

Serum Concentration of Specific IgE Antibody against *Aspergillus fumigatus* and Identification of the Fungal Allergen

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The concentration of specific IgE antibody against whole *Aspergillus fumigatus* antigens was quantitatively measured in 60 sera from patients with anti-*A. fumigatus* precipitins and compared with total IgE level. Raised IgE levels were observed in 31 patients. By the use of an immunosorbent prepared with *A. fumigatus*, IgE antibody against the fungus was quantified. Mean concentration of 10.3 IU/ml of anti-*Aspergillus* IgE was observed. The mean ratio of anti-*Aspergillus* IgE to total IgE was 4.8%. Radioimmunoelectrophoresis allowed identification of the allergen in *A. fumigatus* extracts combined with the IgE antibody. This allergen was distinct from the specific antigen C₂ with chymotryptic activity previously shown to be specific for the species *A. fumigatus*.

INTRODUCTION

Elevated IgE levels have been reported in patients infected with *Aspergillus fumigatus*, mainly in those with bronchopulmonary allergic aspergillosis (1, 2). IgE antibody against *A. fumigatus* antigen can be demonstrated in sera from patients with allergic aspergillosis, though no quantitative measurement of antibody concentration was provided (3). Many antigenic specificities, however, are identified in *A. fumigatus* extracts (4). One of them, called C₂ and supporting chymotryptic activity, was previously shown to be specific for *A. fumigatus* while others cross-reacted with various *Aspergillus* species (5). The present study was undertaken to compare the respective concentrations of IgE antibody against whole *A. fumigatus* or the pure C₂ antigen and to identify the allergen eliciting IgE antibody production.

MATERIALS AND METHODS

Human serum samples and serological study. Sixty sera from patients demonstrating precipitating antibodies against *A. fumigatus* were included in the study. *A. fumigatus* antigen (AF-Ag) was obtained from a sputum culture of *A. fumigatus* (Longbottom strain), cultured in Sabouraud's medium and prepared as previously described (4) in order to obtain both somatic and metabolic antigens in the extract.

Precipitating antibodies were demonstrated by means of immunoelectrophoresis carried out with AF-Ag (4-7). One to fourteen bands were identified in the patients' sera. Thirty-two patients had precipitating antibody against the particular antigen C₂, identified from its chymotryptic activity and electrophoretic mobility (4). All sera were stored at -20°C prior to IgE determination.

Measurement of total IgE level. The radioimmunosorbent test (RIST) was performed with the commercial kit of Pharmacia (Uppsala, Sweden). Results were obtained in international units per milliliter from preparation 68/341 (WHO). Owing to the wide variation observed in the healthy population, IgE levels over 700 IU/ml were assumed to be significantly elevated ($P < 0.05$).

Quantitative measurement of IgE antibody against A. fumigatus. An immunosorbent technique was used for the isolation of anti-*Aspergillus* antibodies which were then assayed for IgE by the RIST, according to a method previously used for measurement of IgE antibody to other parasite antigens (8, 9). Briefly, the immunosorbent was prepared with AF-Ag cross-linked to bovine serum albumin by glutaraldehyde (10). Isolation of anti-AF antibodies was carried out by means of a batchwise procedure. Nonspecific radiolabeled human (myeloma) IgE was added to every serum studied to estimate the recovery of nonspecific IgE. Washing of the immunosorbent with phosphate-buffered saline, pH 7.4, was repeated until the recovered radioactivity equalled the background. The absorbed antibodies were then eluted with a glycine-HCl buffer, pH 2.8, containing 1% albumin. The eluate was immediately neutralized and concentrated to the original volume of serum, i.e., 1 ml, for IgE determination by the RIST.

Nonspecific absorption was further determined by the use of 10 control sera from healthy or atopic individuals without aspergillosis to estimate the background of nonspecifically eluted IgE.

The specific antigen of *A. fumigatus* called C₂ was purified by affinity chromatography (11). Polystyrene tubes were coated with 2 ml of a standard preparation of C₂ antigen, 2 µg/ml, for 3 hr, then incubated with 0.5 ml of patient's serum. Washing and elution of the antibody was performed as above.

Radioimmuno-electrophoresis. A previously described method (12, 13) was used to identify the antigenic fraction of *A. fumigatus* combined with IgE antibody. Immuno-electrophoresis was carried out with whole AF-Ag and a reference immune serum against *A. fumigatus* raised in rabbit, which reacted with the whole antigen with strong precipitin lines (5, 11). A patient's serum was then incubated with the gel containing *A. fumigatus* antigens precipitated by means of rabbit antibodies. A second incubation was carried out with radioiodinated rabbit anti-human IgE antibody (Pharmacia, Uppsala). Autoradiography (Kodirex film, Eastman Kodak, Sevrin) allowed the identification of radioactive precipitation lines from their electrophoretic mobilities and enzymatic activities.

RESULTS

Total IgE levels. Serum IgE levels over 700 IU/ml were found in 31 patients. Mean serum IgE was significantly elevated when compared to normal values (Student's *t* test: $t = 7.132$; $P < 0.001$). No relation was obtained between the concentration of total IgE in serum and the number of precipitation lines in immuno-electrophoresis ($r = 0.042$; $P > 0.10$).

Concentration of anti-AF IgE antibody. To every serum sample, 0.5 IU of radiolabeled nonspecific human IgE was added. The recovery was estimated from the radioactivity in collected washing solutions. In any case, the recovery was not below 97% of the added ¹²⁵I-labeled IgE. Mean recovery was $99 \pm 1\%$ (mean $\pm$

SD). In 10 uninfected patient's sera with the total IgE level ranging up to 2250 IU/ml, the background concentration of IgE eluted from the immunosorbent prepared with AF-Ag ranged up to 6 IU/ml; the geometric mean $\pm$ SD was 3.0 ± 2.8 IU/ml. No relation was observed between serum IgE and background IgE eluted from the immunosorbent in control sera ($r = 0.509$; $P > 0.10$).

In patients' sera, the level of anti-AF IgE antibody eluted from the immunosorbent ranged up to 231 IU/ml (Table 1). A significant difference was observed between the concentration of anti-*A. fumigatus* IgE antibody in infected patients and background IgE determined in uninfected controls ($t = 2.63$; $0.02 > P > 0.01$).

No difference was found between anti-AF IgE antibody levels in patients with or without anti- C_2 precipitating antibody ($t = 0.322$; $P > 0.10$). No relation was observed between anti-AF IgE antibody levels and number of precipitation lines in immunoelectrophoresis ($r = 0.18$; $P > 0.10$).

A highly significant correlation was found, however, between levels of total serum IgE and IgE antibodies against whole AF-Ag ($r = 0.70$; 58 *df*; $P < 0.001$). The relation between both concentrations was linear as demonstrated by regression analysis (Fig. 1).

The percentage of anti-AF IgE related to overall IgE ranged up to 22%. However, the mean percentage $\pm$ SD was only $4.8\% \pm 2.8$.

IgE antibody against the specific antigen C_2 . The amount of IgE eluted from the antigen C_2 -coated tubes was very low, ranging up to only 5 IU/ml, even when the patients had anti- C_2 precipitin. Moreover, the difference between results in patient and control sera was not significant ($t = 0.289$; $P > 0.10$).

*Identification of the allergen in *A. fumigatus* by means of radioimmuno-electrophoresis (Fig. 2).* The allergen of *A. fumigatus* combined with IgE antibody in infected patients' sera was identified by autoradiography with ^{125}I -labeled anti-human IgE. It can be seen from Fig. 2 that the radioactive precipitation line was distinct from the C_2 -anti- C_2 band, as identified from its electrophoretic mobility and chymotryptic activity, which confirms the results obtained with the C_2 immunosorbent.

DISCUSSION

The total serum IgE level was determined in 60 patients infected with *A. fumigatus* and compared with the concentration of specific IgE antibody against

TABLE 1
TOTAL SERUM IgE LEVEL AND CONCENTRATION OF IgE ANTIBODY AGAINST AF-Ag IN PATIENTS
DEMONSTRATING ANTI-AF PRECIPITINS

Patients	Total IgE (IU/ml)		IgE antibody (IU/ml)	
	Range	Geometric mean	Range	Geometric mean
With precipitins	49-31,145	850	1-231	10.3
Controls without precipitins	16- 2,250	195	0- 6	3.0

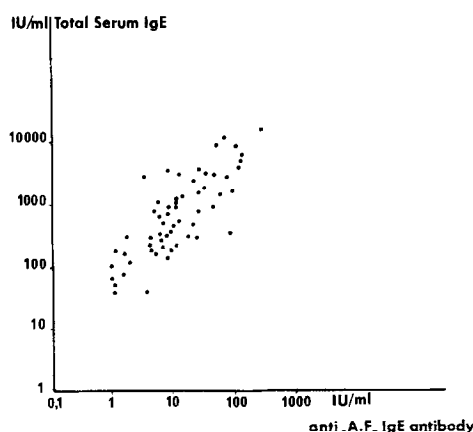


FIG. 1. Linear relationship between total serum IgE and anti-AF IgE antibody in sera from patients with anti-AF precipitins.

fungal antigens. By the RIST, marked variation in IgE levels was observed. Approximately one-half of the infected patients had a serum IgE concentration over 700 IU/ml, but no relation was obtained to the number of precipitation lines in immunoelectrophoresis. Increased IgE concentration in allergic aspergillosis has been reported by Heiner and Rose (1) as being in excess of 800 ng/ml and by Patterson *et al.* (2). In patients with asthma and precipitins to *A. fumigatus* the latter found raised IgE levels in two of five subjects, while two of six individuals with various lung diseases and precipitins had elevated IgE concentrations. These patients had serological features resembling the ones included in the present study. The marked overlap between healthy and infected patients therefore limits the diagnostic usefulness of overall IgE determination.

Specific IgE antibody against AF-Ag was isolated by means of an immunosorbent prepared with the antigen under study. Preliminary experiments showed that pouring the serum repeatedly onto the AF-Ag immunosorbent did not increase the yield of IgE antibody. A glycine-HCl buffer containing albumin was used to elute the antibodies since extraneous protein allows a good yield (14, 15) and exerts a stabilizing effect on the eluted reagents (16). Washing of the immunosorbent prepared with *A. fumigatus* was repeated until the recovery of ^{125}I -labeled nonspecific IgE is almost complete. Thus low-affinity anti-*Aspergillus* antibodies might have been washed off. It can be assumed, therefore, that the amount of IgE antibody measured in the concentrated eluate would be only the highest-affinity antibodies against fungal antigens.

The present results are consistent with the data reported by Patterson and Roberts (3) which provided evidence that most of IgE in sera from patients with allergic aspergillosis is not directed against *A. fumigatus*, by the use of double-antibody diffusion and Polystyrene tube solid-phase radioimmunoassay, though they did not establish the absolute level of serum IgE antibody. As much as 231 IU/ml of IgE antibody against *A. fumigatus* was observed with the present method.

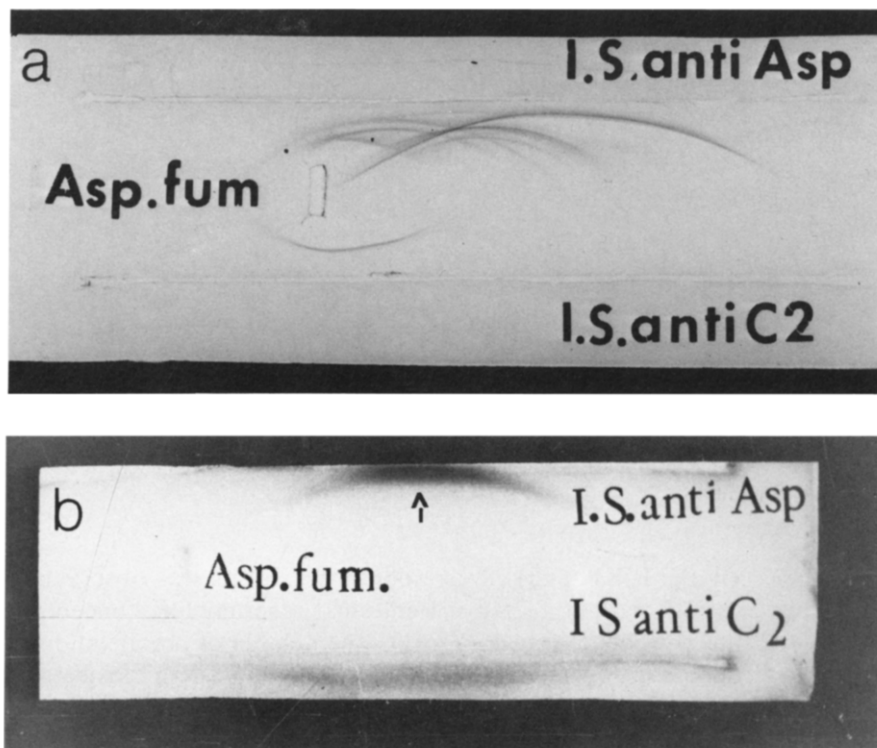


FIG. 2. Radioimmuno-electrophoretic identification of the allergen in *A. fumigatus*, combined with IgE-type antibody in serum from a patient infected with the fungus. (a), Immunoelectrophoretic pattern of a whole *A. fumigatus* extract allowed to react with: (i, upper) a rabbit immune serum against the whole fungus, prepared according to Biguet *et al.* (4); (ii, lower) a monospecific rabbit immune serum against the characteristic antigen C_2 , prepared according to Bout *et al.* (11). (b), Autoradiography of the same preparation which had been incubated first with an infected patient's serum then with radiolabeled rabbit anti-human IgE γ -globulin. A radioactive band is observed in the upper part of the preparation, i.e., with anti-whole *A. fumigatus* antigens only (arrow). The radioactive band demonstrates an anodic migration and is distinct from the particular antigen C_2 which is not combined with the human IgE antibody—radiolabeled anti-IgE complex.

The level of IgE antibody correlated with the total IgE level in serum, but no relation was obtained to incidence of anti- C_2 precipitin. Radioimmuno-electrophoresis showed that both precipitating and IgE antibodies combine with the same antigenic fraction of *A. fumigatus*, distinct from the specific antigen C_2 of the *A. fumigatus* species. The major portion of the IgE in sera from infected patients appears therefore to be the result of unrelated or nonspecific stimulation of IgE-producing cells. In many infections, also, the elevated amount of immunoglobulin is associated with only a small proportion of it having affinity for the pathogen (17, 18). Though no data have yet been provided in fungal infections, it was demonstrated that only 5% of IgG combines specifically with malarial (17) or schistosome antigens (19). The ratio of anti-parasite (*Schistosoma mansoni* or *Echinococcus granulosus*) IgE antibody to total IgE in serum was also dem-

onstrated to be below 10% in most of the patients (8, 9). It appears therefore that only a small proportion of IgE in serum is directed against the antigens of any pathogen.

The potential role of specific IgE antibody against *A. fumigatus* has still to be investigated. This points out the interest of quantitative measurements of IgE antibody. As studied by radioimmuno-electrophoresis, IgE antibody is directed against the antigenic fraction of the fungus that supports cross-reactivity with other *Aspergillus* species, a fact which may explain the common features of immediate-type hypersensitivity associated with infection by the various *Aspergillus* species.

This identification allowed the purification of the allergen in *A. fumigatus* extracts (Bout, D., and Capron, A., in preparation). Such a purified allergen might be of value for skin-test examinations of patients with aspergillosis and will provide a more precise investigative approach to the disease.

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