A Comparison Study of the Staphylococcal Exotoxins and Staphylococcal Enterotoxin A-specific IgE Antibody between Childhood and Adulthood Atopic Dermatitis

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Background: The skin of patients with atopic dermatitis (AD) exhibits a striking susceptibility to colonization with Staphylococcus aureus (S. aureus). Superantigens produced by S. aureus and their specific IgE antibodies are thought to be important precipitating factors of AD, but there are few reports evaluating these 2 factors at the same time, particularly in adult AD patients.

Object: Our purpose was to investigate the differences in the culture degree of S. aureus from the lesion, non-lesion, and control group of child and adult AD patients, to research the correlation between the exotoxin production, total IgE, anti-SEA IgE and the disease severity by SCORAD index, to ascertain the differences between child and adult AD patients.

Methods: The clinical severity of 30 child (2 to 15 years of age) and 30 adult patients (16 to 40 years of age) with AD was evaluated by using SCORAD index. S. aureus was isolated from lesional and non-lesional skin of AD patients, and from healthy controls. Staphylococcal exotoxins were detected by using reversed passive latex agglutination toxin detection kits. Anti-SEA IgE antibody was determined by using AlaSTAT* assay.

Results: S. aureus colonizations were found in 11 (36.7%) of the lesional skin, in 5 (16.7%) of the non-lesional skin of 30 child AD patients, and in 26 (86.7%), in 20 (66.7%) of 30 adult AD patients, respectively. The colonization rates of S. aureus in child patients were much lower than those in adult patients, both form lesional skin and non-lesional skin. Staphylococcal exotoxins were detected in 5 (45.5%) of the 11 colonizations from lesional skin, in 2 (40%) of the 5 colonizations from non-lesional skin of children, and in 10 (38.5%) of the 26 colonizations, in 9 (45%) of the 20 colonizations of adults, respectively. Staphylococcal enterotoxin A (SEA) was most frequently detected in both groups. S. aureus colonization was correlated with the severity of AD in childhood, but not in adulthood. However, there were no statistical significances between severity of AD and others such as exotoxin production, and the level of total IgE and anti-SEA IgE in both groups.

Conclusion: The colonization of S. aureus was more common in adult AD patients than child AD patients. Anti-SEA IgE level was much higher in adult AD patients than in child AD patients. It is tempting to speculate that the colonization of S. aureus and exotoxin production

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Staphylococcus aureus (S. aureus) is thought to be an important precipitating factor of atopic dermatitis (AD). S. aureus colonizes eczematous lesions in 78-100% of children and adults with AD. The bacterium is isolated from unaffected skin of these subjects in 51-100% of cases, but is found only in 2-25% of cases on the skin of healthy subjects. Several studies have been reported concerning

Key Words: Atopic dermatitis, Staphylococcus aureus, Staphylococcal exotoxin

the action of exotoxins as superantigens in AD. Superantigenic exotoxins activate T cells and stimulate production of IL-1 and TNF-α. Specific IgE antibodies against staphylococcal exotoxins are also thought to precipitate AD by mediating type I allergy. Some authors have recently reported that the severity of AD correlates with presence of specific IgE antibodies. It is speculated that staphylococcal exotoxins worsen AD and activate T cells by acting as superantigens or allergens.

Until recently, studies of staphylococcal toxin in AD has mainly been performed in children with AD, but studies in adults with AD have rarely been reported. Therefore, we performed this study to investigate the differences in the culture degree of S. aureus from the lesion, non-lesion, and control group of child and adult AD patients, to research the correlation between the exotoxin production, total IgE, anti-SEA IgE and the disease severity by SCORAD index, and also to ascertain the differences between child AD patients and adult AD patients.

MATERIAL AND METHODS

Subjects
30 child AD patients (16 males and 14 females; mean 6.8 years; range 2-15 years), 30 adult AD patients (15 males and 15 females; mean 22.8 years; range 16-40 years), 15 children in the control group (7 males and 8 females; mean 5.6 years; range 2-14 years), and 15 adults in the control group (7 males and 8 females; mean 27.9 years; range 23-52 years) were enrolled into our study. They were suitable for the diagnosis criteria of Hanifin and Rajka. All the patients had not taken the systemic antihistamine nor steroid as well as application of the topical steroid for more than a week before the examination. They also had not taken any medications for treating other diseases, and had not had a history of the skin infections. Among each 30 child AD patients and adult AD patients, the past history of the allergic rhinitis appeared in 5 children (16.7%) and 7 adults (23.2%), bronchial asthma in 3 children (10%) and 2 adults (6.7%), and family history of more than one above three atopic diseases in 6 children (20%) and 9 adults (30%).

The disease severity of AD was measured in three items such as the extent of skin invasion, the intensity of disease, and the degree of itching through the history taking and inspection of the same dermatologist using the SCORAD index, and divided into a higher group and a lower group on the basis of SCORAD index 50 scores.

Culture and isolation of S. aureus
We swabbed the 2 × 2 cm sized skin each in the lesion and non-lesion of child and adult AD patients and inoculated them on the blood agar culture medium. We cultivated them at 35℃ for a night and isolated the S. aureus using the coagulase and mannitol test. The culture degree, when observed by the naked eyes, was written as many (+++, more than three sections on the basis of the medium), moderate (+++, more than two sections but less than three sections), few (+, more than one section but less than two sections), and no growth (growth was not found in any sections).

Measurement of S. aureus exotoxin
With the subculture of a colony of S. aureus, we examined the type of the enterotoxin (SEA, SEB, SEC, SED) and exfoliative toxin (ETA, ETB) and the formation of TSST-1 according to the manual using the reversed passive latex agglutination toxin detection kit (SET-RPLA, EXT-RPLA, TST-RPLA; Denka Seiken, Tokyo, Japan).

Measurement of Total IgE and anti-SEA IgE
With the sera of each 30 child and adult AD patients and each 15 child and adult control groups, we measured the total IgE using the PRIST kit of Behring (Marburg, Germany), and examined the anti-SEA IgE using the liquid-phase enzyme immunoassay (the AlaSTAT® assay, DPC Los An-
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Table 1. The frequency of staphylococcal colonization on the lesion and non-lesion in childhood and adulthood patients with atopic dermatitis and controls

<table>
<thead>
<tr>
<th></th>
<th>Childhood</th>
<th></th>
<th>Adulthood</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion (%)</td>
<td>Non-lesion (%)</td>
<td>Control (%)</td>
<td>Lesion (%)</td>
</tr>
<tr>
<td>No growth</td>
<td>19(63.3%)</td>
<td>25(83.3%)</td>
<td>14(93.3%)</td>
<td>4(13.3%)</td>
</tr>
<tr>
<td>Few</td>
<td>2(6.7%)</td>
<td>4(13.3%)</td>
<td>1(6.7%)</td>
<td>7(23.3%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>3(10.0%)</td>
<td>1(3.3%)</td>
<td>0(0.0%)</td>
<td>10(33.3%)</td>
</tr>
<tr>
<td>Many</td>
<td>6(20.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>9(30.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>30(100%)</td>
<td>30(100%)</td>
<td>15(100%)</td>
<td>30(100%)</td>
</tr>
</tbody>
</table>

No growth: growth can not be found in any sections on the basis of the medium
Few: more than one section but less than two sections
Moderate: more than two sections but less than three sections
Many: more than three sections

Table 2. Comparison of SCORAD index with staphylococcal colonization, exotoxin production, and anti-SEA IgE between childhood and adulthood AD patients

<table>
<thead>
<tr>
<th></th>
<th>Childhood AD patient+</th>
<th>Adulthood AD patient+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive culture group</td>
<td>66.0 ± 12.7*</td>
<td>52.3 ± 16.4</td>
</tr>
<tr>
<td>Negative culture group</td>
<td>53.5 ± 13.3</td>
<td>50.1 ± 22.8</td>
</tr>
<tr>
<td>Non-lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive culture group</td>
<td>68.0 ± 11.5*</td>
<td>52.3 ± 17.9</td>
</tr>
<tr>
<td>Negative culture group</td>
<td>56.8 ± 12.7</td>
<td>51.5 ± 15.8</td>
</tr>
<tr>
<td>Staphylococcal exotoxins producing group</td>
<td>59.7 ± 9.8</td>
<td>53.3 ± 21.2</td>
</tr>
<tr>
<td>Staphylococcal exotoxins non-producing group</td>
<td>58.4 ± 14.9</td>
<td>51.0 ± 13.3</td>
</tr>
<tr>
<td>Pairing positive group</td>
<td>62.6 ± 11.2</td>
<td>63.4 ± 23.8</td>
</tr>
<tr>
<td>Pairing negative group</td>
<td>58.2 ± 14.4</td>
<td>50.3 ± 15.6</td>
</tr>
</tbody>
</table>

+ Mean SCORAD±SD

* The SCORAD index between the positive culture group and negative culture group in childhood shows statistically significant difference (p < 0.05).

geles, CA, USA). With the anti-SEA IgE was more than 0.35kU/L, we judged it as positive.

Statistical analysis
We inspected our data using SPSS 10.0 for Windows program, the relations between the disease severity (SCORAD index) and the culture degree of S. aureus, the exotoxin production, the total IgE and anti-SEA IgE through the Independent-Sample's t-test, and the differences of the SCORAD index according to the culture degree through one-way ANOVA. The significant level of all the statistical difference was defined when the p-value was less than 0.05.

RESULTS

1. Culture of S. aureus

S. aureus was isolated from 11 swabs (36.7%; few 2, moderate 3, many 6) of lesion and from 5 swabs (16.7%; few 4, moderate 1) of non-lesion of 30 child AD patients. S. aureus was isolated from 26 swabs (86.7%; few 7, moderate 10, many 9) of lesion and from 20 swabs (66.7%; few 15, moderate 5) of non-lesion of 30 adult AD patients. In one case (6.7%) of each child control group and adult control Group, S. aureus was cultivated (Table 1). The positive culture rate of the lesion was higher than that of the non-lesion in both child and adult AD patient groups (p < 0.05). Also, the positive culture rates of the lesion and non-lesion were all higher than that of the control in both child and adult AD patient groups (p < 0.05).

The score of SCORAD index was 66.0 ± 12.7 in child AD patients showing positive culture in the lesion and 53.5 ± 13.3 in patients showing negative
Table 3. The detection rate of staphylococcal exotoxins between lesion and non-lesion in staphylococcal colonizing childhood and adulthood patients with atopic dermatitis

| Character Exotoxins | Childhood AD patients | |          | Adulthood AD patients | |          |
|---------------------|-----------------------|----------------|-----------------------|----------------|----------------|
|                     | Lesion (n=11) Number (%) | Non-lesion (n=5) Number (%) | Lesion (n=26) Number (%) | Non-lesion (n=20) Number (%) |
| SEA                 | 3 (27.3)               | 1 (20.0)       | 4 (15.4)               | 4 (20.0)       |
| SEB                 | 0 (0.0)                | 0 (0.0)        | 3 (11.5)               | 2 (10.0)       |
| SED                 | 0 (0.0)                | 0 (0.0)        | 0 (0.0)                | 1 (5.0)        |
| TSST-1              | 1 (9.1)                | 0 (0.0)        | 2 (7.7)                | 1 (5.0)        |
| SEA + TSST-1        | 1 (9.1)                | 1 (20.0)       | 0 (0.0)                | 0 (0.0)        |
| SED + TSST-1        | 0 (0.0)                | 0 (0.0)        | 1 (3.8)                | 1 (5.0)        |

SEA: staphylococcal enterotoxin A, SEB: staphylococcal enterotoxin B
SED: staphylococcal enterotoxin D, TSST-1: toxic shock syndrome toxin-1
SEC, ETA, and ETB exotoxins were not detected in the culture of lesion and non-lesion.

culture (p < 0.05). The score of SCORAD index was 68.0 ± 11.5 in child AD patients showing positive culture in the non-lesion and 56.8 ± 12.7 in patients showing negative culture (p < 0.05). On the other hand, the score of SCORAD index was 52.3 ± 16.4 in adult AD patients showing positive culture in the lesion and 50.1 ± 22.8 in patients showing negative culture (p > 0.05). The score of SCORAD index was 52.3 ± 17.9 in adult AD patients showing positive culture in the non-lesion and 51.5 ± 15.8 in patients showing negative culture (p > 0.05) (Table 2).

In terms of culture degree in child AD patients, the score of SCORAD index was 70.2 ± 14.2 in patients showing many, 62.7 ± 11.7 in moderate, and 60.8 ± 9.6 in few, but there were no significant differences between them (p > 0.05). In case of adult AD patients, the score of SCORAD index was 60.0 ± 19.3 in patients showing many, 49.8 ± 14.6 in moderate, and 47.8 ± 14.5 in few, but there were also no significant differences between them (p > 0.05).

2. Staphylococcal exotoxin

Staphylococcal exotoxins were detected in 5 cases (45.5%) among 11 cultures of S. aureus in the lesion of child AD patients and 2 cases (40%) of 5 cultures in the non-lesion. Among the various staphylococcal exotoxins, SEA was detected in 6 cases (4 cases in lesion, 2 cases in non-lesion), and TSST-1 could be detected in 3 cases (2 cases in lesion, 1 case in non-lesion) in child AD patients. Whereas staphylococcal exotoxins were detected in 10 cases (38.5%) among 26 cultures of S. aureus in the lesion of adult AD patients and 9 cases (45%) of 20 cultures in the non-lesion. Among them, SEA was detected in 8 cases (4 cases in lesion, 4 cases in non-lesion), SEB in 7 cases (4 cases in lesion, 3 cases in non-lesion), SED in 1 case (non-lesion), TSST-1 in 5 cases (3 cases in lesion, 2 cases in non-lesion) (Table 3).

The score of the SCORAD index in staphylococcal exotoxins producing child AD patients was 59.7 ± 9.8, while 58.4 ± 14.9 in exotoxin non-producing patients (p < 0.05), and 53.3 ± 21.2 in producing group and 51.0 ± 13.3 in non-producing group of adult AD patients (p < 0.05) (Table 2).

3. Total IgE

The average total IgE of the child patient group was 297.23 ± 420.24 IU/ml, and that of the adult patient group was 1098.9 ± 1077.9 IU/ml. The average total IgE value in the lower SCORAD index group of children was 476.45 ± 453.96 IU/ml, which was higher than that of the higher SCORAD index group 220.42 ± 209.80 IU/ml, but there were not any statistically significant differences (p > 0.05). In case of adult patients, 1335.0 ± 1021.4 IU/ml of the higher SCORAD index group was higher than 941.5 ± 1120.1 IU/ml of the lower SCORAD index group, but there was also not any statistically significant difference (p > 0.05). In the comparison of the positive and negative group of the exotoxin production, the positive group of children showed 700.92 ± 684.04 IU/ml, which was higher than 216.49 ± 205.95 IU/ml of the negative group (p < 0.05), and the positive group of adults showed 1674.7 ± 1151.5 IU/ml, which was
also higher than $646.5 \pm 789.9$ IU/ml of the negative group ($p < 0.05$).

4. Anti-SEA IgE
The child patient group showed $0.334 \pm 0.272$ kU/L and the child control group $0.401 \pm 0.156$ kU/L ($p > 0.05$). The adult patient group showed $5.54 \pm 8.36$ kU/L and the adult control group $0.46 \pm 0.16$ kU/L. The figure of the adult patient group was higher than that of the adult control group ($p < 0.05$). The anti-SEA IgE level higher than cut-off value of 0.35 kU/L was observed in 9 children (30%) in the patient group, 6 children (40%) in the control group, 30 adults (100%) in the patient group, and 10 adults (66.7%) in the control group. In the SCORAD index of the anti-SEA IgE positive group and negative group of child AD patients, the positive group showed $54.1 \pm 15.6$ and the negative group showed $60.6 \pm 13.2$, but there was not significant difference ($p > 0.05$). And in the case of that of adult AD patients, all the patients belong to the positive anti-SEA IgE group. In the patient group, the pairing positive group showing the SEA production and positive anti-SEA IgE simultaneously, contained 3 children (10%) and 4 adults (13.3%), and the SCORAD index of the child pairing positive group and negative group were $62.6 \pm 11.2$ and $58.2 \pm 14.4$ ($p > 0.05$), and those of the adult positive and negative group were $63.4 \pm 23.8$ and $50.3 \pm 15.6$ ($p > 0.05$) (Table 2). In the comparison of the positive group and negative group of the anti-SEA IgE, the average total IgE of the positive group of children showed $643.40 \pm 536.73$ IU/ml, which was higher than $148.87 \pm 157.59$ IU/ml of the negative group ($p < 0.05$), and all the adult patients belonged to the positive group.

DISCUSSION
There is various evidence that S. aureus contributes to the pathogenesis of AD. The skin of many 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. But Seymour et al suggested that S. aureus was not the dominant organism found on the skin of patients with atopic dermatitis.

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. Since the degree of adherence is related to keratinization, being maximal for fully keratinized cells, it was hypothesized that corneocytes of the adults and children with AD have an increased number of receptors for S. aureus. The mechanism of worsening AD by S. aureus are thought to be that (1) α-toxin and other cytolytic toxins directly destroy the skin tissue, (2) cellular proteins of S. aureus act as allergens, and (3) superantigenic exotoxins act as superantigen and allergen. Many strains of S. aureus isolated from AD often produce various exotoxins, which can act as superantigens. It might stimulate Langerhans cells or macrophages to produce proinflammatory cytokines such as IL-1 and TNF-α. IL-1 and TNF-α then may contribute to skin inflammation by induction of vascular endothelial leucocyte adhesion molecules such as ICAM-1, ELAM-1, ICAM-2, ELAM-2. Besides their action as superantigens, staphylococcal exotoxin can act as allergens to stimulate production of specific IgE in AD patients. Further studies revealed that 70-80% of all the patients tested had a significantly higher incidence of circulating IgE antibodies specific for SEA and/or SEB. Because the staphylococcal enterotoxins seem to penetrate more easily the injured skin barrier in patients with AD than 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.

Our study was performed to compare the differences of the prevalence of S. aureus between child and adult AD patients, and to investigate the correlation between the exotoxin of S. aureus, specific IgE antibody and the disease severity.

The positive culture rate of S. aureus was 36.7% from lesion and 16.7% from non-lesion in the child patient group. The positive culture rate was 86.7% from lesion and 66.7% from non-lesion in the adult patient group. Consequently the culture rate of the lesion showed a higher rate than that of the non-lesion. And the culture rates of the lesion and non-lesion showed higher rates than that of control in both children and adults. In particular, the adult patient group showed much higher positive rate.
than the child group. Monti et al. suggested that corneocytes of children with AD have fewer receptors for *S. aureus* than corneocytes of subjects with AD in older age, thus explaining lower *S. aureus* skin colonization on skin of our children with AD with respect to adult patients.

Comparing the SCORAD indices between the culture positive and negative group in the child patients, the culture positive group showed higher SCORAD index than the negative group in both lesion and non-lesion. But there were not any statistically significant differences in SCORAD indices between positive culture group and negative group in both lesion and non-lesion of adult AD patients. In addition, there was no significant correlation between the culture degree and the SCORAD index in both child and adult patient groups. These results suggested that the colonization of *S. aureus* might be related to the chronicity rather than the clinical severity of AD. Our result was not consistent with the results of Higaki et al. that the number of *S. aureus* isolated increased in correlation to the increase in severity of the AD lesion.

*S. aureus* isolated from the patients with AD can produce superantigenic exotoxins, such as SEA, SEB, SEC, SED, SEE, TSST-1, ET-A, and ET-B. Several studies have reported that the existence of the staphylococcal exotoxins is correlated with AD severity, but Akiyama et al. have reported that the existence of the exotoxins is not reflective of AD severity. In our study, there was no significant difference in the exotoxin production rate of *S. aureus* between the lesional and non-lesional skin of AD patients. Matsue et al. assessed that the number of *S. aureus* present is more important in the formation of eczematous lesion of AD patients than the presence of superantigenic exotoxin-producing *S. aureus* strains. But, in our data, there were no significant differences between groups showing many, moderate, few culture.

Among the staphylococcal exotoxins produced in child AD patients, SEA showed the highest production rate, but among the staphylococcal exotoxins produced in adult AD patients, SEA and SEB showed similar production rate with the highest rate. Interestingly, the SEB production rate in the adult group was higher than that of the child group. The SCORAD index of the exotoxin production group had few differences from that of the non-production group in both the child and adult patient groups in our study, Nomura et al. have also mentioned that there was no statistically significant difference in disease activity scores of AD patients with or without exotoxin-producing *S. aureus*. The total IgE of the exotoxin production group was higher than that of the non-production group in both the child and adult patient groups in our study. It is why IgE production can be enhanced in response to various exotoxins in exotoxin production group of AD patients.

Besides their action as superantigens, staphylococcal exotoxins can act as allergens. AD patients have IgE antibodies against SEA, SEB and/or TSST-1 in 34 to 96 % of sera whereas relevant of those antibodies are extremely rare in healthy controls or psoriasis patients although the skin of these individuals is also colonized with *S. aureus* capable of exotoxins production. In our study, 30% of the child AD patients and 100% of the adult AD patients showed positive anti-SEA IgE (>0.35 kU/L). Anti-SEA IgE of the children was 0.334 ± 0.272 kU/L, and that of the adults was 5.54 ± 8.36 kU/L, thus anti-SEA IgE of adults was much higher than that of the children. "paring(+)" means that a patient has both exotoxin-producing *S. aureus* on the skin and specific serum IgE to the same exotoxin at the same time, and "paring(+)" is suggested that exotoxins may worsen AD through type I allergy. In our study, 10% of the child group and 13.3% of the adult group showed the pairing positive about SEA, and the positive group showed a little higher SCORAD index than in the negative group, but there were not any statistically significant differences between them, and the child and adult patient groups showed similar figures each other. Our data suggested that anti-SEA IgE was related to the chronicity rather than clinical severity of AD. In addition, anti-SEB IgE levels correlate well with the severity scores of patients with AD and are thought to be as important as house dust mite-specific IgE or total IgE in the evaluation of AD severity, but we did not measure anti-SEB IgE because of unavailability in our country.

In conclusion, our data showed that the colonization of *S. aureus* was more common in adult AD patients than child AD patients, and was not related to the clinical severity. Anti-SEA IgE level was much higher in adult AD patients than in child AD patients, and was not related to the clinical
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severity of AD. It is tempting to speculate that the colonization of S. aureus and exotoxin production might be related to the disease duration rather than clinical severity of AD.

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