

Immunological studies in cystic fibrosis

M. W. TURNER, J. O. WARNER, C. R. STOKES,* AND A. P. NORMAN

From the Department of Immunology, Institute of Child Health, and the Respiratory Unit The Hospital for Sick Children, London

SUMMARY Evidence is presented to support the concept that much of the allergy in cystic fibrosis (CF) is IgE mediated. Total IgE levels were higher in allergic than in nonallergic CF patients. Levels were also higher in those patients who had had the greatest number of chest infections in the preceding 12 months. IgE antibody levels to *Dermatophagoides pteronyssinus*, Timothy grass pollen, and *Aspergillus fumigatus* were higher in those with positive results from skin tests to these allergens. The serum IgG, IgM, and IgA levels of allergic and nonallergic CF patients did not differ but the overall mean values for IgG and IgM were higher than those reported for healthy British children. The highest levels tended to be present in patients with the greatest number of recent major chest infections and the difference was significant for IgG. 16 patients had IgA levels 72SD below the reported means for age-matched controls and 11 of these were nonallergic. IgA levels were also higher in patients who had recently experienced major chest infections. 45 of the patients were tissue types for HLA A and B antigens but no significant clinical associations with single antigens were observed. The antigen phenotype A1 + B8 was more common in patients with multiple allergic symptoms than in those with a single allergy or merely a positive result from a skin test. Nonsignificant increases of W19 in patients with frequent infections and of A2 in patients presenting with meconium ileus were also noted. The data presented do not permit a choice to be made between the alternative concepts of allergy as a primary abnormality in CF, and allergy arising secondary to infection.

There is an increased incidence of immediate skin hypersensitivity to various inhalent allergens, particularly moulds, in patients with cystic fibrosis (CF) (Mearns *et al.*, 1967; Allan *et al.*, 1975; Warren *et al.*, 1975; Warner *et al.*, 1976b). Warner *et al.* (1976a, b) suggested that the allergy arises as a result of defective allergen elimination at mucosal surfaces, because of abnormal mucociliary clearance in the respiratory tract, either secondary to infection or as a primary abnormality in CF. We report here the results of several immunological investigations and their relationships to allergy and recent chest infections in a large group of children with CF.

Patients and methods

Children with CF (sweat Na >70 mEq/l) attending the CF outpatient clinic at this hospital were studied. The patients were included in the study sequentially as they attended the clinic and no

attempt was made to be selective. Their ages ranged from 4 months to 18 years and none had serious or acute infection at the time of testing, although the extent of their chest disease ranged from very severe to slight. The number of major chest infections in the 12 months preceding the study was recorded. A major infection was one that required being in hospital and at least three of the following criteria being present: fever, increased cough with purulent sputum, breathlessness, and increased lung shadowing on x-ray. Prick skin testing was done on the forearms or back using 12 Bencard skin test antigens (house dust extract (150%), *Dermatophagoides pteronyssinus* (1.2%) and *Dermatophagoides farinae* (1.2%), feathers (150%), cat fur (150%), dog hair (150%), whole egg (6%), milk (10%), Timothy grass (2.5%), rye grass (2.5%), *Aspergillus fumigatus* (5%), and *Cladosporium* (10%)) and a control solution. Reactions were examined between 15 and 20 minutes later and considered positive if there was a weal of 3 mm diameter or greater.

No patient reacted to the control solution. Blood samples were obtained at the time of skin testing and serum was stored at -70°C until required.

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*Present address: Department of Animal Husbandry, University of Bristol

Serum IgE was measured by a modification of the double antibody procedure (Nye *et al.*, 1975) using rabbit antihuman IgE (Hoechst Pharmaceuticals, England) in the first stage and donkey antirabbit serum (Wellcome Reagents Ltd) in the second stage; results were expressed as international units per ml using a commercial standard (Pharmacia) calibrated against the WHO IgE standard 58/341. The threshold of detectability in this assay was 4 IU/ml.

IgE antibodies were assayed by a modification of the procedure of Wide *et al.* (1967). Extracts of *D. pteronyssinus*, Timothy grass pollen, and *A. fumigatus* (Bencard) were coupled to cyanogen-bromide activated microcrystalline cellulose particles (Sigma). 0.5 ml of a 1 mg/ml suspension of cellulose particles with bound antigen was mixed with 50 μ l serum and incubated at room temperature for 16 hours with continuous mixing. The cellulose particles were then washed 6 times with physiological saline containing 1% Tween-20 and 100 μ l of 125 I-labelled specific rabbit antihuman IgE (Fc-immunosorbent purified) added. After overnight incubation with agitation at room temperature, the supernatants were removed and the particles washed 5 times with physiological saline containing 1% Tween-20. Radioactivity was counted in a γ counter and results expressed as a percentage of the total radioactive counts which became bound. All sera were analysed in a single batch and positive and negative controls were included.

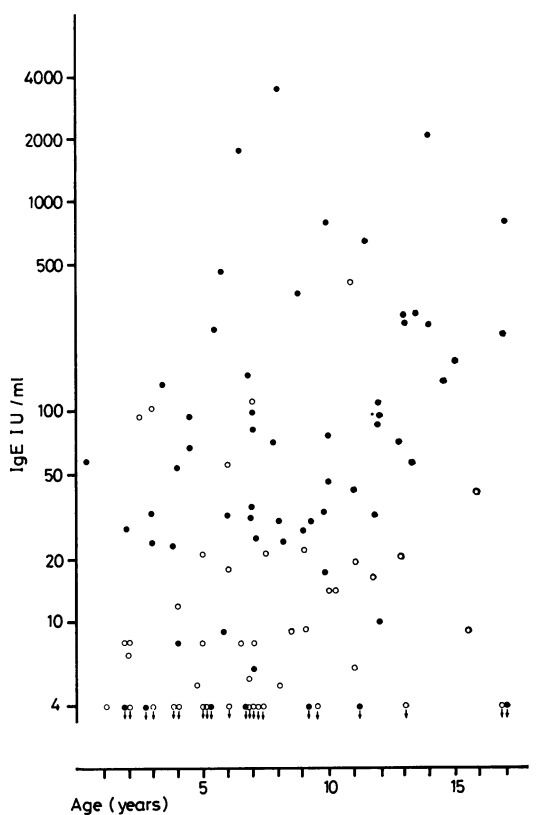
Serum IgG, IgA, and IgM levels were measured by the single radial diffusion method (Fahey and McKelvey, 1965) using antisera supplied by Wellcome Reagents Ltd. A WHO reference standard for immunoglobulins (67/99) was used throughout and results expressed in IU/ml.

HLA tissue typing was done by the microcytotoxicity method (Mittal *et al.*, 1968) on patients' lymphocytes separated on a Ficoll-Triosil density gradient. Eight antigens of the A series and 14 antigens of the B series were tested using 108 antisera kindly provided by the National Organ Matching and Distribution Service, Bristol.

Differences in IgE antibody and total IgE, IgG, and IgM levels were analysed by Student's *t* test on log-transformed data. The frequencies of individual HLA antigens and certain antigen combinations were analysed by χ^2 tests (with Yates's correction) or by Fisher's exact test.

Results

IgE levels. The IgE levels of 103 patients with CF plotted according to age are shown in Fig. 1. With the assay used, the nonatopic adult mean value is 17 IU/ml (2 SD, range <2-195) and peak values are

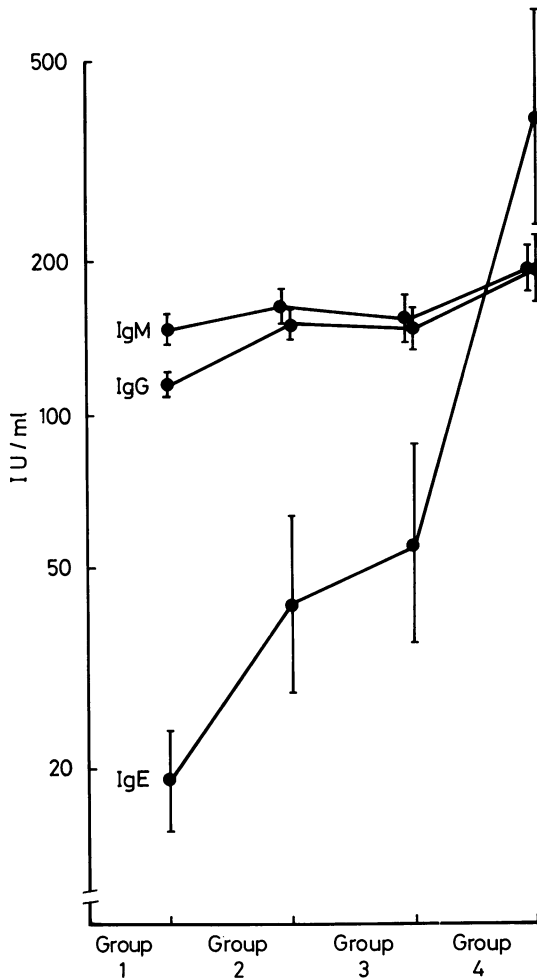


○ = patients who were skin test negative to all allergens; ● = patients with one or more positive skin tests.

Fig. 1 Serum IgE levels of patients with CF.

reached by approximately 5 years of age (unpublished observations). Patients were classified as allergic if one or more skin test gave positive results. Children who were allergic and at least 4 years old had a significantly higher mean IgE (75 IU/ml) than non-allergic children of the same age (10 IU/ml) ($t = 5.69$; $P < 0.001$). The percentage of children with a total IgE > 20 IU/ml increased from 43% in the 5- to 10-year-old group to 66% in those children older than 10 years, but this was not significant.

The relationship between IgE level and recent chest infections was studied in children over the age of 5 years. Patients were grouped according to the number of major chest infections in the preceding 12 months (group 1, no infections; group 2, 1 infection; group 3, 2-4 infections; group 4, > 4 infections). Fig. 2 shows the mean IgE level of patients in each of these groups and Table 1 gives the *t* test comparisons for the four groups and for patients with no infections (group 1) compared with patients having



Group 1 = 0 infections; group 2 = 1 infection; group 3 = 2 to 4 infections; group 4 = >4 infections. Each vertical bar represents the s.e. mean.

Fig. 2 Mean IgE, IgG, and IgM levels of patients with CF, classified according to the number of major chest infections in the preceding 12 months. IgE data for patients aged 5 years and above; IgG and IgM data for patients aged 2 years and above.

infections. IgE levels are clearly related to the number of recent infective episodes.

IgE antibodies. The levels of IgE antibodies to *D. pteronyssinus*, Timothy grass pollen, and *A. fumigatus* are plotted in Fig. 3. For all three allergens IgE antibody levels were significantly different between those patients who gave positive results and those who gave negative results to the skin test (*D. pteronyssinus*, $t = 7.95$, $P < 0.001$; Timothy grass pollen, $t = 6.66$, $P < 0.001$; *A. fumigatus*, $t = 5.97$, $P < 0.001$). This is consistent with the view that the patient's allergy is, at least partly, IgE mediated.

IgG, IgA, and IgM levels. The total serum IgG, IgA, and IgM levels in 106 patients with CF are plotted according to age in Fig. 4. The mean serum IgG and IgM levels for CF patients older than 2 years were 131 IU/ml and 162 IU/ml respectively, both higher than the mean values reported by Hobbs (1970) for healthy British children (87 IU/ml and 106 IU/ml respectively). Some of the children below the age of 2 years had surprisingly low levels of IgG and IgM.

There was no significant difference between serum IgG and IgM levels in allergic and nonallergic CF children but a relationship between level and the number of recent infections was noted (Fig. 2 and Table 1). However, whereas children with no infections had significantly lower IgG levels than those who had had one or more infections, there was no significant difference between the IgM levels in the groups of patients.

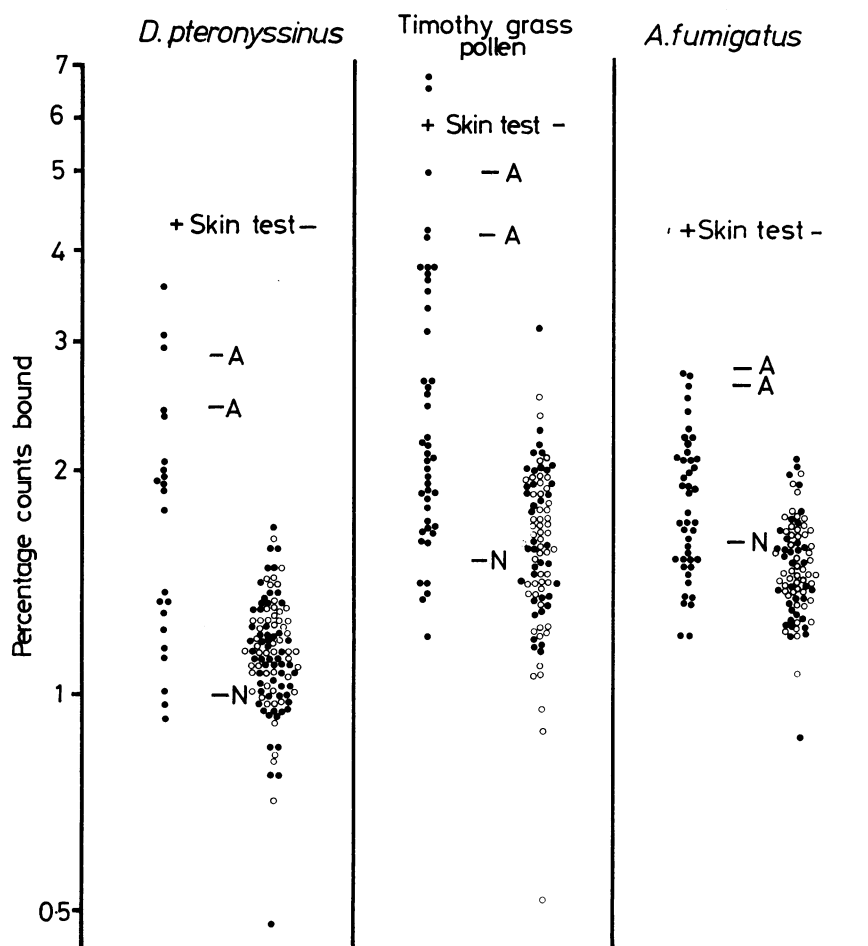
As IgA levels rise progressively with age, analysis of the data is more difficult. Overall, there was no obvious difference in the IgA level of allergic and nonallergic CF children. A surprising number of children had rather low levels of IgA. 16 were more than 2 SD below the mean reported for healthy British children (Hobbs, 1970) and 11 of these were nonallergic, a significantly higher proportion than in the rest of the CF children ($\chi^2 = 4.3$; $P < 0.05$). In 3 of the 16 patients IgA was not detected (< 4 IU/ml).

Table 1 Student's *t* test comparisons of immunoglobulin concentrations in CF patients (grouped according to the number of major chest infections in the preceding 12 months*).

	IgE			IgG			IgM		
	n	t	P	n	t	P	n	t	P
*Group 1 v. 2	60	1.8558	NS	75	3.0334	<0.005	76	0.7195	NS
1 v. 3	51	2.0545	<0.05	70	2.3447	<0.025	71	0.2631	NS
1 v. 4	53	5.9630	<0.001	68	4.2242	<0.001	69	1.6756	NS
2 v. 3	27	0.4342	NS	31	0.2465	NS	31	0.4112	NS
2 v. 4	29	3.5303	<0.005	29	1.7448	NS	29	1.3273	NS
3 v. 4	20	2.8793	<0.01	24	1.6385	NS	24	1.5210	NS
1 v. 2+3+4	82	4.0968	<0.001	101	4.5537	<0.001	102	1.3330	NS

*Group 1 = 0 infections, group 2 = 1 infection, group 3 = 2-4 infections, group 4 = >4 infections.

NS = not significant.



● in a skin test negative column indicates an individual with one or more positive skin tests to other allergens; ○ individuals who were skin test negative to all allergens. For each allergen the binding observed for two skin test positive (non CF) individuals is indicated (A) as well as a skin test negative control value (N).

Fig. 3 Levels of serum IgE binding to immunosorbents of *D. pteronyssinus*, Timothy grass pollen, and *A. fumigatus* (% counts bound) in patients with CF, classified as skin test positive or negative for each allergen.

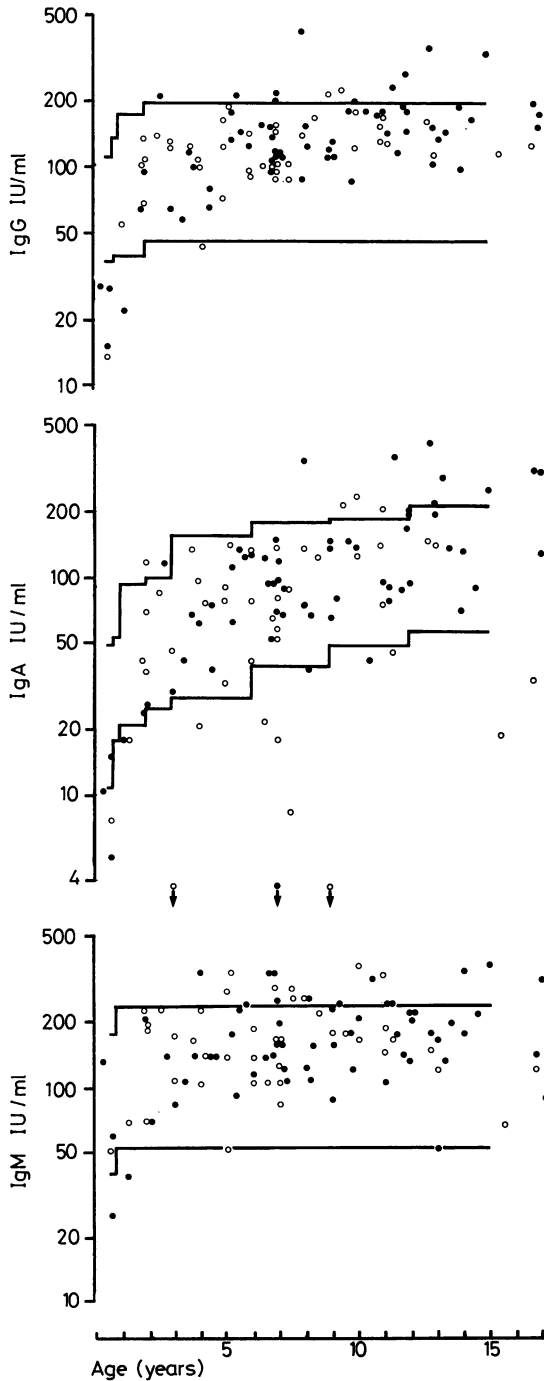
The relationship between IgA level and number of recent chest infections was studied by comparing the number of patients with serum IgA levels above and below the published age-related mean values for healthy British children Hobbs, (1970). These data, presented in Table 2, show that IgA levels are also significantly related to the number of recent infective episodes.

HLA typing. The frequencies of the HLA A and B antigens in 45 of the children with CF are presented in Table 3. The antigens W19, B5, and B12 were detected more often in the patients than in a control

Table 2 Numbers of patients with CF with serum IgA levels above and below age-related British mean values (Hobbs, 1970). (Patients grouped according to the number of infections in the preceding 12 months.)

Group*	Above mean	Below mean	Fisher's exact test	χ^2 test
1	20	39	} P < 0.01	} P < 0.01 } P < 0.001
2	13	5		
3	8	8	} P < 0.025	
4	11	1		
2, 3, & 4	32	14		

*See classifications of groups in footnote to Table 1.



○ = patients who were skin test negative to all allergens; ● = patients with one or more positive skin tests. Mean \pm 2 SD data for healthy British children (Hobbs, 1970) is indicated for each immunoglobulin.

Fig. 4 Serum IgG, IgA, and IgM levels of patients with CF.

Table 3 Frequency of HLA tissue types in 45 patients with CF

Antigen	Patients with CF		Normal blood donors	
	No. of times observed (n = 45)	% occurrence	No. of times observed (n = 1036)	% expected*
A series				
A1	16	35	348	34
A2	26	58	536	52
A3	11	24	265	26
A9	10	22	190	18
A10	3	7	87	9
A11	1	2	106	10
A28	0	0	38	4
'W19'	12	27	175	17
B series				
B5	9	20	87	8
B7	9	20	265	26
B8	13	29	260	25
B12	17	38	254	24
B13	1	2	45	4
Bw35(W5)	4	9	114	11
Bw40(W10)	7	15	116	11
B14	4	9	72	7
Bw15	4	9	50	5
Bw17	3	7	79	8
B18	1	2	35	3
Bw21	0	0	18	2
Bw22	1	2	55	5
B27	4	9	77	7

*Nelson *et al.* (1974).

population but the differences were not significant after correction for the number of antigen comparisons. This correction is used because in typing a group of patients for 20 different HLA antigens a spurious association with one of the 20 is likely to be significant at the 5% level (Svejgaard *et al.*, 1975). Similarly, the frequencies of the three common HLA phenotypes A1+B8 (24%), A2+B12 (24%), and A3+B7 (13%) did not differ significantly from the frequencies of these antigens in the normal population (G. H. Tovey, 1976, personal communication).

The children with CF had the full range of allergic symptoms (Warner *et al.*, 1976b) and as the HLA phenotypes A1+B8 and A3+B7 are associated with different allergic manifestations in patients without CF (Turner *et al.*, 1977), we examined these antigen combinations in the 28 CF patients who gave a positive skin test result to one or more allergens. Of the 18 allergic patients with a single clinical symptom only 2 had the A1+B8 combination and 3 had A3+B27. Of the 10 allergic patients with two or more clinical symptoms, 4 had the A1+B8 combination and none was typed positive for A3+B27 (Fig. 5). These differences are not statistically significant in this small series but the trend is similar to that seen in our earlier study of non CF patients (Turner *et al.*, 1977).

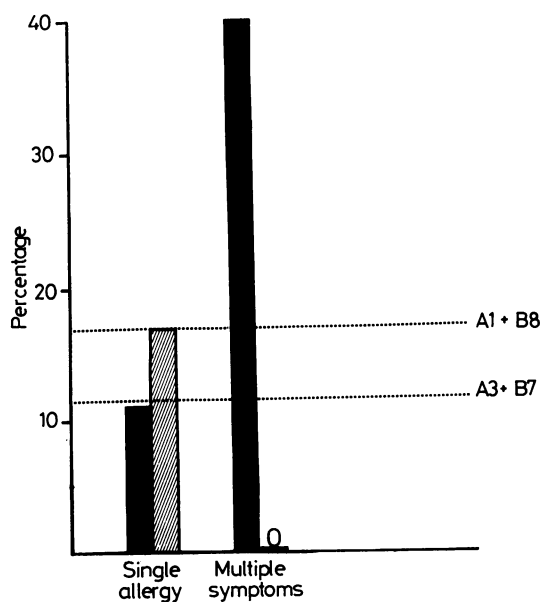


Fig. 5 Frequencies of the HLA antigen combinations A1 + B8 (solid column) and A3 + B7 (hatched column) in 18 CF patients with a single allergy (one allergic symptom and/or positive skin test) and 10 CF patients with two or more clinical symptoms of allergy. The incidence in a general population for these antigens is indicated by the horizontal lines.

The relationship between tissue type and recent chest infections was studied using the four groups described above. No significant associations were noted but there was a trend for W19 to be increased in those with the most infections. This antigen was present in 4 of the 6 individuals who had >4 infections but in only 8 of 35 individuals with fewer than 4 infections in the previous 12 months. Similarly W19 was observed in 8 of the 19 patients with recent infections but in only 4 of the 22 with no chest infections.

Patients were also classified according to presentation with or without meconium ileus and the tissue types of these two groups compared. The antigen A2 was present in 9 of 11 patients presenting with meconium ileus compared with 15 of 30 without. This trend was not significant by Fisher's exact test.

Discussion

We have previously reported on the incidence of allergy in this CF population (Warner *et al.*, 1976b). Unlike most atopic individuals CF homozygotes often have a large number of positive skin test reactions to the moulds *A. fumigatus* and *C. herbarum* (Mearns *et al.*, 1967; Warner *et al.*, 1976b).

Nevertheless, our combined IgE and IgE antibody results suggest that much of this allergy is reaginic (Type 1 immediate hypersensitivity (Coombs and Gell, 1975).

In a study of 40 patients with CF, Wallwork *et al.* (1974) also noted elevated IgE levels but Weeke *et al.* (1976), using the same radioimmunosorbent technique (RIST), found 'normal' IgE levels in 30 of 32 CF patients. These apparent discrepancies are probably due to the relative insensitivity of the original RIST technique at levels of IgE below 100 IU/ml. Using the double antibody procedure we found clear differences (even below 100 IU/ml) in the IgE levels between allergic and nonallergic CF patients.

In a study of 35 CF patients Warren *et al.* (1975) reported elevated IgE antibodies to *D. pteronyssinus* in 3 of 5 patients with positive results to prick skin tests with this antigen. Similarly, elevated IgE antibodies to grass pollen and *A. fumigatus* were found in 7 of 9 and in 15 of 21 patients, respectively, with positive results to prick skin tests to these antigens. We have confirmed these associations in our larger series of 106 patients.

IgG anaphylactic antibodies have been implicated in some types of immediate hypersensitivity (Parish, 1970), and Shakib *et al.* (1976) have claimed that IgG4 levels were selectively elevated in 8 out of 18 patients with CF. The authors suggested that this might be significant in view of the observations of Stanworth and Smith (1973) that IgG4 myeloma proteins can block baboon PCA reactions mediated by IgE reaginic antibodies. However, Shakib *et al.* (1976) also found an elevation of IgG1 in 5 of 18 patients and that, in general, those with high IgG4 levels had evidence of recent infections. We have not investigated a possible anaphylactic role for IgG antibodies in the allergy of CF patients but in our series high levels of IgG were associated with frequent infection but not necessarily allergy. Before IgG4 reaginic antibodies can be implicated in CF, subclass specific antibody studies with appropriate allergen preparations will be required.

IgA was not detected in 3 of 106 patients, a much higher incidence than that reported for the general population (1 in 500, *Lancet*, 1975). In view of the association between transient IgA deficiency and allergy (Taylor *et al.*, 1973; Soothill *et al.*, 1976), it is surprising that most of our patients with CF and low levels of IgA were not allergic.

There is evidence that allergic CF patients have worse chest disease, a greater degree of bronchial lability, and are more likely to respond favourably to bronchodilators than are nonallergic CF patients (J. F. Price *et al.*, 1978, unpublished). In the present study IgG, IgA, and IgE levels were significantly

higher in the CF patients with several recent chest infections. A similar trend was noted for IgM but this was not significant. Wallwork *et al.* (1974), Warren *et al.* (1975), and Weeke *et al.* (1976) have also reported raised levels of IgG and IgA in smaller groups of CF patients but none of these authors has analysed data in terms of recent episodes of infection.

Our failure to observe any abnormal frequency of HLA antigens in patients with CF is in agreement with another study of 24 patients (Polymenidis *et al.*, 1973) but it differs from a recent report (Kaiser *et al.*, 1976) in which the antigen B18 was said to be more common in both patients and heterozygotes. However, the numbers studied were small (12 patients and 32 heterozygotes) and only the patients differ significantly from the normal frequency if a correction for the number of antigen comparisons is made (Svegaard *et al.*, 1975). In our previous study of HLA antigens in various groups of allergic individuals (Turner *et al.*, 1977) we found an increased incidence of the antigen combination A1+B8 in patients who had presented with eczema and subsequently developed other allergic diseases. Patients with a single allergic disease did not show such an increase although the A1: B8 haplotype is known to be associated with many other nonallergic diseases—coeliac disease (McNeish *et al.*, 1973), dermatitis herpetiformis (Gebhard *et al.*, 1973), chronic active hepatitis (Mackay and Morris, 1972), and myasthenia gravis (Pirkanen *et al.*, 1972), as well as with a high graft rejection rate in recipients of parental kidney grafts (Mickey *et al.*, 1971). This has led to the suggestion that individuals with these antigens have a hyper-reactive immune response. Our observations that A1+B8 occurs more often in CF patients with multisystem allergic disease than in CF patients with a single disease symptom is in agreement with our earlier findings and could be explained by such a hyper-reactive mechanism. The nonsignificant associations between W19 and recent infections, and A2 and meconium ileus are of interest but require confirmation in a larger series.

The data obtained in this study extend our knowledge of the immunological changes characteristic of CF and highlight the close association between recent infections and serum levels of the immunoglobulins, including IgE. Whether the high incidence of allergy in CF reflects IgE sensitisation secondary to infections with adjuvanting organisms or arises through some unknown mechanism related to the underlying defect remains to be determined. We found no evidence to suggest linkage of the CF gene to the HLA complex and the strongest association observed (between A1+B8 and multiple allergic symptoms) is also commonly found in nonCF atopics.

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Correspondence to Dr M. W. Turner, Institute of Child Health, Department of Immunology, 30 Guilford Street, London WC1N 1EH.