

Long-term falls in antibodies to dust mite and pollen allergens in patients with asthma or hay fever

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Summary

'Spontaneous' improvement in, or alteration of, allergic symptoms is a common occurrence, and the immunological basis is of interest in attempts to develop effective specific therapy. In the present study we measured levels of serum antibodies to *Dermatophagoides pteronyssinus* in patients diagnosed as having house-dust-allergic asthma up to 40 years previously. The results show a progressive fall in both IgG and IgE antibodies to antigen P₁ and RAST binding to crude *D. pteronyssinus* extract. By contrast changes in total serum IgE were not marked. Within each of the groups of patients diagnosed 20, 30 and 40 years previously, 70% no longer suffered severe symptoms. However, the absence of detectable IgE antibody in serum was neither a necessary nor a sufficient condition for loss of symptoms. A group of patients who had spontaneously recovered from hay fever had significantly lower IgG and IgE antibody for the major grass pollen allergen Rye I and also lower total IgE than current hay fever sufferers. In neither hay fever nor asthma was there evidence to link spontaneous improvement in symptoms with an increase in IgG antibodies.

Introduction

Many allergic patients show a switch in symptoms, e.g. from rhinitis to asthma, or eczema to asthma, or simply 'grow out' of their symptoms with increasing age (Rackemann & Lamsa, 1965; Smith, 1971; Broder *et al.*, 1974). Understanding the immunological basis of these changes in symptom patterns might help to shed light on the factors controlling the severity of allergic symptoms and might also help in the development of specific therapy for allergic diseases. The presence of allergic symptoms caused by pollen, animal dander or dust mite allergens correlates well with the presence of specific IgE antibodies on skin mast cells and in the serum. However,

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there is little relationship between the severity of symptoms and the quantity of IgE antibodies in the serum (Norman, Lichtenstein & Ishizaka, 1973) and during desensitization treatment marked reductions in symptoms can occur without any reduction in IgE antibodies (Yunginger & Gleich, 1973). Although it has been suggested that blocking antibodies are responsible for the effects of desensitization, the evidence for this is poor (Irons, Pruzansky & Patterson, 1975; Chapman *et al.*, 1980; Norman, 1980). It has been reported that IgG antibodies to allergens decline with age (Delespesse *et al.*, 1977) and Hill *et al.* (1981) have reported significant falls in IgG and IgE antibodies to *Dermatophagoides pteronyssinus* in a 5-year follow up of children with asthma.

A prospective study comparing changes in antibody levels and symptoms with time would be most informative but very difficult because of the long time involved. Using the records of an allergy clinic it was decided to study retrospectively serum antibodies, skin tests and symptoms in patients who had been diagnosed as having house-dust-allergic asthma up to 40 years previously. We have also studied serum antibodies in a group of patients who had 'spontaneously' recovered from hay fever.

Materials and methods

Subjects

Using the records of an allergy clinic in Cardiff it was possible to identify and trace patients who had been diagnosed as having asthma with positive skin tests to house dust in 1940–41; 1950–51; 1960–61 and 1970–71. Ten patients from each period were studied except that for the 1950–51 period twenty patients were included, half of whom had received desensitizing injections. A group of ten similarly diagnosed patients visiting the Cardiff clinic for the first time during 1981, and a group of twenty such patients visiting the Northwick Park Hospital clinic for the first time during 1979–81 were also included. All patients were aged between 5 and 20 years when first seen.

Current hay fever sufferers were patients attending the Northwick Park allergy clinic for treatment of hay fever. Subjects who had 'recovered' from hay fever included: hospital staff, parents of patients and also some patients identified during the Cardiff study. In each case they had required medical treatment for hay fever for at least three seasons in the past, but had suffered no symptoms for at least 3 years.

All subjects were skin prick tested with house dust, *D. pteronyssinus*, grass pollen and cat dander (Bencard, U.K.) and a clinical history and a serum sample were obtained.

Symptom classification

Patients were classified according to present symptoms of asthma: 0 = no wheezing; 1 = occasional wheeze, whether requiring treatment or not; 2 = current asthma requiring treatment.

Assays for total IgE- and class-specific binding activity

Total serum IgE was measured by fluid phase double antibody inhibition radioimmunoassay (RIA) (Gleich, Averbach & Svedlund, 1971). Allergen specific antibodies were measured by antigen binding RIA as described previously for antigen P₁ from *D. pteronyssinus* (Chapman & Platts-Mills, 1980) and Rye I from rye grass pollen (Platts-Mills *et al.*, 1978). Briefly, 100 μ l of a suitable serum dilution was incubated for

4 hr at room temperature with ^{125}I -labelled purified allergen. The counts bound by antibodies were then precipitated using monospecific goat anti-human IgG or IgE. For IgE assays a small quantity of myeloma IgE was used in order to make the precipitate. The precipitate was allowed to form overnight at 4°C , washed and counted, and the binding activity was calculated by reference to a dilution curve for IgG Ab using a standard serum run in parallel with each assay.

Dermatophagoides pteronyssinus radioallergosorbent test (RAST)

A crude extract of *D. pteronyssinus* culture (prepared from the redissolved precipitate of a 50% ammonium sulphate cut of an aqueous extract, concentrated by negative pressure dialysis over an Amicon PM10 filter) was coupled to cyanogen-bromide-activated filter paper discs, 0.6 cm diameter, by a standard technique (Chapman, 1981) at approximately 10 mg protein/g of discs. Excess reactive groups were blocked with β -ethanolamine. Discs were incubated with 100 μl of a suitable dilution of serum in 250 μl flat-bottomed microtitre wells overnight at room temperature. The diluent used throughout was horse serum (Wellcome, No. 3). Discs were washed five times with borate buffered saline (BBS) containing 0.5% Tween20. Fifty microlitres of ^{125}I -labelled goat anti-IgE, specific activity 8 $\mu\text{Ci}/\mu\text{g}$, diluted to 50 000 ct/min in 50 μl (kindly provided by Dr D.M. Kemeny) was added to each disc and incubated overnight at room temperature. Unbound counts were washed away and the discs were counted. Binding activity units were read from a standard curve of doubling dilutions of a strongly positive serum with low total IgE, which was arbitrarily assigned as having 1000 RAST units/ml.

Results

House-dust-allergic asthma

Assaying sera obtained in 1981 from patients who had presented with asthma up to 40 years previously and were found to be allergic to house dust, we observed that overall there was a marked fall in the mean levels of both IgG and IgE antibodies to the major *D. pteronyssinus* allergen, antigen P_1 (Fig. 1), and in RAST binding activity for crude *D. pteronyssinus* extract (Fig. 2) with time since diagnosis. However, this fall was only clear in the groups from 1950–51 and 1940–41, the levels in the other groups being remarkably similar apart from an increased *D. pteronyssinus* RAST binding activity in the 1970–71 group (Fig. 2). The specific antibody levels in the 1980–81 groups from Harrow and Cardiff were not markedly different, both groups showing 20% of patients with negative binding activity for antigen P_1 and low *D. pteronyssinus* RAST binding (Figs 1 and 2). The mean total serum IgE was highest in the 1970–71 group and lowest in the 1950–51 desensitized group, but was very similar in the other groups (Fig. 3). The two 1950–51 groups showed no difference in their *D. pteronyssinus* RAST binding but the desensitized group has lower levels of IgG and IgE antibodies to antigen P_1 , despite having a higher level of total IgE (Figs 1, 2 and 3).

There was a clear tendency to lose symptoms with time, 70% of the patients in the 1960–61, 1950–51 and 1940–41 groups suffering minimal or no symptoms (Figs 1 and 2). Overall, patients not suffering severe symptoms had lower specific antibody levels, but within the groups there was no obvious relationship between symptoms and antibody levels.

As we have reported before (Chapman & Platts-Mills, 1980), there is an excellent

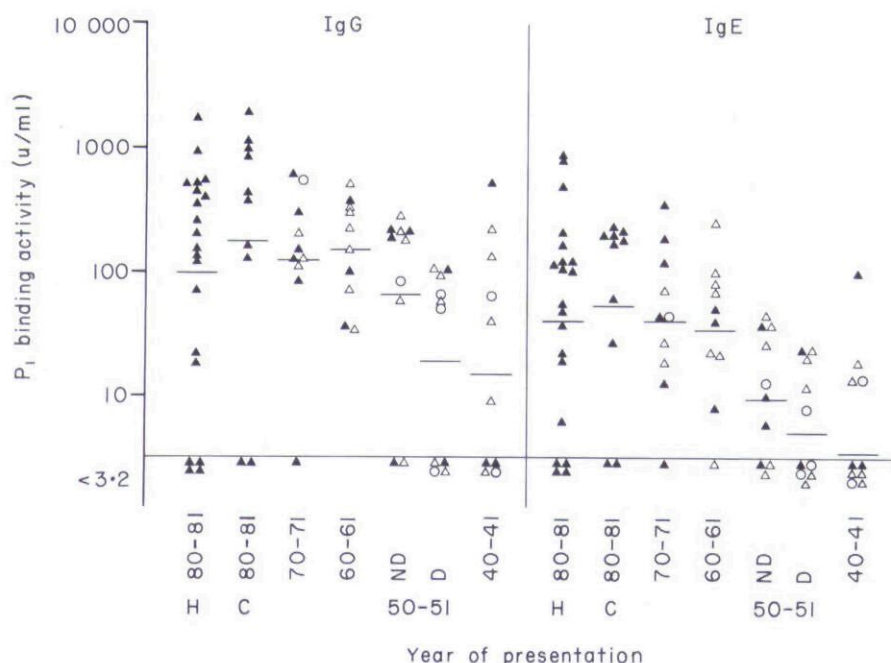


Fig. 1. Variation in levels of class specific antibodies to the major *D. pteronyssinus* allergen P_1 with time since initial diagnosis in dust allergic asthma patients diagnosed up to 40 years before the present study. Bars indicate geometric means. H, Harrow; C, Cardiff; ND, Not desensitized; D, desensitized; ▲, patients with current severe asthmatic symptoms; △, patients currently suffering mild symptoms; ○, patients with no remaining symptoms.

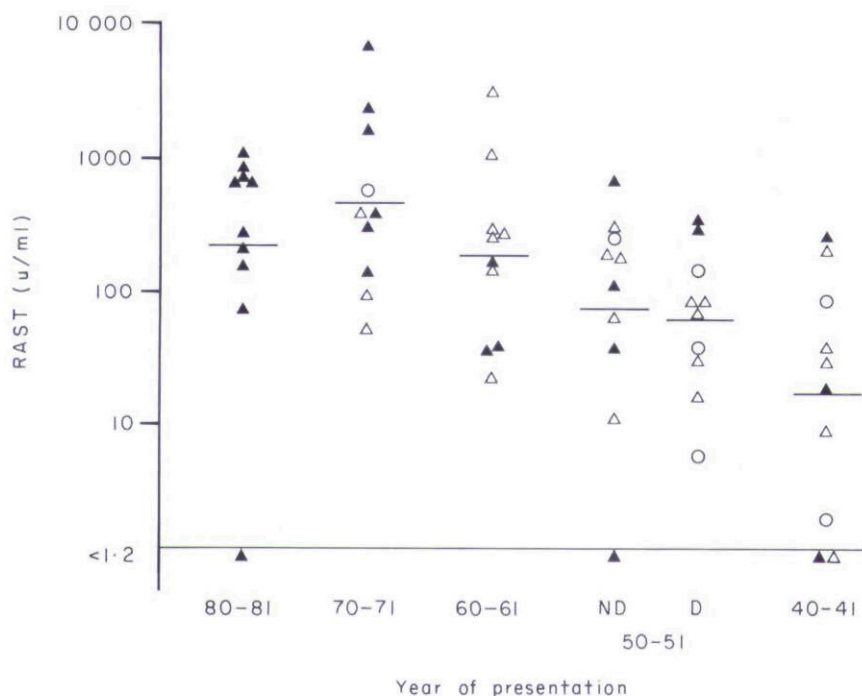


Fig. 2. Variation of *D. pteronyssinus* RAST binding activity (arbitrary units) with time since initial diagnosis of dust allergic asthma. ND, Not desensitized; D, desensitized; ▲, patients with current severe asthma; △, patients currently suffering mild symptoms; ○, patients with no remaining symptoms; Bars indicate geometric means.

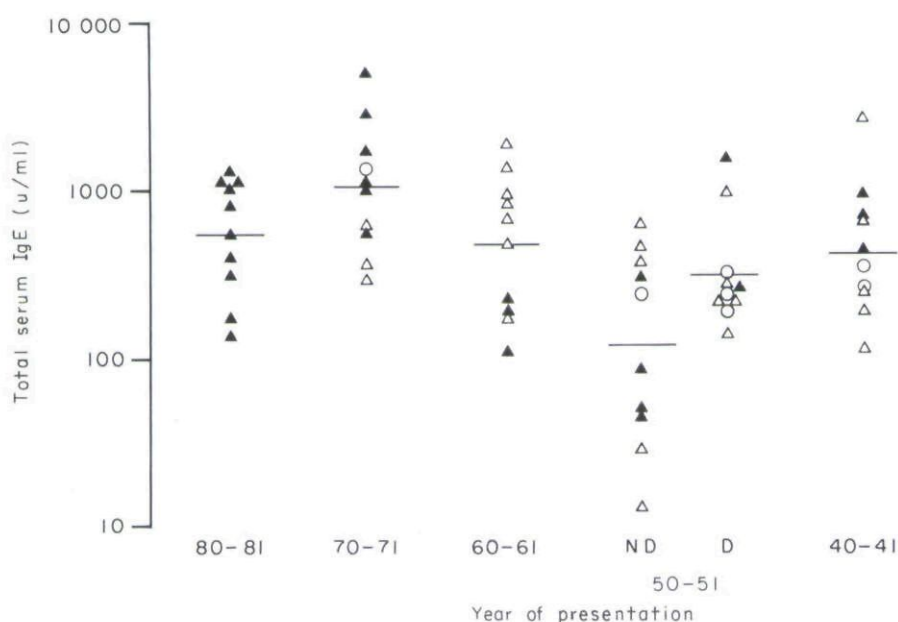


Fig. 3. Total serum IgE levels in dust allergic asthmatic patients grouped according to time of initial diagnosis. ▲, patients with current severe asthma; △, patients currently suffering mild symptoms; ○, patients with no remaining symptoms; Bars indicate geometric means.

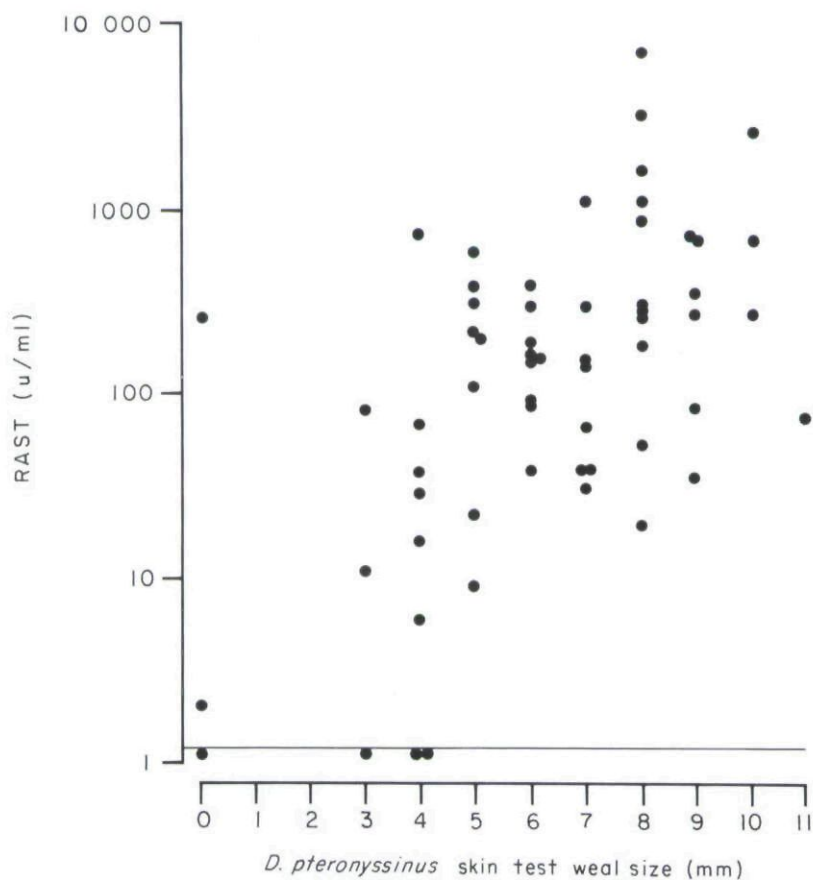


Fig. 4. Correlation of *D. pteronyssinus* RAST binding activity (arbitrary units) with the size of the skin prick test weal to *D. pteronyssinus*.

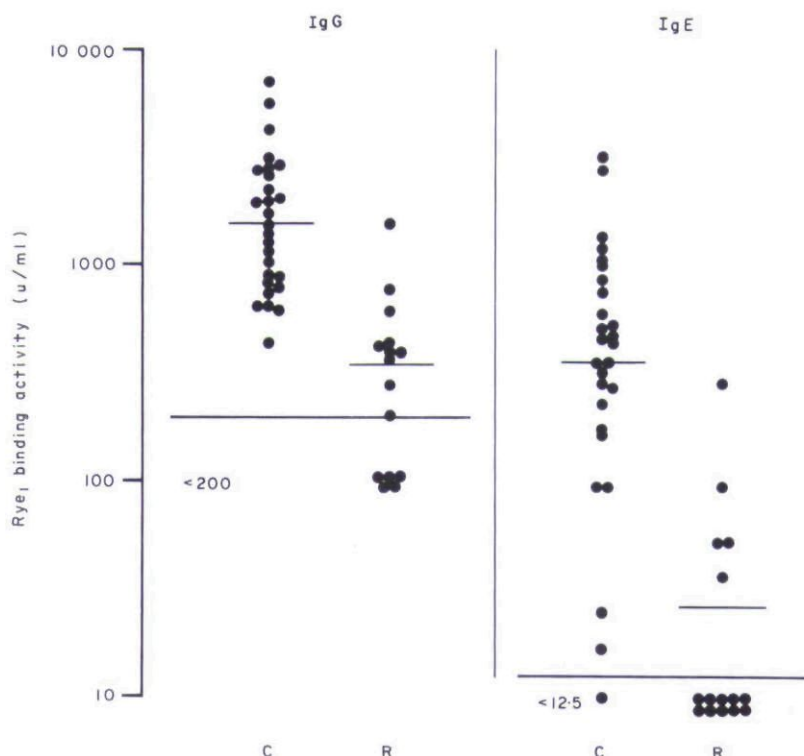


Fig. 5. Immunoglobulins class specific binding activity for the grass pollen allergen Rye I in current (C) and recovered (R) hay fever sufferers.

correlation between *D. pteronyssinus* RAST and IgE anti- P_1 binding activities ($r = 0.770$). Both values correlate significantly with the *D. pteronyssinus* skin test weal size, the correlation for *D. pteronyssinus* RAST binding (Fig. 4; $r = 0.564$, $P < 0.001$) being better than that for IgE binding activity for antigen P_1 ($r = 0.392$, $P < 0.01$). There was no apparent tendency for skin test reactivity to decline with time since diagnosis (data not shown).

Hay fever

Serum from patients who had recovered from hay fever contained significantly lower levels of IgG and IgE binding activity for Rye I (Fig. 5) and also lower total IgE than current hay fever patients (Fig. 6).

Discussion

Both IgG and IgE binding activity for antigen P_1 decreased in parallel with time. There was no single case of IgE Ab being present without IgG Ab in either asthma or hay fever subjects, nor of IgG Ab being grossly elevated in comparison with IgE Ab. These results agree with those of Chapman *et al.* (1980) and give no support for specific-IgG-blocking antibodies having a role in spontaneous improvement of symptoms. Comparison of the two groups from 1950–51 also shows no evidence for long-term blocking antibodies. The desensitized group had a slightly lower incidence of persistence of severe symptoms (two out of ten *v.* four out of ten) and on average a

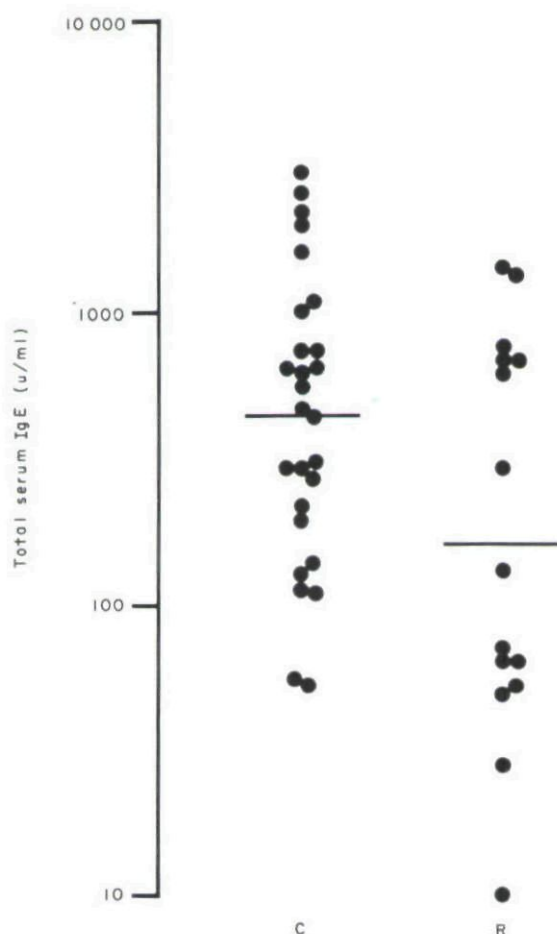


Fig. 6. Total serum IgE levels in current (C) and recovered (R) hay fever sufferers.

considerably lower proportion of the total IgE was directed against *D. pteronyssinus*, particularly P_1 . However, these differences may not result solely from desensitization, as the criteria used to decide for or against desensitization in 1950–51 were not clear from the notes. The higher *D. pteronyssinus* RAST binding and total IgE in the 1970–71 group compared with the 1980–81 group may indicate that the humoral response to *D. pteronyssinus* was not at its maximum at the time of first diagnosis, and may reflect developing sensitivity to house dust proteins other than those originally causing symptoms.

Some patients had lost their symptoms within 10 years of presentation, and the 1960–61, 1950–51 and 1940–41 groups each had 70% of patients suffering minimal or no symptoms. However, the drop in antibody levels is only marked in the 1950–51 and 1940–41 groups. In the 'recovered' hay fever group there are also patients who still have specific IgE antibodies to Rye I long after their symptoms had disappeared. The data from both studies was not sufficient to answer whether all patients who stop having symptoms will eventually lose their specific humoral response.

High levels of total serum IgE are maintained for a remarkably long time after the

specific antibody response has disappeared, and this cannot be accounted for by an increased incidence of other allergic symptoms in the older group. Previous studies have shown a decrease in total serum IgE with age in allergic patients (Delespesse *et al.*, 1977) but it is unlikely that the oldest group in the present study (mean age 52 years) was sufficiently old to show this effect. In contrast, the 'recovered' hay fever group had a lower mean total serum IgE level than the 'current' hay fever group, but it is possible that there was a bias towards more severe cases in the 'current' group, as the 'recovered' group, while requiring medical treatment, had not necessarily been referred to a specialist clinic. Certainly, a greater proportion of the 'recovered' group had total serum IgE values within the normal range.

When the patients, excluding the 1980–81 group (mean age 15 years) were grouped according to their smoking habits, it appeared that smoking had no effect on the mean level of total serum IgE, contrary to some previous reports (Gerrard *et al.*, 1980). Most of the patients who smoked heavily (fifteen out of nineteen) suffered few symptoms, while of those who did not smoke at all, twelve out of twenty-two had persistent symptoms requiring treatment. These results could suggest that smoking protects against asthmatic symptoms, but could equally indicate that persistent asthma is a disincentive towards smoking.

The obvious implication of these results is that the severity of allergic symptoms is controlled by factors other than the level of IgE or IgG antibody. Possible factors include the recruitment of basophils and/or mast cells to the nose or lung (Hastie, Heroy & Levy, 1979; Patterson *et al.*, 1977) and the direct or indirect effects of sensitized T-cells. While there seems little doubt that IgE antibodies contribute to the symptoms of hay fever and acute bronchospasm, the role they play in the prolonged or chronic symptoms of both diseases is not clear. We assume that allergens make a major contribution to the development of bronchial hyper-reactivity (Altounyan, 1970; Kerrebijn, 1970; Platts-Mills *et al.*, 1982). The persistence of bronchospasm in some asthmatic patients who have no detectable serum antibodies may reflect the observation that bronchial hyperreactivity, once established, may be very difficult to reverse in a proportion of the patients (Chan-Yeung, 1977). This data fits in with the idea that IgE Ab is part of an immune response which includes IgG and IgA antibodies, and also a form of delayed hypersensitivity. This could be either classical delayed hypersensitivity masked by immediate hypersensitivity (Brostoff & Roitt, 1969) or cutaneous basophil hypersensitivity, which certainly appears to be present in patients with atopic eczema (Mitchell *et al.*, 1982). A reliable method for testing cellular immunity to allergens is obviously required, to see whether this correlates with symptoms better than does IgE antibody. A study of cellular responses in patients who have recovered from allergic symptoms is an obvious area for future research.

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