

Anti-*Staphylococcus aureus*-specific IgE in atopic dermatitis

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Serum samples from 60 adults and 64 children with atopic dermatitis were tested for antistaphylococcal IgE antibodies with RAST discs coupled to cellular proteins from Wood 46 strain S. aureus. Anti-S. aureus IgE antibodies were detected in 19 (29.6%) of the children and 14 (23.3%) of the adult patients. Anti-S. aureus IgE-positive adults had more severe and prolonged disease than those who were negative. Two groups of children comprising 10 who were anti-S. aureus IgE positive and 10 who were negative were compared. Children with anti-S. aureus IgE antibodies had more severe and more extensive disease ($p < 0.05$), a greater prevalence of cutaneous S. aureus infections ($p < 0.05$), higher mean total serum IgE level ($p < 0.05$), a greater prevalence of specific IgE responses to food allergens ($p < 0.05$), and a higher percentage of helper T cells ($p < 0.05$) than children who were negative for these antibodies. (J ALLERGY CLIN IMMUNOL 78:583-9, 1986.)

Certain bacteria, *S. aureus* in particular, are believed to play a role in the clinical expression of atopic dermatitis.¹

S. aureus may contribute to the course of atopic dermatitis by its ability to invade areas of active inflammation and produce suppuration. In addition, *S. aureus* may play a basic pathophysiologic role inducing or sustaining mediator release from mast cells and macrophages via an IgE-mediated reaction. Specific anti-*S. aureus* IgE antibodies have previously been reported in atopic dermatitis²⁻⁴ and in patients with the hyper-IgE syndrome.^{4,6} Although reports of the prevalence of anti-*S. aureus* IgE antibodies on atopic dermatitis have been high in some studies,^{6,7} a low prevalence has been reported in other studies⁸ in which different solid-phase immunosorbents were used to detect specific anti-*S. aureus* IgE antibodies. By use of a RAST system in which cellular proteins of Wood 46 strain *S. aureus* were used as the immunosorbent, we have determined the prevalence of anti-*S. aureus*

Abbreviations used

OKT3: Total T cells (Ortho diagnostics)
OKT4: Helper T cells (Ortho diagnostics)
OKT8: Suppressor T cells (Ortho diagnostics)

IgE antibodies in 64 children and 60 adults with atopic dermatitis and investigated whether or not the presence of anti-*S. aureus* IgE antibodies held any clinical significance.

METHODS AND PATIENTS

S. aureus RAST

The study was approved by the Ethical and Research Committees of the University of Cape Town Medical School, and of the Red Cross War Memorial Children's Hospital. Informed consent was obtained from all adult patients participating in the study and from the parents of all children in the study.

The *S. aureus* RAST was performed with RAST discs to which cellular proteins from Wood strain 46 *S. aureus* had been coupled by Dr. Hasse Schröder of Pharmacia, Uppsala, Sweden. In brief, the method used to prepare these discs involved sonication of the cultured organisms, removal of the cell walls and nuclear material by centrifugation ($4000 \times g$ for 25 minutes), and coupling of the proteins in the supernatant solution to CNBr-activated paper discs.

A standard RAST procedure^{9,10} was used to measure specific IgE antibodies in 50 μ l samples of patients' sera. RAST classes were defined according to a standard curve obtained with Birch reference sera ABC and D (Pharmacia)

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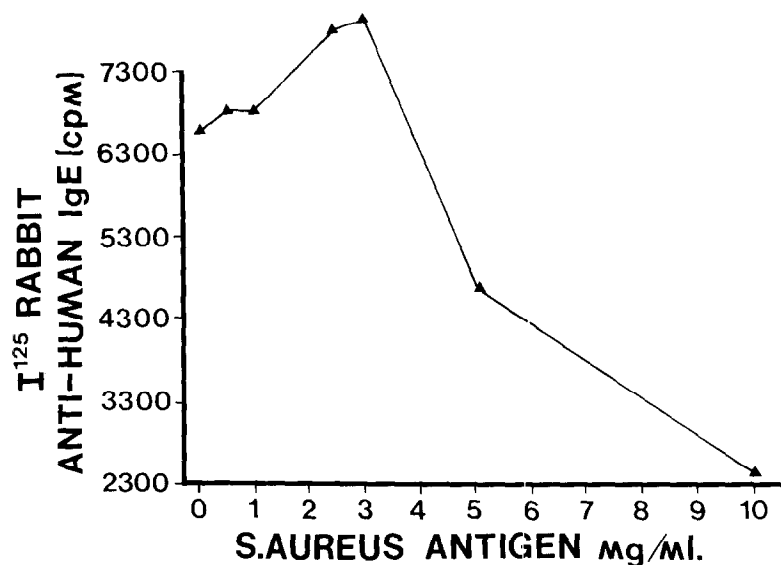


FIG. 1. Saturable binding of patient's serum to *S. aureus* RAST discs according to the method of Friedman et al.¹¹

TABLE I. The incidence of positive and negative anti-*S. aureus* IgE RAST in adult patients

	Positive <i>S. aureus</i> RAST	Negative <i>S. aureus</i> RAST
Extensive eczema (arms, legs, trunk, and head) since childhood	12 (85.7%)	9 (19.5%)
Mild, moderate, or localized eczema	2 (14.2%)	37 (80.4%)
Total	14	46

and Birch reference discs. A RAST class ≥ 1 was considered positive. RAST classes obtained on individual sera by use of this procedure were completely reproducible on different runs.

Specificity of the anti-*S. aureus* RAST was established by demonstrating saturable binding and by demonstrating that binding of patient IgE could be inhibited by the presence of soluble Wood strain 46 cellular proteins prepared as above. The inhibitor assays were performed by mixing 40 μ l samples of patient serum with 10 μ l volumes of a solution of *S. aureus* varying in concentration from 1 to 10 mg/ml of protein in phosphate-buffered saline. The mixtures were incubated on a Braun shaker (Infors AG, Basel, Switzerland) for 3 hours, and the *S. aureus* RAST procedure was then performed. The results are presented in Fig. 1.

Saturable binding of the RAST discs was demonstrated according to the method of Friedman et al.¹¹ in which increasing volumes of patients' sera were incubated with the RAST discs. The results are presented in Fig. 2.

Wood stain 46 *S. aureus* was selected because it is known to contain only trivial amounts of protein A in its cell membrane⁵ and therefore does not bind immunoglobulin nonspecifically. This fact was confirmed by assays performed at Pharmacia in which the *S. aureus* antigen preparation was tested before and after IgG affinity purification in the RAST procedure. The anti-*S. aureus* RAST was reproducible up to 1 year after testing individual patient's serum. Patients' RAST scores ranged between class 1 and class 3. No patient scored class 4 according to the Birch reference standard used.

Patients

Serum samples for 60 adults and 64 children with atopic dermatitis were assayed for anti-*S. aureus* antibodies. The history and the findings on physical examination were used to assess the relationship between the *S. aureus* RAST results and the duration, severity, or extent of the eczema in adult patients. The results (Table I) demonstrated a significant correlation between anti-*S. aureus* IgE RAST positivity and severity and duration of the disease in adults.

From the 64 children with atopic dermatitis, the first 10 who were anti-*S. aureus* RAST positive were chosen for comparison, in clinical and laboratory detail, with the first 10 who were *S. aureus* RAST negative. None were receiving antibiotics at the time of the study, and all were free of parasitic infestation as judged by fecal examination.

The severity of the disease in these children was graded by allocating a score to the following well-recognized features of atopic dermatitis: erythema, edema, pruritus (day and night), vesiculation, crusting, excoriation, scaling, lichenification, pigmentation, and loss of sleep caused by itching and scratching. The scores ranged from 0 to 4 for each feature according to the scale 0 = absent; 1 = slight; 2 = moderate; 3 = marked; 4 = severe. The extent of the

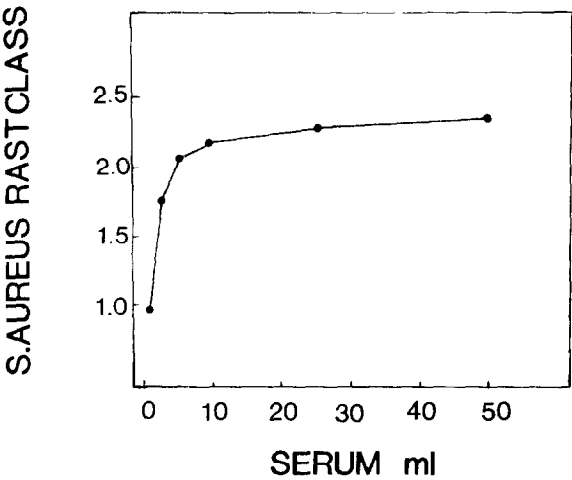


FIG. 2. Specific inhibition of IgE binding to *S. aureus* RAST discs by free *S. aureus* antigen.

disease was expressed as a percentage of the surface area by use of the National Institute for Burn Medicine (United States) charts.¹² The patients were also examined for the presence or absence of cutaneous pustules. Weight and height, age of onset of atopic dermatitis, and the presence or absence of any coexisting respiratory tract allergic condition were recorded.

Other laboratory investigations

Total serum IgE levels. The Phadebas IgE PRIST technique (Pharmacia) was used to measure total serum IgE levels.

RAST for common allergens. Commercially available RAST discs (Pharmacia) were used to detect specific IgE directed against *Dermatophagoides pteronyssinus*, Bermuda grass, *Alternaria*, cow's milk, egg white, wheat, and maize.

Immunologic investigations. Lymphocyte transformation to phytohemagglutinin, lymphocyte subset analysis (OKT3, OKT4, OKT8), immunoglobulin levels (IgA, IgM, IgG), total hemolytic complement, nitroblue tetrazolium test, full blood count, and differential white blood counts were performed on both groups.

Bacteriologic studies. Swabs were taken for culture from the pustules, areas of active dermatitis, and normal unaffected skin in all patients. The anterior nares and the external auditory canals were also swabbed. The swabs obtained were cultured on McConkey agar (Oxoid Ltd., Basingstoke, Hants, England) and 2% and 4% blood agar plates (Biolab Chemicals, Verwoerdburg, S. Africa). Repeat bacteriologic cultures were performed 1 month after the initial assessment.

Statistical methods. The two-tailed t test and Fisher exact probability tests were used to analyze the results.¹³

RESULTS

Prevalence of anti-*S. aureus* IgE

Anti-*S. aureus* IgE antibodies were detected in 19 of the 64 children (29.6%) and in 14 of the 60 adults

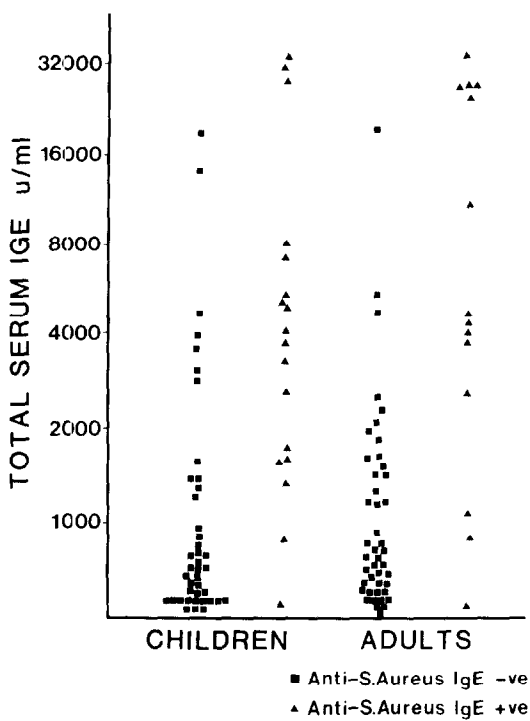


FIG. 3. Total serum IgE levels in children with eczema and in adults with or without anti-*S. aureus*-specific IgE antibodies.

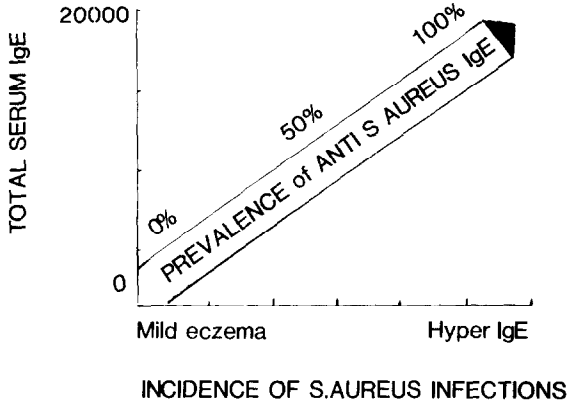


FIG. 4. Schematic representation of the relationship between *S. aureus* infection, total serum IgE, and anti-*S. aureus* IgE.

(23.3%) who had atopic dermatitis. The type of eczema encountered in the adult patients with or without a positive anti-*S. aureus* RAST result is presented in Table I.

Total serum IgE levels of adults and children with and without anti-*S. aureus* positive RAST are presented in Fig. 3. The levels of total serum IgE in patients positive for anti-*S. aureus* RAST ranged from 200 to 30,000 U/ml. The eight patients who had total

TABLE II. Clinical assessment in 20 children with atopic dermatitis

	Anti- <i>S. aureus</i> IgE positive	Anti- <i>S. aureus</i> IgE negative	<i>p</i> Value
Age (mo)	71.8 (47)	67.6 (43.8)	NS
Duration of disease (mo)	64.2 (26.1)	56.8 (28.9)	NS
Sex			
Male	4	10	
Female	6	0	
Severity of disease (scored)			
1 to 10	1	2	
11 to 20	4	8	
21 to 30	5	0	<0.05
Extent of disease (% of body surface area)			
1% to 10%	0	3	
11% to 25%	4	7	
26% to 50%	2	0	
50%	4	0	<0.05
Cutaneous pustules	8	3	<0.05

NS = not significant.

serum IgE levels >20,000 U/ml had positive anti-*S. aureus* RAST. No patient with a serum IgE level of <200 U/ml had a positive anti-*S. aureus* RAST.

Assessment of children with and without anti-*S. aureus* IgE antibodies

Clinical. Children with anti-*S. aureus* IgE antibodies had more severe and more extensive disease than those without IgE antibodies (Table II).

Furthermore, significantly more patients from this group had cutaneous pustules from which *S. aureus* was cultured ($p = <0.05$), both at the initial assessment and at the 1-month follow-up assessment. The mean age of patients with anti-*S. aureus* positive RAST was 6 years, and 5 years and 8 months for negative RAST.

There was no significant difference between the groups with regard to duration of disease ($p > 0.05$), coexisting respiratory allergic disorders ($p > 0.05$), or weight and height percentiles ($p > 0.05$).

Total serum IgE. The geometric means of the total serum IgE concentration in the children with and without anti-*S. aureus* IgE antibodies were 8722 U/ml and 863 U/ml, respectively ($p = <0.05$).

RAST for common allergen. Significant differences in specific IgE responses between the two groups were found for cow's milk protein ($p < 0.05$), egg white ($p < 0.05$), wheat ($p < 0.05$), and maize ($p < 0.05$). No differences were found for house dust mite, Bermuda grass, or for *Alternaria* ($p > 0.05$) (Table III).

Immunologic investigations. Lymphocyte prolif-

erative responses to phytohemagglutinin were normal in both groups. OKT4 numbers were higher in the group with positive RAST for anti-*S. aureus* IgE ($p < 0.05$), but no significant difference was found in suppressor (OKT8) T cell numbers ($p > 0.05$) (Table IV) and immunoglobulin levels ($p > 0.05$) between groups. Serum hemolytic complement activity was normal in both groups. The generation of hydrogen peroxide by the nitroblue tetrazolium test was normal in both groups. Full blood count and differential white blood counts demonstrated no differences between the two groups.

Bacteriologic studies. *S. aureus* was isolated from all the pustular lesions. Pustular lesions were found in eight of anti-*S. aureus* IgE positive children and three of anti-*S. aureus* IgE negative children ($p = <0.05$). There were no significant differences between the numbers of *S. aureus* isolates from affected areas of the skin and unaffected areas of the skin nor between isolates obtained from the auditory canals of children in both groups. *S. aureus* was cultured from the anterior nares in seven of the children with positive anti-*S. aureus* RAST compared with only three children who were *S. aureus* IgE negative ($p = >0.05$) (Table V).

DISCUSSION

The prevalence of anti-*S. aureus* antibodies

Several authors have attempted to assess the prevalence of the presence of specific IgE antibodies to staphylococcal proteins in atopic dermatitis.^{2, 4-6}

Abramson et al.,⁵ by use of bacterial pellets as the

TABLE III. Positive RAST: Common inhalant and ingestant allergens in 20 children with atopic dermatitis

Allergen	Anti- <i>S. aureus</i> IgE positive	Anti- <i>S. aureus</i> IgE positive	<i>p</i> Value
<i>D. pteronyssinis</i>	10 (0)	9 (1)	NS
Bermuda grass	10 (0)	7 (3)	NS
<i>Alternaria</i>	7 (3)	5 (5)	NS
Cow's milk protein	8 (2)	2 (8)	<0.05
Egg white	9 (1)	1 (9)	<0.05
Wheat	10 (0)	4 (6)	<0.05
Maize	10 (0)	5 (5)	<0.05

Figures in parentheses indicate *negative* rast; positive rast = 1+ or higher.

solid-phase antigen, detected anti-*S. aureus* IgE in patients with atopic dermatitis with a high serum IgE in the absence of serious *S. aureus* infection.

Hauser et al.⁷ found that 10 of 21 patients with atopic dermatitis had IgE directed against purified *S. aureus* cell wall and protoplast antigens and that there was a positive association between elevated anti-*S. aureus* IgE and regional lymphadenopathy. Schopfer et al.⁹ reported specific anti-*S. aureus* IgE antibodies against cell wall proteins of *S. aureus* but not of *S. capitis*, *S. sciuri*, *S. simulans*, *S. xylosus*, or strains of RBII or Armour. Recently, Friedman et al.⁸ found that only three of 25 patients with atopic dermatitis and superimposed impetiginization and none of 44 patients who had no impetiginized lesions had anti-*S. aureus* IgE to purified *S. aureus* cell wall antigens but that 60% of the patients with atopic dermatitis with impetiginized lesions had anti-*S. aureus* IgE when whole organisms were used as the immunosorbent.

Thus, there is a discrepancy in the reported prevalence of anti-*S. aureus* IgE antibodies in atopic dermatitis ranging from 12% to 60%, depending on the antigen used as the immunosorbent.

A partial explanation for such a wide discrepancy is that the use of whole organisms as immunosorbent produces high levels of nonspecific binding,¹¹ and thus, purified cell walls have been favored as the immunosorbent.⁸ The use of cell walls alone would preclude the detection of specific IgE antibodies to other *S. aureus* cellular proteins.

We have used a RAST system in which the supernatant proteins of sonicated *S. aureus* cells are coupled to CNBr-activated paper discs. This system produces saturable binding and is inhibitable by free antigen. RAST is simple to perform, highly reproducible, and applicable to the study of large numbers of patients. With this system, approximately 30% of patients with atopic dermatitis make IgE antibodies to staphylococcal cellular proteins other than protein A, which

TABLE IV. T lymphocyte subsets in 20 children with atopic dermatitis

Subset (%)	Anti- <i>S. aureus</i> IgE positive (n = 10)	Anti- <i>S. aureus</i> IgE negative (n = 10)	<i>p</i> Value
	Mean $\pm$ 1 SD	Mean $\pm$ 1 SD	
OKT3	57 (12.08)	56.7 (14.62)	NS
OKT4	30.7 (12.53)	20.2 (8.96)	<0.05
OKT8	11.3 (4.64)	11.4 (4.32)	NS
4/8 Ratio	3.32 (2.08)	1.92 (1.09)	NS

NS = not significant.

is secreted by *S. aureus*. Less than 12% of the patients of Friedman et al.⁸ made specific IgE to cell wall proteins. Antibody to noncell wall proteins may be more important, since antibodies to these proteins correlated with the severity of the atopic dermatitis. Although all patients with total IgE levels >16,000 IU had anti-*S. aureus* IgE antibodies, we encountered patients with total serum IgE as high as 15,000 units without any detectable anti-*S. aureus* IgE.

Clinical significance of anti-*S. aureus* IgE antibodies

The relationship between anti-*S. aureus* IgE antibodies and clinical, immunologic, and bacteriologic aspects of atopic dermatitis is unclear. Walsh et al.² observed that these antibodies were found to be elevated in patients with atopic dermatitis only in association with *S. aureus* infections. Abramson et al.⁵ reported that these antibodies may be elevated in patients with atopic dermatitis with or without significant *S. aureus* infections. In this study the group of patients with anti-*S. aureus* IgE antibodies had a higher prevalence of superficial pustules. The 70% nasal carriage of *S. aureus* in the group with anti-*S. aureus* IgE antibodies was higher than reported in normal subjects

TABLE V. Cutaneous infection and carriage of *S. aureus* in 20 children with atopic dermatitis

	Anti- <i>S. aureus</i> IgE positive (n = 10) (%)	Anti- <i>S. aureus</i> IgE negative (n = 10) (%)	p Value (Fisher exact)
Cutaneous pustules	80	30	<0.05
<i>S. aureus</i> isolates			
Eczematous lesion	100	80	NS
Skin unaffected by eczema	50	20	NS
Anterior nares	70	30	NS
External auditory canal	70	40	NS

NS = not significant.

(30% to 40%).¹⁴ This may be a contributing factor to the high prevalence of skin sepsis in these patients. The eradication of *S. aureus* from the nasal reservoir and eczematous skin would appear to be a logical step in the treatment of these patients. Our results also indicate that patients with atopic dermatitis who have developed these antibodies tend to have more severe disease. Adult patients who had anti-*S. aureus* IgE invariably had a history of long-standing severe disease since childhood. It is likely that the presence of anti-*S. aureus* IgE antibodies may predict a more chronic course and less favorable outlook for patients with atopic dermatitis. *S. aureus* may act as a flare factor in atopic dermatitis by initiating the itch-scratch-excoriation sequence via an IgE-mediated reaction. Once the skin has been breached by scratching, the natural cutaneous barriers are disrupted, *S. aureus* invades the skin, and overt infection follows. A self-perpetuating mechanism could then be set up with further sensitization via IgE receptors on mast cells or macrophages. The consequence of this is further mediator release, cellular recruitment, and chronic inflammation. The total serum IgE levels in patients with anti-*S. aureus* IgE antibodies were generally extremely high. This probably reflected the severe and extensive dermatitis, as well as the high prevalence of IgE antibodies to food allergens. The difference in IgE levels between the groups could not be attributed to OKT8 dysfunction since no significant difference was found for OKT8 function between the groups ($p > 0.05$). The finding of increased OKT4 numbers in the group with anti-*S. aureus* IgE antibodies (Table IV) was unexpected, and its significance remains speculative.

Our findings suggest that patients with atopic dermatitis with anti-*S. aureus* IgE antibodies may constitute a subgroup. Patients who make specific anti-*S. aureus* IgE may have atopic dermatitis with associated *S. aureus* skin sepsis or may have the more severe hyper-IgE syndrome in which deep abscesses

occur, possibly because of the associated chemotactic defects in this disorder (Fig. 4).

Why certain patients with chronic atopic dermatitis produce anti-*S. aureus*-specific IgE antibodies, whereas other patients do not, is not clear. Friedman et al.⁸ suggested that IgE antibodies against *S. aureus* are simply an epiphenomenon concurrent with the development of *S. aureus* infections.

This possibility is not an unreasonable one because the patients with specific anti-*S. aureus* IgE antibodies were also those with a higher incidence of skin sepsis in this study. However, even if the initial induction of anti-*S. aureus* antibodies is an epiphenomenon, the possibility that *S. aureus* may subsequently initiate and sustain mast cell activation or macrophage degranulation via an IgE mechanism remains a viable hypothesis. If this hypothesis has validity, antibacterial therapy used to control flare-ups of active dermatitis in patients with eczema with specific anti-*S. aureus* IgE antibodies may be an important adjunct to therapy.

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