Thorax, 1977, **32**, 269–274

Studies in chronic allergic bronchopulmonary aspergillosis
3 Immunological findings

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Malo, J. L., Longbottom, J., Mitchell, J., Hawkins, R., and Pepys, J. (1977). *Thorax*, **32**, 269–274. **Studies in chronic allergic bronchopulmonary aspergillosis. 3 Immunological findings.** Precipitin tests by two different methods, double-diffusion (DD) and counterimmunoelectrophoresis (CIE), and measurements of total and specific IgE against *Aspergillus fumigatus* were made in 50 patients with chronic allergic bronchopulmonary aspergillosis and in three control groups—atopics with a positive immediate prick test to *A. fumigatus* but no evidence of allergic aspergillosis, atopics with a negative prick test to *A. fumigatus*, and non-atopics.

Precipitins were found in 84% and 78% of the patients with aspergillosis by the DD and CIE methods respectively. Precipitins were also found in 6 out of 27 (22%) patients with a positive prick test to *A. fumigatus* but no evidence of aspergillosis and in 1 of 24 patients with a negative prick test to *A. fumigatus*.

The means of specific and total IgE values were significantly higher in the group of patients with aspergillosis than in the three other groups of patients. The increase in specific but not total IgE showed a statistically significant correlation with positive precipitin tests in the patients with aspergillosis. Total IgE but not specific IgE values were significantly higher ($0{\cdot}02<P<0{\cdot}05$) in patients who had had a transient radiographic shadow in the previous three months. Positive precipitin tests were also significantly correlated with the number of transient shadows in the past and with the interval of time since the last transient shadow.

Precipitin tests by double-diffusion (DD) (Longbottom and Pepys, 1964) and counterimmunoelectrophoresis (CIE) (Ward and Kohler, 1973; Flaherty *et al.*, 1974) are helpful tools in supporting the diagnosis of allergic bronchopulmonary aspergillosis. Marked elevation of serum total IgE (Patterson *et al.*, 1973; Patterson and Roberts, 1974) may suggest the diagnosis in acute episodes though it is not clear how much is attributable to specific IgE against *A. fumigatus* (Patterson and Roberts, 1974; Turner *et al.*, 1974).

The aim of this report was to compare the incidence of precipitins by the DD and CIE methods and the levels of total and specific IgE against *A. fumigatus* in 50 patients with chronic allergic bronchopulmonary aspergillosis and to compare these patients with three groups of subjects—atopics with a positive prick test to *A. fumigatus* but without evidence of aspergillosis, atopics with a negative prick test to *A. fumigatus*, and non-atopics. The immunological findings were also correlated with the clinical and radiological findings in the patients with aspergillosis.

[1]Supported by a fellowship grant from the Medical Research Council of Canada

Material and methods

PATIENTS

Four groups of patients were tested:

Group I: 50 patients with chronic allergic bronchopulmonary aspergillosis in whom the diagnosis had been made from 2 to 25 years before (mean duration 10·9 years). The criteria for the diagnosis were asthma with at least one episode of fleeting radiographic shadow, and blood eosinophilia, together with a positive immediate prick reaction to an extract of *A. fumigatus*. The clinical, physiological, and radiological features of these patients have been reported (Malo *et al.*,

1977a, b). All the patients had skin prick test with a routine battery of 23 common allergens and with two different extracts of *A. fumigatus*, one provided by Bencard (5% w/v extract) and another prepared in our department (dialysed, freeze-dried preparation at 1 mg/ml).

Group II: 27 asthmatic patients, all atopics, ie, with positive prick test reactions to common allergens as well as a positive prick test to *A. fumigatus*. The diagnosis of allergic aspergillosis was carefully excluded by the absence of the following: suggestive past history; fleeting shadows in chest radiograph; blood eosinophilia; and precipitins. The chest radiographs of 18 of these were assessed 'blind' by a radiologist (Dr. George Simon) and were regarded as normal in 16 instances whereas two had limited tubular shadows.

Group III: 12 atopic patients with a history of asthma, rhinitis or urticaria and with a negative prick test to the two extracts of *A. fumigatus*.

Group IV: 12 non-atopic individuals with negative skin tests to the routine allergens and to the two extracts of *A. fumigatus*. They consisted of three healthy subjects and nine patients with cryptogenic asthma, rhinitis or urticaria.

ANTIGEN PREPARATIONS

Four different antigen preparations of *A. fumigatus* were used. Three of these were prepared from freeze-dried, dialysed culture filtrates grown at room temperature for five weeks on an histoplasmin synthetic medium. One of these was included for the prick testing and all the immunological tests. The fourth extract was commercially available (Bencard).

SEROLOGICAL TESTS

The following tests were done on the sera of all the patients:

(1) Precipitin tests by the DD method (Longbottom and Pepys, 1964) against the four different extracts of *A. fumigatus*. Two extracts were used at 10 and 30 mg/ml and the other two at 20 and 30 mg/ml respectively. The serum was concentrated four-fold and the test repeated when the neat serum gave negative results.

(2) Precipitin tests by the CIE method (Culliford, 1964) modified as follows: the tests were performed in 1% agarose, 1 mm deep, in Veronal buffer, pH 8·6, 0·05 molar. The wells were cut 6 mm apart and contained 13 μl of serum and antigen. The neat sera only were tested against two or three different antigens, the concentrations of two being 30 mg/ml and of the third 15 mg/ml. Electrophoresis was carried out at 4 V/cm for 90 minutes. The plates were washed in citrate buffered isotonic saline solution overnight, dried, and stained with Coomassie blue. The sera of 46 of the 50 patients with allergic aspergillosis and all the sera of the other groups of patients were tested.

(3) Specific IgE measurements against *A. fumigatus* were made by the radioallergosorbent test (RAST) using cellulose discs coupled with one of our own extracts of *A. fumigatus* according to the technique of Wide *et al.* (1967).

(4) Total IgE measurements were made by the Mancini method modified by Rowe (1969).

The results of precipitin tests by the DD method were scored in the following way:

Grade 6: neat serum reacting to the 6 extracts with 3 lines or more
Grade 5: neat serum reacting to the 6 extracts with 1 to 2 lines
Grade 4: neat serum reacting to 3 to 5 extracts
Grade 3: neat serum reacting to 1 to 2 extracts
Grade 2: serum reacting only after concentration to 4 to 6 extracts
Grade 1: serum reacting only after concentration to 1 to 3 extracts
Grade 0: no reaction with concentrated serum.

The results of precipitin tests by the CIE method were also scored by measuring the ratio between the number of extracts to which the serum reacted and the total number of extracts tested.

Unpaired *t* test and linear correlation tests were used for statistical analysis of the results.

Results

PRECIPITIN TESTS

Table I shows that 32 patients (64%) with allergic aspergillosis (group I) had evidence of precipitins by the DD method when the neat serum was tested and 10 others only after the serum was concentrated. A total of 42 patients (84%) in this

Table 1 *Incidence of precipitins by double-diffusion*

Group	Neat serum	Concentrated serum	Total	
			No. of patients	%
I Allergic aspergillosis	32/50	10/18	42/50	84
II Atopics with + skin test to *A. fumigatus*	4/27	2/23	6/27	22
III Atopics with − skin test to *A. fumigatus*	0/12	0/12	0/12	0
IV Non atopics	1/12	0/11	1/12	9

group were thus shown to have evidence of precipitins. Four of the 27 patients (15%) with a positive prick test to *A. fumigatus* but not aspergillosis (group II) had precipitins in the tests on neat serum and two others in tests with concentrated serum, giving a total of six (22%) with positive precipitin reactions. The single patient in the two other groups of patients (groups III and IV) who had a positive precipitin test with neat serum was an asthmatic in whom the diagnosis of allergic aspergillosis was excluded. Concentration of the serum did not increase the incidence of positive precipitin tests in groups III and IV.

Table 2 shows that positive precipitin tests by the CIE method were obtained in 36 of the 46 (77·8%) sera of the patients with allergic aspergillosis (group I) and in only two of the 27 (7·5%) patients in group II with a positive prick test to *A. fumigatus* but not aspergillosis. Except for the serum of the non-atopic asthmatic patient mentioned above, no reactions were shown in the two other groups (groups III and IV).

Table 2 *Incidence of precipitins by counter-immunoelectrophoresis*

Group	Positive to		Total	
	All extracts	*Only one extract*	*No. of patients*	*%*
I Allergic aspergillosis	29/46	7/46	36/46	78
II Atopics with + skin test to *A. fumigatus*	0/27	2/27	2/27	8
III Atopics with − skin test to *A. fumigatus*	0/12	0/12	0/12	0
IV Non atopics	1/12	0/12	1/12	8

TOTAL AND SPECIFIC IgE TESTS

The figure shows a considerable overlap of the values of total IgE in the four groups, the standard deviations being wide. The values for specific IgE against *A. fumigatus* show better separation of the four groups. Considering the values within one standard deviation of the mean, there was a small overlap between the patients with aspergillosis (group I) and the atopic patients with a positive prick test to *A. fumigatus* but not aspergillosis (group II) and no overlap between the patients with aspergillosis (group I) and the two groups with a negative prick test to *A. fumigatus* (groups III and IV).

The values of specific IgE against *A. fumigatus* were significantly higher ($P<0.001$) in patients with allergic aspergillosis (group I) than in the three other groups of patients respectively. The patients with a positive prick test to *A. fumigatus* but no aspergillosis (group II) had significantly higher

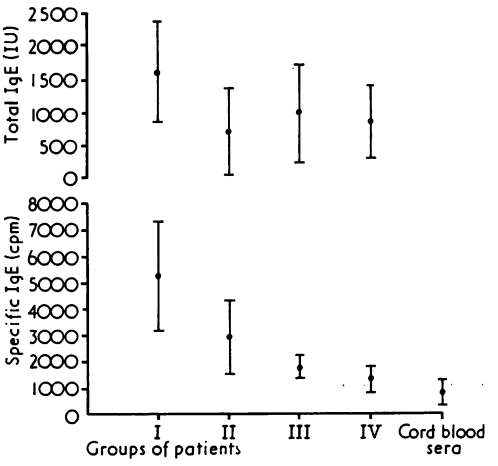

Figure *Means and standard deviations of total and specific IgE. One standard deviation above and below the mean value is illustrated. See text for levels of statistical differences. Group I: patients with allergic aspergillosis; group II: atopic patients with a positive prick test to* A. fumigatus *but without aspergillosis; group III: atopic patients with a negative prick test to* A. fumigatus*; group IV: non-atopic individuals.*

values of specific IgE than the atopics with a negative prick test to *A. fumigatus* (group III) ($P<0.05$) and than the non-atopics (group IV) ($P<0.01$). Finally, atopics with a negative prick test to *A. fumigatus* (group III) had higher values for specific IgE than the non-atopics (group IV) ($P<0.05$).

Considering the values for total IgE shown in the Figure, patients with allergic aspergillosis (group I) were found to have significantly higher levels than the atopics with a positive skin test to *A. fumigatus* (group II) ($P<0.001$), than the atopics with a negative skin test to *A. fumigatus* (group III) ($P<0.05$), and, finally, to the non-atopics (group IV) ($P<0.01$).

CORRELATION BETWEEN IMMUNOLOGICAL FINDINGS

Table 3 shows good agreement between the results of precipitin tests by the two different methods. The increase in specific IgE bore a strong correlation with the degrees of positivity of precipitin reactions as measured by the CIE method but less so by the DD method. The total of IgE values bore no correlation with the specific IgE values or the precipitin tests.

CORRELATION BETWEEN IMMUNOLOGICAL, CLINICAL, AND RADIOLOGICAL FINDINGS

In the allergic aspergillosis group (group I), there

Table 3 *Relationship between values of immunological tests**

	Total IgE	*Specific IgE*	*Precipitins by counterimmuno-electrophoresis***
Precipitins by double-diffusion	NS	$0{\cdot}05 < P < 0{\cdot}1$	$P < 0{\cdot}001$
Precipitins by counterimmuno-electrophoresis	NS	$0{\cdot}001 < P < 0{\cdot}01$	
Specific IgE	NS		

*Values for P using the linear correlation test.
**Graded degree of positivity. See text for gradation.

was no correlation between any of the immunological and clinical findings, such as age, ages at onset of asthma and at diagnosis of aspergillosis, nor with the duration of the asthma or the aspergillosis. Table 4 shows that positive precipitin tests were related to the activity of the disease as assessed by the interval since the last transient shadow and the total number of past radiographic shadows. There was no linear correlation between the values of total and specific IgE and the radiological features. Twelve patients who had had a transient shadow in the previous three months were found to have significantly ($0{\cdot}02 < P < 0{\cdot}05$) higher total IgE values than the other patients. This was not the case for specific IgE values.

Table 4 *Relationship between radiological and immunological findings**

	*Precipitins***		*Specific IgE*	*Total IgE*
	Double diffusion	*Counterimmuno-electrophoresis*		
Interval since last transient shadow	$0{\cdot}01 < P < 0{\cdot}02$	$0{\cdot}02 < P < 0{\cdot}05$	NS	NS
Total number of past transient shadows	$0{\cdot}01 < P < 0{\cdot}02$	$0{\cdot}02 < P < 0{\cdot}05$	NS	NS

*Values for P using the linear correlation test.
**Graded degree of positivity. See test for gradation.

Discussion

The 84% incidence of precipitin reactions to *A. fumigatus* as measured by the double-diffusion method in the patients with allergic aspergillosis is close to the 89% reported by McCarthy and Pepys (1971) and the 82% reported by Safirstein *et al.* (1973). The double-diffusion and counter-immunoelectrophoresis methods showed a good correlation, in agreement with the findings of Ward and Kohler (1973). The sensitivity of the counter-immunoelectrophoresis method is greater than the double-diffusion method in tests on unconcentrated serum but less than the latter method when the serum is concentrated. As previously found (Longbottom and Pepys, 1964; Flaherty *et al.*, 1974), no one extract reacted with all the sera using either method. In the two groups of patients who had a positive prick test to *A. fumigatus* (groups I and II), it was found that some sera (12) gave a positive precipitin test only after concentration. By contrast, in the two other groups of patients with a negative prick test to *A. fumigatus* (groups III and IV), concentration of serum did not increase the incidence of positive precipitin tests. It seems that concentration of the serum increases the sensitivity of the test without reducing its specificity, as reported previously by Longbottom *et al.* (1968).

Among the 27 patients with a positive prick test to *A. fumigatus* but without evidence of aspergillosis, precipitins were found in six by the double-diffusion test and in two by the counter-immunoelectrophoresis method. It is not known whether these patients are more at risk of developing allergic aspergillosis, and regular follow-ups with chest radiographs are needed to see if this is so. In the two other control groups with a negative prick test to *A. fumigatus* (groups III and IV), only one asthmatic patient in whom the diagnosis of aspergillosis was excluded gave a positive precipitin reaction. The presence of precipitins against *A. fumigatus* has been reported in asthmatics without evidence of aspergillosis (Campbell and Clayton, 1964; Longbottom and Pepys, 1964; Mearns *et al.*, 1967; Coleman and Kaufman, 1972). Positive precipitin tests do not in themselves make the diagnosis. Precipitins to the relevant antigen are found in exposed but apparently unaffected subjects, for example, 20% of farmers (Pepys and Jenkins, 1965) and 45% of pigeon breeders (Barboriak *et al.*, 1965). The results of precipitin tests have to be interpreted in the light of the clinical findings.

The specific and total IgE values were significantly higher in patients with allergic aspergillosis (group I) than in any of the three control groups. The separation between the groups was far better in the case of the specific IgE against *A. fumigatus*. There was no overlap of values between the patients with aspergillosis and the two groups with a negative prick test to *A. fumigatus* (groups III and IV), and only small overlap was found between the patients with aspergillosis (group I) and the atopic patients with a positive prick test to *A. fumigatus* but without aspergillosis (group II). Raised specific IgE values against *A. fumigatus*

may thus be helpful in suggesting allergic aspergillosis in between episodes of pulmonary eosinophilia.

The atopic patients with a negative prick test to *A. fumigatus* (group III) had significantly higher values of specific IgE against *A. fumigatus* than the non-atopics (group IV) even though the patients in both groups gave a negative prick test to *A. fumigatus*. This may perhaps be attributable to cross-reactions of the IgE antibodies against other allergens which are contained in the sera of the atopic patients.

There was no correlation between the increases of specific IgE against *A. fumigatus* and total IgE. Thus, in chronic allergic aspergillosis, there appears to be both specific and a non-specific stimulation of IgE production. This IgE response has been mainly studied in acute aspergillosis where it has been argued that the majority of the total IgE increase is due either to specific (Turner *et al.*, 1974) or to non-specific (Patterson and Roberts, 1974) stimulation of IgE. Our findings that the total IgE but not the specific IgE values were higher in patients who had had a radiographic shadow in the previous three months tend to suppport the latter.

The increase in total IgE was not linearly correlated with the interval since the last radiographic transient shadow or with the number of past shadows but was nevertheless found to be significantly higher ($0{\cdot}02<P<0{\cdot}05$) in patients who had had an episode of pulmonary eosinophilia in the previous three months in comparison with the other patients. This suggests that there is a rapid fall in the serum levels of this immunoglobulin after the acute episode. The fact that the degree of positivity of precipitin tests was correlated with the activity of the disease, as assessed by chest radiographs, suggests that increase in IgG antibodies which are assumed to play a part in precipitating immune-complex reactions persists for longer periods.

The degree of positivity of the precipitin test by the counterimmunoelectrophoresis method, and less so by the double-diffusion method, showed a correlation with the increases in specific IgE against *A. fumigatus*, suggesting that both classes of antibody are stimulated concurrently in chronic allergic aspergillosis, and further studies of total and specific IgG in acute and chronic aspergillosis may help to clarify the role of these immunological responses in the disease.

We are grateful to Mrs. J. Neyron-Malo and to Mrs. M. Rehahn for help with the statistical work and to the personnel of the Medical Records Department.

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