

Evidence That *Aspergillus fumigatus* Growing in the Airway of Man Can Be a Potent Stimulus of Specific and Nonspecific IgE Formation

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Serum IgE levels in patients with allergic bronchopulmonary aspergillosis are elevated but the degree of elevation varies markedly. Serum IgE levels in patients with aspergillomas may be strikingly elevated or normal. Absorption of serums with antigens of *Aspergillus fumigatus* combined with a solid phase radioimmunoassay technic demonstrated that both immunoglobulin E (IgE) and immunoglobulin G (IgG) antibody activity against *A. fumigatus* were markedly reduced without a parallel reduction in serum total IgE. These results indicate that the very high levels of serum IgE found in allergic bronchopulmonary aspergillosis and aspergilloma are not all specific IgE. These results are similar to those observed in rats infested with *Nippostrongylus brasiliensis*. An explanation for the elevations of IgE levels in infestations with *A. fumigatus* may be analogous to the postulate that parasite-produced materials may result in T cell stimulatory factors for IgE-producing cells. Alternatively, *A. fumigatus* organisms may produce materials that inhibit T suppressor lymphocytes.

We previously reported that serum IgE levels were elevated in allergic bronchopulmonary aspergillosis [1] and suggested that this might provide a diagnostic aid in this disease state [2]. Initial attempts to demonstrate that the majority of the markedly increased serum IgE was antibody against *Aspergillus fumigatus* antigen were unsuccessful, and it was suggested that IgE might be nonspecific (or against unknown specificities).

As a result of experience with an increasing number of cases of allergic bronchopulmonary aspergillosis, we were able to extend these studies and here provide several types of evidence that *A. fumigatus*, growing in the respiratory tract without tissue invasion [3], can provide a potent stimulus to the production of IgE not directed against antigenic materials of the organism and, in addition, specific IgE against *A. fumigatus*.

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MATERIALS AND METHODS

Serum Samples. Allergic bronchopulmonary aspergillosis serums: These were obtained from patients with established characteristics of allergic bronchopulmonary aspergillosis: recurrent pulmonary infiltrates, eosinophilia, immediate-type skin reactivity to *A. fumigatus* and precipitating antibodies against *A. fumigatus* [4]. These patients were either seen at the Northwestern Clinics or serums of patients with these clinical characteristics were sent from other university clinical services.

TABLE I IgE Concentrations in Serum from 35 Patients with Characteristics of Allergic Bronchopulmonary Aspergillosis and Four with Aspergillomas

Patient No.	Serum IgE (ng/ml)*	Patient No.	Serum IgE (ng/ml)	Patient No.	Serum IgE (ng/ml)
1	72,000	14	25,440	27	11,280
2	71,300	15	24,768	28	8,800
3	62,400	16	20,160	29	8,160
4	53,280	17	17,280	30	7,680
5	52,800	18	16,800	31	7,440
6	50,400	19	15,600	32	7,200
7	45,600	20	14,400	33	6,960
8	40,800	21	14,400	34	6,000
9	36,000	22	14,160	35	2,880
10	32,400	23	13,920	36†	50,400
11	31,200	24	13,440	37†	31,200
12	30,000	25	12,480	38†	60
13	27,600	26	12,480	39†	60

* Normal level approximates 100 ng/ml [6].

† Patients with aspergilloma.

Aspergilloma serums: Four serums were from four patients with characteristics of aspergillomas on chest films and positive sputum cultures for *A. fumigatus*. Two of these serums had been shown to have increased IgE concentrations.

Control serums: These were from normal laboratory workers known to have none of the characteristics of allergic bronchopulmonary aspergillosis.

Post-treatment allergic bronchopulmonary aspergillosis serums: Patients with allergic bronchopulmonary aspergillosis observed at this institution were treated with alternate day regimens of prednisone (0.5 mg/kg). Post-treatment serums were obtained after a minimum of six months of corticosteroid therapy and after a slow tapering of the dose of prednisone had been initiated.

A. fumigatus antigen: A stock pool of *A. fumigatus* antigen was prepared from four sources. One was a culture preparation of *A. fumigatus* obtained from the sputum of a patient with allergic bronchopulmonary aspergillosis. The second and third preparations were from Dr. Charles Reed, Madison, Wisconsin, Dr. John Thurston, Ames, Iowa, respectively, and the fourth preparation was from Hollister-Stier Corporation, Seattle, Washington. The final *A. fumigatus* antigen preparation had a protein concentration of 4.1 mg/ml as estimated by optical density using a Beckman DU spectrophotometer at 280 nm. This pool of *A. fumigatus* antigen gave precipitin bands with all 35 serums of patients with allergic bronchopulmonary aspergillosis and with the four serums of patients with aspergilloma which were tested.

Polystyrene tube radioimmunoassay (RIA): IgE and IgG antibodies against *A. fumigatus* were evaluated by the polystyrene tube solid phase RIA technic as used previously in this laboratory [3]. The following reagents are added sequentially to the polystyrene tubes with appropriate washes and incubation periods; *A. fumigatus* antigen to form a solid phase immunoabsorbent; human serum albumin, patient or control serum in appropriate dilutions; heterologous anti-immunoglobulin specific for the heavy chain of the respective immunoglobulin under study and, lastly, ^{125}I -labeled immu-

noglobulin of the same class as that against which the heterologous antiserum is directed.

IgE assay: This was carried out utilizing the double antibody radioimmunoassay technic of Gleich and associates [5].

Absorption experiments: Tenfold dilutions of serum were added to polystyrene tubes coated with *A. fumigatus* antigen as described in the RIA method. After incubation of the serum in the *A. fumigatus*-coated polystyrene tubes overnight at 4°C, the same serum dilutions were transferred to a second *A. fumigatus*-coated tube and finally a third *A. fumigatus*-coated tube. The IgE and IgG antibody bound during these successive absorptions was determined using the method described. Subsequent to the final absorption, the total IgE remaining in the absorbed serum samples was again measured.

RESULTS

Serum IgE in Patients with Allergic Bronchopulmonary Aspergillosis and Aspergilloma. The serum IgE concentrations in patients meeting the criteria for allergic bronchopulmonary aspergillosis are listed in Table I as are the results in the four patients with aspergillomas.

All of the serums from patients with allergic bronchopulmonary aspergillosis contained increased IgE concentrations, but only two of the serums from patients with aspergilloma contained significantly increased IgE concentrations. Although IgE levels may vary widely in normal people, the average IgE concentration in their serum is on the order of 100 ng/ml [6].

Detection of IgE and IgG Antibodies Against *A. fumigatus* and Absorption Experiments. Serums from three patients with allergic bronchopulmonary aspergillosis and from the two patients with aspergilloma with

increased IgE were used for detailed study by the RIA technic. Typical binding curves for IgE and IgG antibody demonstrated by the RIA technic are shown in Figure 1. Both IgE and IgG antibody activity was demonstrated in this serum. Antibody activity was absorbed by successive exposure of the serum dilutions to polystyrene tubes coated with *A. fumigatus* antigen. The IgE and IgG antibody activity was determined after two successive absorptions, and the decline in both IgE and IgG antibody activity remaining after absorption is shown in Figure 1. IgE antibody activity was demonstrated at a 10-fold higher concentration of serum than IgG antibody. Absorption of this serum by repeated exposure to *A. fumigatus*-coated tubes reduced IgE antibody activity at the 10^{-1} dilution to 24 per cent of original (unabsorbed) activity (Figure 1A). The absorption reduced IgG to 15 per cent of original (unabsorbed) activity at the 10^{-3} dilution (Figure 1B). The decline in IgE antibody activity was not paralleled by a decline in serum IgE concentration determined at the 10^{-1} dilution because the

absorbed serum retained 92 per cent of original IgE (Figure 1A).

The absorption experiments described were carried out using two other serums from patients with allergic bronchopulmonary aspergillosis and the two serums from patients with aspergillomas which had markedly increased IgE concentrations. The results shown in Table II demonstrate that the total IgE absorbed from these serums ranged from a minimum of 2.7 per cent to a maximum of 29 per cent. The IgE antibody activity against *A. fumigatus* remaining after absorption was no less than 72 per cent of the initial unabsorbed activity, and the IgG antibody activity remaining after absorption was of similar magnitude or less. These results demonstrate that a majority of the IgE and IgG antibody activity of these serums was removed by the two absorptions. The amount of total IgE removed did not parallel the absorption of the IgE antibody indicating that the majority of the IgE was not directed against *A. fumigatus*.

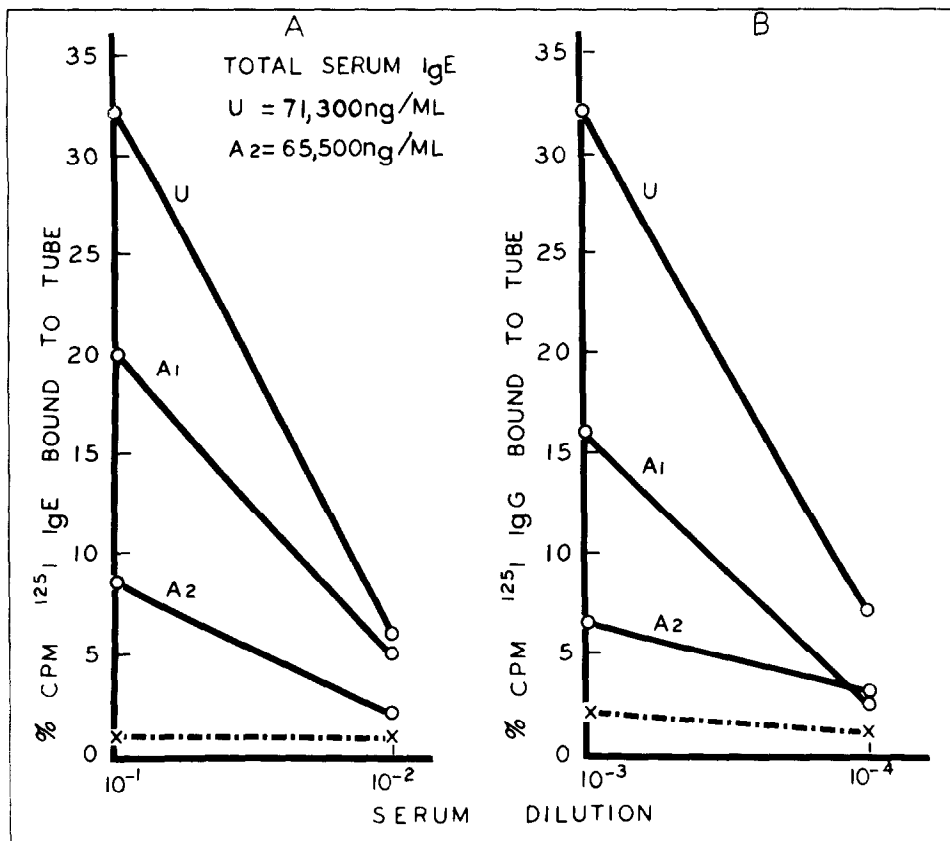


Figure 1. Serum total IgE levels and IgE and IgG antibody activity against *A. fumigatus* antigen before and after absorption with *A. fumigatus* using the serum of a patient with allergic bronchopulmonary aspergillosis. **Left,** IgE antibody against *A. fumigatus*; **right,** IgG antibody against *A. fumigatus* detected by a solid phase polystyrene tube radioimmunoassay. U = unabsorbed serum, A1 serum, absorbed once with *A. fumigatus*; A2 serum absorbed twice with *A. fumigatus*. (See Table II.) Normal control serum (x-x-x).

TABLE II The Effect of Absorption of Serum from Patients with Allergic Bronchopulmonary Aspergillosis and Aspergillomas Using a Solid Phase *A. fumigatus* Immunosorbent

Diagnosis	IgE Concentration (ng/ml)		Per Cent Reduction in Total IgE*	Per Cent Reduction in IgE Antibody-Binding Activity Against <i>A. fumigatus</i> *	Per Cent Reduction in IgG Antibody-Binding Activity Against <i>A. fumigatus</i> *
	Preabsorption	Postabsorption			
ABPA	27,370	25,760	6	78	81
A	20,585	14,605	29	77	74
ABPA	28,750	21,850	24	87	89
ABPA	71,300	65,550	8	76	85
A	29,900	29,095	2.7	72	82

NOTE: ABPA = allergic bronchopulmonary aspergillosis; A = aspergilloma.

* After absorption twice with polystyrene tubes coated with *A. fumigatus*. Calculated for 10^{-1} dilution of serum.

Comparison of Serum Total IgE and IgE Specific for *A. fumigatus*. Specific IgE directed against *A. fumigatus* was measured by RIA using selected serum samples. Serum total IgE was measured in the same samples. The comparison of the level of IgE ($\mu\text{g/ml}$) with the level of specific IgE expressed as per cent of counts per minute bound at the 10^{-1} dilution for all serums are shown in Figure 2. These results demonstrate that although elevations in serum total IgE may

be associated with IgE antibodies against *A. fumigatus*, there is no direct correlation between the serum total IgE and the specific IgE against *A. fumigatus*.

Comparison of Serum Total IgE and Specific IgE Before and After Therapy with Prednisone and Clinical Improvement. Serum samples from patients with allergic bronchopulmonary aspergillosis obtained at time of diagnosis and prior to therapy were compared with serum samples obtained after therapy with prednisone. Clinical improvement had occurred in all patients studied here as evidenced objectively by reduction or disappearance of pulmonary lesions on x-ray examination. At the time of collection of the second (post-treatment) serum sample, the patients were either not receiving prednisone or the daily dose had been tapered to no more than an average daily dose of 5 mg (given as alternate day, single dose therapy).

The results of two comparisons of the change in total and specific IgE are shown in Figure 3, and the results in five subjects studied are shown in Table III. These results show that both serum total IgE and specific IgE against *A. fumigatus* declined significantly after treatment and coincidentally with clinical improvement.

COMMENTS

The results reported here demonstrate that *Aspergillus* organisms growing in the respiratory tract can provide a potent stimulus to the production of IgE. The absorption experiments indicate that a majority of this IgE is not specific for *A. fumigatus* antigen since the decline in the serum total IgE did not parallel the decline in antibody activity after sequential absorption of serums with *A. fumigatus*. Further, the comparisons of serum total IgE with specific IgE in the serum of several patients which showed a lack of correlation of total and specific IgE is best explained by the suggestion that *A. fumigatus* is a stimulus to nonspecific IgE or IgE specific for antigens other than *A. fumigatus*.

The studies showing the decline in total IgE and IgE specific for *A. fumigatus* after therapy and clinical im-

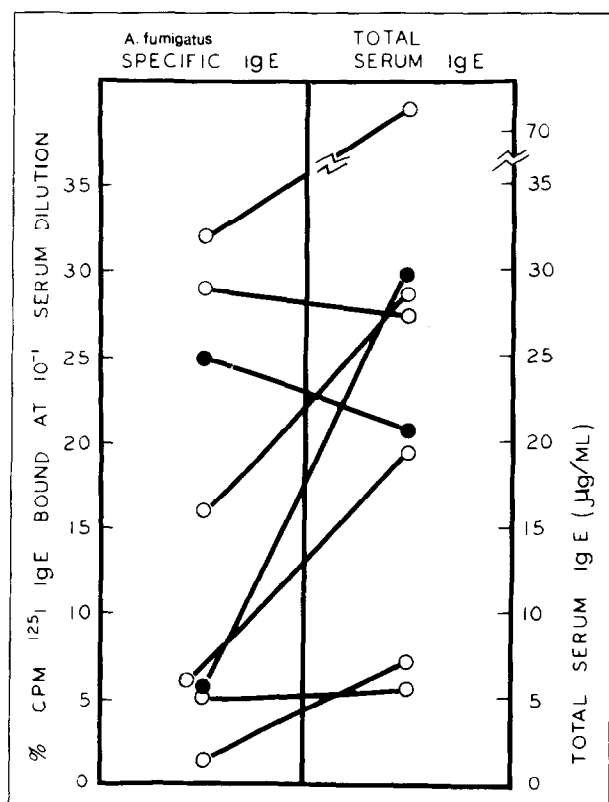


Figure 2. Relationship between serum total IgE and IgE specific for *A. fumigatus* antigen in serum of patients with allergic bronchopulmonary aspergillosis (O—O) and aspergillomas (●—●).

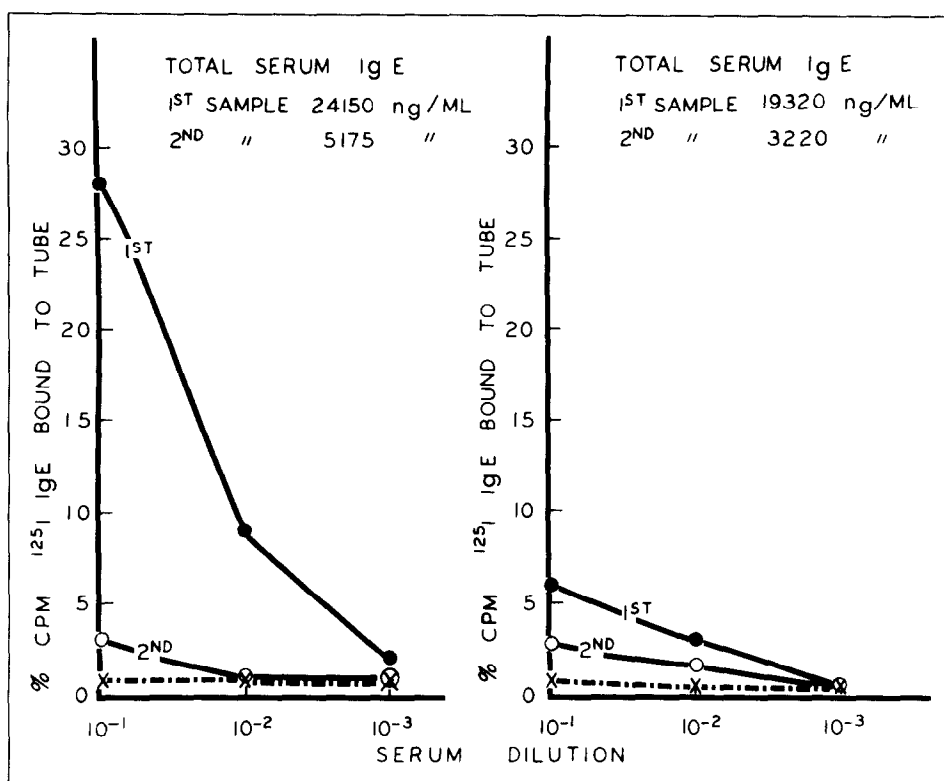


Figure 3. Changes in serum total IgE and IgE specific for *A. fumigatus* antigen in serum of two patients with allergic bronchopulmonary aspergillosis. First sample (●—●) before prednisone therapy; the second sample (O—O) was collected after months of decreasing prednisone therapy and diminution of chest lesions evident on roentgenograms. Normal control serum (x—x).

provement also indicate that *A. fumigatus* is a stimulus to both nonspecific and specific IgE. Allergic bronchopulmonary aspergillosis has been recognized as a disease characterized by the growth of the organism in the bronchi of asthmatic persons with no or minimal invasion [4]. Aspergillomas have been recognized as opportunistic growths of the organism in cavities in the lung resulting, generally, from other causes and are also noninvasive [4]. We propose that the presence of the organism in the bronchi in patients with allergic bron-

chopulmonary aspergillosis or in a cavity in some patients with an aspergilloma can provide a potent stimulus both for specific IgE, specific IgG and also for hyperproduction of IgE not directed against the organism.

The possibility that the experimental results showing a decline in specific IgE after absorption with *A. fumigatus* without a parallel decline in serum total IgE might be due to a deficiency of antigens in the *A. fumigatus* preparation used. We believe this unlikely because (1)

TABLE III Change in Serum Total IgE and IgE Specific for *A. fumigatus* After Treatment of Patients with Allergic Bronchopulmonary Aspergillosis with Prednisone

Patient No.	IgE Concentration (ng/ml)		Per Cent Reduction in Total IgE	Per Cent Reduction in Specific IgE*
	Pretreatment	Post-Treatment		
1	46,000	6,900	85	88
2	24,150	5,175	79	93
3	5,750	1,840	68	75
4	29,900	10,580	65	29
5	19,320	3,220	83	59
6	7,360	2,990	59	56

* Calculated from reduction of binding of serum IgE to Aspergillus antigen coated tubes at a serum dilution of 10^{-1} (see Figure 3).

the *A. fumigatus* preparation used was a combination of preparations from four sources and (2) all 39 serums from patients with allergic bronchopulmonary aspergillosis or aspergillomas gave precipitin reactions against this *A. fumigatus* antigen. Both the multiple sources of *A. fumigatus* antigen and the reaction rate in all serums tested provide support (but not proof) of adequate antigenic content.

We have previously observed that the serum total IgE may not correlate with the specific IgE response against the immunologic stimulus. In two patients with trichinosis in whom the exposure to raw pork was clearly defined, serial studies of serum total IgE and serum IgE specific for *Trichinella* antigen were evaluated. These studies showed that the peak total IgE response did not correlate with the specific IgE response [7].

An experimental system in rodents which may be analogous to the status of IgE in allergic bronchopulmonary aspergillosis and certain aspergillomas has been studied in detail [8–10]. Infection of rats with the nematode, *Nippostrongylus brasiliensis*, results in production of reaginic antibody against antigens of the parasite and a great increase of total IgE in rat serum. All of this IgE in the rat is not specific for antigens of *N. brasiliensis* and some of the IgE is directed against unrelated antigens. In this rodent species, it has been postulated [9] that the total IgE and the potentiated IgE response which forms a small component of it results from the release of a nonspecific IgE-stimulating factor

produced by *N. brasiliensis*-specific T cells. A similar explanation may apply to human subjects with *A. fumigatus*-specific T cells producing the IgE-stimulating factor. Further, it was shown in the rat system that far different levels of both specific and nonspecific IgE occurred in different strains of rats. Similar genetic mechanisms may explain the marked differences in the serum IgE levels in subjects with aspergillomas (Table I) and also the marked variation in the range of serum total IgE levels in subjects with allergic bronchopulmonary aspergillosis (Table I). The latter would also likely be influenced by the duration and degree of involvement of the respiratory tract with *A. fumigatus*. An alternate hypothesis is that *A. fumigatus* antigens may specifically inhibit T suppressor cells for IgE-producing B lymphocytes.

The reduction in both total and specific IgE which accompanies prednisone therapy and clinical remission in allergic bronchopulmonary aspergillosis is thought to be due to the decrease in the organism in the respiratory tract. The possibility that these serologic changes are due to prednisone alone is possible. However, the determinations of the serologic changes were made after the administration of prednisone had been discontinued or was at such a low level that this explanation is thought to be less likely. Prednisone therapy in other clinical situations has not been shown to markedly affect serum IgE levels [11].

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