

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA): STUDIES ON THE GENERAL AND SPECIFIC HUMORAL RESPONSE*

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Abstract

Serum specimens from 138 patients suffering from chronic respiratory disorders including 63 with allergic bronchopulmonary aspergillosis (ABPA), 20 with suspected ABPA, 25 with pulmonary tuberculosis, 14 with bronchial asthma, 10 with chronic bronchitis and 6 with miscellaneous pulmonary conditions were studied for circulating antibodies to *Aspergillus*. The ammonium sulfate test was employed with an iodine-125 labeled mycelial component derived from *Aspergillus fumigatus*. When compared to normal controls from the same area, this test indicated that sera from 82 per cent of patients with ABPA had elevated binding titers to the radiolabeled antigenic component. Immunodiffusion using a culture filtrate antigen from *A. fumigatus*, revealed precipitating antibody to this fungus in 89 per cent of sera from ABPA patients. The majority of patients with ABPA demonstrated marked elevations of total serum IgE, moderate elevations of serum IgA and IgD and slightly increased levels of IgG and IgM.

Introduction

Allergic bronchopulmonary aspergillosis (ABPA) represents a hypersensitivity disorder usually associated with dual antibody response involving as yet a poorly understood interplay of IgE, IgG and IgM antibodies (2, 26, 27). Most patients with ABPA have elevated levels of specific and non-specific IgE, and by Ouchterlony technique it is

often possible to demonstrate precipitins against antigenic preparations of *A. fumigatus* in about 70-90 per cent of the cases (17, 20, 25). Intracutaneous skin test with *A. fumigatus* antigens characteristically elicits a dual response with an immediate (Type I) wheal and flare reaction which is followed by an Arthus (Type III) oedematous swelling at the same site 4-6 hours later (25). In as much as of these tests represent secondary or tertiary capacities of antigen and antibody interaction, several variables are likely to influence them, e.g., presence of precipitating immunoglobulin, antigen-antibody ratio, availability of complement, histamine content of the skin, therapy with corticosteroids or antihistamines, etc. (13, 15, 25, 26, 29, 32). Recently, a quantitative technique has been developed to measure primary binding of antibody to a radiolabeled mycelial component derived from *A. fumigatus*, which has revealed a universal occurrence of aspergillus antibodies with significantly higher levels in cases of aspergillosis (2, 4, 5).

During a survey of allergic bronchopulmonary aspergillosis in Delhi, a large number of cases have been diagnosed (17, 28). This communication presents observations on the levels of *Aspergillus* antibodies in sera of patients with ABPA and other disease groups using immunodiffusion and the ammonium sulfate tests. In addition, levels of serum immunoglobulins in these patients are also presented.

Materials and methods

Test sera and patients

Sera from 138 cases included in this study were selected from 1007 patients suffering from respiratory disorders

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Table 1. Clinical classification of 149 subjects studied for antibodies to *Aspergillus*.

Clinical category	Number of patients
1. Allergic bronchopulmonary aspergillosis (ABPA)	63*
2. Suspected ABPA (Two patients had hypogammaglobulinemia)	20*
3. Pulmonary tuberculosis (One patient had sputum cultures positive for <i>A. fumigatus</i>)	25
4. Bronchial asthma (One patient had Type I skin hypersensitivity, 2 had precipitins and 3 had sputum cultures positive for <i>A. fumigatus</i>)	14
5. Chronic bronchitis	10
6. Miscellaneous Lung abscess -3 Diffuse pulmonary fibrosis -3 (One of the latter patients had Type I skin hypersensitivity, precipitins and positive sputum cultures for <i>A. fumigatus</i>).	6
7. Normal healthy adults from Delhi	11

*All patients with ABPA or suspected ABPA developed dual reaction to intracutaneous test with aspergillin.

who were investigated for bronchopulmonary aspergillosis 1971-1974 at the V. P. Chest Institute, Delhi (16). The sera examined represented five categories of disease, i.e., ABPA, suspected ABPA, bronchial asthma, pulmonary tuberculosis and chronic bronchitis (Table 1). A patient giving dual skin reaction to intracutaneous test with aspergillin was diagnosed as ABPA if positive evidence was available for at least 4 of the 5 following criteria: i) extrinsic asthma, ii) pulmonary infiltrations, iii) raised blood eosinophil count, iv) sputum culture for fungus and v) precipitins against *A. fumigatus*. If, however, two or more of the above features were lacking the patient was categorized under suspected ABPA (16). Clinical and laboratory characteristics of patients with ABPA and suspected ABPA are summarized in Table 2. Besides, sera from 11 normal subjects from Delhi were included as controls. Specimens of venous blood were taken from fasting patients and the sera were either stored frozen at -20 °C or lyophilized until used. Aliquots of serum were mailed unfrozen from Delhi to the United States where a portion of the immunological studies was conducted.

Aspergillus antibody measurement

Crude and relatively purified antigens were isolated from sonically disrupted mycelia of *A. fumigatus*, as previously described (4, 5). The crude sonic supernatant (SS-Fum) was radiolabeled with iodine 125 and was precipitated with equal amounts of 20% trichloroacetic acid (TCA); the

Table 2. Summary of principal clinical and laboratory findings in 63 patients with ABPA and 23 with suspected ABPA

Clinical parameters	ABPA		Suspected ABPA	
	Number positive	Per cent positive	Number positive	Per cent positive
History of episodic airways obstruction	63	100.0	23	100.0
Onset of respiratory symptoms below 30 years	40	63.5	16	69.5
Forced expiratory volume in 1 sec. < 70% vital capacity	37/47*	78.7	5/9	55.5
Recurrent Pulmonary infiltrations	59	93.6	4/22	18.1
Blood eosinophilia > 500 cells/cu. mm	55	87.3	11	47.8
Dual skin hypersensitivity against <i>A. fumigatus</i>	63	100.0	23	100.0
Dual skin hypersensitivity against <i>A. flavus</i>	31/55	56.3	9	39.1
Dual skin hypersensitivity against <i>A. niger</i>	17/55	30.9	3	13.0
Sputum cultures repeatedly positive for <i>A. fumigatus</i>	48	76.1	5	21.7
Precipitin bands by immunodiffusion against <i>A. fumigatus</i>	56	88.8	3	13.0
Precipitin bands by immunodiffusion against <i>A. flavus</i>	15/62	24.2	0	—
Precipitin bands by immunodiffusion against <i>A. niger</i>	2/62	3.1	1	4.3
Primary binding 20% or above	52	82.0	4/18	22.0

*Numerator denotes number of positive patients and denominator number investigated

supernatant, TCA-S(Fum), was used in an ammonium sulfate precipitation test (12). The per cent ^{125}I -TCA-S antigen at $0.01 \mu\text{g N}$ per ml that was bound by 0.5 ml of a 1 : 5 dilution of each test serum was determined by precipitation of ^{125}I -TCA-S(Fum) : anti-*Aspergillus* complexes with 50% saturated ammonium sulfate (SAS/2). Calculations were made on the basis of the per cent of radiolabeled antigen in the SAS/2 precipitates, less the background counts, without correction for precipitation by normal human serum and/or TCA-precipitable material (4, 6).

The immunodiffusion technique and the antigens employed were the same as previously described (17). The culture filtrate antigen (aspergillin) was prepared from 5 strains of *A. fumigatus* and one each of *A. flavus* and *A. niger*. The fungi were grown in synthetic glucose-asparagine broth (30) for 4 weeks at $27 \pm 1^\circ\text{C}$. The culture filtrates were dialysed and then concentrated about 20 folds by exposing the dialysis bags to an air current. The potency of these antigens was tested against hyperimmune sera and stored refrigerated. The protein content varied from 9–12 mg per ml. The immunodiffusion test was carried out in 50 mm. Petri dishes containing 4 ml of 1.0% Difco Noble agar in McIlvaine citrate buffer, pH 7.3 to eliminate non-specific reactions due to C-reactive proteins requiring Ca^{++} ions. For skin test about 0.01 ml culture filtrate antigen diluted to its original strength was injected intracutaneously to give 2 mm bleb. The immediate and late reactions were recorded at 20 minutes and 6 hours, respectively.

Immunoglobulins

Serum IgE levels were measured by a commercial radioassay (Pharmacia Laboratories, Inc., Piscataway, New Jersey, U.S.A.) based on the radioimmunoabsorbent technique of Johansson, Bennich & Wide (14). Radial immunodiffusion (22) was used for the quantification of serum levels of IgG, IgA, IgM and C3 components of complement. Monospecific antisera to purified IgG, IgA, IgM and C3 were raised in pygmy goats using incomplete Freund's adjuvant. IgD antiserum was obtained from Melpar, Inc., U.S.A. The IgG, IgA, IgM, IgD and C3 standards were those of Hoechst Pharmaceuticals Ltd. and Hyland Laboratories, Inc., U.S.A. Normal values for these immunoglobulin and complement determinations were independently established by assaying 100 normal Red Cross blood donors. Mean values with standard deviation were as follows: IgG— $1,274 \pm 280 \text{ mg}\%$; IgA— $227 \pm 53 \text{ mg}\%$; IgM— $127 \pm 46 \text{ mg}\%$; IgD— $5.5 \pm 3.3 \text{ mg}\%$; IgE— $199 \pm 211 \text{ WHO I.U./ml}$; and C3— $145 \pm 30 \text{ mg}\%$.

Results

Data obtained from primary binding to ^{125}I -TCA-S(Fum) in 138 sera from patients with established ABPA, five pulmonary disease categories and 11 healthy control subjects are summarised in Table 3 and schematically shown in Figure 1. Sera from patients with ABPA demonstrated the highest per cent mean binding values to the

Table 3. Summary of data on binding of ^{125}I -TCA-S(Fum) mycelial antigen by serum from patients with ABPA and other respiratory disorders

Clinical category	No. sera tested	Per cent ^{125}I -TCA-S(Fum) antigen at $0.01 \mu\text{g N/ml}$ bound by 1:5 dilution of test sera	
		Range	Mean $\pm$ S.D.*
Normal	11	6.5 – 18.6	10.9 ± 4.4
ABPA	63	12.0 – 55.4	36.6 ± 13.8
Suspected ABPA	20**	6.9 – 32.2	16.3 ± 6.3
Pulmonary tuberculosis	25	10.4 – 33.1	20.3 ± 5.8
Bronchial asthma	14	4.1 – 28.4	16.0 ± 5.8
Chronic bronchitis	10	10.0 – 20.8	14.2 ± 3.4
Miscellaneous	6	8.0 – 29.9	16.8 ± 12.5

* S.D. = Standard deviation

** Data from 2 patients with hypogammaglobulinemia excluded

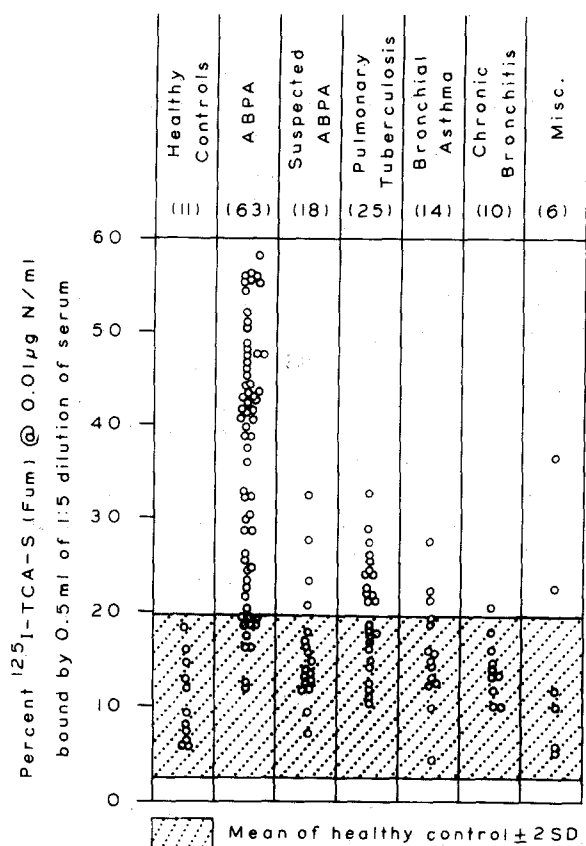


Fig. 1. Graphic representation of primary binding values in sera of patients with ABPA and other categories. Figures in parenthesis denote number of sera tested.

labeled *Aspergillus* antigen (36.6 ± 13.8), followed by pulmonary tuberculosis (20.3 ± 5.8), suspected ABPA (16.3 ± 6.3), bronchial asthma (16.0 ± 5.8) and chronic bronchitis (14.2 ± 3.4). The mean binding value in 11 normal individuals from Delhi was 10.9 ± 4.4 . Sera from patients with ABPA had significantly higher binding values than those found in any other disease category ($p < 0.001$). Fifty two of 63 sera (82%) from ABPA patients had elevated binding titers. However, 11 ABPA patients had binding values within two standard deviations of the normal individuals. Apart from ABPA, sera from 13 of 25 patients with pulmonary tuberculosis demonstrated elevated binding values. In addition, sera from 6 individuals in the remaining three disease categories had abnormally elevated binding values.

The immunodiffusion test revealed one or more precipitin bands in sera from 56 (89%) of 63 ABPA patients. Some degree of correlation was evident between the number

of precipitin bands, level of antigen binding, and per cent sputum samples culturally positive for *A. fumigatus* (Table 4). Patients with ABPA having two or more precipitin bands demonstrated significantly higher levels of binding (mean values: 41 ± 12.9) and positive sputum cultures (70%) than patients with a single or no precipitin band ($p < 0.05$). In patients suspected for ABPA on the basis of dual response to an intracutaneous test with aspergillin, precipitins and elevated binding to *A. fumigatus* were observed in sera from 13% and 22% of the cases, respectively (Table 2). Precipitins were also obtained in 2 (14%) of the sera from patients with bronchial asthma and one of the 3 sera from patients with diffuse pulmonary fibrosis. Fifteen (24%) and 2 (3%) sera from ABPA patients reacted with *A. flavus* and *A. niger* culture filtrates. The remaining sera, with one exception from suspected ABPA category, did not reveal any positive immunodiffusion test for these two species.

Determination of serum IgG, IgA, IgM, IgD and IgE immunoglobulin levels as well as C3 component of complement were carried out in 99 patients including 61 with ABPA, 20 with suspected ABPA, five with bronchial asthma, three each with pulmonary tuberculosis, lung abscess and diffuse pulmonary fibrosis, and four healthy controls (in addition to the normal 100 blood donors cited above). Results of serum immunoglobulin determinations in the sera from patients with ABPA and suspected ABPA are schematically depicted in Figure 2.

Total serum IgE levels in 61 patients with ABPA ranged from 270 to 30,000 I.U./ml with a mean value of 6626 ± 6541 I.U./ml. This was significantly higher than in any other disease category studied ($p < 0.001$). Serum IgE levels were elevated in the majority of patients with suspected ABPA with a mean value of 1261 ± 823 I.U./ml and a range of 400 to 3100 I.U./ml. Of 57 patients with ABPA, 39 had serum IgG levels exceeding the upper limits of normal range (1834 mg%) with a mean value of 2239 ± 935.6 mg%. Hypogammaglobulinemia IgG was not found in this group, but two patients in the suspected ABPA category had abnormally reduced levels of IgG. Thirty ABPA patients had substantially elevated levels of IgA (> 333 mg%) with a mean value of 351 ± 135 mg%. Eighteen patients with ABPA had elevated levels of IgM (> 219 mg%) with a mean of 185 ± 65 mg%. All but six of the 28 ABPA patients studied had elevated levels of serum IgD with a mean of 52 ± 71 mg%. Levels of C3 component of the complement were essentially normal with a mean value of 155 ± 37 mg%.

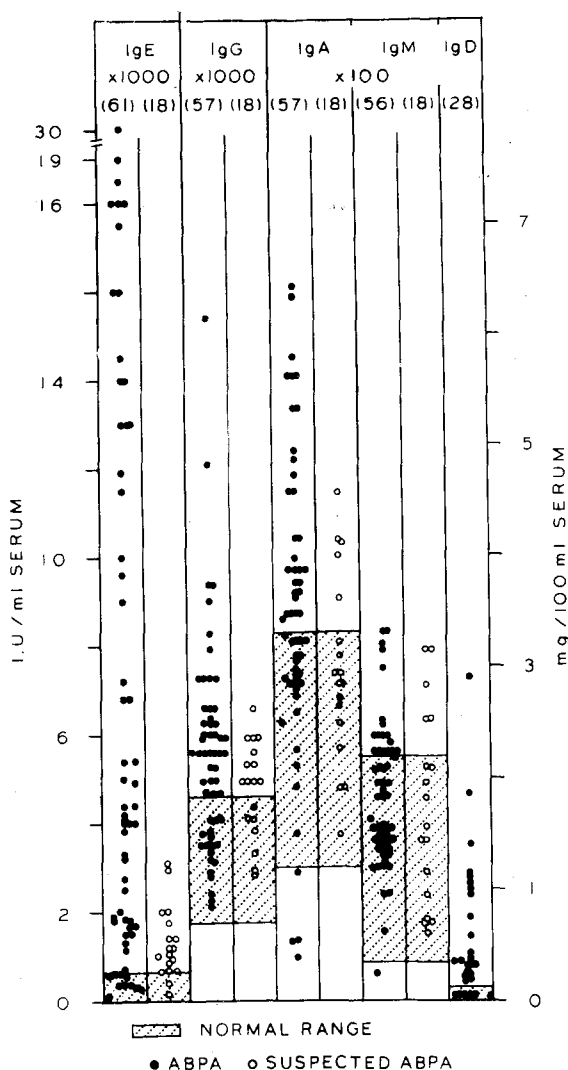


Fig. 2. Serum immunoglobulin levels in patients with ABPA and suspected ABPA. Figures in parenthesis denote number of sera tested. Normal range (shaded area) reflect two standard deviation from the mean (± 2 S.D.). No patient with suspected ABPA was assayed for IgD.

Discussion

The precipitating antibodies have been ascribed a pathogenic role in Arthus reaction involved in ABPA (25, 26). Although, unlike in aspergilloma, the precipitin bands are weaker and fewer in this condition (9, 10, 20) a positive immunodiffusion test is usually included among its diagnostic criteria (17, 28). In the present study 89% of the ABPA patients revealed one or more immunodiffusion bands against *A. fumigatus* which is considered to be the chief casual fungus of this disease. In comparison, *A. flavus* and *A. niger* gave only 25% and 3% positive immunodiffusion tests respectively. Furthermore, the positive tests with *A. fumigatus* in other disease conditions were very low, that is, 13% in suspected ABPA and not more than 5% in the rest of the disease groups.

The deployment of a more sensitive ammonium sulphate test has revealed varying quantities of antibodies to *A. fumigatus* in all the 147 patients. Significantly, 52 of the 63 (82%) manifested binding values in excess of two standard deviations of the mean normal value thus further corroborating the diagnosis of ABPA in a great majority of the cases. It would appear that both these test systems and the antigenic components employed to perform them, complement each other in the demonstration of *Aspergillus* antibody, thus facilitating a more precise diagnosis. Whereas five of 11 ABPA patients with normal binding values had demonstrable precipitins, two sera with elevated binding values failed to demonstrate precipitating antibody. This situation is probably the result of antibodies directed to components better represented in antigens employed in the immunodiffusion test in the former sera, and to the TCA-S (Fum) antigen in the later sera. Simultaneous utilization of both tests increases the probability of verifying the presence of specific antibody, utilises the quantitative nature of the primary binding test to serially measure increasing levels of antibody in the early phases of ABPA, and diminishing antibody levels as a result of treatment (3). It is of interest that some degree of correlation was evident between

Table 4. Comparison of precipitating antibody, binding values and sputum culture yield in patients with ABPA

Number of patients	Number of precipitin bands by immunodiffusion	Per cent binding of ¹²⁵ I-TCA-S(Fum) antigen Mean $\pm$ S.D.	Sputa yielding <i>A. fumigatus</i> in culture % positive
18	2 or more	41.1 $\pm$ 12.9	70.1
38	one	36.6 $\pm$ 12.5	36.8
7	nil	24.7 $\pm$ 10.2	36.8

binding values observed, number of precipitin bands and the number of positive sputum cultures for *A. fumigatus* in a given patient (Table 4). It is noteworthy that both the techniques have revealed positive serological evidence in only a limited number of suspected ABPA cases. This would suggest that sensitization of the host as indicated by dual skin reaction to aspergillin, is not sufficient for full manifestation of the syndrome unless pathogenetic circulating antibodies to *A. fumigatus* are simultaneously present in detectable amounts. Elevated binding values and/or precipitins to *A. fumigatus* in sera from some patients in other disease categories (fig. 1) may reflect saprophytic colonization by this fungus or possible sub-clinical aspergillosis in such patients. Earlier workers have reported precipitins in cystic fibrosis and a variety of chronic pulmonary diseases (7–10, 23, 31), suggesting a persistent or frequent contact with this highly immunogenic fungus.

Elevated total levels of serum IgE in great majority of patients with ABPA are in complete agreement with earlier reports (1, 6, 24, 35). Although determination of Aspergillus-specific IgE may be a more informative parameter, elevated levels of total IgE frequently parallel Aspergillus-specific IgE and together with other clinical and immunological parameters can be very helpful in arriving at a correct diagnosis (36). The few instances of normal IgE values (under 621 units/ml) in serum from patients with ABPA in this series may represent a favourable response frequently observed following corticosteroid therapy, e.g., Turner et al. (36) reported wide fluctuations of serum IgE (500 to 18,000 units/ml) during an 18 months follow up period. These investigators also noted higher levels of serum IgE in association with fresh pulmonary infiltrations.

Serum from patients with ABPA manifested normal to slightly increased levels of IgG and IgM and a majority of patients manifested a more pronounced increase in concentrations of IgA and IgD. Elevation of serum IgG and IgM is consistent with the presence of chronic infection and with the previous observations that the majority of antibody activity detected by the ammonium sulfate test is attributable to these two classes of immunoglobulins (5). Serum IgA levels are frequently elevated in a variety of disorders involving secretory surfaces, and may reflect increased amounts of circulating exocrine IgA (34, 37). Deficiency of IgA has also been considered by some investigators to be causally related to atopy (11) in that it may alter mucosal permeability with resultant enhanced production of IgE in pre-disposed subjects (19, 33).

Although three patients were noted to have abnormally low serum concentrations of IgA, it would appear unlikely that this was related to ABPA. The finding of elevated levels of serum IgD is consistent with previous observations that this immunoglobulin tends to be elevated in patients afflicted with atopic disorders (18). A recent study uncovered a striking elevation of serum IgD in approximately 50 per cent of patients with ABPA. Mycetomal and invasive forms of aspergillosis did not demonstrate this abnormality (21). Further work with immunoadsorbant techniques should reveal the specificity of these IgD antibodies.

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