

Staphylococcal and human dander IgE antibodies in superinfected atopic dermatitis

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Summary

Ten patients (Group 1) with *Staphylococcus aureus* superinfected atopic dermatitis (SAD) were compared with three control groups without SAD. One control group (Group 2) was comprised of ten atopic patients with total IgE levels of > 1000 u/ml, another control group, of six atopic patients (Group 3) with total IgE levels of < 1000 u/ml and a third group, of ten non-atopics (Group 4), five of whom were infected with *Staphylococcus aureus*. Total and specific anti-staphylococcal IgE levels and anti-human dander IgE were high in Group 1. Specific IgE levels to *S. aureus* were slightly increased in some Group 2 patients but were normal (< 7%) for Group 3. No specific IgE to human dander antigen was detected in either Group 2 or Group 3.

In the non-atopic control group (Group 4), six out of ten patients had both low total, and specific IgE antibodies to both staphylococcal, and human dander antigens. Two had increased total IgE and specific anti-staphylococcal IgE antibodies, and two more with a severe, chronic infection, also had anti-staphylococcal IgE antibodies.

In SAD patients, a good correlation was seen between cutaneous basophil hypersensitivity and high specific IgE antibodies against staphylococcal and human dander allergens. Delayed type hypersensitivity reactions to staphylococcal antigen were negative or poorly positive in the SAD group by comparison to controls.

A paradoxical anaphylactic type of response to the offending bacterial agent in SAD patients, associated with 'auto-atopy' to human dander, is discussed.

Introduction

Many authors have described an association between high IgE levels, recurrent skin infection and defective cell-mediated immunity in superinfected atopic dermatitis (SAD) (Buckley, Wray & Belmaker, 1972; Gottlieb & Hanifin, 1974; Hill & Quie, 1974; McGeady & Buckley, 1974),

Recently, we have shown that patients with SAD, infected with *St. aureus* and without respiratory symptoms had little or no cutaneous response to intracutaneous injections of staphylococcal antigen after 48 hr. Moreover, biopsy specimens of skin tests showed a prevalence of basophilic cells, intensely degranulating among the histio-lymphocytic perivascular infiltrates of the usual delayed type. Biopsy specimens in diseased eczematous skin also showed numerous basophilic cells in a permanent degranulating process, indicating an unusual 'releasability', as if a self-perpetuating antigen stimulus was constantly present (Hénocq & Gaillard, 1981). Several authors have associated the human dander allergen with the atopic condition (Garcelon, Oppenheim & Hénocq, 1977; Hampton & Cooke, 1941; Hénocq, Bazin & Girard, 1966; Keller, 1925; Simon, 1948; Storm van Leewen & Van Niekerk, 1929).

In the present study we have measured the staphylococcal (Schopfer *et al.*, 1979) and human dander (Berrens & Guikers, 1980) IgE antibodies by radioimmunoassay in patients with SAD, comparing the results with those of non-atopic patients with staphylococcal infections and atopic patients with above normal and low total IgE concentrations, but without SAD.

Materials and methods

Patients

Group 1. This group consisted of ten patients with SAD, determined by bacteriological examination, and IgE levels 280 u/ml to >2000 u/ml (PRIST). They presented skin lesions with a generalized, maculopapular, lichenified, pruritic skin-rash. Areas of the skin had erythema and papulo-vesicular changes; adenopathies were associated with areas of exoserosis with superinfection.

Group 2. This group consisted of ten serum samples from atopic patients without SAD and with total IgE >1000 u/ml.

Group 3. This group consisted of six serum samples from atopic patients without SAD and with total IgE of <1000 u/ml.

Group 4. This group consisted of ten non-atopic patients, eight of whom had low IgE levels of 0.5–250 u/ml. Five were suffering from chronic staphylococcal infection, two had a non-identifiable, chronic infection and one had chronic urticaria. The remaining two patients had high IgE levels (1500 and 2000 u/ml) but without atopic dermatitis; one had asthma and the other, chronic urticaria.

Skin tests

These were performed in patients of Groups 1 and 4. Groups 2 and 3 were only used to compare specific serum IgE levels.

St. aureus was tested intracutaneously on the volar surface of the forearm with the following suspension: heat-killed staphylococci, 9×10^9 organisms/ml; (ethylmercurithio)-2-sodium benzoate, 0.15 mg maximum; purified staphylococcal toxoid (codex) q.s.p., 1.5 ml (Vaccin 'Divasta', Institut Pasteur, Paris). The volume injected was 0.1 ml adjusted to a concentration of 10^9 organisms/ml.

Human dander allergen (a commercial preparation, a gift from HAL Laboratories, Haarlem, The Netherlands) was also tested intracutaneously on the volar surface of the forearm at 0.1, 0.01 and 0.001% w/v concentration.

Skin tests were read after 20 min and 48 hr.

Histologic examinations

Skin biopsy specimens were fixed in Bouin and embedded in paraffin. For each specimen, tissue sections were stained (a) with haemalum-eosin-safron, (b) with a special stain for basophil granules composed of a nuclear stain (basic fuchsin) and a cytoplasmic stain (Azur blue II) which stains basophilic granules dark blue (Gaillard & Ravisse, 1979).

Staphylococcal and human dander IgE antibodies

St. aureus. A heat-killed mixture of staphylococcus coagulase + bacteria 2×10^9 organisms/ml (Stallergenes Lab. Ltd.).

Human dander allergen. 30% (w/v) aqueous extract (HAL Laboratories).

Insolubilized allergens

St. aureus. 10^{10} organisms suspended in 5 ml 0.1 M NaHCO_3 buffer containing 0.5 M NaCl were rotated overnight with 1 g cyanogen bromide activated Sepharose 4B (Pharmacia Ltd.) at 4 °C. Unbound allergen was removed by washing the gel three times in 5 ml 0.1 M NaHCO_3 /0.5 M NaCl. Uncoupled sites were reacted with 1 M ethanolamine for 1 hr at room temperature. The gel was then washed four times in 5 ml 0.1 M acetate buffer (pH 4.0) and once in 5 ml 0.1 M borate buffer (pH 8.0). Finally, the gel was suspended in 4 ml phosphate-buffered saline. 100 μl aliquots of this suspension were used in the RAST.

Human dander. 1 ml 30% extract was rotated with sixty cyanogen bromide activated paper discs (Brighton, Lampard & Fordham, 1975) suspended in 3 ml 0.1 M NaHCO_3 buffer overnight at 4 °C. Unbound allergen was removed by washing the discs in 0.1 M NaHCO_3 . Uncoupled sites were reacted with epichlorhydrin-dipropylene-triamine polymer (EPA 33). 50 μl per disc, for 1 hr at room temperature. The discs were then washed four times in 0.1 M acetate buffer and two times in phosphate-buffered saline. The discs were then dried and stored in airtight containers at -25 °C, ready for use.

RAST. The modified technique of Tee (1980) and Schopfer *et al.* (1979) was used, in which 50 μl of undiluted serum was incubated with insolubilized allergen for 3 hr at room temperature. Unbound serum was removed by washing four times in 300 μl phosphate-buffered saline containing 1% Tween 20. 50 μl ^{125}I rabbit anti-human IgE, D ϵ 2 specific (Pharmacia Ltd.) was then added to the insolubilized allergen and incubated at room temperature overnight. Unbound antiserum was removed by washing five times in 300 μl buffered saline/Tween 20 (1%). All assays were performed in triplicate and in parallel, with a reference in which 0.1% human serum albumin was substituted for the patients' serum. The activity bound to both sera and reference was determined using an Inter technique CG 2000 gamma counter. The mean activity bound to the insolubilized allergen, incubated with the serum, was expressed as a percentage of the total activity added to each sample in the assay determined by counting a sample of 50 μl ^{125}I anti-IgE only. Binding was considered significant when greater than 7% (Hénocq & Gaillard, 1981).

The specificity of the bacterial RAST was tested by inhibition studies, in which sera of known specificity to grass pollen, house dust and house dust mite were first incubated with bacterial allergen to determine the capacity to inhibit binding in a second incubation of the serum with specific allergen.

Results

Skin tests with staphylococcal antigen

Group 1. At 48 hr the skin reactions were positive in only four out of the ten patients. Moreover, the skin reaction was weak, consisting of a transient erythema without induration. The six other patients had no reaction at all, of delayed onset. The four patients who had a positive skin reaction, experienced a flare up of eczema after the tests.

The distribution of cellular infiltrates was irregular and was either irregularly dispersed, or perivascular in the same microscopic preparation. This wide dispersion throughout the preparation made cell counting extremely difficult (Hénocq & Gaillard, 1981). In skin tests sites numerous intensely degranulating cells were obtained. In all the SAD cases the diseased skin similarly showed basophilic cells intensely degranulating, but they were fewer in number.

With haemalum-eosin-safron, a histio-lymphocytic, perivascular infiltration was observed on skin test sites, with a secondary invasion by polymorphonuclear leucocytes.

Group 4. In Group 4, patients' skin tests were obviously of the delayed type in nine patients with a 'cocarde' type reaction at 48 hr, consisting of a central papule, indurated, surrounded by pale erythema with oedema.

With haemalum-eosin-safron, the inflammatory changes observed included a perivascular infiltration with polymorphonuclear leucocytes and oedema of the superficial dermis.

With fuchsin-blue, no basophilic infiltrates were observed in any patient. One patient, suffering from a generalized recurrent furunculosis, developed a weak skin reaction of delayed onset, comparable to the reactions described in Group 1.

All patients of Groups 1 and 4 had an immediate type reaction to the staphylococcal antigen, but as this is frequently observed in normal persons injected with *St. aureus* this finding was not considered significant.

Skin tests with human dander

Group 1. The ten patients had a weal and flare reaction of the immediate type, at 0.1 and 0.01% w/v concentration; four of them also reacted positively to the 0.001% concentration. Flare-ups of eczema were observed in the majority of patients after an intracutaneous injection of 0.1% concentration. No delayed type reaction was observed.

Group IV. No reaction was observed with human dander allergen after 20 min or 48 hr.

Specific anti-staphylococcal and anti-human IgE antibodies

Group 1. The majority of patients had increased amounts of specific IgE against both antigens. Patient No. 7 had suffered from eczema in childhood, but at the time of this investigation he was in a quiescent state (Table 1).

Group 2. Elevated levels of specific IgE against staphylococcal antigen were found for some patients in this group (Table 2), though they were significantly lower ($P < 0.02$) than the levels found in Group 1. High total IgE, however, had no influence ($r = 0.26$) on the specific staphylococcal IgE titres. Specific IgE to human dander was detected in only one serum in this group.

Group 3. Low levels of specific IgE against staphylococcal antigen were found in all

Table 1. Group 1; % specific IgE binding to two antigens and total IgE in patients with SAD

Serum	Specific IgE (% binding)		Total IgE
	<i>St. aureus</i>	Human dander	
1 Eczéma	17	23	> 2000
2 Eczéma	20	16	> 2000
3 Eczéma	10	6	1400
4 Eczéma	11	8	> 2000
5 Eczéma	11	8	1400
6 Eczéma	15	15	> 2000
7 Eczéma	1	3	280
8 Eczéma	5	3	740
9 Eczéma	7	19	> 2000
10 Eczéma	20	6	> 2000

* The specificity of the human dander RAST was tested using an absorption test in which the positive sera were first incubated with *St. aureus* antigen and then with human dander antigen. No inhibition in binding to the human dander antigen was observed.

Table 2. Group 2; % specific IgE binding to two antigens and total IgE in atopic patients without SAD

Serum	Specific IgE (% binding)		Total IgE
	<i>St. aureus</i>	Human dander	
1	1	2	> 2000
2	11	13	1020
3	6	3	> 2000
4	7	4	> 2000
5	1	2	1000
6	10	4	1000
7	6	3	> 2000
8	4	3	1100
9	7	2	> 2000
10	2	1	> 2000

the sera giving less than 5% binding of specific IgE. No specific IgE to human dander was detected in this group.

Group 4. Seven of the ten non-atopic patients, who were suffering from recurrent infections, had low specific IgE for both antigens. One patient had a severe cutaneous infection with, and increased IgE against *St. aureus* and was in generally poor condition. These patients were the only two of Group 4 to develop a weak and transient skin reaction when tested with staphylococcal antigen. Two other patients of Group 4 had elevated levels of anti-staphylococcal IgE.

The results for all four groups are summarized in Table 3.

Table 3. Summary of the results of examination of the four groups of patients

	1	2	3	4
No. of persons	10	10	6	10
Staphylococcus	+	—	—	+
Total IgE serum (u/ml)	280–2000	> 1000	< 1000	0.5–2000
Specific IgE staphylococcus*	7	2	0	4
Specific IgE dander*	6	1	0	0
Staphylococcus skin test (20 min, 48 hr)	10, 4			10, 9
Dander skin test (20 min, 48 hr)	10, 0			0, 0
Basophils in staphylococcus skin test sites	numerous			nil

* No. of persons with specific IgE binding above 7%; the threshold significance binding being 5%

Discussion

The lower degree of delayed type hypersensitivity to staphylococcal antigen in patients suffering from SAD with *St. aureus* is peculiar to this kind of patient, being frequently exposed to large numbers of pyococci (Rajka, 1976). It parallels other defects of cell-mediated immune responses towards common antigens (Candidin, P.P.D., DNCB) (Rogge & Hanifin, 1976). In the present study concordance between cell-mediated immune deficiency against the staphylococcal antigen, and a high IgE antibody response to the same antigen has been observed in patients suffering from SAD.

All our data indicate that the RAST for IgE anti-*St. aureus* was specific for that organism. Firstly, there was no evidence of non-specific binding by sera with large amounts of total IgE, as has been seen in some RAST procedures when suspensions of uncoupled *St. aureus* or *Candida albicans* are used as antigen (W. E. Parish, personal communication). A comparison of the individual results in Tables 1 and 2 show that large amounts of IgE may not be related to anti-staphylococcal IgE. Furthermore, non-specific binding of IgE-Fc to staphylococcal Protein A, recently reported by Johansson (1980), does not apply in our tests, because we found no reduction in RAST titres for sera positive for grass pollen, house dust or mites when these sera were first incubated with the staphylococcal antigen, before incubation with the specific insoluble antigen.

The binding of IgE antibodies to *St. aureus* antigen detected by ¹²⁵I anti-IgE was considered significant at 5% or greater.

In the six atopic control patients with no SAD (Group 3), all of whom had IgE concentrations < 1000 u/ml, some showed 5% binding of IgE to staphylococcal antigen. We report, in Table 3, the incidence of persons in each group with a binding above 7%. It is evident that, in the patients of Group 1 with SAD, seven of ten had significant amounts of IgE antibodies to staphylococci, matched by only four patients in Group 4.

The findings of numerous basophils, intensely degranulating in the sites of skin

tests made with staphylococcal antigen, correlates with the specific anti-staphylococcal IgE antibodies: when skin-tested with staphylococcal antigen, SAD patients have no reaction at all, or develop a transient reaction of delayed onset comparable to the Jones-Mote reaction (Dvorak, 1976). Such cutaneous, basophil hypersensitivity seems to be the only response shown to the offending bacteria by patients with SAD in contrast to normal controls where the response is always of the delayed type both macroscopically and in skin biopsy specimens. The presence of spontaneous, degranulating basophils in diseased eczematous skin could be related to an auto-allergic response to human dander: IgE antibodies, determined by RAST against human dander, are generally positive only in cases of severe atopic dermatitis and not in asthma without eczema, or in hay fever or rhinitis patients. Exacerbations of eczema have been observed after skin test with human dander. Specific desensitization has already been attempted in some patients with favourable results (Hénocq *et al.*, 1966). The allergen in skin flakes of the human scalp has long been associated with the atopic condition, most particularly with atopic dermatitis (Parish & Rook, 1964; Voorhorst, 1977). It is conceivable that a number of cell-damaging factors might release auto-allergens, particularly in infected Group 1 patients (Table 1), thus permitting them to react with the specific IgE fixed to the local mast cells, leading in turn to the liberation of several biologically active substances (Askenase *et al.*, 1978).

An anaphylactic type of response to the offending bacterial agent in SAD could thus replace, or be additional to the delayed type hypersensitivity usually observed in non-atopic patients against infectious agents (Jarrett, 1976).

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