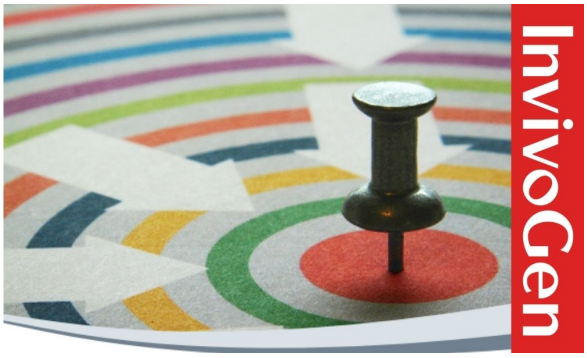

The Journal of Immunology
RESEARCH ARTICLE | DECEMBER 01 1980

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M Berger; ... et. al

J Immunol (1980) 125 (6): 2437–2443.
https://doi.org/10.4049/jimmunol.125.6.2437

0022-1767/80/1256-2437$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 125, No. 6, December 1980
Printed in U.S.A.

IgE ANTIBODIES TO *STAPHYLOCOCCUS AUREUS* AND *CANDIDA ALBICANS* IN PATIENTS WITH THE SYNDROME OF HYPER-IMMUNOGLOBULIN E AND RECURRENT INFECTIONS

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Staphylococcal abscesses and chronic mucocutaneous candidiasis are the primary infectious problems in patients with the hyper-immunoglobulin E (hyper-IgE) syndrome. In order to better understand the pathogenesis of these infections, we investigated the antimicrobial specificity of the IgE antibodies in 17 patients with this syndrome. Radioiodinated anti-IgE was used to detect IgE that bound to microorganisms serving as a solid phase. Thirteen of 17 patients had IgE that bound to *Staphylococcus aureus*, and 12 of 17 had IgE that bound to *Candida albicans*. In contrast, none had IgE that bound to *Escherichia coli*, and only 4 had IgE that bound to *Streptococcus pneumoniae*. Four of the hyper-IgE syndrome patients' sera did not contain IgE antibodies to either *Staphylococcus* or *Candida*. IgE binding to *Staphylococcus* or *Candida* was not detected in the sera of patients with parasitic infections and high IgE, of patients with primary neutrophil defects and recurrent *Staphylococcus* infections, or from normal subjects. Adsorption of sera from patients with the hyper-IgE syndrome with *Candida* resulted in an 85% reduction in binding of IgE to *Candida* ($p < 0.001$) and a 5% reduction in binding to *Staphylococcus* ($p > 0.05$). Adsorption with *Staphylococcus* resulted in a 76% reduction in binding of IgE to *Staphylococcus* ($p < 0.001$) and a 28% reduction in binding of IgE to *Candida* ($p < 0.001$). The total IgE concentrations in the sera were not reduced by these adsorptions, indicating that nonspecific binding did not occur. The concordance in production of IgE against both *Staphylococcus* and *Candida* and the finding of cross-reactivity may indicate partial antigenic similarity between these 2 organisms. We speculate that the production of IgE antibodies against *Staphylococcus* and *Candida* but not other common pathogens may contribute to the increased susceptibility of patients with the hyper-IgE syndrome to infections with these particular organisms.

The syndrome of extreme elevation of serum IgE, chronic pustular dermatitis, and recurrent infections is now well recognized (1–3). Since staphylococcal abscesses and chronic mucocutaneous candidiasis are the predominant infectious problems in these patients, studies of the pathogenesis of this syndrome primarily have concerned disorders of the phagocytes and lymphocytes. Although reduced neutrophil chemotaxis has been reported in many of the patients (1–4), the severity of the defect has been variable among different patients and even in the same individual at different times. In some subjects, normal *in vitro* chemotactic activity has been observed on occasion (1–5).

The role of the IgE in this syndrome remains unexplained. The total serum IgE concentration does not correlate with the clinical status of an individual patient or with the severity of the *in vitro* neutrophil chemotactic defect (1–3). Many patients have manifestations of atopy and have IgE that binds to common allergens (6, 7). It has recently been reported that 3 young children (5) and 7 older patients (8) with this syndrome have anti-staphylococcal IgE antibodies. Thus, it is possible that specific antimicrobial IgE antibodies play an important role in the pathophysiology of the host defense defect.

In the present study, a solid-phase radioimmunoassay (5) was used to determine the antimicrobial specificity of the IgE from the sera of 17 patients with elevations of this antibody class and chronic, recurrent infections with staphylococci and/or *Candida*. We found that 13 of 17 patients had IgE antibodies that bound to *Staphylococcus aureus* and that 12 patients had IgE antibodies to *Candida albicans*. In contrast, none had IgE antibodies to *Escherichia coli*, and only 4 patients had IgE antibodies to *Streptococcus pneumoniae*. Adsorption studies suggest that there is some cross-reactivity and/or antigenic similarity between *Staphylococcus* and *Candida*. We postulate that the excessive production of IgE antibodies to these antigens is a consequence of a disturbance in the normal mechanisms of immunologic regulation and that these antibodies in turn may be responsible for the unique spectrum of infections in patients with this syndrome.

MATERIALS AND METHODS

Patients. All patients were referred to the National Institute of Allergy and Infectious Diseases for evaluation of unusual susceptibility to infections. Patients with staphylococcal infections were included in this study if they had recurrent subcutaneous abscesses requiring multiple incision and drainage procedures and/or x-ray evidence of pulmonary infection with abscesses or persistent pneumatoceles. Patients with severe mucocutaneous candidiasis were screened for elevated serum IgE levels and were included in the study even if they had not had staphylococcal abscesses.

Four healthy laboratory workers served as controls. Other controls included 6 patients with elevated serum IgE concentrations due to parasitic or chronic fungal infections but who

Received for publication April 28, 1980.
Accepted for publication August 18, 1980.
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did not have the hyper-IgE syndrome. We also studied 2 populations of patients with recurrent or chronic infections and normal concentrations of serum IgE. The latter groups were composed of 7 patients with defective neutrophil function (5 with chronic granulomatous disease and 2 with Chediak-Higashi syndrome) and recurrent staphylococcal infections and 25 other patients with chronic mucocutaneous candidiasis. Clinical characteristics of the patients with the hyper-IgE syndrome and controls are given in Tables I and IV. All patients had normal levels of IgG, IgA, IgM, and total hemolytic complement. Sera for IgE determinations and binding studies were stored at −20°C until used. Neutrophil chemotaxis and lymphocyte proliferation assays were performed as previously described (9, 10).

Microorganisms. S. aureus was isolated from a subcutaneous abscess in the hand of patient 8 (see Table I). *E. coli, S. pneumoniae* type VII, and *S. aureus* Wood 46 were obtained from the American Type Culture Collection, Rockville, MD. All bacteria were cultured in serial overnight passes at 37°C in trypticase-soy broth. Pneumococci were incubated at 56°C for 30 min to prevent autolysis. Cultures were harvested by centrifugation at 3000 × G for 10 min at 4°C, washed twice with 0.15 M phosphate-buffered saline (PBS), pH 7.4, and stored in suspension at −20°C. For use in IgE-binding studies, the bacteria suspensions were diluted in PBS to a protein concentration of 1 mg/ml as determined by a microbiuret procedure (11).

C. albicans was a laboratory strain that was cultured in Sabouraud's liquid medium with 5% dextrose at 37°C for 48 hr in a CO_2 incubator. The yeast phase organisms were handled as described for the bacteria except that the suspension was standardized to an optical density of 0.35 at 650 nm in a 1-cm light path cuvette.

All suspensions were free from contamination as judged by Gram's stain and reculture.

IgE reagents. Serum IgE concentrations were determined using Phadebas PRIST reagent kits obtained from Pharmacia, Inc., Piscataway, NJ. ^{125}I-anti-human IgE produced in rabbits was also purchased from Pharmacia as the radio-allergosorbent test (RAST)[1] reagent and was reconstituted as directed and used without further processing. Chromatographically purified human IgE myeloma protein PS was the kind gift of Dr. Michael Kaliner, N.I.H. IgE was purified from the serum of patient 10 by affinity chromatography on anti-IgE-Sepharose. These proteins were diluted in IgE-free horse serum from Pharmacia. Immune serum globulin (human) U.S.P. (ISG) was purchased from Cutter Laboratories, Berkeley, CA, or Armour, Inc., Phoenix, AZ.

Binding of IgE to microorganisms. Aliquots of 1 ml of the suspension of microorganisms were placed into 12 × 75 mm plastic tubes and washed twice with 2 ml of PBS by vigorous vortexing, then centrifugation at 2500 × G for 5 min. The pellet was resuspended in 0.5 ml of PBS, and 0.1 ml of serum was added. The mixture was vortexed vigorously, then incubated at room temperature for 3 hr with occasional mixing. After this incubation, the pellets were washed 3 times with PBS, as described above. When *S. aureus* was used, it was necessary to consider the possibility of nonspecific binding of the ^{125}I-rabbit anti-IgE (an IgG antibody) to staphylococcal protein A. For this reason, 0.1 ml of ISG was added to each tube, and the contents were carefully mixed, then incubated at room temperature for another 3 hr. In other experiments, *S. aureus* strain Wood 46, which lacks protein A, was substituted for the clinical isolate. In all experiments, the pellets were then washed 3 times with PBS, and then 0.05 ml anti-IgE (8 ng bearing 0.05 μCi of ^{125}I) was mixed with the pellet in each tube and incubation was continued overnight, allowing the tubes to stand at room temperature. The pellets were finally washed 3 times with PBS, and the bound radioactivity was determined using a Packard Autogamma spectrometer. The results are expressed as the percentage of total ^{125}I added that remained bound to the pellets. All determinations were performed in duplicate, and values for controls in which PBS was used in place of serum were subtracted to correct for nonspecific binding (see Fig. 1). When organisms other than the clinical isolate of *S. aureus* were used, the ISG incubation and second set of 3 PBS washes were omitted.

Adsorption experiments. Pellets containing 10 times more organisms than used in the binding assays were prepared as above, washed twice with PBS, and drained of excess fluid by inverting the tubes. One and two-tenths milliliters of a 20-fold dilution of each serum (in PBS) was mixed with the pellet of organisms by vortexing vigorously for 30 sec. The mixtures were incubated at room temperature for 45 min with occasional vortexing and then centrifuged at 3500 × G for 10 min. The supernatant was carefully pipetted off and mixed with a fresh pellet of the same organism. This adsorption procedure was performed 3 times for each organism, and the final supernatant was given an additional 15-min centrifugation at 3500 × G. IgE concentrations and binding to microorganisms were determined on duplicate aliquots of the adsorbed sera and on equivalent dilutions of unadsorbed sera, as described above.

Statistical analyses. The 2-tailed Student's *t*-test was used unless otherwise indicated.

RESULTS

Clinical characteristics of patients with hyper-IgE and recurrent infections (Table I). As can be seen in Table I, the patients ranged from 7 to 34 yr of age at the time of these studies, but in many cases the history of recurrent infections began in early childhood. Although there was a predominance of Caucasians (13 of 17) and females (13 of 17), Blacks were also afflicted (4 of 17), indicating that this syndrome is not limited to fair-skinned, red-haired females as suggested in the original report (12). Fifteen patients had staphylococcal infections of the lungs and subcutaneous abscesses that are characteristic of the syndrome; 7 of these patients also had chronic mucocutaneous candidiasis. One patient (No. 13) had disseminated candidiasis, and granulomas containing *Candida* were found at autopsy. The other 2 patients with candidiasis were selected from a larger group of patients with that diagnosis because they had extreme elevations of serum IgE, but these 2 patients did not have histories of increased susceptibility to staphylococcal infections.

Neutrophil chemotaxis was abnormal (less than 70% of control) in 9 of the 14 patients tested. Lymphocyte proliferation responses to concanavalin A were depressed to below 70% of the mean of concurrently run controls in 54% (7 of 13) of those tested. Responses to *Candida* were similarly depressed in 57% (8 of 14).

Binding of IgE to S. aureus. The ^{125}I-anti-human IgE employed for these assays was a rabbit IgG, and the possibility that this antibody could bind nonspecifically to the staphylococcal A protein (13) and give falsely elevated results for "bound" IgE had to be considered. Three sets of experiments were done to determine the possible effects of this type of nonspecific binding in our system. First, staphylococci were

[1] Abbreviations used in this paper: ISG, immune serum globulin (human) U.S.P.; RAST, radio-allergosorbent test.

pelleted and washed as described above and incubated either with the serum of a patient with the syndrome of hyper-IgE and recurrent staphylococcal infections, the serum of a patient with schistosomiasis and elevated IgE, serum from a normal control, or with PBS alone. Binding studies were carried out as described in the *Materials and Methods* section, but in 1 set of tubes the incubation with ISG was omitted. As can be seen in Figure 1, there was marked binding of the labeled antibody when the bacteria were incubated with PBS in the absence of serum or ISG (first bar of left panel), but this was reduced to less than 5% of the ^{125}I added when the ISG incubation was carried out (first bar of middle panel) ($p < 0.01$). These results indicate that the A protein of our isolate is capable of binding the radiolabeled rabbit IgG, but this binding capacity can be saturated by the excess IgG present during the ISG incubation. Binding of anti-IgE was minimal when the *Staphylococcus* was incubated with control sera, indicating that there was no IgE specific for *Staphylococcus* in these sera and there was sufficient IgG to saturate the A protein even when the ISG incubation was omitted. When the *Staphylococcus* was incubated with serum containing specific anti-staphylococcal IgE (see below), 32% of the ^{125}I was bound regardless of whether the ISG incubation was performed, indicating that this did not interfere with specific binding. In all the binding studies with the clinical isolate of *S. aureus*, the ISG incubation was included to insure that nonspecific binding of ^{125}I-antibody would not occur.

The second control experiment used the Wood 46 strain of *S. aureus*, which lacks the A protein (14). As shown in the right panel of Figure 1, less than 5% of the anti-IgE was bound when PBS alone or control sera were incubated with this *Staphylococcus*, but 20% of the ^{125}I was bound when the incubation was with serum from a patient with elevated IgE and recurrent staphylococcal infections ($p < 0.01$).

Control tubes with PBS instead of patient serum were run in all studies, and the binding in these tubes, which we assumed was nonspecific, was subtracted from all other results. When organisms other than the clinical isolate of *Staphylococcus* were used, the binding in these control tubes was less than 5% without the ISG incubation; hence, the incubation with ISG was omitted.

To assure that the binding of the IgE to the microorganisms represented a specific property of the antigen-combining sites rather than nonspecific binding mediated by the Fc region, we

TABLE I

Clinical characteristics and cellular responses of patients with hyper-IgE and Staphylococcus and/or Candida infections

Patient No.	Age	Sex	Race	Infections	Chemotaxis	Lymphocyte Stimulation	
						Candida	Con A
					% nl[a]	% of control mean[b]	
1	20	M	W	*Staph.*: s, l[c]	70	N.D.	N.D.
2	7	F	W	*Staph.*: s, l, n; MCC[d]	71	76	22
3	16	F	W	*Staph.*: s, l, n; osteomyelitis; MCC	28	56	46
4	7	F	W	*Staph.*: s; MCC	N.D.[e]	51	30
5	22	F	W	*Staph.*: s, l; MCC	51	69	18
6	25	M	W	*Staph.*: s, l, n; peritonitis; MCC	48	58	16
7	28	M	W	MCC	109	100	100
8	34	F	B	*Staph.*: s, n	91	N.D.	N.D.
9	10	F	W	*Staph.*: l	119	193	139
10	16	F	B	*Staph.*: s, l, n	60	6.2	64
11	29	F	W	*Staph.*: l	57	213	27
12	25	F	W	*Staph.*: s, n, kidney, joint *Candida*: vaginitis	54	311	204
13	26	M	B	*Staph.*: s; MCC disseminated candidiasis	N.D.	22	20
14	16	F	B	*Staph.*: l	57	N.D.	N.D.
15	11	F	W	*Staph.*: s, n	66	44	181
16	16	F	W	*Staph.*: s, l	49	98	N.D.
17	10	F	W	MCC; dermatophytosis	N.D.	58	150

[a] Chemotaxis expressed as percentage of control.

[b] Results given are for ^{3}H-thymidine incorporation by lymphocytes cultured in the presence of antigen or mitogen divided by incorporation in unstimulated cultures. Values are expressed as percentage of means for 30 normal controls which were 6.5 for *Candida* and 100 for concanavalin A.

[c] Staphylococcal infection sites: l, lung (includes pneumonia, abscess, and empyema); s, subcutaneous abscesses; n, lymph node abscesses.

[d] Mucocutaneous candidiasis.

[e] Not determined.

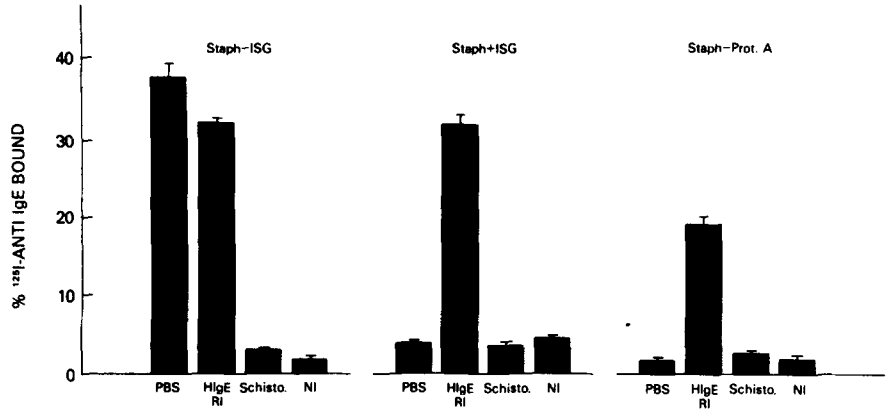

Figure 1. Binding of IgE to *S. aureus*. In the experiment shown in the left panel (Staph − ISG), 1-mg pellets of a clinical isolate of *S. aureus* were incubated with: 100 μl each of PBS, serum from patient 5 with the hyper-IgE-recurrent infection syndrome and total IgE = 21,500 (HIgE, RI), serum from a patient with schistosomiasis and total IgE = 4800 (Schisto.), or serum from a normal control with total IgE = 84 (NI). After 3 hr at room temperature, the pellets were washed, mixed with anti-IgE, incubated overnight at room temperature, and then washed again with PBS. Radioactivity remaining bound to the pellets is expressed as the percentage of total ^{125}I added. The experiment in the center panel (Staph + ISG) was done identically except that each pellet was incubated with 100 μl of immune serum globulin for 3 hr at room temperature after the serum incubation and first set of PBS washes. They were then washed again before addition of the ^{125}I-anti-IgE. The experiment on the right (Staph − Prot. A) was conducted in exactly the same manner as the Staph − ISG experiment, but the Wood 46 strain of *S. aureus* was used rather than the clinical isolate. The means ± S.E.M. for duplicate determinations are shown. The differences between the values for PBS with Staph − ISG and Staph + ISG and for HIgE-RI with Staph − ISG and Staph − Prot. A were significant at $p < 0.01$.

studied the interactions of the purified IgE myeloma protein PS with the organisms. The myeloma protein or purified IgE from patient 10 was incubated at the same concentration (2.4 μg/ml) with *Staphylococcus* or *Candida*. When the patient's IgE was used with *Staphylococcus*, 14.3% of the anti-IgE was bound, and with *Candida*, 21.2% was bound. In contrast, when the myeloma protein was used, there was no detectable binding to *Staphylococcus*, and the binding to *Candida* was only 0.3%. To further study this question, the organisms were preincubated with a 10-fold excess of the purified myeloma protein to saturate any nonspecific binding sites. The organisms were then washed, and their ability to bind the patient's IgE was determined. No decrease in binding to *Staphylococcus* (14.3 ± 0.6% for nontreated *vs* 12.2 ± 1.0% for pretreated, $p > 0.2$) or *Candida* (21.2 ± 2.8 *vs* 21.6 ± 0.6) was caused by this treatment. The integrity of the Fc portion of the myeloma protein was confirmed by preincubating it with the ^{125}I-anti-IgE used to detect the patient's IgE, which becomes bound to the organisms. This treatment totally neutralized the labeled antibody, and no ^{125}I could be detected in the pellets, although identical incubations with non-preincubated ^{125}I-anti-IgE resulted in binding of 12.7% with *Staphylococcus* and 20.3% with *Candida*.

IgE levels and binding to microorganisms in sera from patients with hyper-IgE and recurrent infections and controls. As shown in Table II, the IgE concentrations in the sera of the patients in the hyper-IgE and recurrent infection group were extremely elevated—all were over 1000 IU/ml, and 6 were 10,000 IU/ml or greater. The results of the binding studies are shown in Table II and are summarized in Table III. Of the 7 patients (Nos. 2 through 6, 12, and 13) who had both staphylococcal and *Candida* infections, all had IgE antibodies against staphylococci and 6 had IgE antibodies against *Candida*. Eight of the hyper-IgE syndrome patients had significant staphylococcal but not *Candida* infections (Nos. 1, 8 through 11, and 14 through 16), and 2 had infections with *Candida* but not staphylococci (Nos. 7 and 17). Of these 10 patients who had clinically apparent infections with only 1 of the 2 organisms, 6 nevertheless had IgE antibodies against both, and 4 had IgE antibodies against neither. These results were significantly different from our expectation that the patients not infected with *Candida* would not have IgE antibodies against *Candida* and that the patients not infected with *Staphylococcus* would not have IgE antibodies against *Staphylococcus* ($p < 0.01$, Kendall's V test (15)). There was no apparent correlation between the total IgE concentration and the degree of binding to either of the 2 organisms. Three of the 4 patients (Nos. 14 through 17) who did not have IgE antibody activity against either of the 2 organisms had comparatively low IgE concentrations and had been free from symptomatic infections for a prolonged period at the time of these studies. In contrast, no patient had elevated levels of IgE binding to *E. coli*, and only 4 had greater than 5% binding when pneumococcus was used as the test organism.

TABLE II

Total IgE and IgE binding to microorganisms in patients with hyper-IgE and recurrent infections[a]

Patient No.	Total IgE	Organisms Causing Infection	% ^{125}I-Anti-IgE Bound When Patient's Serum Was Incubated with			
			Candida	*S. aureus*	*Escherichia coli*	*Streptococcus pneumoniae*
	IU/ml					
1	3,900	S[a]	27.5	5.7	0.8	0.9
2	6,300	S, C[b]	2.4	27.8	1.6	1.6
3	10,000	S, C	32.6	28.0	4.1	2.6
4	3,300	S, C	27.0	24.0	1.7	2.2
5	21,500	S, C	30.0	31.0	4.3	2.6
6	15,750	S, C	37.0	32.6	3.1	8.4
7	1,450	C	5.0	7.7	3.0	6.9
8	8,400	S	22.4	17.4	3.2	9.9
9	17,000	S	37.5	13.5	2.0	2.9
10	37,500	S	33.6	27.0	3.5	5.7
11	6,250	S	29.0	21.4	0.9	0.9
12	6,000	S, C	30.3	6.1	0.5	1.4
13	6,750	S, C	35.6	10.2	1.3	0.9
14	1,200	S	1.7	0	N.D.	N.D.
15	1,000	S	0.1	0	N.D.	N.D.
16	2,400	S	1.3	0	N.D.	N.D.
17	10,000	C	0	0	N.D.	N.D.

[a] Results are expressed as the percentage of total ^{125}I added that remains bound to the pellet and are the means of duplicate determinations. When *Staphlococcus* was used as the adsorbent, an additional 3-hr incubation with human immune serum globulin and washes with PBS were performed before the ^{125}I-anti-IgE was added.

[b] S, *Staph.*; C, *Candida*.

TABLE III

Correlation between clinical infections and production of IgE antibodies against Staphylococcus and Candida in 17 patients with the hyper-IgE syndrome

No. of Patients Infected with	No. of Patients with IgE Antibodies against[a]			
	Both *Staphylococcus* and *Candida*	*Staphlococcus* only	*Candida* only	Neither
Staphylococcus and Candida				
7	6	1	0	0
Staphylococcus alone				
8	5	0	0	3
Candida alone				
2	1	0	0	1

[a] Positive IgE antibodies defined as greater than 5% binding of ^{125}I-anti-IgE.

Sera from 3 groups of control patients were also tested for the presence of IgE antibodies against *Staphylococcus* and *Candida*. The clinical characteristics of the patients and the results are presented in Table IV. The first group (patients 18 through 23) included patients with elevated IgE levels and a variety of conditions such as parasitic or chronic fungal infections but with no history of serious staphylococcal or *Candida* infections. None of these patients had more than background binding of IgE or *Staphylococcus*. One patient (No. 22) with aspergillosis had slight binding to *Candida*.

The second group consisted of patients with defined neutrophil defects—chronic granulomatous disease (patients 24 through 28) or the Chediak-Higashi syndrome (patients 29 and 30). Although each of these patients had recurrent staphylococcal infections, none had elevated IgE concentrations and none had detectable IgE binding to *Staphylococcus* or *Candida*. The same was true of 25 other patients with chronic mucocutaneous candidiasis (data not shown). Similarly, the sera of 4 healthy laboratory workers contained normal total IgE concentrations and had no detectable binding to *Staphylococcus* or *Candida*.

Adsorption studies with Staphylococcus and Candida. Because of the concordance of IgE binding to both *Staphylococcus* and *Candida* in the patients' sera, we performed adsorptions with these microorganisms to further define the specificity of the IgE antibodies. In these experiments, the sera of patients 1 through 13 were diluted 20-fold in PBS. As shown in Figure 2, the binding with each patient's unadsorbed, diluted serum was

TABLE IV

IgE levels and binding to microorganisms in control patients[a]

Patient No.	Diagnosis	Age	Sex	Race	Total IgE	% ^{125}I-Anti-IgE Bound When Patient's Serum Is Incubated with	
						Candida	*Staphylococcus*
					IU/ml		
18	Schistosomiasis	30	M	Hispanic	3800	2.4	0.7
19	Schistosomiasis	26	M	Hispanic	4800	0.4	0
20	Schistosomiasis	25	M	Hispanic	3400	2.1	1.1
21	Tropical eosinophilia	30	M	Indian	5000	1.4	1.2
22	Bronchopulmonary aspergillosis	18	M	White	3000	5.0	0
23	Severe pustular acne, eczema	22	M	Black	1000	0	0
24	Chronic granulomatous disease (CGD)	23	M	White	182	0	0
25	CGD	14	M	White	42	0.9	0
26	CGD	16	F	White	8	0	0.2
27	CGD	12	M	White	7.5	0	0
28	CGD	4	M	White	12.5	0	0.5
29	Chediak-Higashi syndrome	28	M	White	20	0	0
30	Chediak-Higashi syndrome	29	M	White	105	0	0.8
31	Normal	29	M	White	84	0	0
32	Normal	44	F	White	100	0	0.3
33	Normal	36	M	White	23	0	0
34	Normal	39	F	White	18	0	0.2

[a] Total IgE levels and binding to microorganisms were determined as described above. All values are means of duplicate determinations.

used as the reference value, and the binding with the adsorbed serum was expressed as the percentage of this value. Total IgE concentrations were determined on all of the diluted sera, and these results were also expressed as the percentage of the value of the unadsorbed serum. Binding of IgE to *Staphylococcus* was diminished by 76% after adsorption of the sera with *Staphylococcus* ($p < 0.001$) but by only 5% after adsorption with *Candida* ($p > 0.05$). In contrast, binding to *Candida* was diminished by 85% after adsorption with *Candida* ($p < 0.001$) but was also diminished by 28% after adsorption with *Staphylococcus* ($p < 0.001$). Thus, it appears that in addition to specific IgE, some antibodies that cross-react with *Candida* and *Staphylococcus* are also present.

In all but 2 of the patients, the total IgE concentrations were not significantly changed by the adsorptions, indicating that nonspecific adsorption of IgE did not occur. Two of the patients (Nos. 9 and 10), however, had 20 to 25% reductions of total IgE after adsorption with *Candida*, suggesting that this proportion of the total IgE was anti-*Candida* antibody.

DISCUSSION

In this report, we describe the application of a solid-phase radioimmunoassay for specific antimicrobial IgE antibodies. This method, which uses whole microorganisms as the solid-phase antigen, has 2 advantages over the traditional RAST procedure in which purified antigens are coupled to an insoluble support. First, the entire surface of the organism is available for antigen binding rather than a limited repertory of selected

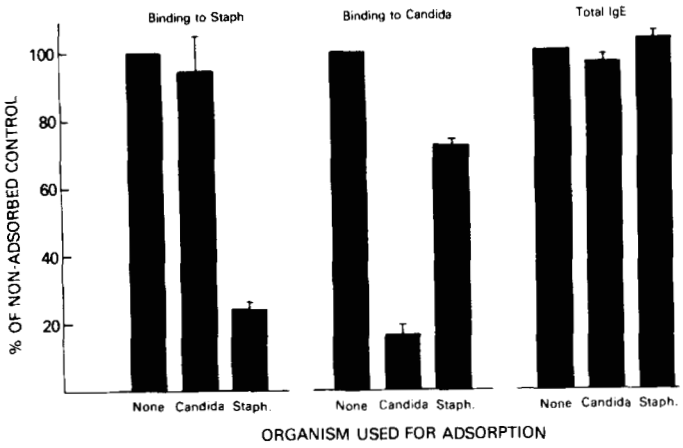

Figure 2. Effect of adsorption with *Staph.* and *Candida* on specific and total IgE. Results are expressed as percentage of the value obtained for the same patient's diluted but nonadsorbed serum. For the *Staph.*-binding assays with patients 1, 7, and 12 and the *Candida*-binding assays with patient 7, undiluted serum was used for adsorption. All determinations were done in duplicate, and the mean ± S.E.M. for the group of patients (Nos. 1 to 13, Table I) are shown. The difference in binding to *Staph.* between unadsorbed sera and sera adsorbed with *Staph.* was significaint ($p < 0.001$). The differences in binding to *Candida* by unadsorbed sera *vs* sera that had been adsorbed with *Staph.* or *Candida* were both significant ($p < 0.001$). There was no significant change in the total IgE levels ($p > 0.05$).

antigens. Second, the surface structures are not treated by materials that could cause denaturation or chemical modification; hence, they retain their natural antigenicity.

Because of the possibility that particular strains of *Staphylococcus* might be responsible for the infections in patients with the hyper-IgE-recurrent infection syndrome, a clinical isolate of *Staphylococcus* from 1 of these patients was used. Preliminary experiments (Fig. 1) demonstrated that this organism possessed the A protein but that the presence of the A protein did not interfere with the assay procedure. In order to obtain valid control values and to insure that correct results would be obtained regardless of the immunoglobulin content of the patient's serum, we took the additional precaution of incubating all the *Staphylococcus*-containing tubes with an excess of human IgG. This did not reduce binding of specific IgE antibody, but prevented nonspecific adsorption of the ^{125}I-anti-IgE, which itself is rabbit IgG. In the experiments shown in Figure 1, less IgE was bound to the Wood 46 strain of *Staphylococcus*, which lacks the A protein (14), than to the clinical isolate. This may reflect the lack of binding of IgE to the A protein itself, or it may be indicative of other antigenic differences between the strains. Nonspecific adsorption of IgE to the A protein is an unlikely explanation for this difference, since binding of IgE from the sera of patients with parasitic diseases did not occur. We interpret our results as indicating that if the IgE is binding to the A protein under these conditions, it is doing so as a specific antibody binding to its antigen and not by nonspecific interactions as has been claimed by others (16). The lack of binding of the IgE myeloma protein to *Staphylococcus* or *Candida* and the inability of excess myeloma protein to block binding of the patient's IgE to these organisms confirm that the binding of the patient's IgE to the organisms is mediated through the antigen-binding sites rather than through the Fc portion.

Patients with the syndrome of hyper-IgE and recurrent infections have major clinical problems with staphylococcal "cold" subcutaneous abscesses and pulmonary infections, and

many also have chronic mucocutaneous candidiasis. In contrast to patients with antibody-deficiency syndromes, they are not unusually susceptible to infection with other microorganisms, such as pneumococci. It is interesting that most (12 of 17) of our patients had IgE antibodies against both staphylococci and *Candida* even though some patients had not had clinical infections with both organisms. This could, in part, be in response to antigen exposure during subclinical infection or from the skin or gut. The concordance of IgE antibody production suggested that these patients may recognize a similar antigenic structure on both organisms. The adsorption experiments indicated partial cross-reactivity and support this hypothesis. Although both organisms may bear *N*-acetylglucosamine on their surfaces (17), we have found no previous report of cross-reactivity between *Staphylococcus* and *Candida*.

The production of high titers of antimicrobial IgE in these patients may be a primary defect and is probably not the consequence of abnormal or persistent antigenic stimulation *per se*, since similar IgE antibodies were not found in patients with chronic granulomatous disease or Chediak-Higashi syndrome despite their similar frequencies of staphylococcal infections and antibiotic treatment. It is possible that the latter groups of patients and/or normal individuals may produce some IgE that can react with *Staphylococcus* or *Candida* and that such antibodies are present at concentrations below the limits of detection by our assays.

Regulation of IgE synthesis is a complex phenomenon requiring multiple lymphocyte interactions (2, 4, 18–20). Recent *in vitro* experiments have demonstrated increased IgE synthesis in lymphocytes of patients with the hyper IgE syndrome, suggesting abnormal regulation of B cells producing this class of antibody (2). It has also been suggested that some IgG antibody responses may be deficient in these patients (2, 4). We have not as yet tested our patients' sera for IgG antibodies against *Staphylococcus* or *Candida* and cannot exclude the possibility that deficiencies of other protective antibodies may be related to their host defense defect. Most of our patients had impaired lymphocyte responses to *Candida* antigens, and in 7 patients there were impaired responses to concanavalin A. The mechanism of the lymphocyte defect has not been conclusively defined, but the current data suggest defects in either the numbers or functions of at least 2 subclasses of T cells—those that regulate specific IgE antibody synthesis and those that regulate or effect cellular immune responses.

Although many of the patients have life-long histories of staphylococcal abscesses and candidiasis, several seem to have acquired these problems with no antecedent history of unusual infections or abnormal immune responses. Similarly, some of the patients (Nos. 14 through 16) who previously had typical "cold" *Staphylococcus* abscesses have now been free of these infections for many months. These patients have somewhat lower IgE concentrations than most of the other patients in the study and did not have antimicrobial IgE. This may be indicative of heterogeneity in the population of patients with the clinical diagnosis of hyper-IgE recurrent-infection syndrome.

The role of IgE in the host defense against infection is unknown. A previous study reported anti-*Candida* IgE in patients with vaginal candidiasis and questioned a role for IgE in local immunity at the mucosa (21). It seems more likely that production of IgE antibodies against microorganisms is a pathologic response that may impede the prompt eradication of infection. IgE antibodies are probably not present in sufficient quantity to block binding of antibody of other classes, since even at the extreme elevations reported in this study the total IgE levels are below 10 mg per 100 ml of serum, and only a small fraction of this was shown to be specific antibody against *Staphylococcus* or *Candida*. IgE antibodies do, however, have the unique capacity to bind to mast cells and basophils (19). Upon reaction with antigen, such cells release potent pharmacologic mediators, including histamine, slow-reacting substance, and others (22, 23). Release of mast cell products at a site of infection could lead to diminished chemotactic responsiveness of neutrophils (1, 8, 24, 25). This mechanism may explain the predominance of skin and lung infections in these patients, since these 2 tissues are rich in mast cells (8, 22, 24). Although inhibition of chemotaxis by IgE or histamine could not be demonstrated *in vitro* (26), recent *in vivo* studies have shown that leukocytes from the venous effluent of an extremity with an experimentally induced angioedematous lesion had markedly impaired chemotactic responsiveness (27). This chemotactic defect was subsequently found in leukocytes collected from the nontreated extremity, indicating that it had become systemic (27). Systemic effects of factors produced at a local site of IgE-mediated reactions may thus account for the variable results of chemotactic assays in these patients and may also account for the eosinophilia that is often observed.

Thus, the abnormal production of IgE may play a pathogenic role in this syndrome, possibly by leading to the release of substances that inhibit normal neutrophil function. Alternatively, it is possible that the lymphocyte abnormality that allows the inappropriate IgE production simultaneously leads to production of an independent factor capable of altering phagocyte function.

The observations of specific antimicrobial IgE may thus be directly related to the unique pattern of infection in these patients. Model systems for the further study of the hyper-IgE-recurrent infection syndrome should thus include antigen and tissue specific components. *In vivo* measurements of neutrophil mobilization, such as the use of skin windows with specific bacterial extracts, may give more pertinent results than those obtained with standardized, defined chemoattractants *in vitro*.

Acknowledgments. The use of ISG in the radioimmunoassay of IgE binding to *Staphylococcus aureus* was developed with the assistance of Dr. Raif Geha in his laboratory at Children's Hospital Medical Center, Boston, MA, using a different clinical isolate than that used in the present study. A preliminary report of a study using that strain was submitted to the 1979 meeting of the Society for Pediatric Research and is cited here (5).

We are indebted to Dr. Richard DeShazo, Allergy Research Laboratory, Walter Reed Army Medical Center, for determining the serum IgE concentrations in the patients with chronic mucocutaneous candidiasis; to Mr. Terrill Smith and Ms. Lynn Greenberg for excellent technical assistance; and to Dr. David Alling for assistance with the statistical analyses.

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