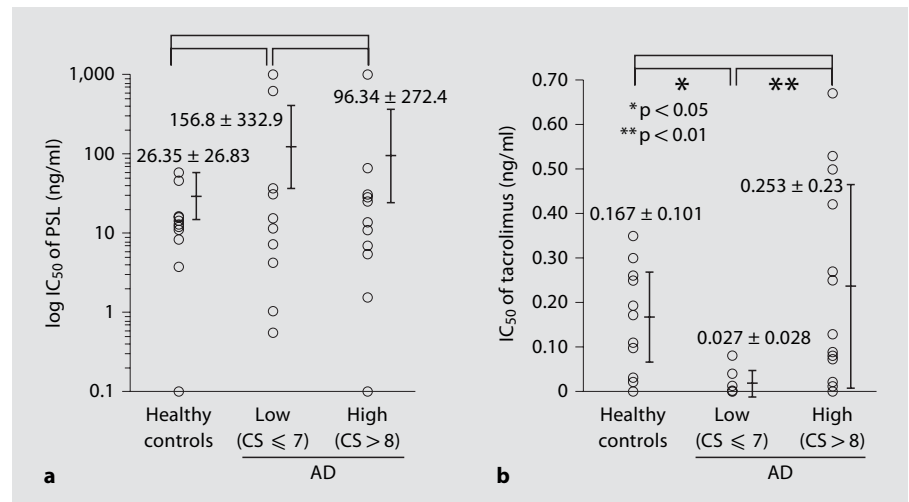


Fig. 4. Typical dose-response curves for PSL and FK506 on the proliferation of PBMCs stimulated with ConA in 1 AD patient.

Fig. 5. Individual PBMC sensitivities to PSL (a) and FK506 (b) in healthy subjects, and AD patients with low and high clinical scores. PBMC sensitivities were evaluated as the IC_{50} values for each agent against the ConA-induced proliferation of PBMCs. A statistically significant difference in FK506 IC_{50} values was observed between the two AD subgroups.

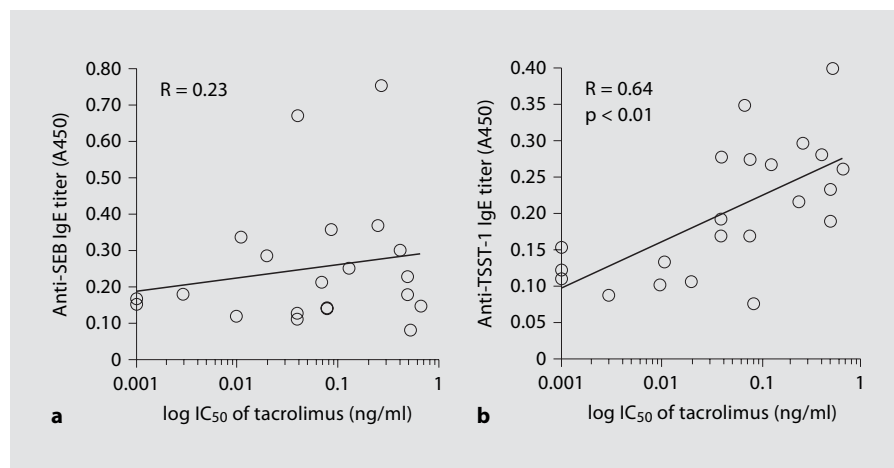


95.2 ng/ml, while those of the AD patients also showed wide deviations, ranging from 0.1 to 1,000 ng/ml. In particular, 3 AD patients exhibited extremely low PBMC sensitivity to PSL, and their IC_{50} s of PSL were far above the upper limit of PSL IC_{50} of healthy PBMCs (fig. 5a). Plasma levels of anti-TSST-1 IgE in these 3 patients were also highest among the AD patients and healthy subjects presently examined. FK506 IC_{50} values in the PBMCs of the healthy subjects ranged from 0.001 to 0.35 ng/ml, while those of the AD patients ranged from 0.001 to

0.67 ng/ml. However, no statistically significant difference was observed in the median PSL and FK506 IC_{50} values between the healthy subjects and AD patients.

Patients in the low- and high-score groups were classified and their PBMC sensitivities to PSL and FK506 in vitro were compared on the basis of the clinical scores as described above. All of the 22 patients examined here had already been treated with FK506 ointments for more than 6 months. The means $\pm$ SD of PSL IC_{50} s in the low- and high-score groups were 156.8 ± 332.9 and $96.3 \pm$

Fig. 6. Relationship between PBMC sensitivities to FK506 (IC_{50} values) and the plasma titers of anti-SEB IgE (**a**) or anti-TSST-1 IgE (**b**) in AD patients treated with FK506 ointment.



272.4 ng/ml, respectively, and there was no significant difference in the IC_{50} s between these two subgroups (fig. 5a). The means $\pm$ SD of FK506 IC_{50} s in the low- and high-score groups were 0.027 ± 0.028 and 0.253 ± 0.23 ng/ml, respectively, and there was a significant difference in the IC_{50} s between these two subgroups ($p < 0.01$; fig. 5b). Thus, PBMC sensitivity to FK506 was significantly lower in the high-score AD group than that in the low-score group. FK506 IC_{50} values in the PBMCs of the high-score AD group, however, were not significantly different with those in the PBMCs of the healthy subjects (fig. 5b). When we divided these AD patients into two groups, according to their PBMC sensitivities to FK506 by the median FK506 IC_{50} (0.087 ng/ml), clinical scores in the high-sensitivity groups ($IC_{50} < 0.087$ ng/ml) were significantly lower than those in the low-sensitivity groups ($IC_{50} \geq 0.087$ ng/ml; $p < 0.01$). We also examined the relationship between PBMC sensitivity to FK506 and plasma anti-TSST-1 IgE levels in the AD patients. The IgE titers in these patients significantly correlated with FK506 IC_{50} s as shown in figure 6b, while the anti-SEB IgE titers did not correlate with the IC_{50} s (fig. 6a). These data suggest that the AD patients with higher plasma anti-TSST-1 IgE levels, but not anti-SEB IgE levels, showed relatively lower PBMC sensitivity to FK506, as compared to the patients with lower plasma anti-TSST-1 IgE levels.

Finally, a statistical evaluation was performed by multiple analyses of variances (MANOVA) with the variance: disease severity, anti TSST-1 IgE levels, and PBMC response to FK506 in AD patients. The data showed that the IC_{50} values of FK506 had significant effects on the two parameters, disease severity ($p = 0.041$) and TSST-1-specific IgE ($p = 0.012$).

Discussion

The pathogenesis of AD is known to be associated with skin colonization by SAg-producing *S. aureus*, increased IgE synthesis, and SAg-specific IgE, which can be detected in the plasma of AD patients as shown in the present study, and may contribute to exacerbating clinical AD symptoms. Though we did not measure the presence of *S. aureus* on the skin of AD patients in the present study, many studies have already shown that the extent of *S. aureus* colonization correlates with AD disease severity, and, in more than 90% of AD patients, massive skin colonization with *S. aureus* can be found [25]. It has also been reported that *S. aureus* produces immunomodulatory toxins such as SAGs in up to 65% of AD patients [26]. Thus, in the present study, we focused on the relationship between the exposure of patients to SAGs by measuring the specific plasma SAg IgE and their respective AD clinical score. In this study, we have found that plasma titers for anti-SEB and TSST-1 IgE in the high clinical AD score group were significantly higher than those in the low clinical score group. Especially, plasma anti-TSST-1 IgE concentrations in the severe AD patients were markedly high, as compared to the other AD patients with moderate or mild severity. Tada et al. [19] found specific IgE to SEA and SEB in sera of 80 out of 96 adult AD patients, but they detected no correlation between these antibody titers and AD severity. Breuer et al. [27] also reported no difference of AD severity between patients having SAg-specific IgE and those without such IgE. However, several recent articles have reported that colonization with SEB-producing *S. aureus* is associated with increased severity of AD [7, 21, 28]. Nomura et al. [28] were able to

show that the severity of AD is related to the level of SEB-specific IgE, and not to the level of SEA- or TSST-1-specific IgE in children and adolescents with AD. Thus, while the correlation between SAg-specific IgE and severity in AD is controversial, our findings strongly support the possibility that SEB and TSST-1 produced from *S. aureus* at the AD skin region influence immune responses and AD symptoms.

On the other hand, Yoshino et al. [29] assessed the correlation between clinical disease severity and proliferative response of PBMCs to SEB in vitro in AD. They found that PBMCs from their severe patients showed a markedly suppressed proliferative response to SEB. König et al. [30] also showed that proliferative responses to SEB in T cells from AD were greatly suppressed compared to those from healthy controls. In contrast to these findings, other reports showed that the proliferative responses of PBMCs from AD patients to SEB were higher than those of healthy subjects [31, 32], whereas Tanaka et al. [33] reported that proliferation of PBMCs against SEB stimulation did not significantly differ between AD patients and normal subjects. These discrepant results may be explained by the variability of clinical severity often observed in AD patients in different studies. In this study, we observed that PBMC responses to SEB and TSST-1 stimulations significantly decreased in severe AD patients as compared to healthy subjects. In addition, a statistically significant difference in proliferation ability of PBMCs in response to TSST-1 was also observed between the low- and high-score AD groups. However, the molecular mechanisms for such differences in PBMC function associated with AD severity remain to be elucidated.

Yoshino et al. [29] found that T cell viability decreased and apoptosis increased in the high-score AD group when compared with the low-score AD group and the control group. These observations raise the possibility that PBMCs from severe AD patients have been exposed to various antigens, and are likely to undergo apoptosis after being exposed to SAg a second time. König et al. [30] suggested that the expression of IL-2 in SEB-stimulated T cells was suppressed in AD. These observations suggest that bacterial toxins are able to induce antigen-specific nonresponsiveness or a state of anergy in human PBMCs. In particular, our data showed that PBMCs in severe AD patients exhibit markedly lower responses to TSST-1, as compared to ConA or SEB. In addition, AD patients with PBMCs exhibiting low proliferation responses to TSST-1 in vitro had higher plasma levels of anti-TSST-1 IgE. This tendency was not found when ConA or SEB stimulation was used for PBMC stimulation

in our study. We assume that constitutive hyperstimulation with TSST-1 might exacerbate AD symptoms, and that repeated exposure to TSST-1 may reprogram immunocytes including T cells to enter an anergic state. Thus, TSST-1 might induce 'nonresponsiveness' in AD T cells, and the degree of such 'nonresponsiveness' might be relevant to AD severity. Florquin et al. [34], Gimeno et al. [35], and Schols et al. [36] reported that IL-10 and IFN- γ are newly produced after a secondary exposure to SEB despite the anergy of T cells. These immune changes observed after a second challenge of SEB affected not only the secretion of IL-2, IL-6, IL-10, IL-12, and IFN- γ cytokines, but also the intracellular response to the cytokines, STAT1 and 3 [37]. Understanding the changes in immune response patterns would contribute to establishing a therapeutic strategy to controlling the pathological processes implicated with SAg-producing staphylococcal strain infections on the skin of AD patients.

FK506 ointment is used for the treatment of AD patients, especially those exhibiting low responses to GC therapy [38, 39]. In most cases, the drug is useful in suppressing severe clinical symptoms of AD; however, individual variations in tacrolimus efficacy, adverse effects from tacrolimus including skin burning, pruritus, varicella and blisters [40], and/or recurrence of AD after cessation of tacrolimus use have been reported. Pharmacodynamic approaches with the use of PBMCs of the patients could be an efficient strategy for the evaluation of the individual therapeutic potentials of tacrolimus, as has been shown for cyclosporine use in the treatment of psoriasis [41] and GC use in kidney transplantations [23, 41].

In the present study, we evaluated the individual therapeutic efficacy of tacrolimus using PBMCs obtained from AD patients treated with tacrolimus ointment for more than 6 months. FK506 is effective in suppressing the proliferative response of PBMCs from AD patients, and its potency was revealed to be approximately 120-fold superior to that of PSL. For the measuring of PBMC sensitivities to immunosuppressive drugs, in the present study, we used ConA for stimulation of PBMCs instead of SAg. As indicated in figure 2, PBMCs obtained from the AD patients, especially those with severe AD, exhibited little response to SAg, and therefore we could not examine the effect of immunosuppressive drugs under SAg stimuli. The PSL or FK506 IC₅₀ values were not significantly different between the healthy subjects and severe AD patients (fig. 5). However, PBMC response to immunosuppressive drugs exhibited wide individual variations, and the differences in IC₅₀ values of these drugs

among the AD patients. PSL IC₅₀ values on the proliferation of AD PBMCs deviated from 0.1 to 1,000 ng/ml, whereas FK506 IC₅₀ values deviated from 0.001 to 0.67 ng/ml. However, the molecular bases for these individual deviations are not clear. We showed that PBMCs in severe AD patients exhibited lower sensitivity to FK506, and had higher plasma levels of anti-TSST-1 IgE as compared to the mild AD patients. It is assumed that TSST-1 changes the immune response pattern in AD patients, which might be implicated in PBMC sensitivity to FK506. In this study, our data showed that the PBMC sensitivities to PSL in AD patients did not differ significantly as compared to those in healthy subjects. However, 3 AD patients exhibited extremely low PBMC sensitivity to PSL, and their IC₅₀s for PSL were far above the upper limit of PSL IC₅₀s in healthy PBMCs. Plasma levels of anti-TSST-1 IgE in these 3 patients were also highest among the AD patients and healthy subjects examined here.

In our study, GC markedly inhibited ConA-induced PBMC proliferation, but they scarcely inhibited TSST-1-induced PBMC proliferation in human subjects (data not shown). Hauk et al. [8] suggested that SEB may contribute to dexamethasone insensitivity through induction of GC receptor β . Li et al. [42] reported that phosphorylation of GC receptors triggered by SEB prevents nuclear translocation of GC receptors, thereby inducing dexamethasone resistance. Taken together, these results suggest that SAgS might be associated with the molecular basis for the individual deviations of PBMC responses to GC in AD. On the other hand, although FK506 is known to be extremely effective in the treatment of AD and reduce staphylococcal colonization of skin in AD patients [43], we suggest that immune stimulation by SAgS might be important in the development of insensitivity to FK506.

Although the main genomic actions of GCs in their anti-inflammatory and immunomodulatory effects have been suggested to be different from those of FK506, TSST-1-induced stimulation of PBMCs in AD might partially attenuate the inhibitory efficacy of these drugs via disturbance of at least a common pathway. The molecular bases of FK506 in its immunomodulatory effects are much more specific than those of GCs, and, therefore, TSST-1-induced insensitivity might result from FK506 implicated for its immunosuppressive pathway. The physiologic mechanism should be clarified at the cellular level by further studies.

Consequently, it is thought that differences in the amount of SAgS produced from *S. aureus* colonized in the skin, the period of immune stimulation by SAgS, and/or the intensity of SAgS to modulate immunity in AD patients could be causes of the exhibited individual differences in PBMC sensitivity to FK506. It may be useful to measure PBMC sensitivity to FK506 when measuring plasma levels of anti-SAgS IgE for individualized immunosuppressive therapy in patients with AD. According to the PBMC sensitivity data in vitro and the antibody titer against SAgS, the concomitant use of antibiotics combined with immunosuppressive drugs may be effective for the treatment of AD patients exhibiting insensitivity to immunosuppressive drugs and *S. aureus* colonization in the skin.

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