Presence of IgE Antibodies to Staphylococcal Exotoxins on the Skin of Patients with Atopic Dermatitis

Evidence for a New Group of Allergens

Donald Y. M. Leung,* Ronald Harbeck,* Paul Bina,‖ Raoul F. Reiser,‖ Evelyn Yang,¶ David A. Norris,‡ Jon M. Hanifin,§ and Hugh A. Sampson¶

**Department of Pediatrics, The National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; ‡Department of Dermatology, University of Colorado Health Sciences Center, Denver, Colorado 80262; §Department of Dermatology, University of Oregon Health Sciences, Portland, Oregon 97201; ‖Toxin Technology, Sarasota, Florida 34231; and ¶Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21218*

Abstract

In the current study, we investigated whether *Staphylococcus aureus* grown from affected skin of atopic dermatitis (AD) patients secreted identifiable toxins that could act as allergens to induce IgE-mediated basophil histamine release. The secreted toxins of *S. aureus* grown from AD patients were identified by ELISA using antibodies specific for staphylococcal enterotoxin (SE) exfoliative toxin (ET), or toxic shock syndrome toxin (TSST-1). *S. aureus* isolates from 24 of 42 AD patients secreted identifiable toxins with SEA, SEB, and TSST accounting for 92% of the isolates. 32 of 56 AD sera (57%) tested contained significant levels of IgE primarily to SEA, SEB, and/or TSST. In contrast, although SEA, SEB, or TSST secreting *S. aureus* could be recovered from the skin of psoriasis patients, their sera did not contain IgE antitoxins.

Freshly isolated basophils from 10 AD patients released 5–59% of total histamine in response to SEA, SEB, or TSST-1 but only with toxins to which patients had specific IgE. Basophils from eight other AD patients and six normal controls who had no IgE antitoxin failed to demonstrate toxin-induced basophil histamine release. Stripped basophils sensitized with three AD sera containing IgE to toxin released 15–41% of total basophil histamine only when exposed to the relevant toxin, but not to other toxins. Sensitization of basophils with AD sera lacking IgE antitoxin did not result in release of histamine to any of the toxins tested.

These data indicate that a subset of patients with AD mount an IgE response to SEs that can be grown from their skin. These toxins may exacerbate AD by activating mast cells, basophils, and/or other Fcε-receptor bearing cells armed with the relevant IgE antitoxin. (*J. Clin. Invest.* 1993. 92:1374–1380.)
Key words: atopic dermatitis • staphylococci • toxin • immunoglobulin E • allergen

Introduction

Atopic dermatitis (AD)[1] is a chronic pruritic inflammatory skin disorder characterized by the infiltration of T cells and monocyte macrophages into the local skin lesion (1, 2). Although it is thought that immunologic mechanisms contribute to the pathogenesis of this skin disease, the actual events that result in this common skin condition are poorly understood (3). Several observations, however, suggest that IgE and/or allergens may play a role: First, the majority of patients with AD have a personal or family history of atopic disease, e.g., asthma or allergic rhinitis. Second, serum IgE levels are elevated in about 80% of patients with AD (4). In this regard, serum IgE levels have been reported to correlate with extent of skin disease (5). Third, most patients with AD have positive immediate skin tests and radioallergosorbent tests for IgE to various food and inhalant allergens (6). Fourth, double-blinded placebo-controlled food challenge studies have demonstrated that food allergies can trigger cutaneous reactions in a subset of children with AD (7). In the latter studies, serial skin biopsies and sampling of plasma for histamine release suggested that the inflammatory cell infiltrate associated with food challenges was the result of an allergen-induced IgE-dependent late phase cutaneous reaction (8, 9). Finally, mite-specific Th_2-type lymphocytes have been cloned from skin lesions of AD patients with IgE-specific antibody to dust mites (10, 11).

Although clinically relevant food-induced dermatitis has been convincingly demonstrated, it affects only a minority of patients with AD. In this regard, it is of interest that there are numerous reports suggesting that *S. aureus* contributes to the exacerbation of this skin disease (12–15). Leyden and coworkers (12) first demonstrated that *S. aureus* could be isolated from the affected skin of over 90% of patients with AD. In contrast, the skin of normal subjects harbored this organism in only 5% of their cases, and its localization was mainly in the nose and intertriginous areas. Using quantitative methods, the density of *S. aureus* on inflamed AD lesions without clinical superinfection can reach up to 10^7 colony-forming units/cm^2 (13). Clinically, it is also well known that not only patients with impetiginized AD but also patients without superinfection show a better clinical response to combined treatment with antistaphylococcal antibiotics and topical corticosteroids than to corticosteroids alone (16).

Patients with the hyperimmunoglobulin E (HIE) and recurrent infection syndrome produce high levels of IgE antibody directed against the cell wall of *S. aureus* (17–19). Although recurrent superficial staphylococcal pustulosis can be a significant problem in AD, the invasive *S. aureus* infections, e.g., recurrent pneumonias, seen in HIE syndrome, do not occur

Address correspondence to Donald Y.M. Leung, M.D., Ph.D., Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

Received for publication 19 January 1993 and in revised form 19 April 1993.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/93/09/1374/07 $2.00
Volume 92, September 1993, 1374–80

1. *Abbreviations used in this paper:* AD, atopic dermatitis; ET, exfoliative toxin; HIE, hyperimmunoglobulinemia E; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin.

(20). *S. aureus* is known to secrete toxins with potent immunogenic properties (21). Since such toxins could be absorbed from the surface of inflamed AD skin, the current study was carried out to determine whether *S. aureus* isolated from AD skin secretes identifiable toxins that could act as allergens to induce histamine release from the basophils and mast cells of AD patients armed with IgE to staphylococcal toxins.

Methods

Patients. 56 patients with moderate to severe AD (greater than 20% skin involvement) and elevated serum IgE (serum IgE; median = 9847, range 880–40,682 IU/ml) were studied. Sera were available for analysis of IgE antitoxin from all 56 patients. 42 of these 56 patients were available for culture of their skin, all of whom yielded *S. aureus* isolates for characterization of exotoxin production (see below). Their diagnosis was based on the criteria of Hanifin and Rajka (1). All patients had a history of requiring oral antibiotics on at least one occasion for superficial skin infections, e.g., pustulosis or impetigo. None of these patients, however, had a history of deep-seated *S. aureus* skin infections. Four control groups were studied: first, 15 healthy, nonatopic donors (serum IgE; median = 81, range: < 5–198 IU/ml); second, 10 patients with respiratory allergy (asthma and allergic rhinitis; serum IgE; median = 285, range: 45–1004 IU/ml); third, 16 patients with psoriasis, (serum IgE; median = 42, range: < 4–4600 IU/ml); and 7 patients with HIE syndrome. The diagnosis of HIE syndrome (17–20) was based on a history of recurrent sinusitis, pneumonia, deep-seated skin and pulmonary abscesses predominantly caused by *S. aureus,* chronic eczematoid dermatitis, and marked elevation of serum IgE (median IgE = 22,000, range: 5500–34,000 IU/ml) with an increased proportion of IgE directed to *S. aureus* strain Wood 46 (protein A–deficient). Informed consent was obtained from all subjects before performing these studies.

Analysis of toxins secreted by S. aureus grown from AD skin. In these experiments, *S. aureus* isolated from AD lesional skin were grown overnight at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, MI). 0.1 ml of the overnight growth was then applied and spread over the surface of a brain heart infusion agar plate overlaid with a 6–8,000 mol wt cut off dialysis membrane (Spectra/Por, Los Angeles, CA) as previously described (22). The membrane over agar plates was then incubated overnight at 37°C. The next day the bacteria were harvested by resuspending them in 0.5 ml of 0.01 M Na_2HPO_4, 0.9% NaCl, pH = 7.4. The bacteria suspension was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected for identification of toxin secretion.

The ELISA for detection of staphylococcal toxin was carried out as previously described (23). 96 well flat-bottomed, polystyrene microtiter plates (Nunc, Roskilde, Denmark) were coated with 0.1 ml of staphylococcal toxin-specific polyclonal rabbit IgG (Toxin Technology, Sarasota, FL) diluted to a 10 μg/ml concentration in 0.05 M Na_2CO_3, pH = 9.5. After that, the plates were covered and incubated at 37°C for 12–14 h. The plates were then washed three times with PBS (PBS-0 0.1 M NA_2HPO_4, 0.9%, NaCl, pH = 7.4) with 0.05% Tween 20 (PBS-Tween).

Before exotoxin assay, the *S. aureus* culture supernatants were diluted 1:4 in PBS-Tween. Normal rabbit serum containing no antibodies to staphylococcal exotoxins (Toxin Technology) was added to a concentration of 1% and the mixture was incubated for 30 min at 25°C. 100 μg of these mixtures was then added to the IgG antitoxin coated wells and shaken 100 rpm for 2 h at 37°C. The plates were then washed three times with PBS-Tween. 100 μl of toxin-specific polyclonal rabbit IgG conjugated to horseradish peroxidase (Toxin Technology) were then added to each well. The plates were shaken at 100 rpm for 1 h at 37°C. and washed 10 times with PBS-Tween. 100 μl of substrate solution consisting of 3% 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma Immunochemicals, St. Louis, MO 22,23) and 0.001% H_2O_2 in 0.05 M citrate buffer, pH = 4.0 was added to each well. After 30 min, the wells were read at 410 nm. In these assays, serial dilutions of purified toxin solutions (range: 0.6–10 ng/ml) are used as standards. The sensitivity of these assays was approximately 1 ng/ml.

Assay of IgE anti–S. aureus exotoxins. As previously described (24), flat-bottomed, polystyrene microtiter plate (Nunc) wells were filled with 100 μl of a 10-μg/ml solution of highly purified staphylococcal exotoxin (SE) (either SEA, SEB, SEC, SED, ET, or TSST-1; Toxin Technology) in 0.05 M Na_2CO_3 buffer, pH = 9.5. The plates were covered and shaken overnight at 100 rpm at 37°C. The following day the plates were washed three times with PBS-Tween. Serum to be tested was diluted 1:4 and 1:8 in PBS-Tween, then 100 μl of each dilution were placed into a precoated well. The plates were then washed three times with PBS-Tween. 100 μl of a 1:1000 dilution of goat anti–human IgE (Sigma Chemical Co.) were placed in the wells. The plates were incubated at 37°C for 1 h while being shaken at 100 rpm. The plates were then washed 10 times with PBS-Tween. 100 μl of a 1-μg/ml solution of *p*-nitrophenyl phosphate (Sigma Chemical Co.) in 9.7% diethanolamine buffer, pH = 9.8 was placed in each well. The plates were incubated at 25°C for 1 h, then read at 410 nm. In all experiments, background was < 0.05 and was subtracted from the values obtained with test samples. Specificity of the assay was demonstrated by the loss of IgE binding in the presence of soluble toxin added to test samples.

Preparation of peripheral-blood basophils. Venous blood was collected in sterile plastic syringes containing EDTA (pH 7.2) at a final concentration of 25 mM. The syringes were inverted several times for proper mixing, and then the anticoagulated samples were added to detergent-free polycarbonate tubes containing 6% Dextran 70 (Pharmacia Fine Chemicals, Uppsala, Sweden). The erythrocytes were sedimented at room temperature for 90 min, and the leukocyte-containing plasma was removed. After centrifugation at 300 *g* for 12 min at 4°C, the cell pellet was washed three times in cold Hepes-buffered saline (0.01 M, pH 7.4) containing 0.3 mg HSA/ml. The final cell pellet was resuspended and adjusted to 10–15 × 10^6 cells/ml in Hepes-buffered saline containing albumin, 2 mM calcium, and 1 mM magnesium (Hepes-ACM) for use in the histamine release assay.

Leukocyte histamine-release assay. Duplicate 50-μl aliquots of the leukocyte suspension (~ 1.5–2 × 10^4 basophils) were incubated in polystyrene tubes containing 50 μl of prewarmed Hepes-ACM for 45 min at 37°C to determine the rate of spontaneous release of histamine by basophils. Similar duplicate samples were heated at 100°C for 10 min to measure the total histamine content. To determine the rate of histamine release by basophils that was induced by staphylococcal exotoxins, either 1 ng/ml, 10 ng/ml, 100 ng/ml, or 1 μg/ml of the respective toxins was added to 50 μl of the leukocyte preparation (1.5–2 × 10^4 basophils), and the cell suspension was incubated for 45 min at 37°C. The cell suspensions were spun down, the supernatants were removed, and the histamine content was determined utilizing a sensitive radioenzymic assay, modified from the method of Verburg et al. (25) This assay uses purified histamine methyltransferase and gives results that were highly reproducible within the range of 30 pg to 10 ng/ml. The release of histamine, either spontaneously or as induced by staphylococcal toxin, was recorded as a percentage of the total leukocyte histamine content. The basophils were counted by means of toluidine blue (26) staining.

Lactic acid stripping of basophil IgE. Surface-bound IgE was dissociated from the basophils with the use of the lactic acid elution method of Pruzansky et al. (27). The leukocytes were washed once in buffer and once in normal saline, resuspended in 5 ml of 0.01 M lactic acid (pH 3.9) containing 140 mM sodium chloride and 5 mM potassium chloride, and incubated for 3.5 min at 23°C. Cells were then diluted in 30 ml of Pipes buffer containing 25 mM Pipes, 110 mM sodium chloride, 4 mM potassium chloride, 0.003% HSA, and 0.1% D-glucose and adjusted to pH 7.4 (Pipes-AG). After two washes in Pipes-AG, cells were resuspended in Pipes-AG containing 4 mM EDTA and 10 μg heparin/ml.

Passive sensitization of lactic acid-treated basophils. The method of Levy and Osler (28) was used to sensitize the stripped basophils passively. Acid-treated leukocytes from three donors were incubated with serum diluted in Pipes-AG containing EDTA and heparin for 90 min

Table 1. Characterization of Identifiable Toxins Secreted by Staphylococcus aureus from Skin Isolates of Patients with Atopic Dermatitis and Psoriasis

Toxin	No. of AD patients	No. of psoriasis patients
SEA	7 (29%)	4 (40%)
SEB	8 (33%)	2 (20%)
SEC	0 (0%)	1 (10%)
SED	1 (4%)	0 (0%)
ET	1 (4%)	0 (0%)
TSST-1	7 (29%)	2 (20%)

at 37°C. Unless indicated, serum samples high in IgE from patients with myeloma or patients' serum samples were diluted to contain 417 IU IgE/ml, or 250 ng/leukocyte-cell pellet. The leukocytes were then washed twice before use in the leukocyte histamine-release assays.

Statistical analysis. The data were analyzed with the nonparametric Wilcoxon signed-ranks test for paired data or the Wilcoxon nonpaired rank-sum test for nonpaired data. The in vitro basophil-stripping experiments were analyzed with the paired t test, after normality was confirmed by the Wilk-Shapiro test.

Results

Toxins secreted by S. aureus grown from AD skin lesions. Skin cultures were obtained from the lesional skin of 42 patients with AD and 16 patients with psoriasis. *S. aureus* was isolated from all 42 AD patients. They were then characterized for their capacity to secrete the following exotoxins: SEA, SEB, SEC, SED, ET, and TSST-1. As shown in Table I, *S. aureus* isolates from 24 of the 42 AD patients secreted identifiable toxins. Furthermore, SEA, SEB, and TSST-1 accounted for 22 of the 24 (92%) identifiable toxins. *S. aureus* was isolated from 9 of the 16 patients with psoriasis. These staphylococcal organisms were also found primarily to secrete either SEA, SEB, or TSST-1 (Table I).

Presence of IgE antitoxin in AD and HIE syndrome. In these experiments, we determined whether serum from 56 AD patients contained IgE antibodies directed against *S. aureus* exotoxins. 32 of the 56 AD (57%) sera tested had increased IgE binding to either SEA, SEB, SEC, SED, ET, or TSST-1 (Fig. 1). These AD sera were, however, predominantly positive for IgE to SEA, SEB, and TSST1. Of note, 22 of the AD sera reacted with only one of the six toxins tested, nine reacted with two of these toxins and only one of these sera reacted with all three toxins. Furthermore, sera from 21 of the 24 patients in Table I who had *S. aureus*–secreting exotoxins were found to have IgE antibodies directed against at least one *S. aureus* exotoxin. These data suggest a high correlation between presence of exotoxin on AD skin and IgE sensitization.

Control sera from 16 patients with psoriasis, 7 patients with HIE syndrome, 10 patients with respiratory allergy, and 15 normal controls were also tested for IgE against SEA, SEB, SEC, SED, ET, and TSST-1. None of the sera from psoriasis patients, respiratory allergy patients, or normal controls demonstrated any reactivity with these toxins. In contrast, sera from all seven of the HIE patients contained IgE antibodies

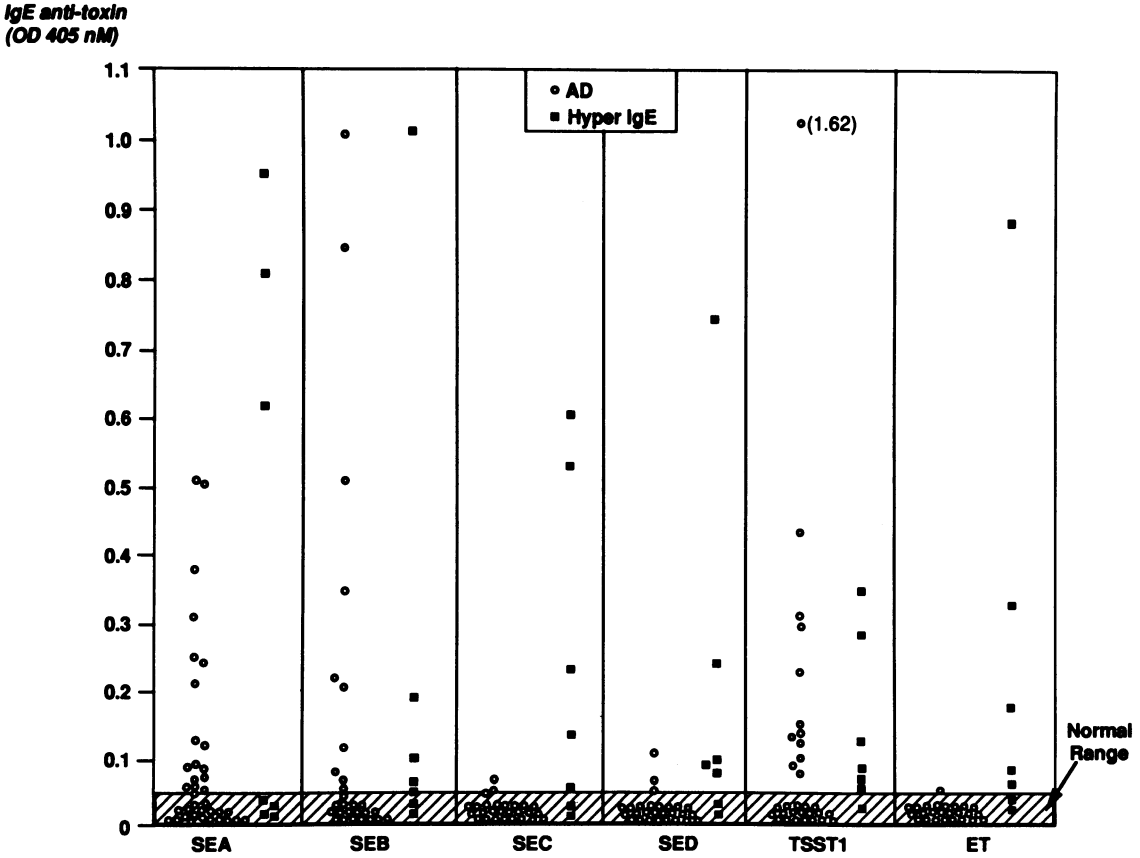

Figure 1. Atopic dermatitis and hyper IgE syndrome are associated with increased serum IgE anti–*S. aureus* toxin.

against at least three toxins (Fig. 1). Furthermore, unlike the AD sera, five of the seven HIE patient sera tested contained IgE against SEC, SED, and ET.

Since patients with AD contain high levels of total serum IgE, we were concerned with the possibility that the high frequency of IgE against staphylococcal toxins was due to nonspecific IgE binding. This is unlikely for several reasons: first, the ELISA assay using staphylococcal toxins as the capture antigen was unable to detect binding of myeloma IgE (PS) at levels of IgE up to 200,000 IU/ml) (data not shown). Second, binding of patient serum IgE to plastic wells coated with the various staphylococcal toxins was completely inhibited by preincubation of positive sera with the corresponding staphylococcal toxin at a concentration of 1 μg/ml. Finally, there was no correlation between IgE antitoxin level and total IgE level. In this regard, the total IgE level of sera from patients who had IgE antitoxin (mean serum IgE = 7152±1741 IU/ml) was similar to the total IgE level of sera from patients without IgE antitoxin (mean serum IgE = 5258±1797 IU/ml; $P = 0.4$).

S. aureus toxin-induced histamine release from AD basophils. To study the potential functional role of IgE antistaphylococcal toxins in AD, we assessed the capacity of staphylococcal toxins to induce histamine release from freshly isolated basophils of AD patients and normal controls. In these experiments, basophils were incubated either with medium to determine spontaneous histamine release or with SEA, SEB, and TSST-1. The data shown in Tables II and III were derived from experiments in which the toxins were used at 100 ng/ml. Similar data, however, was obtained when these toxins were used at 10 ng/ml and 1 μg/ml. Net basophil histamine release (spontaneous histamine release subtracted from toxin-induced histamine release) ≥ 5% was considered significant.

As depicted in Table II, no control subject's basophils released > 2% histamine to any staphylococcal toxin: mean±1 SD of SEA = 0.30%±0.53%; SEB = 0.70%±0.72%; and TSST-1 = 0.14%±0.22%. Similarly, basophils from AD patients lacking identifiable toxin-specific IgE (group 1 in Table III) failed to release any significant histamine: mean±1 SD of SEA = 1.00%±0.93%; SEB = 1.38%±1.30%; and TSST-1 = 1.51%±1.40%. In contrast, patients in group 2 of Table III, who had serum IgE antibodies against staphylococcal toxins, released significant amounts (5–59%) of total basophil histamine in response to at least one staphylococcal toxin: six patients to SEA, six to SEB, and four patients to TSST-1; mean±1 SD of SEA = 7.50%±7.27%; SEB = 12.10%±10.02%; and TSST-1 = 9.58±17.76%. As a group, AD patients in group 2 of Table III released significantly more basophil histamine to all staphylococcal toxins tested compared with AD group 1 ($P < 0.01$). Basophil histamine released from individual donors, however, only occurred when the respective donor had circulating IgE against the relevant staphylococcal toxin. Furthermore, there was a significant correlation between the presence of serum IgE antitoxin and positive toxin-induced basophil histamine release ($P = 0.52$ by McNemar test for association between the two assays).

*Table II. Net Percent Histamine Release by Basophils from Nonatopic Donors in the Presence of Staphylococcal Toxins**

Donor	SEA	SEB	TSST-1	Anti-IgE
1	0.5	0.6	0.5	ND
2	1.3	1.0	0.0	ND
3	0.0	0.3	0.2	ND
4	0.0	0.3	0.0	ND
5	0.0	0.0	0.0	53
6	0.0	2.0	0.0	35

* Net percent histamine release = exotoxin-induced basophil histamine release minus spontaneous basophil histamine release divided by total basophil histamine content. Median spontaneous histamine release by basophils from these six donors was 2.0% (range 0.9–3.2%).

Table III. Net Percent Histamine Release by Basophils from Patients with AD in the Presence of Staphylococcal Toxins

Group*	Patient	SEA	SEB	TSST-1	Anti-IgE
1	1	2.0	2.0	0.0	31
	2	0.0	2.0	0.0	28
	3	2.0	1.0	3.0	52
	4	1.0	0.0	1.0	70
	5	1.0	3.0	3.0	45
	6	0.0	0.0	0.1	37
	7	0.0	0.0	3.0	11
	8	2.0	3.0	2.0	39
2	9	0.0	19.0	12.0	22
	10	5.0	1.0	3.0	52
	11	6.0	3.0	2.0	11
	12	0.0	11.0	0.8	6
	13	21.0	29.0	59.0	23
	14	2.0	1.0	8.0	63
	15	4.0	14.0	4.0	14
	16	17.0	4.0	0.0	36
	17	7.0	25.0	6.0	40
	18	13.0	14.0	1.0	84

* Group 1 contains AD patients with no serum IgE antitoxin antibodies, whereas group 2 patients have serum IgE antitoxin antibodies. Median spontaneous histamine release basophils was 3.0% (range 1.6–40%) in group 1 and 8.0% (range 0.2–70%) in group 2.

To demonstrate that the histamine release by AD basophils in response to staphylococcal toxins occurred through an IgE-dependent mechanism, basophils from an unrelated atopic donor were stripped of surface-bound IgE and passively sensitized with various AD sera. Table IV shows that stripped basophils obtained from an atopic donor sensitized with myeloma IgE did not respond to SEA, SEB, or TSST1. Sensitization of stripped basophils with AD sera lacking IgE antitoxin (AD1 and AD2 in Table III) did not result in the release of histamine to any of the toxins tested. However, when these stripped human basophils were sensitized with three AD sera (3–5 in Table III) containing IgE to toxin, increased levels of histamine release was observed, but only when exposed to the relevant toxin but not to other toxins. More important, we have found that removal of IgE from AD sera depletes the activity that mediates toxin-induced basophil histamine release (data not shown). Furthermore, toxin-induced histamine release in a dose-dependent manner from stripped human basophils that were sensitized with sera containing IgE antitoxin. As shown in Table V, maximum histamine release was observed at toxin concentrations of 10 and 100 ng/ml.

*Table IV. Percent Histamine Release by Lactic Acid–stripped Basophils Sensitized with Sera from Five AD Patients**

Stimuli	Percent histamine release by basophils sensitized with:					
	Myeloma IgE	AD-1	AD-2	AD-3	AD-4	AD-5
Medium	7.0	2.0	6.0	5.0	11.3	2.0
SEA (100 ng/ml)	7.8	3.0	0.0	15.0	9.3	2.0
SEB (100 ng/ml)	6.0	1.0	1.5	24.0	10.7	24.0
TSST-1 (100 ng/ml)	5.5	3.0	0.0	37.0	41.0	2.0
Anti-IgE	79.9	24.0	17.0	20.0	88.0	18.0

* These data represent typical results from 5 of 12 patients with AD and/or HIE tested in the presence and absence of (100 ng/ml) SEA, SEB, or TSST-1. This analysis includes AD donors (1, 2) who lacked IgE antitoxin, and AD donor 3 with IgE anti-SEA, IgE anti-SEB, and IgE anti-TSST-1; AD donor 4 with IgE anti-TSST-1; and AD donor 5 with IgE anti-SEB.

We have found that removal of IgE from AD sera depletes the activity that mediates toxin-induced basophil histamine release. In the experiments shown in Table VI, we removed IgE from sera of patients with IgE anti-TSST by affinity chromatography over an anti-IgE column with a decrease in serum IgE level for patient M.E. from 30,000 to 269 IU/ml, and from 12,855 to 234 IU/ml for patient W.W. As shown in Table VI, before removal of IgE, sensitization of stripped basophils with sera from patient M.E. and W.W. resulted in significant levels of TSST-induced histamine release. However, sensitization of stripped basophils with IgE-depleted sera resulted in no TSST-induced histamine release. Taken together, these data indicate that toxin-induced histamine release from stripped basophils sensitized with AD sera containing IgE antitoxin involves the recognition of IgE on the basophil surface.

Discussion

It is well established that *S. aureus* can be an important trigger for the exacerbation of AD (10–14). The current study documents that *S. aureus* isolated from the skin of more than half of AD patients secrete identifiable toxins, primarily SEA, SEB, and TSST. Recent studies demonstrating that these staphylococcal toxins can act as superantigens (21) provide potential mechanisms by which *S. aureus* could mediate an inflammatory skin lesion that consists predominantly of activated T cells and monocytes (29). In particular, it has been shown that staphylococcal enterotoxins can engage HLA-Dr on macrophages to induce the release of cytokines and cause the selective stimulation of T cells expressing specific TCR Vβ gene segments.

This capacity of staphylococcal toxins to bind to MHC class II molecules or stimulate T cells bearing particular TCR Vβs provide several mechanisms by which *S. aureus* could exacerbate AD. First, staphylococcal exotoxins secreted at the skin surface could penetrate inflamed skin and engage HLA-Dr on epidermal macrophages or Langerhans cells to stimulate the production of IL-1 and TNF. IL-1 and TNF have potent proinflammatory properties that can contribute to skin inflammation. Indeed the skin lesion in AD and elicited late-phase allergic reactions are associated with the expression of IL-1 and TNF inducible vascular endothelial leukocyte adhesion molecules such as ICAM-1 and ELAM-1 (30, 31). Second, staphylococcal toxins could stimulate a high proportion of T cells via the variable region of the T cell receptor β chain to divide and produce cytokines that modulate tissue inflammation (21). Third, bacterial toxins can stimulate activated T cells via their surface HLA-DR (32) and thus, play an important role in maintaining the activation of T cells that infiltrate into the AD skin lesion.

The current study investigated an additional novel mechanism by which staphylococcal toxins could mediate skin in-

Table V. Toxin Induces Histamine Release, in a Dose-dependent Manner from Stripped Basophils Sensitized with Sera Containing IgE Antitoxin

Stimulus	Percent of histamine release by stripped basophils sensitized with			
	Medium	Myeloma IgE	AD Serum DM	Serum DAF
Medium	6.7	10.8	6.3	4.7
SEA (1 ng/ml)	—	—	8.4	16.7
SEA (10 ng/ml)	—	—	16.9	48.3
SEA (100 ng/ml)	—	—	33.3	40.5
SEA (1 μg/ml)	—	—	20.0	27.6
α IgE	4.3	79.7	73.9	78.8

Basophils from an atopic donor (nonresponsive to staphylococcal exotoxins) were stripped of IgE and passively sensitized with IgE myeloma protein or IgE from AD patients with IgE antistaphylococcal exotoxin antibodies. Basophils were then incubated with different concentrations of SEA.

Table VI. Percent Histamine Release by Lactic Acid–stripped Basophils Sensitized with AD Sera, before and after IgE Depletion

AD patient	Serum IgE depletion	Percent histamine release induced by		
		TSST-1*	TSST-1	Anti-IgE
		10 ng/ml	*100 ng/ml*	
WW	Before	31	34	67
WW	After	0	0	5
ME	Before	36	18	83
ME	After	0	0	0

* Percent of total histamine content minus spontaneous histamine release. Stripped basophils were sensitized with AD sera, before and after depletion of IgE from the sera by affinity chromatography over an anti-IgE column.

flammation in AD. We have found that the majority of AD patients have circulating IgE directed to staphylococcal exotoxins, particularly SEA, SEB, and TSST-1. Furthermore, their own basophils release histamine on exposure to the relevant exotoxin but not in response to exotoxins in which there is no IgE response. Although nearly 40% of patients with AD had *S. aureus,* which did not secrete an identifiable toxin, we cannot exclude the possibility that other, yet to be identified, staphylococcal toxins are involved.

These findings suggest the possibility that local production of exotoxin at the skin surface could cause IgE-dependent mast cell degranulation. This could have several important consequences. First, the acute release of histamine and other mediators could trigger the itch-scratch cycle which can exacerbate AD. More important, mast cell degranulation results in the local release of mediators, cytokines, and leukocyte chemotactic factors that result in late-phase inflammatory reactions (30, 31). Since patients with AD are colonized with *S. aureus,* the continuous release of exotoxins into the skin may promote the chronic inflammation found in AD.

The specificity of our current observations for AD is supported by the lack of IgE antitoxin in normal controls and patients with respiratory allergy. More important, patients with psoriasis and chronic *S. aureus* colonization did not have any detectable levels of IgE antistaphylococcal toxins in their sera. It is now well documented that patients with HIE syndrome, a syndrome characterized by chronic dermatitis and recurrent invasive *S. aureus* infections, e.g., pneumonia and deep-seated abscesses, mount an IgE response against the cell wall of *S. aureus* (15–18). In contrast, patients with AD do not produce IgE antibodies against the cell wall of *S. aureus* (18, 33). The divergent IgE response between these two diseases may be due to the fact that HIE syndrome is associated with deep-seated *S. aureus* infections. In contrast, AD is associated with superficial *S. aureus* skin infections and colonization. In the latter situation, only the secreted exotoxins would be expected to penetrate the skin. These data strongly suggest that patients with AD are exposed to *S. aureus* exotoxins in a clinically and immunologically significant manner and that these exotoxins may participate in several mechanisms to trigger AD.

Interestingly, there also appears to be a difference between these two diseases in their spectrum of IgE responses directed to staphylococcal toxins. The majority of AD patients produce IgE against either SEA, SEB, or TSST. In contrast, patients with HIE syndrome produce IgE against multiple staphylococcal toxins including SEC, SED, and ET. Although the basis of these observations are not clear, the differences in IgE responses between HIE syndrome and AD may reflect the increased frequency of different *S. aureus* infections in HIE syndrome as well the more invasive nature of these infections (34, 35).

In summary, our current data indicate that the majority of patients with AD and all patients with HIE syndrome mount an IgE response to staphylococcal exotoxins that can be grown from their skin. In particular, these toxins may exacerbate AD and perpetuate tissue inflammation by inducing degranulation of mast cells, basophils, Langerhans cells, and/or other Fcε-receptor bearing cells armed with the relevant IgE antitoxin. These data may also explain the clinical observation that many flares of eczema correlate with high colonization counts of *S. aureus* on the skin and that the skin rash frequently resolves when *S. aureus* is eradicated or drastically reduced following antibiotic therapy. New strategies for the treatment of this increasingly common skin disease may include ones that interfere with the production of toxin by *S. aureus.*

Acknowledgments

The authors wish to thank Maureen Plourd-Sandoval for her assistance in the preparation of this manuscript.

This work was supported in part by grants AR-41256, HL-37260, AI-24439, and RR-00052 from the National Institutes of Health.

References

1. Hanifin, J. M., and G. Rajka. 1980. Diagnostic features of atopic dermatitis. *Acta Dermato.-Venereol.* 92:44–47.

2. Leung, D. Y. M., A. R. Rhodes, and R. S. Geha. 1986. Atopic dermatitis. *In* Dermatology in General Medicine. T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freeberg, and K. F. Austen, editors. New York. 1385–1408.

3. Leung, D. Y. M., and R. S. Geha. 1986. Immunoregulatory abnormalities in atopic dermatitis. *Clin. Rev. Allergy.* 4:67–86.

4. Wittig, H. J., J. Belloit, and I. DeFillippi. 1980. Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. *J. Allergy Clin. Immunol.* 66:305–313.

5. Jones, H. E., J. C. Inouye, J. L. McGerity, and C. W. Lewis. 1975. Atopic disease and serum immunoglobulin E. *Br. J. Dermatol.* 92:17–25.

6. Hoffman, D. R., F. Y. Yamamoto, B. Geller, and Z. Haddad. 1975. Specific IgE antibodies in atopic eczema. *J. Allergy Clin. Immunol.* 55:256–267.

7. Sampson, H. A., and C. C. McCaskill. 1985. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J. Pediatr.* 107:669–675.

8. Sampson, H. A., and P. L. Jolie. 1984. Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. *N. Engl. J. Med.* 311:372–376.

9. Sampson, H. A. 1983. Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J. Allergy Clin. Immunol.* 71:473–480.

10. Van der Heijden, F., E. A. Wierenga, J. D. Bos, and J. L. Kapsenberg. 1991. High frequency of IL-4 producing $CD4^+$ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J. Invest. Dermatol.* 97:389–394.

11. Sager, N., A. Feldmann, G. Schilling, P. Kreitsch, and C. Neumann. 1992. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: frequency and lymphokine profile in the allergen patch test. *J. Allergy Clin. Immunol.* 89:801–810.

12. Leyden, J. E., R. R. Marples, and A. M. Kligman. 1974. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br. J. Dermatol.* 90:525–530.

13. Hauser, C., B. Wuethrich, L. Matter, J. Wilhelm, W. Sonnabend, and K. Schopfer. 1985. *Staphylococcus aureus* skin colonization in atopic dermatitis patients. *Dermatologica (Basel).* 170:35–39.

14. Aly, R., H. Maibach, and H. Shinefield. 1977. Microbial flora of atopic dermatitis. *Arch Dermatol.* 113:780–782.

15. Hanifin, J. M., and J. L. Rogge. 1977. Staphylococcal infections in patients with atopic dermatitis. *Arch. Dermatol.* 113:1383–1386.

16. Lever, R., K. Hadley, D. Downey, and R. Mackie. 1988. Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy. *Br. J. Dermatol.* 119:189–198.

17. Schopfer, K., K. Baerlocher, P. Price, U. Krech, P. G. Quie, and S. D. Douglas. 1979. Staphylococcal IgE antibodies, hyperimmunoglobulinemia E and *Staphylococcus aureus* infections. *N. Engl. J. Med.* 300:835–838.

18. Berger, M., C. H. Kirkpatrick, P. K. Goldsmity, and J. I. Gallin. 1980. IgE antibodies to *Staphylococcus aureus* and *Candida albicans* in patients with the syndrome of hyperimmunoglobulin E and recurrent infections. *J. Immunol.* 125:2437–2442.

19. Geha, R. S., E. L. Reinherz, D. Y. M. Leung, S. Scholossman, and F. S. Rosen. 1981. Suppressor T cell deficiency in the hyper IgE syndrome. *J. Clin. Invest.* 68:783–791.

21. Marrack, P., and J. Kappler. 1990. The staphylococcal enterotoxins and their relatives. *Science (Wash. DC).* 248:705–711.

22. Robbins, R., S. Gould, and M. Bergdoll. 1974. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. *Appl. Microbiol.* 28:946–950.

23. Freed, R. C., M. L. Evenson, R. F. Reiser, and M. S. Bergdoll. 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Appl. Environ. Microbiol.* 44:1349–1355.

24. Abramson, C. 1987. Immunoserology of Staphylococcal Disease. American Society of Microbiology, Washington, D.C. 1–5

25. Verburg, K. M., R. R. Bowsher, and D. P. Henry. 1983. A new radioenzymatic assay for histamine using purified histamine N-methyltransferase. *Life Sci.* 32:2866–2867.

26. Moore, J. E. I., and G. W. I. James. 1953. A simple direct method for absolute basophil leukocyte counts. *Proc. Soc. Exp. Biol. Med.* 82:601–603.

27. Pruzansky, J. J., L. C. Grammer, R. Patterson, and M. Roberts. 1983. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J. Immunol.* 131:1949–1953.

28. Levy, D. A., and A. G. Osler. 1966. Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen. *J. Immunol.* 97:203–212.

29. Leung, D. Y. M., A. K. Bhan, E. E. Schneeberger, and R. S. Geha. 1983. Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J. Allergy Clin. Immunol.* 71:47–56.

30. Leung, D. Y. M., R. S. Cotran, and J. S. Pober. 1991. Expression of an endothelial leukocyte adhesion molecule (ELAM-1) in elicited late phase allergic skin reactions. *J. Clin. Invest.* 87:1805–1810.

31. Groves, R. W., M. H. Allen, D. O. Haskard, and D. M. MacDonald. 1991. Endothelial leukocyte adhesion molecule-1 in acute and chronic eczema. *In* Immunological and Pharmacological Aspects of Atopic and Contact Eczema. Pharmacol. Skin. J. M. Czernielewski, editor. Karger Basel, Switzerland. 85–88.

32. Spertini, F., H. Spits, and R. S. Geha. 1991. Staphylococcal toxins deliver activation signals to human T-cells via major histocompatibility complex class II molecules. *Proc. Natl. Acad. Sci. USA.* 88:7533–7537.

33. Friedman, S. J., A. L. Schroeter, and H. A. Homburger. 1984. Whole organisms and purified cell walls compared as immunosorbents for the detection of IgE antibodies to *Staphylococcus aureus. J. Immunol. Methods.* 66:369–374.

34. Jozefczyk, Z. 1974. Specific human antibodies to entertoxins A, B, and C_1 of *Staphylococcus:* their increased synthesis in staphylococcal infection. *J. Infect. Dis.* 130:1–7.

35. Noleto, A. L. S., E. D. Cesar, and M. S. Bergdoll. 1986. Antibodies to staphylococcal enterotoxins and toxic shock syndrome toxin 1 in sera of patients and healthy people in Rio de Janeiro, Brazil. *J. Clin. Microbiol.* 24:809–811.