
Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects

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Background: The aggravating role of *Staphylococcus aureus* superantigens is well known in atopic dermatitis (AD) but has not yet been proven in psoriasis (PS).

Objective: We investigated the distribution of *S aureus* in the skin and nares of patients with AD, PS vulgaris, erythroderma, skin infections, and sepsis, and in healthy control subjects. A Staphylococcal enterotoxin test—reversed passive latex agglutination (SET-RPLAR) test was performed to determine Staphylococcal enterotoxins A, B, C, and D.

Results: *S aureus* was cultivated from lesional skin of 22 of 25 patients with AD and 15 of 25 patients with PS. Isolated strains were toxigenic in 44% for patients with AD and in 36% for patients with PS. The activity of disease in AD and PS according to the Severity Scoring of Atopic Dermatitis (SCORAD) or Psoriasis Area and Severity Index score, respectively, correlated significantly ($P = .001$) with an isolated toxigenic strain in both diseases. *S aureus* from skin infections was toxigenic in half of the patients. All patients with erythroderma harbored *S aureus*, mostly on their skin. In AD, sepsis and skin infections, toxin C and in PS toxin B was most often detected. *S aureus* was cultured in 12% of healthy persons. These strains were toxin negative. The limitations of these investigations are that other potentially acting enterotoxins, such as toxic shock syndrome toxin-1, which may play a role in aggravating disease, were not investigated with our latex agglutination test.

Conclusion: In this study, *S aureus* was present in more than 50% of patients with AD and PS. We found that the severity of AD and PS significantly correlated to enterotoxin production of the isolated *S aureus* strains. (J Am Acad Dermatol 2005;53:67-72.)

The skin is colonized by various micro-organisms and this microbial environment is necessary for skin homeostasis. *Staphylococcus aureus* is one of the saprophytic bacteria that can be detected in 5% to 30% of healthy persons' skin and in 20% of their nares.¹ Colonization with *S aureus*, however, can cause skin infections such as pyoderma, erysipelas, and folliculitis. Further, colonization with *S aureus* has also been detected in a higher percentage of chronic skin diseases. The carriage rate of *S aureus* in patients with atopic dermatitis (AD)

is 76% for the uninvolved skin, 93% for lesions, and 79% for the anterior nares.^{2,3} In psoriasis (PS), *S aureus* was detected in nonlesional skin of 27% of patients and in 46% of lesions.⁴ *S aureus* has also been isolated from skin of patients with erythroderma.⁵ In a study of 42 patients, 76% were positive for *S aureus* in culture from blood or skin.⁶

An important role of *S aureus* colonization of the skin is its potential to modify the course of dermatologic diseases. In particular, *S aureus* enterotoxins A through E and the toxic shock syndrome toxin (TSST)-1 have been shown to trigger exacerbation of AD, PS, and cutaneous T-cell lymphoma.⁶⁻⁹ T-lymphocyte dysfunction is common in all these diseases. Enterotoxins A through E and TSST-1 act as superantigens by binding and activating certain V-chains of the T-cell receptor.¹⁰ In contrast, conventional peptide antigens require interaction with all 5 (ie, V- β , D- β , J- β , V- α , and J- α) variable chains of the T-cell receptor.

Superantigens belong to a group of microbial antigens that differ from conventional peptide antigens in several respects. Unlike peptide antigens,

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Table I. Patient demographics

	No. of patients	Male	Female	Median age, y (range)	SA negative no./ (%)	SA positive no./ (%)	Toxin negative	Toxin positive
Atopic dermatitis	25	16	9	24 (3 mo-60)	3/(12)	22/(88)	11	11
Psoriasis	25	18	7	54 (21-80)	10/(40)	15/(60)	6	9
Erythroderma	6	5	1	65 (47-79)	0	6/(100)	5	1
Sepsis	9	5	4	59 (37-80)	0	9/(100)	5	4
Skin infections	25	10	15	55 (11-93)	0	25/(100)	12	13
Healthy control subjects	25	12	13	31 (20-58)	22	3/(12)	3	0
Total	115	66	49		35	80	42	38

SA, *Staphylococcus aureus*.

superantigens bind as intact proteins to T-cell receptor and major histocompatibility complex class II molecules outside their binding site.¹¹ Superantigen induced T-cell activation leads to the production of elevated amounts of inflammatory cytokines. Superantigens have a much higher excitatory potential than conventional peptide antigens. Superantigens also stimulate keratinocytes to produce cytokines for T-cell-activation.^{12,13}

The purpose of our study was to investigate the role of *S aureus* enterotoxins A through D in correlation to the severity of disease in PS in comparison with their well-known influence in AD and for patients with skin infections, erythroderma, and healthy skin.

METHODS

Patients

To investigate skin colonization with *S aureus* and its enterotoxin profile, we performed a prospective study in 25 patients with AD, 25 with PS, 6 with erythroderma, and in 25 healthy control subjects between January 2001 and March 2002 (Table I). For control purposes, *S aureus* strains isolated from 25 patients with skin infections and 9 patients with staphylococcal sepsis were investigated. Patients with *S aureus* skin infection had impetigo (n = 5), leg ulcer (n = 4), basal cell carcinoma (n = 3), erysipelas (n = 3), furuncle (n = 4), folliculitis (n = 2), prurigo simplex (n = 2), morphea (n = 1), or pyoderma (n = 1). The 6 patients with erythroderma had AD (n = 2), PS (n = 2), Norwegian scabies (n = 1), or mycosis fungoides (n = 1). Patients with sepsis were from the intensive care department. Healthy control subjects had no clinical signs of infection or dermatologic disease. This study was accepted by the local ethics committee. Patients and control subjects gave informed consent before enrollment in the study.

Diagnosis of AD was made according to the criteria of Hanifin and Rajka¹⁴ as modified by Sampson,¹⁵ and for PS diagnosis was made clinically

and confirmed by histology. Disease severity in AD was measured according to the Severity Scoring of Atopic Dermatitis (SCORAD) and in PS according to Psoriasis Area and Severity Index score.¹⁶ All topical and oral medications were registered and a data entry form was used for each patient. Topical medication for patients with AD included corticosteroids, emollients, skin disinfectants, and antibiotic ointments. Systemically, oral antihistamines were administered and two patients with AD were taking systemic antibiotics. Patients with PS used topical corticosteroids, the vitamin D analogue calcipotriene, and ointments containing salicylic acid, or received psoralen-UVA therapy with local emollients or a combination of treatments. None of the patients in any group were taking systemic immunosuppressive drugs.

Materials and methods

Isolation of *S aureus*. For the isolation of *S aureus*, skin swabs were taken from lesional skin, in healthy control subjects from the volar site of the elbow, and in addition from both nares in patients and control subjects. For this procedure, the skin area was briefly wiped with disinfectant to remove contaminating bacteria and residues of ointments (Dodesept-N-farblos, Merck, Vienna, Austria). Then a sterile cotton swab (Copan Innovation, Brescia, Italy) dipped in sterile distilled water was passed repeatedly over the affected skin area. Smear material was applied on blood agar plates (Heipha Diagnostics GmbH, Heidelberg, Germany) and incubated at 37°C for 24 hours. *S aureus* was identified by testing colonies for their coagulase activity (Staphylase, Oxoid Diagnostics, Basingstoke, United Kingdom). For patients with sepsis, blood was cultured for *S aureus*.

Culture samples were frozen in microbank storage boxes according to the manufacturer's instructions (Pro-Lab Diagnostics, Westwood Court, United Kingdom). An antibiogram was used for phenotyping (Sensi Disc, Becton Dickinson, Meylan Cedex, France; Mueller-Hinton-Agar, Bio Mérieux, Marcy l'Etoile, France).

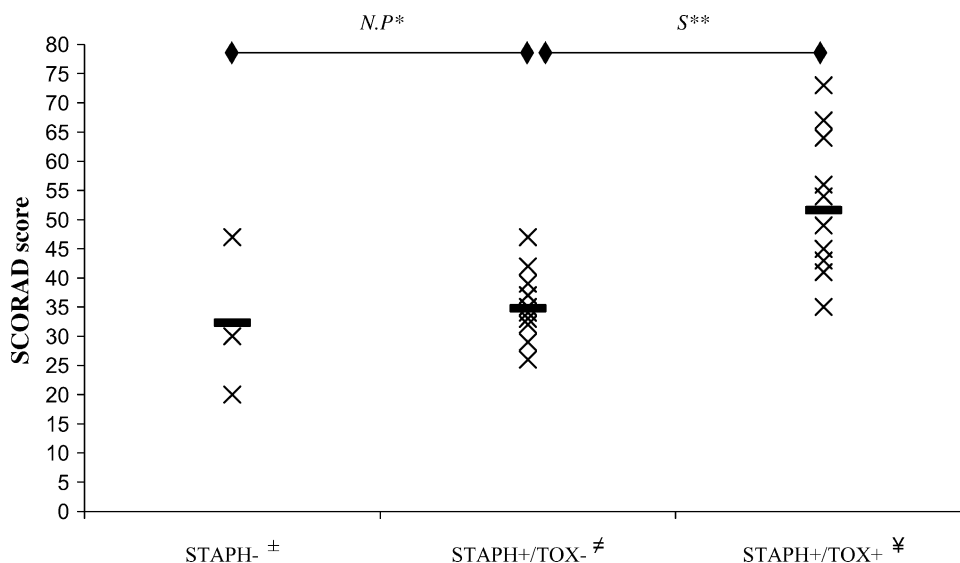


Fig 1. Severity Scoring of Atopic Dermatitis (SCORAD) in patients with atopic dermatitis in relation to *Staphylococcus aureus* enterotoxin-producing strains. *Significance test not possible to less subjects; **significance $P = .001$; $\pm$ *S aureus* negative; $\neq$ *S aureus* positive and no enterotoxin production; $\forall$ *S aureus* positive and enterotoxin production.

Detection of staphylococcal enterotoxins.

Samples of frozen *Staphylococcus* were thawed, inoculated into sterile Tryptone Soya broth (Oxoid Diagnostics, Basingstoke, United Kingdom) and incubated at 37°C for 24 hours. After growth, the culture was filtered through a 0.2- to 0.45- μ m pore membrane filter. To identify the staphylococcal enterotoxins in the filtrate, a Staphylococcal enterotoxin test—reversed passive latex agglutination (SET-RPLAR) test (Oxoid Diagnostics) was performed on microtiter plates (V-well). A total of 25 μ L diluent was pipetted in each well. Then, 25 μ L of *S aureus* filtrate was added to the first well in each row. Starting at the first well, 25 μ L was drawn up and added to next, producing 1:64 dilutions by the seventh well. Afterwards, in the first row, 25 μ L of latex solution sensitized with antienterotoxin A was added. The same procedure was continued with latex sensitized with antienterotoxin B for the second row, with antienterotoxin C for the third, with antienterotoxin D for the fourth, and with latex control for the fifth. To mix the contents of each well, the plate was gently shaken by hand for 3 minutes. V-plates were covered with a lid to prevent evaporation and left undisturbed for 24 hours. The experiments were repeated once and 10 randomized samples were investigated for a second time to test the reproducibility of the method.

Statistical methods

Statistical analyses were performed with 1-way analysis of variance combined with Tukey post hoc test to identify significant differences in the 3 groups (*S aureus* negative, *S aureus* positive/toxin negative,

S aureus positive/toxin positive) of AD and PS. Software (SPSS 11.01 for Windows; SPSS Inc, Chicago, Ill) was used for calculations.

RESULTS

S aureus was cultivated from lesional skin of 22 of 25 patients with AD and 15 of 25 patients with PS (Table I). Isolated strains were toxigenic in 44% for patient with AD and in 36% for patients with PS. The activity of disease in AD and PS according to the SCORAD or Psoriasis Area and Severity Index score, respectively, correlated significantly with an isolated toxigenic strain in both diseases (Figs 1 and 2). *S aureus* was further isolated in all 6 patients with erythroderma and in 2 of 25 healthy control subjects (Table II). For patients with AD, the cultivated strains were derived from nares alone in 32% and from nares and skin in 68% (Table II). For patients with PS, *S aureus* was cultivated from the nares alone in 27%, from the skin alone in 7%, and from both in 66%. Patients with erythroderma harbored *S aureus* in their nares alone in 83% and on their skin and nares in 17%. For patients with skin infections, *S aureus* was always isolated from the skin and from the anterior nares. In healthy persons, *S aureus* was cultured in 3 of 25 individuals, twice from the nares and once from the skin. The isolated strains were either *S aureus* toxin positive or negative in persons with disease but toxin negative in all healthy control subjects (Table I). Enterotoxin B was the toxin most often produced in PS and erythroderma (Fig 3). In AD, sepsis and skin infections, toxin C was most

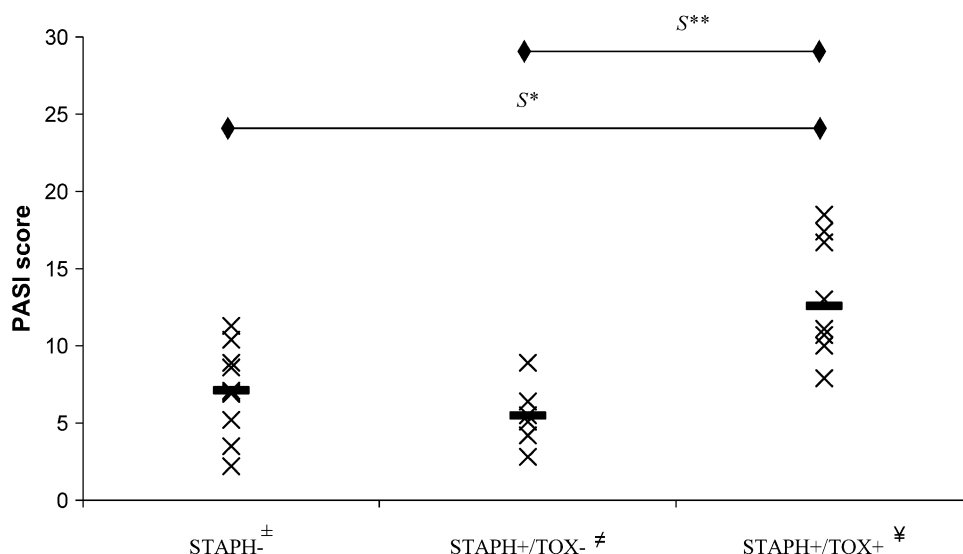


Fig 2. Psoriasis Area and Severity Index (PASI) in patients with psoriasis in relation to *Staphylococcus aureus* enterotoxin-producing strains. *Significance $P = .003$; **significance $P = .001$; $\pm S aureus$ negative; $\neq S aureus$ positive and no enterotoxin production; $\yen S aureus$ positive and enterotoxin production.

Table II. Distribution of *Staphylococcus aureus* in different dermatoses and healthy control subjects

No. of patients with SA	Skin only No. of patients/%	Nares only No. of patients/%	Skin and nares No. of patients/%
Atopic dermatitis (n = 22)	0/0	7/32	15/68
Psoriasis (n = 15)	1/7	4/27	10/66
Erythroderma (n = 6)	1/17	0/0	5/83
Infectious skin diseases (n = 25)	3/12	2/8	20/80
Healthy control subjects (n = 3)	1/33	2/67	0/0

SA, *Staphylococcus aureus*.

often detected. Toxin D was detected only for patients with PS or skin infections. Strains producing toxin A/D and toxin B/C were present in PS and skin infections, and strains producing toxin A/D in AD. Strains producing only toxin A were not detected. Altogether, the 80 *S aureus* strains isolated from the skin, nares, or blood were toxigenic in 52%. In all, 17% of patients with erythroderma, 44% of patients with sepsis, and 52% of patients with skin infections but none of the healthy control subjects presented a toxigenic strain. A total of 31 strains produced only one toxin and 5 strains, 2 toxins. The corresponding strains for patients isolated from the nares or the skin showed the same characteristics with respect to their antibiotic resistance and toxin composition.

All 6 patients with erythroderma harbored *S aureus*, but only one toxigenic staphylococcus enterotoxin B (SEB)—strain was isolated in a patient with erythrodermic AD. In skin infections, 52% of the strains were positive for enterotoxins; staphylococcus enterotoxin C (SEC) was detected in 20%, SEB in 12%, SED in 12%, SEA/D in 4% and SEB/C in 4% of strains in this patient group. In summary, toxi-

genic strains were mainly seen in skin infections (n = 13) followed by AD (n = 11) and PS (n = 9).

DISCUSSION

S aureus in PS

When we analyzed the distribution of staphylococcal enterotoxins and their potential effect on disease activity, we found colonization of lesional skin with *S aureus* occurred in 60% of patients with PS, consistent with data that have already been reported in the literature.^{4,17} In 60%, these strains were toxigenic. The toxin most often detected was SEB followed by SEC, SED, SEA/D, and SEB/C. Patients with enterotoxin-positive PS had a significantly ($P = .001$) higher Psoriasis Area and Severity Index score than patients who were toxin negative or patients without *S aureus* colonization ($P = .003$). This distribution of *S aureus* enterotoxins in PS confirms the long-standing hypothesis that these toxins play a role in aggravating this disease.^{13,15,18,19}

There is evidence that the induction or triggering of PS by *S aureus* enterotoxins could be directly caused by the stimulation of keratinocytes, perhaps

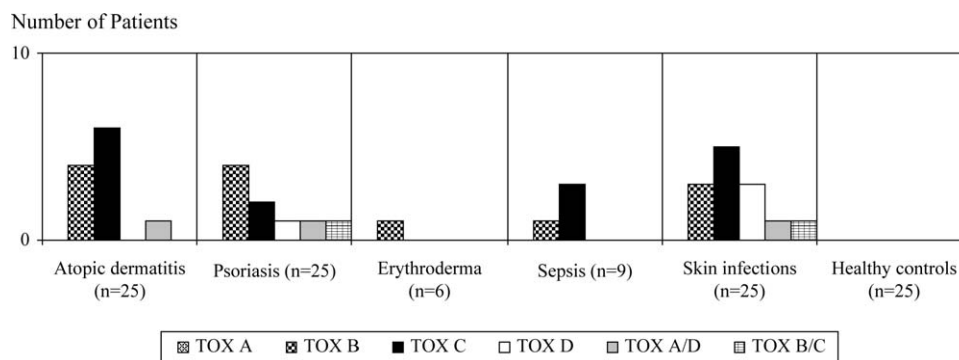


Fig 3. Staphylococcal toxins A-D in different dermatoses and healthy control subjects. *TOX*, Toxin.

through superantigen activity.¹² Staphylococcal antigens applied by patch test for 6 hours onto the skin of those with PS after tape stripping elicited greater inflammatory response in PS skin than in the skin of AD or patients with lichen planus as evidenced by increased tumor necrosis factor- α messenger RNA expression in the epidermis.¹³ In PS, superantigen-stimulated keratinocytes express CD54 and produce T-cell activating cytokines.⁹ Further, HLA-DR expression, which seems to enhance inflammatory skin reactions to superantigens,⁹ was significantly higher in keratinocytes for patients with PS than in healthy control skin.¹² In contrast, mutants of superantigens are not able to induce skin inflammation.¹³

***S aureus* in AD**

S aureus was cultivated from lesional skin in 88% of patients with AD. Of these strains, 50% were toxigenic, producing SEC > SEB > SEA + SED in decreasing frequency. These data are similar to previous reports in which toxigenic strains were isolated from the skin in 31% to 65% of patients.^{7,8,10} Toxin A was never produced as a single toxin in our study, in contrast to the findings of Breuer et al⁷ or Bunikowski et al,⁸ who used the same technique and detected most often SEA or SEC, respectively. The frequency and composition of toxin production varies considerably in other studies but there does seem to be a disease-promoting effect of *S aureus*-derived toxins in AD.^{7,8} The mean SCORAD score in our patients with toxin-positive AD was 52, significantly ($P = .001$) higher than the score of 35 in patients who were toxin negative, consistent with observations from previous studies by Bunikowski et al^{8,20} and Breuer et al,⁷ in which toxigenic *S aureus* strains correlated significantly with a higher SCORAD score. Breuer et al⁷ reported significant improvement in their SCORAD score from 52.1 before to 41.4 after treatment with antibiotics. In addition, *S aureus* colonization density proved to be

significantly higher in affected than in unaffected skin of patients with AD and to be significantly higher for patients with severe than with moderate to mild AD.⁸ Lending support to these clinical observations are the findings that in AD, specific IgE antibodies to *S aureus* contribute significantly to disease severity²¹ and SEB applied on the skin in a Finn Chamber can produce a superantigen-driven dermatitis.²²

The implication of toxigenic *S aureus*

Although the mechanisms of action and the significance of toxigenic *S aureus* in AD have been proven in numerous studies,^{7,8,23} the influence and the disease-aggravating role of *S aureus* enterotoxins in PS are not fully understood.¹³ TSST-1 and SEB applied to the involved skin of those with PS can trigger skin inflammation significantly more than in the skin of healthy subjects.¹³

In this study all patients with erythroderma harbored *S aureus*; only one strain was toxigenic in a patient with AD-associated erythroderma. Alternatively, colonization with *S aureus* but not its enterotoxins might play a role in the development of erythroderma, or other staphylococcal antigens, such as TSST-1, might be responsible for the development of erythroderma or propagation of disease.⁶

Similarly, only 4/9 strains isolated from patients with sepsis were toxigenic, with SEC as the most frequently detected toxin. However, it seems to be clear that staphylococcal superantigens are among the most lethal toxins leading to toxic shock.²³ With our agglutination test not all potentially acting toxins were investigated, although the identification of *S aureus* enterotoxin by agglutination test is an established method also used by Breuer et al⁷ and Bunikowski et al.⁸

Our findings support previous data that toxigenic strains of *S aureus* isolated from the skin not only of patients with AD but also with PS may have a disease-initiating or -promoting effect.²⁴ Strategies to

reduce bacterial colonization could also be a mainstay in managing the disease not only in patients with AD but in those with PS as well.

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