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Encoding a superantigen by *Staphylococcus aureus* does not affect clinical characteristics of infected atopic dermatitis lesions

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Summary

Background—Bacterial infection with *Staphylococcus aureus* is a known trigger for the worsening of atopic dermatitis (AD). Staphylococcal superantigens have been theorized to potentially contribute to this worsening of AD seen with infection.

Objectives—We sought to assess whether encoding a superantigen by *Staphylococcus aureus* affected the inflammatory characteristics of an impetiginized AD skin lesion

Methods—Fifty-two children with clinically impetiginized lesions of AD which were positive for *Staphylococcus aureus* were enrolled in this study. A lesion was graded clinically using the Eczema Area and Severity Index (EASI), and then wash fluid obtained from the lesion for quantitative bacterial culture, and measurement of bacterial products lipoteichoic acid (LTA) and staphylococcal protein A (SPA) and cytokines. The staphylococcal isolate was tested for antibiotic susceptibilities and the presence of a superantigen.

Results—Fifty-four percent (28 of 52) of the staphylococcal isolates encoded a superantigen. The presence of a superantigen had no significant effect on EASI score, amounts of bacterial products or inflammatory cytokines in the AD lesion.

Conclusions—These studies suggest that the expression of a superantigen by *Staphylococcus aureus* alone does not play an important role in the increased skin inflammation associated with staphylococcal infection in childhood AD.

Keywords

Atopic Dermatitis; *Staphylococcus aureus*; Superantigen; Lipoteichoic acid; Staphylococcal Protein A

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Introduction

Staphylococcal skin infections are common triggers for worsening of atopic dermatitis (AD)[1]. These effects could be due to either direct invasion by the bacteria or by bacterial products. Potential soluble mediators that could worsen AD include the cell wall lipoprotein lipoteichoic acid (LTA) that can act as an agonist for the toll-like receptor 2[2] as well as the platelet-activating factor receptor[3,4]. Proteins including staphylococcal protein A (SPA) and alpha toxin have also been demonstrated to have biological effects[5,6]. Staphylococcal bacteria can also encode proteins that act as superantigens. Through the ability of these toxins to activate large numbers of T cells and class II antigen-expressing cells, superantigens can evoke potent immune responses[7]. Many subjects with AD have been shown to have IgE antibodies that recognize superantigens that could result in immediate hypersensitivity reactions[8]. Thus, accumulating evidence has implicated superantigens as playing a role in the worsening of AD following a staphylococcal skin infection[9,10]. The objective of the present study is to assess whether infection with a *Staphylococcus aureus* encoding a superantigen results in enhanced clinical evidence of inflammation in pediatric AD subjects.

Materials and Methods

Atopic Dermatitis Subjects

In these studies, we enrolled 52 children (age 4 months to 6 years; three less than or equal to 6 months) with clinically impetiginized AD diagnosed using criteria of Hanifen and Rajka using our previously published protocol[11]. These studies were approved by the Indiana University Institutional Review Committee. Subjects were not exposed to oral antibiotics for a period of one month before the study. Subjects enrolled into the study underwent a clinical assessment of a clinically-infected lesion of dermatitis using the Eczema Area and Severity Index (EASI)[12], as well as an EASI scoring of entire body. Wash fluid derived from lesions was removed and aliquotted for measurement of bacterial products and cytokines exactly as previously outlined[4,11]. Briefly, a sterile 2.5 cm diameter ring of PVC tubing (Nalgene® Labware, Rochester, NY) was placed over the skin lesion of patient, then, 1 ml sterile rinse solution (0.069M Na_2HPO_4, 0.0064M NaH_2PO_4, and 0.1% Tx-100) was administered inside the ring chamber that was held tightly on the skin to prevent leakage. The rinse solution was stirred around in the chamber with a sterile Teflon® rod (Scientific Commodities Inc., Lake Havasu City, AZ) for 15–20 times and collected. This collection was repeated and 2 ml total rinse solution was obtained. *S. aureus* colonies were quantified by limiting dilution assay, and antibiotic susceptibilities assessed by standard methodology.

Measurement of bacterial superantigens

Qualitative assessment of bacterial superantigens Staphylococcal enterotoxin (SE) type A, B, C, D, E, H and Toxic shock toxin-1 (TSST-1) and Epidermolytic toxin (ET) A on *S. aureus* isolates was performed by P.B. at Toxin Technologies, Inc., by specific ELISA as previously reported[8].

Measurement of bacterial products and cytokines in wash fluid specimens

Quantitative measurements of LTA protein used immunoblotting exactly as previously described[4,11]. Quantitative measurement of SPA was performed using ELISA (Assay Designs Inc., Ann Arbor, MI). Levels of cytokines of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ and TNF-α were measured using the Multiplex Bead Immunoassays as per manufacturer's protocol (Millipore, Billerica, MA)[11]. Cytokines and staphylococcal products were quantified based upon area (ng/cm^2) of the chamber that then

was converted to volume (ng/cm^3) based upon estimation of 0.1 cm effective epidermal thickness.

Statistical analysis

Wilcoxon rank sum test was applied to compare EASI scores, staphylococcal CFU, LTA, SPA and cytokine levels between superantigen-positive and negative staphylococcus groups. Association between antibiotic sensitivities and presence of a superantigen was tested by Fishers exact test.

Results

Clinical characterization of infected AD lesions

Previously, our group published data from 89 children (ages 3 months–6 years) with AD with clinically impetiginized lesions to clinically define eczema severity and quantitatively or qualitatively determine the levels of lesional bacterial products before and after treatment[11]. Lesion wash fluid was obtained from an infected lesion and used for quantitative *S. aureus* culture or aliquotted and stored at −80 °C before it was used for measurement of other proteins/cytokines. An isolate of the *S. aureus* was assessed for antibiotic sensitivities and 52 of the isolates tested for presence of a superantigen at first visit. The eight superantigens tested represent the majority of exotoxins found, particularly on AD patients[7,13]. The present studies examined whether or not the *S. aureus* expressed a superantigen was positively correlated with the level of inflammation. To that end, 52 subjects whose *S. aureus* isolates were tested for the presence of a superantigen were examined. As shown in Table I, of the 52 isolates that were tested for the presence of a superantigen, 28 (54%) were positive. The superantigens expressed by the 28 staphylococcal isolates were as follows: SEA (8), SEB (20), SED (2), TSST-1 (3), and ET (2). Two isolates expressed both SEA and TSST-1 and five isolates expressed both SEA and SEB. Of the 52 isolates, 21% were methacillin-resistant (MRSA). There was not an association between antibiotic sensitivities and presence of a superantigen, as 82% of the isolates positive and 75% of those negative for a superantigen were methacillin-sensitive ($p = 0.73$). The presence of a superantigen did not result in differences in lesional or total EASI or staphylococcal CFU in the lesions (Table I). These studies indicate that the expression of a superantigen in the *S. aureus* did not affect the lesional or whole body dermatitis clinical characteristics, nor resulted in a selective advantage to allow increased bacterial CFU.

Levels of lesional bacterial proteins and cytokines do not correlate with presence of superantigen

To quantitate the inflammatory responses in the lesions, we next examined the levels of bacterial products LTA and SPA and a panel of cytokines in the 52 subjects whose staphylococcal isolate was tested for the presence of a superantigen. It should be noted that our previous studies demonstrated that the amounts of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α correlated with EASI levels[11]. Levels of LTA, SPA and pro-inflammatory cytokines IL-8 and TNF-α were not appreciably different in lesions derived from a superantigen-expressing versus non-expressing staphylococcus (Table I). Moreover, levels of other cytokines (IL-4, IL-5, IL-10, IL-12, IL-13, IL-17 and IFN-γ) also were similar between superantigen-expressing versus non-expressing *S. aureus*-infected AD lesions (*data not shown*).

Discussion

Through their ability to stimulate tremendous levels of cytokine production in immune cells, superantigens have been implicated in staphylococcal-mediated worsening of AD. Indeed, a

significant body of work exists demonstrating that superantigens have potent effects on cell types involved in atopic dermatitis[8,9,14]. Moreover, it has been reported that the topical application of microgram amounts of the superantigen SEB can trigger inflammatory skin reactions[15], and lesser amounts can synergize with topical allergens to induce skin inflammation in AD subjects[16]. Of interest, Schlievert and colleagues recently demonstrated that staphylococcal strains encoding a superantigen were more commonly associated with steroid-resistant AD[13]. Surprisingly, the current studies indicate that infection with bacteria that encoded a superantigenic toxin alone did not seem to result in worse features than non-expressing *S. aureus.* Since these studies did not attempt to quantify superantigens in the wash fluid specimens, nor examine patients for the presence of anti-superantigen IgE antibodies, nor examine the steroid-responsiveness of the subjects, it is indeed plausible that superantigens could still play an important role in staphylococcal infection-mediated worsening of AD.

What's already known about this topic?

Superantigens encoded by S. aureus have been hypothesized to be significant pathogenic factors for worsening of atopic dermatitis, though no studies have directly examined whether encoding a superantigen results in a perceptible clinical effect.

What does this study add?

The presence or absence of a staphylococcal superantigen alone does not affect clinical and inflammatory characteristics of impetiginized atopic dermatitis, suggesting that these potent toxins do not play a primary role in the clinical worsening seen following staphylococcal secondary impetiginization.

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Table I

Comparison of clinical assessment of inflammation, amounts of *S. aureus*, and levels of staphylococcal products LTA and SPA and cytokines IL-8 and TNF-α with the presence or absence of a superantigen in staphylococcal-positive AD lesions.

Variable	Superantigen-negative (24 isolates)	Superantigen-positive (28 isolates)
EASI-lesional	10.4 (1.7) [11.0, 9.0–12.0]	10.1 (1.8) [10.0, 9.0–12.0]
EASI-total	20 (11) [18, 12–28]	24 (6) [25, 11–32]
Log[S.aureus](CFU/ml)	6.4 (1.5) [6.9, 5.7–7.4]	5.9 (1.3) [6.2, 5.4–6.8]
[LTA] (ng/cm^3)	1230 (1839) [648, 0–1447]	1106 (2171) [266, 0–1104]
[SPA] (ng/cm^3)	24 (47) [6.8, 0.6–23.5]	23 (69) [1.5, 0.3–15.8]
[IL-8] (pg/cm^3)	7906 (8670) [4696,1772–11037]	13174 (19608) [4299, 1449–14444]
[TNF-α] (pg/cm^3)	45 (35) [32, 14–68]	52 (57) [35, 10–75]

The EASI score of the tested lesion and total body EASI scores, concentration of *S. aureus* bacteria (in CFU/ml) and LTA, SPA and cytokine levels were compared in samples which the bacterial isolate tested positive or negative for a superantigen. The data represent the mean (standard deviation) and [median, 1st quartile–3rd quartile].