

SHORT COMMUNICATION

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Prevalence of producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strains isolated from atopic dermatitis lesions

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Eczematous skin lesions of atopic dermatitis (AD) are usually colonized densely with *Staphylococcus aureus* [1, 2]. A correlation between the severity of eczematous lesions and colonization with *S. aureus* has been demonstrated [2]. *S. aureus* can produce superantigenic exotoxins which may be involved in the exacerbation of AD. Exotoxins with superantigenic properties have been shown to be produced in *S. aureus* isolates from 24 of 42 AD patients (57.1%) in one study [3] and 24 of 37 AD patients (64.9%) in another study [4]. The strains isolated from severe AD have been shown to produce α -toxin [5] in 20 of 20 patients (100%), β -toxin in 12 of 20 patients (65%), and δ -toxin in 17 of 20 patients (85%) [6]. We examined the prevalence of producers of enterotoxins (SE) and toxic shock syndrome toxin-1 (TSST-1) production among strains of *S. aureus* isolated from AD patients and compared this with the clinical symptoms exudation, swelling and erythema.

Patients who fulfilled the criteria of Hanifin and Rajka for AD [7] were examined for bacteria, and only patients with positive *S. aureus* culture were enrolled. A group of 76 patients with AD (34 males and 42 females; mean age ($\pm$ SD) 21.9 $\pm$ 9.6 years, range 2–48 years) were studied, of whom 61 were available for culture of lesions of the face only and 15 were available for culture of lesions of the neck only. The lesions were classified as with or without exudation, with or without swelling, and red or light/dark-red erythema.

We scrubbed a 3-cm diameter circular area of the AD lesions five times with sterilized swabs (Nissui, Tokyo,

Japan) soaked in sterilized saline solution and smeared the swabs on culture dishes (15 $\times$ 60 mm; Sumitomo Bakelite, Tokyo, Japan) containing 10 ml *Staphylococcus* Medium No-110 (Nissui, Tokyo, Japan). The colonies were mature after 48 h incubation at 37°C, and the grown colonies were evaluated. Four colonies were randomly selected from each culture and inoculated onto Mueller Hinton Medium (Difco, Detroit, Mich.). After an additional 24 h at 37°C, an aliquot of each colony was tested by Staphaurex (rapid latex test kit for the identification of *S. aureus*; Murex Diagnostics, Dartford, UK) and the positive strains were regarded as *S. aureus*. The total number of identified *S. aureus* strains was 286, and 18 strains were excluded because the Staphaurex test was negative. After centrifugation of each culture at 500 *g* for 20 min, 25 μ l of the supernatant and 25 μ l of the tenfold diluted supernatant solutions were placed in 96-well tissue culture dishes (Becton Dickinson, NJ.). Standard exotoxins and control latex sensitized by normal rabbit immunoglobulin G (IgG) (Denka Seiken, Tokyo, Japan) were placed in separate wells as positive and negative controls. Latex particles (25 μ l) coated with specific rabbit IgG against staphylococcal enterotoxins (SEA, SEB, SEC, SED) and TSST-1 (Denka Seiken, Tokyo, Japan) were added to the culture wells. After 20 h incubation at room temperature, the wells were checked for agglutination.

Comparison between the production of exotoxins and the clinical symptoms of the AD lesions were analysed using the χ^2 -test with Yates' correction.

The incidence of producers of identifiable exotoxins is shown in Table 1. Of 76 cultures of *S. aureus*, 47 (62%), each from a different skin lesion, produced one or more identifiable exotoxin(s). Examination of four colonies from each of the 76 cultures revealed that 23 of the cultures (30%) exhibited at least two different patterns of exotoxin production. Table 2 shows the total number of positive cultures of *S. aureus*, the number of cultures producing one or more exotoxin(s), and the number of cultures producing SEA and/or SEB at a titre of 1:10. Table 3 shows the total number of positive cultures of *S. aureus* growing more than 101 colony-forming units per lesion, the number

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of cultures producing one or more exotoxin(s), and the number of cultures producing SEA and/or SEB at a titer of 1:10. The correlation between the production of one or

more exotoxin(s) and the presence or absence of swelling was significant ($0.01 < P < 0.05$; Tables 2, 3), but the correlations between the production of exotoxin(s) and the other clinical symptoms were not significant.

It is well known that dense colonization with *S. aureus* can be an important trigger for the exacerbation of AD [1, 2]. *S. aureus* is present at higher density in exudative lesions than in chronic lichenoid plaques of AD [1] and the bacteriological environment in AD shows increasing numbers of *S. aureus* with increasing severity of the dermatitis [2]. These results suggest that the size of the population of *S. aureus* in the skin lesions of AD is an important factor in its aggravation.

In the present study more than half the *S. aureus* isolates from AD produced one or more superantigenic exotoxin(s) [3, 4]. Superantigens [8–10] predominantly acti-

Table 1 The number of positive cultures of *S. aureus* producing superantigenic exotoxin(s) from atopic dermatitis patients. The total number of cultures is 76. Forty-seven out of 76 cultures (62%) of *S. aureus*, each from one skin lesion, produced one or more identifiable exotoxin(s)

Toxin	1 ≤ titer < 10	Titer ≥ 10
SEA	3 (4%)	19 (25%)
SEB	11 (14%)	27 (36%)
SEC	0	1 (1%)
SED	5 (7%)	0
TSST-1	11 (14%)	0

Table 2 The number of cultures of *S. aureus* producing superantigenic exotoxin(s) in relation to clinical symptoms. The total number of cultures is 76

Prevalence of <i>S. aureus</i> producing exotoxins	Clinical symptoms					
	Erythema		Swelling		Exudation	
	Red	Light/dark red	+	–	+	–
No exotoxins	15	14	0	29	8	21
More than one exotoxin	33	14	11	36	18	29

*: $0.01 < P < 0.05$

Prevalence of <i>S. aureus</i> producing exotoxins	Clinical symptoms					
	Erythema		Swelling		Exudation	
	Red	Light/dark red	+	–	+	–
No exotoxins or other exotoxins except SEA and/or SEB with titer ≥ 10	22	14	3	33	13	23
SEA and/or SEB with titer ≥ 10	26	14	8	32	13	27

Table 3 The number of cultures of *S. aureus* producing superantigenic exotoxin(s) in relation to clinical symptoms. The total number of cultures with more than 101 cfu/one lesion is 63

Prevalence of <i>S. aureus</i> producing exotoxins	Clinical symptoms					
	Erythema		Swelling		Exudation	
	Red	Light/dark red	+	–	+	–
No exotoxins	14	8	0	22	8	14
More than one exotoxin	30	11	10	31	17	24

*: $0.01 < P < 0.05$

Prevalence of <i>S. aureus</i> producing exotoxins	Clinical symptoms					
	Erythema		Swelling		Exudation	
	Red	Light/dark red	+	–	+	–
No exotoxins or other exotoxins except SEA and/or SEB with titer ≥ 10	20	8	2	26	12	16
SEA and/or SEB with titer ≥ 10	24	11	8	27	13	22

vate T-cells, and the mechanism of T-cell activation involves superantigen-mediated complexes of accessory cells presenting human leucocyte antigen class II and defined V region of the T-cell receptor beta-chain [8]. Cooper suggested that staphylococcal superantigens released into the epidermis bind to class II major histocompatibility complex-positive Langerhans cells or dermal dendritic cells, and could thereby cause a marked stimulation of several classes of T cells that have been nonspecifically recruited into the lesion [11].

Because we considered the examination of only one colony from each lesion insufficient, we examined four randomly selected colonies from each culture. The finding that 30% of *S. aureus* isolated showed different patterns of exotoxin production among four strains from one skin lesion suggest that the population of *S. aureus* in AD is composed of a heterogeneous group of *S. aureus*.

Røder et al. investigated the production of SEA, SEB, SEC and SED by 196 strains of *S. aureus* isolated from blood cultures and 95 strains from a nasal carrier. Overall, 30.6% of the bacteraemia strains and 40.0% of the nasal strains produced SE, and SEB and SEC were the toxins produced most frequently in both groups [12]. A similar incidence and pattern of SE production has been found among *S. aureus* strains in the presence and absence of endocarditis, and even among strains isolated from nares of healthy people [12]. The amount of exotoxins is considered to increase with an increase in the colony density and the production of exotoxins. Therefore we examined the colony density of each skin lesion and the titre of exotoxins. The incidence of SE and TSST-1 production was similar in every clinical symptom examined except in two cases, regardless of the colony density or the titre of exotoxins. Nevertheless, the possibility that the specific population of *S. aureus* that produces the exotoxins is important in the modification of cutaneous manifestations still remains.

Staphylococcal diseases are usually due to the simultaneous production of several pathogenic factors [5]. Staphylococcal factors, such as α -toxin [5, 6], β -toxin, δ -toxin, various enzymes [6], coagulase [13], exfoliative toxin [14], and superantigenic exotoxins, have previously been implicated in AD. The results of the present study suggest that the size of the specific population of *S. aureus* that pro-

duces the exotoxins is important in the modification of the cutaneous manifestations of AD. Further study is necessary to determine the specific mechanism of this involvement.

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