

Allergy in cystic fibrosis

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SUMMARY

Forty-four patients with cystic fibrosis, sixty-seven parents, and thirty-nine controls were compared with regard to their immediate cutaneous hypersensitivity, total and specific serum IgE and serum IgG₄ concentrations. The patients had increased prick test reactions and specific IgE to *Aspergillus fumigatus*. The development of reactions to *A. fumigatus* correlated with the severity of the disease. There were no other significant differences when serum total IgE, specific IgE and IgG₄ concentrations or skin test reactivity were compared in the patients, parents and controls.

INTRODUCTION

Cystic fibrosis (CF) has been reported to be linked with increased immediate hypersensitivity in the skin (Warren *et al.*, 1975) and this has been associated with increased specific and total IgE in the serum (Warren *et al.*, 1975; MacFarlane, Allan & Van der Zeil, 1977). The IgG₄ subclass, which may have an anaphylactic function (Vijay & Perelmutter, 1977), has also been reported to be elevated in CF patients (Shakib *et al.*, 1976) and may be involved in increased immediate hypersensitivity. In addition, some authors (Warner, Norman & Soothill, 1976a; MacFarlane & Allan, 1976) have reported such an association for obligate heterozygotes of the CF gene and have suggested that it was associated with the development of atopy in the general population.

For these reasons we decided to survey CF patients (homozygous for the recessive CF gene), CF parents (obligate heterozygotes for the CF gene) and control subjects. We examined the serum concentrations of total and specific IgE and IgG₄ and compared the immediate cutaneous hypersensitivity, respiratory function and chest X-ray findings with these results.

MATERIALS AND METHODS

We surveyed all consenting CF patients (forty-four) who lived within the catchment area of the Bristol Children's Hospital. Patients had a firm diagnosis of CF based on an elevated sweat sodium concentration, and/or a reduction in pancreatic trypsin, together with a supporting clinical history. Most were studied during their routine attendance at the Children's Hospital, as were the sixty-seven parents. The thirty-nine controls were unselected medical students. The age and sex of the groups studied are indicated in Table 1. The project was approved by the Bristol Health District Ethical Committee.

Immediate cutaneous hypersensitivity was tested in all the subjects by prick testing using ten standard (Bencard) allergens (*Dermatophagoides pteronyssinus*, mixed grass pollens, *Cladosporium herbarum*, *Alternaria tenuis*, *Aspergillus fumigatus*, *Candida albicans*, milk (cow), egg (whole), cat fur and dog hair), with carbolsaline and 0.05% histamine as controls. If the diameter of the wheal was greater than the diameter of the carbolsaline control at 15 min after pricking, this positive result was recorded. For most comparisons this figure was used, but where prevalences were directly compared with other series (Table 7), only wheals more than 2.0 mm greater in diameter than those of the controls were taken as positive.

Sera were obtained from capillary or venous blood samples. All estimates on sera were performed without knowledge of the subjects' diagnostic group. IgG₄ levels in the serum were estimated by the single radial immunodiffusion method (Shakib *et al.*, 1975). The antiserum and standard were supplied by Dr D.R. Stanworth, Department of Experimental Pathology,

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TABLE 1. Age and sex of population studied

Group	Total number	Males	Age in years (\pm s.d.)
CF patients	44	22	7.3 \pm 4.3
CF parents	67	30	35.7 \pm 8.0
Controls	39	24	22.4 \pm 1.9

University of Birmingham. The mean difference found between duplicate determinations was 0.99 mg% which, with a mean of 64.1 mg%, gave a coefficient of variation of duplicate estimations $\left[\frac{\log \text{ s.d.}}{\log \text{ mean}} \times 100 \right]$ of 2.6%.

Total IgE concentrations were estimated by the paper radioimmunosorbent technique (PRIST) (Kjellman, Johansson & Rooth, 1976; Kjellman, 1976), using immunoassay kits (Pharmacia). IgE antibody to *A. fumigatus*, *D. pteronyssinus* and Meadow fescue pollen were assayed using the radio-allergosorbent technique (RAST) (Johansson, 1975).

A. fumigatus precipitins were tested in the serum of seventeen of the CF patients using the technique of Warnock (1977).

Peak expiratory flow (PEF) and maximum mid-expiratory flow (MMEF) were measured using Wright peak expiratory flow meters and the Vitalograph, respectively. Results were recorded as a percentage of the predicted normal for height (Godfrey, Kamburoff & Nairn, 1970; Cogswell *et al.*, 1975). Chest X-ray scores (CXS) were made (Crispin & Norman, 1974) and the higher the score, the greater the pulmonary disease. The lowest score recorded near the time of survey was used.

Unless otherwise specified, significance testing was by the Mann-Whitney U or Kendall's Tau tests. All statistical analyses of serum concentrations of IgE and IgG₄ used logarithmic transformations.

RESULTS

The immediate hypersensitivity responses on skin testing are shown in Table 2. *A. fumigatus* was the only allergen which showed a significant difference between the groups, with the CF children reacting significantly more often than the controls and the heterozygotes.

Geometric mean values for total serum IgE and IgG₄ concentrations of the population groups are shown in Table 3. There were no significant differences between the three population groups or between

TABLE 2. Percentage of subjects whose cutaneous response to antigen was greater than to carbosaline control

Group	<i>D. pteronyssinus</i>	Grass pollen	<i>A. fumigatus</i>	Any positive excluding <i>A. fumigatus</i>
CF patients	25	34	39*	44
CF heterozygotes	24	18	0	36
Controls	36	31	0*	50

* $P < 0.01$.

TABLE 3. Geometric mean values of total serum IgE and IgG₄ concentrations

Group	IgE (u/ml)	IgG ₄ (mg/100 ml)
CF patient	26	15
CF heterozygotes	21	7
Controls	45	10

the sexes. Sixteen per cent of the CF children had high IgE concentrations according to the limits of Kjellman *et al.* (1976). Fewer of the older children had high concentrations. The CF children with a positive skin test for *A. fumigatus* had significantly ($P < 0.001$) more serum IgE than the others. This was not so for the serum IgG₄ concentration (Table 4).

Forty-three per cent of thirty-seven patients, 17% of sixty-three heterozygotes and 36% of thirty-six controls had serum IgG₄ concentrations higher than the adult upper limit (18.5 mg/100 ml) (Shakib *et al.*, 1975). The IgG₄ concentrations in the patients correlated well with age ($P < 0.001$).

Significant positive correlations ($P < 0.001$) were demonstrated between skin and RAST tests for *D. pteronyssinus* ($r = 0.62$), grass pollen ($r = 0.62$) and *A. fumigatus* ($r = 0.63$). The frequency of positive RAST tests for allergens other than *A. fumigatus* were similar in all groups and between the sexes (Table 5).

TABLE 4. Mean serum IgE and IgG₄ concentrations in thirty-six CF patients compared with cutaneous reactivity to *A. fumigatus*

Immediate cutaneous hypersensitivity to <i>A. fumigatus</i>	IgE (u/ml)	IgG ₄ (mg/100 ml)
Negative ($n = 22$)	8.3*	11.5
Positive ($n = 14$)	132.4*	24.8

* $P < 0.001$.

TABLE 5. Percentage of subjects with specific IgE in serum (RAST score ≥ 1)

Group	<i>D. pteronyssinus</i>	Grass pollen	<i>A. fumigatus</i>
CF patients	16	24	19*
CF heterozygotes	15	20	0
Control	24	29	0*

* $P < 0.01$.

The correlations between maximum mid-expiratory flow (MMEF), peak expiratory flow (PEF) and chest X-ray scores in the CF patients are shown in Table 6. MMEF correlated significantly with positive skin tests for *A. fumigatus*, lowering in PEF correlated significantly with positive skin tests for *A. fumigatus*, positive RAST for *A. fumigatus*, *D. pteronyssinus* and higher total IgE concentrations. Chest X-ray score correlated positively with a raised IgG₄ concentration and with the RAST for *A. fumigatus*.

TABLE 6. Correlations of the reductions in maximum mid-expiratory flow (MMEF) and peak expiratory flow (PEF) and increase in chest X-ray score (CXS) with skin and blood tests in CF patients

	Skin tests			RAST			IgE	IgG ₄
	<i>D. pteronyssinus</i>	Grass pollen	<i>A. fumigatus</i>	<i>D. pteronyssinus</i>	Grass pollen	<i>A. fumigatus</i>		
MMEF	0.03	0.23	-0.28*	-0.11	0.08	-0.16	0.15	0.05
PEF	0.10	0.07	-0.30†	-0.31†	-0.25	-0.32†	-0.32†	-0.06
CXS	-0.34‡	-0.11	0.15	-0.05	-0.06	0.39‡	0.20	0.47‡

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Chest X-ray score correlated negatively with the skin test for *D. pteronyssinus*. There was a positive correlation ($r = 0.39$, $P < 0.01$) between IgG₄ and IgE in the control population, but not in the heterozygote ($r = 0.05$) or homozygous ($r = 0.08$) CF populations.

Two of the seventeen CF patients had precipitins to *A. fumigatus*; six of the CF patients, including the two with positive precipitin tests, had wheals >2 mm diameter on prick testing with *A. fumigatus* extract.

DISCUSSION

We have confirmed previous reports of an increased reactivity of CF homozygotes on immediate hypersensitivity testing against *A. fumigatus* antigen (Table 7). MacCarthy, Pepys & Batten, (1969) reported that there was no higher incidence of personal or family history of eczema, asthma or hayfever

TABLE 7. Percentages of positive immediate cutaneous hypersensitivity reactions

Publication	<i>A. fumigatus</i>	<i>D. pteronyssinus</i>	Grass pollen
Homozygous CF (patients)			
MacCarthy <i>et al.</i> (1969)	49	—	—
Warren <i>et al.</i> (1975)	58	16	28
Allen <i>et al.</i> (1975)	48	44	9
Warner <i>et al.</i> (1976b)	56	25	56
Present series	34	16	25
Homozygous CF (parents)			
Warner <i>et al.</i> (1976a)	—	13	28
Present series	0	21	18
Controls			
Godfrey & Griffiths (1976)	—	26	24
Davis (1976)	—	18	18
Present series	0	28	31

in families with CF. They showed that the patients who had positive skin tests for *A. fumigatus* (49%) or precipitins in the sera (27%) had more severe lung involvement. All patients with positive immediate cutaneous hypersensitivity to *A. fumigatus* reacted to bronchial challenge with the antigen, whilst none of the patients with negative skin tests reacted. These findings were subsequently confirmed by Warren *et al.* (1975). Thirty-seven per cent of CF patients had serum precipitins against *A. fumigatus* and 54% reacted to nasal challenge with this antigen. Patients with cutaneous immediate hypersensitivity to *A. fumigatus*, grass pollen or *D. pteronyssinus* had higher IgE antibody to these allergens in their sera. Total IgE concentrations were higher in patients with skin reactions to *A. fumigatus*. Batten (1967) reported that only 3% (five out of 180) of CF patients had clinical allergic bronchopulmonary aspergillosis.

Other workers (Allan *et al.*, 1975; Warner *et al.*, 1976b) reported similar findings in the CF homozygotes. One interesting feature of all four series is that when the reactions to *A. fumigatus* are removed, the frequency of immediate cutaneous hypersensitivities in the homozygous CF patients approximates to that found in the community at large (Godfrey & Griffiths, 1976; Davis, 1976). Our series show similar reactivities when wheals more than 2.0 mm in diameter are classified as positive (Table 7). Differences in the interpretation of the results of the skin tests in the series quoted arise largely as a result of differences in methods of analysis or in the controls. Our control population was representative of the community at large as no exclusions of subjects with a history of allergy or abnormal test results were made.

Skin reactivity to *A. fumigatus* has been associated with more severe pulmonary damage (MacCarthy *et al.*, 1969; Warner *et al.*, 1976b), as in our results. Severe chest disease was less likely to be associated

with positive skin tests for *D. pteronyssinus*, but was positively correlated with RAST for *A. fumigatus* and with raised IgG₄ concentration. The allergic reactions to *A. fumigatus* shown in the CF patients are not generally found in atopic asthma in childhood (Sarsfield, 1974).

The cutaneous reactivity in CF patients is probably due to IgE, since they have increased serum concentrations of total and specific IgE (Warren *et al.*, 1975; MacFarlane *et al.*, 1977). This hypersensitivity was passively transferrable in the patients' sera (MacFarlane *et al.*, 1977) but such a transfer was prevented by prior heating of the sera, which is known to selectively inactivate IgE. Our findings of significant correlations between serum total IgE, serum IgE antibody to *A. fumigatus* and skin reactivity to *A. fumigatus* support this theory.

However, Shakib *et al.* (1976) reported that 44% (seven out of sixteen) of CF patients had elevated IgG₄ levels and IgG₄ may have an anaphylactic function (Stanworth & Smith, 1973; Vijay & Perelmutter, 1977). Butler & Hiller (1977) did not find high serum IgG₄ concentrations in CF homozygotes. The correlation found between serum IgG₄ concentration and severity of disease (CXS) may merely reflect the general elevation of serum IgG concentration in CF which is probably a consequence of inflammatory chest disease (Warren *et al.*, 1975).

When reactions to *A. fumigatus* were excluded, there were no significant differences between our CF patients, CF heterozygotes and controls with regard to skin test reactivity, RAST tests in serum and mean serum IgE and IgG₄ concentrations. It might be expected that if the CF gene directly, or by some genetic linkage, produced an atopic state there would have been a partial expression of this in the heterozygotes. None was evident. Indeed the response to *A. fumigatus* was confined to the homozygotes in both the skin tests and RASTs. The pattern of skin test reactivity is different from that observed in childhood asthmatics (Sarsfield, 1974). These features suggest that the abnormal hypersensitivity reactions in the CF patients are not a consequence of the gene, but rather arise as a consequence of the pulmonary damage.

Wallwork *et al.* (1974) reported normal concentrations of serum IgE in obligate heterozygotes, but subsequently MacFarlane & Allan (1976) reported that the total serum IgE was elevated in the heterozygotes as well as in the patients. Increased skin test reactivity was also reported (Warner, Norman & Soothill, 1976a) in forty-seven CF heterozygotes (Table 7). These findings led to the suggestion that the CF gene is an important cause of allergy in the community. Our results do not support this theory.

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