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Immunoglobulin E Anti-*Staphylococcus aureus* Antibodies in Atopic Patients

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Sera from 56 patients and normal adults were examined to quantitate total immunoglobulin E (IgE) and IgE antibodies to *Staphylococcus aureus* and *Staphylococcus epidermidis*. Patients were divided into six groups based on clinical symptoms; a seventh group consisted of normal adults. Anti-*S. aureus* IgE binding was significantly higher in three groups of patients (those with eczema, those with or without serious staphylococcal abscesses, and allergic patients with staphylococcal skin infections) than it was in the control group. Patients with high IgE due to allergies or parasitic infections without staphylococcal infections and patients with low or normal IgE and serious staphylococcal infection showed low levels of binding. The assay measured specific binding of IgE to bacterial antigens.

There have been several reports describing patients with chronic atopic dermatitis, hypergammaglobulinemia E, and recurrent abscesses involving *Staphylococcus aureus* (1, 3, 4, 6). The possible relationship between this syndrome and the more frequent staphylococcal disease associated with atopy is unknown (5). To define the spectrum of this syndrome more precisely, anti-*S. aureus* immunoglobulin E (IgE) antibodies were assayed by a modified radioallergoabsorbent test (6). This assay, which can be used to measure specific binding of serum antibodies to any bacterial strain, is a useful diagnostic tool.

MATERIALS AND METHODS

The patients studied were selected from the Dermatology, Pediatric Infectious Disease, and Allergy Clinics at the University of Minnesota Hospitals in Minneapolis. Patients were admitted to the study if they could be classified as experiencing one or more of the following: eczema—chronic or chronically relapsing, severely pruritic dermatitis occurring in characteristic body sites, depending on the age of the patient; allergy—asthma or rhinitis or both, in which the provoking antigen has been identified by history or provocative testing or both, and has been confirmed by an immediate positive wheal-flare reaction skin test; staphylococcal infection—one or more positive cultures for *S. aureus* taken from either a skin or a deep abscess, or history and clinical findings of recurrent staphylococcal abscesses. To study parasitic infection, sera from children (18 months to 18 years) with elevated IgE and multiple intestinal parasites, but otherwise healthy, were obtained from the Nestle Foundation Laboratory, Abidjan, Ivory Coast, West Africa.

Positive control sera were obtained from patients with hypergammaglobulinemia E and recurrent *S. aureus* abscesses (1, 3, 4, 6) and were provided by Paul Quie and Jon Abramsom. Control sera were collected from healthy, nonallergic, adult volunteers. All sera, including the pooled positive and pooled control sera, were divided into 0.5-ml portions and stored at −20°C. Each sample was thawed only once. The patients and controls were placed in one of seven groups for data analysis. These groups were defined as follows: (i) eczema, recurrent staphylococcal abscesses (1, 3, 5); (ii) eczema, recurrent staphylococcal skin infection without abscesses; (iii) allergic, staphylococcal skin infection; (iv) allergic, no staphylococcal infection; (v) parasitic infections, no staphylococcal infection; (vi) nonallergic, recurrent staphylococcal infection; and (vii) control: nonallergic, no staphylococcal infection.

IgE quantitation. Total IgE levels were determined by the paper radioimmunosorbent test (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) (2). Serum levels greater than 300 IU/ml were considered elevated, as previously determined with a randomly selected nonallergic Minnesota ppopulation (unpublished data).

Preparation of staphylococcal antigen. *S. aureus* Wood 46 and *Staphylococcus epidermidis* U1219 and 555b were used. The Wood 46 strain lacks protein A and thus will not bind immunoglobulins via Fc receptors. The bacteria were grown for 18 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C with agitation. The organisms were harvested by centrifugation, washed, diluted to a 15% (vol/vol) suspension in phosphate-buffered saline (pH 7.4) containing 0.1% sodium azide, and used within 24 h.

Antistaphylococcal IgE assay. One milliliter of the bacterial suspension containing approximately 10^{10} organisms was centrifuged at 900 × *g* for 10 min, and the pellet was incubated with 50 μl of serum for 3 h at room temperature with constant stirring. After it was washed, the pellet was mixed with 50 μl of ^{125}I-labeled (6.25 μCi/μg of antibody protein) rabbit antihuman IgE (Pharmacia) overnight at room temperature with constant stirring. The pellet was washed twice with phosphate-buffered saline and was counted in a Beckman gamma 4000 counter (Beckman Instruments, Inc., Fullerton, Calif.). All samples were tested in duplicate. Values were expressed as percentages of total counts added.

RESULTS

Initial studies were performed to assess the reproducibility of the assay (Table 1). Pooled positive and normal control sera were tested in duplicate over a period of several weeks. Once 99% confidence levels for the assay were established, any tests on either the positive or the control sera which gave results not within this confidence range were repeated. The data for the seven groups are presented in Fig. 1 and Table 2.

Groups 1 through 3 had elevated levels of specific *S. aureus* binding. Ninety-seven percent of these values had greater than 15% specific binding, whereas 100% of the binding of groups 4 through 7 were below this value. Group 1 had the highest level of specific binding (34%). Groups 2 and 3 had lower levels of binding (27 and 25%, respectively). No statistical differences between mean values from groups 1 through 3 or from groups 4 through 7 were observed ($P > 0.5$). The mean value for sera in groups 1 through 3 was significantly higher than that for sera in groups 4 through 7 ($P > 0.001$).

There was no significant correlation between total IgE levels and specific binding in any group. With the exception of group 1, the percent binding of all groups to *S. epidermidis* was similar to that of the control group. The percent binding of group 1 to *S. epidermidis* was slightly elevated, but was consistently less than the percent binding to *S. aureus*. The contribution of *S. epidermidis* infection in this group has not been established.

TABLE 1. *Reproducibility of anti-S. aureus IgE assay*[a]

Serum	*n*[b]	Mean ± SD[c]	Confidence limits (%)
Positive	8	30 ± 4	95 ± 5, 99 ± 8
Normal	6	5 ± 1.5	95 ± 1, 99 ± 2

[a] Antistaphylococcal IgE as a percentage of total activity added.
[b] *N*, Number of duplicate determinations of pooled sera.
[c] SD, Standard deviation.

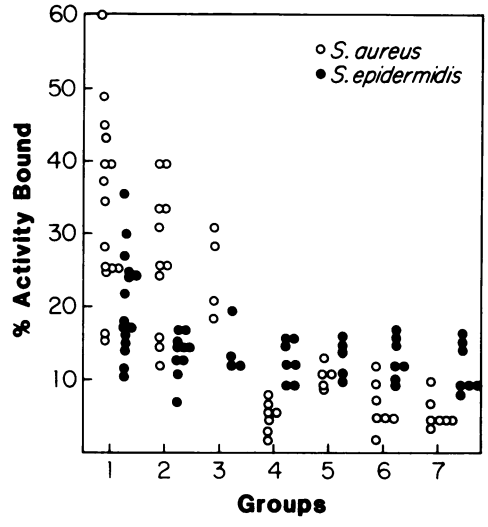

FIG. 1. *Percent binding of antistaphylococcal IgE in subpopulations. Groups: (1) eczema, recurrent staphylococcal abscesses; (2) eczema, recurrent staphylococcal skin infection, no abscesses; (3) allergic, staphylococcal skin infection; (4) allergic, no staphylococcal infection; (5) parasitic infections, no staphylococcal infection; (6) nonallergic, recurrent staphylococcal infection; (7) control: nonallergic, no staphylococcal infection.*

DISCUSSION

By using more comprehensive criteria for classification of atopic and allergic patients, we modified and extended the use of the solid-phase radioallergoabsorbent test to measure specific IgE binding to *S. aureus* (6). The method was found to be reproducible, accurate, and specific. In preliminary experiments, the amount of binding of ^{125}I-labeled anti-IgE remained constant for bacterial suspensions, ranging between 10^6 and 10^{14} organisms per ml. At 10^{10} organisms per ml, decreases in serum concentration resulted in a dose-dependent decrease in binding of anti-IgE.

Elevated antibodies to *S. aureus* were found in patients with eczema, hyperimmunoglobulinemia E, and severe abscesses, patients with eczema, hyperimmunoglobulinemia E, and superficial skin infections, and patients with rhinitis, asthma, and staphylococcal skin infections. Increased anti-*S. aureus* antibodies were observed in allergic patients only when accompanied by staphylococcal infections. Elevated total IgE levels are not directly associated with an increase in antistaphylococcal IgE in the absence of staphylococcal infection. Increased binding to *S. epidermidis* was observed only in group 1.

TABLE 2. *Total and specific IgE levels in subpopulations*

Group[a]	No. of patients	Total serum IgE[b]		Anti-*S. aureus* IgE[c]		Anti-*S. epidermidis* IgE[c]	
		Range	Means ± SD[d]	Range (%)	Mean ± SD	Range (%)	Mean ± SD
1	15	400–1,200	3,985 ± 4,066	16–60	34 ± 12	11–36	20 ± 7
2	11[e]	560–14,000	6,493 ± 186	12–40	27 ± 10	7–17	14 ± 3
3	4	306–720	434 ± 186	18–31	25 ± 6	12–19	14 ± 3
4	7	320–169	497 ± 169	2–8	5 ± 2	9–16	13 ± 3
5	5	320–2,000	864 ± 720	9–13	11 ± 1	10–16	13 ± 3
6	7	6–101	46 ± 33	2–12	6 ± 3	12–17	14 ± 3
7	7	5–145	51 ± 63	4–12	6 ± 2	8–16	11 ± 3

[a] Groups defined as in the legend to Fig. 1.
[b] Total serum IgE in international units per milliliter.
[c] Antistaphylococcal IgE as a percentage of total activity added.
[d] SD, Standard deviation.
[e] Number of patients tested for *S. epidermidis* = 10.

Whether this binding is the consequence of colonization with *S. epidermidis* or antigenic cross-reactivity is unknown. Studies of the binding of antistaphylococcal IgE antibodies to different strains of *Staphylococcus* sp. and cell wall components suggest that the binding site may be related to interpeptide bridges in peptidoglycan (7).

This assay offers a method for evaluation of patients with recurrent staphylococcal infections. The etiology and regulation of the hyperimmunoglobulinemia E response are unknown. The possibility of a genetic defect resulting in a clonal expansion of IgE-producing lymphocytes requires further investigation. This assay may have potential value for the detection of heterozygous carriers.

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