

The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization

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Summary

Total IgG, IgG subclass and IgE antibodies specific for grass pollen allergens were measured by the red cell linked antigen-antiglobulin reaction (RCLAAR) in serum samples from nineteen patients who had undergone a course of hyposensitization. Increases in both specific IgG and IgE antibodies were seen after treatment in most patients. In the IgG subclasses the predominant response was for IgG1 and IgG4 antibodies. Attempts were made to correlate the antibody responses with the clinical response and the results are discussed with reference to the possible mechanisms of hyposensitization.

Introduction

Hypsensitization of hay fever patients with extracts of grass pollen was first described by Noon in 1911. This practice has continued almost unchanged except that it is now usual to reduce the number of doses required by use of various adjuvants. The use of chemically modified allergens may also prove to be an important new development.

Hypsensitization is usually associated with clinical improvement but the mechanism by which this occurs is still far from clear. While there have been many conflicting reports of the immunological changes that take place after hypsensitization it is usually accepted that there is (1) an initial rise in specific IgE antibodies followed by a gradual decrease after several years of therapy (Connell & Sherman, 1969); (2) an increase in specific non-reaginic or so-called 'blocking' antibodies (Cooke *et al.*, 1935), and (3) a decrease in basophil reactivity to allergen in some patients (Levy *et al.*, 1971). Despite many attempts to correlate changes in the titres of specific 'blocking' antibodies with the clinical response no statistically significant association has ever been demonstrated (Irons, Pruzansky & Patterson, 1975). It therefore seems unlikely that the benefit derived from hypsensitization is due entirely to allergen neutralization by non-reaginic antibodies in serum and it is probable that a more complex mechanism is in operation.

The finding of a high incidence of antibodies to castor bean allergen in the IgG4 subclass in patients with allergic asthma and, in particular, high titres of IgG4 antibodies in the absence of IgE antibodies in two patients who were not apparently sensitive to castor bean allergen despite long exposure to the dust (Devey & Panzani, 1975) prompted us to investigate the antibody changes in the IgG subclasses in a group of patients with hay fever undergoing hyposensitization therapy. It has previously been demonstrated that these patients had immediate increases in titres of IgG and IgE antibodies to grass pollen allergens after hyposensitization (Johansson *et al.*, 1974).

Specific IgE antibodies to grass pollen are probably best measured by the radio-allergosorbent test (Wide, Bennich & Johansson, 1967) but unfortunately this is not very satisfactory for antibodies of the other immunoglobulin classes due to non-specific absorption of non-antibody immunoglobulin. Specific antibodies to grass pollen allergens were therefore measured by the red cell linked antigen-antiglobulin reaction (Gell & Coombs, 1974) using specific antiglobulin sera for total IgG, IgE and the four subclasses of IgG and some attempt was made to correlate the changes in specific antibodies with the clinical response to hyposensitization.

Materials and methods

Human sera

Serum samples were obtained from nineteen hay fever patients undergoing clinical trials of various hyposensitization therapies. Most of these patients have been described by Miller & Tees (1974) and Johansson *et al.* (1974). Six serum samples were obtained from each patient during the course of the trial (Table 1).

Clinical responses to therapy were assessed subjectively as 'very good' (minimal symptoms), 'good' (noticeable improvement of symptoms) or 'poor' (no improvement of symptoms).

Hyposensitization therapies

Hyposensitizing preparations were based on the Bencard Group B₂ mixture of pollens from twelve different grasses (Bent, Brome, Cocksfoot, Dogstail, Fescue, Foxtail, Meadow, Rye, Vernal, Timothy, False oat and Yorkshire fog).

Ten patients received pollen-tyrosine adsorbate (glycerinated pollen extract adsorbed onto L-tyrosine crystals, Miller & Tees (1974)); of these, five had 'good' or 'very good' responses and five had 'poor' responses. Five patients received Bencard 'Alavac-P' (alum precipitated pyridine pollen extract); of these, two had 'good' or

Table 1. Times of bleeding of patients undergoing hyposensitization therapies

Serum sample	Times of bleeding
1 Pre-hyposensitization course	(February–March)
2 Mid course or immediately after course	(March–May)
3 One week after end of course	(May)
4 Mid pollen season	(June–July)
5 Immediately after pollen season	(August–September)
6 Late after pollen season	(October)

'very good' responses and three had 'poor' responses. Two patients received 'Polline x (gluteraldehyde treated pollen extract adsorbed onto L-tyrosine crystals) and both had 'very good' clinical responses. Two patients received modified pollen allhydrogel (gluteraldehyde treated pollen extract with allhydrogel); one of these had a 'good' clinical response and one had a 'poor' response.

Antiglobulin sera

Antiglobulin sera to total IgG, IgE and the four subclasses of IgG were those described by Devey & Voak (1974) and Devey & Panzani (1975) and had been shown to be mono-specific in haemagglutination tests. The antiglobulin sera to the IgG subclasses were used at dilutions at which they were approximately equipotent. All sera were heat inactivated and absorbed with human group O red blood cells.

The antibody-coupled grass pollen reagent

This was prepared as described by Wilson & Devey (1973) using a 7S fraction of a rabbit antiserum to human group O red blood cells and an aqueous extract of the Bencard B₂ mixture of grass pollens. In brief, an aminoimidazolyl derivative of the rabbit antibody was converted into a reactive diazo compound by diazotization at 4°C with nitrous acid. Excess nitrous acid was destroyed with 2% ammonium sulphamate. Coupling to the grass pollen extract was carried out at pH 7.8–8.0. After 10 min the solution was adjusted to pH 7.0–7.5 with N NaOH, kept at 4°C overnight and centrifuged to remove insoluble residues.

Characterization of the antibody-coupled reagent and performance of the red cell linked antigen-antiglobulin reaction (RCLAAR)

Serial dilutions of the antibody-coupled grass pollen reagent were incubated with equal volumes of washed 2% human group O red blood cells for 0.5 hr at room temperature. After washing in PBS the cells were resuspended to 2% and added to a microtitre plate containing normal rabbit serum in row 1, a goat antiserum to rabbit IgG in row 2 and a rabbit antiserum to grass pollen in row 3. The optimal dilution of the antibody-coupled reagent for coating red cells in the RCLAAR was the one that gave strong agglutination with the anti-pollen serum without direct agglutination in normal rabbit serum.

The general application of the RCLAAR has been described by Gell & Coombs (1974) as was performed here with the modifications described by Devey & Panzani (1975). Inhibition tests were performed to establish that the antibodies detected in the test were specifically directed to the grass pollen allergens and that the antiglobulin sera were specific for the immunoglobulin classes and IgG subclasses.

Antibody titres were expressed as log₂ values and mean values for each bleeding time in each treatment and response group were calculated. Analysis of variance was performed on the log transformation of the data using a Genstat computer program. Individual values for specific antibody titres in the four IgG subclasses were analysed by Duncan's multiple range test.

Results

It has previously been shown by the RCLAAR that sera from individuals with hay fever contained specific IgG and IgE antibodies to grass pollen allergens whereas sera from non-atopic controls did not have significant titres of antibody (Wilson *et al.*,

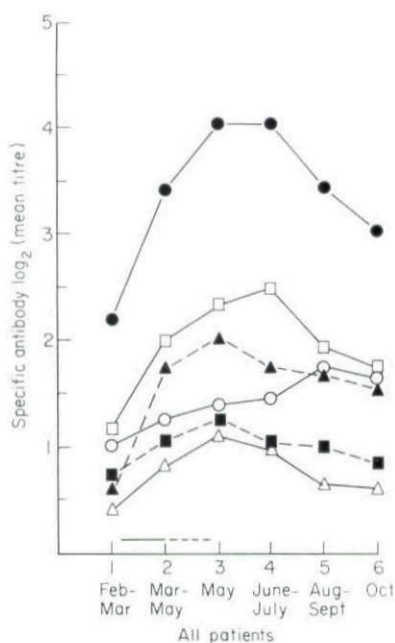


Fig. 1. The mean titres of specific antibodies to grass pollen allergens in nineteen patients after hyposensitization. (●—● IgG antibodies; ○—○ IgE antibodies; □—□ IgG1 antibodies; ■—■ IgG2 antibodies; △—△ IgG3 antibodies; ▲—▲ IgG4 antibodies; — — —, period of hyposensitization therapy.)

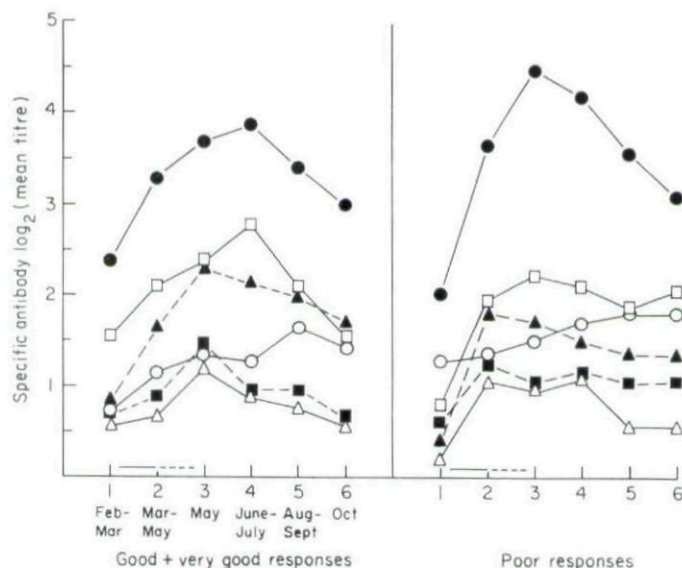


Fig. 2. The mean titres of specific antibodies to grass pollen allergens in ten patients with good or very good clinical responses to hyposensitization and in nine patients with poor clinical responses. (●—● IgG antibodies; ○—○ IgE antibodies; □—□ IgG1 antibodies; ■—■ IgG2 antibodies; △—△ IgG3 antibodies; ▲—▲ IgG4 antibodies; — — —, period of hyposensitization therapy.)

1972). In the serum from the nineteen hay fever patients described in this study there was quite wide individual variation in the response to hyposensitization as reflected by the specific antibody titres but in most cases therapy was associated with an increase in the titres of both specific IgG and IgE antibodies.

The mean specific total IgG, IgE and IgG subclass antibody responses to grass pollen allergens for all nineteen patients regardless of type of therapy and clinical response are shown in Fig. 1. Titres of total specific IgG antibodies increased after

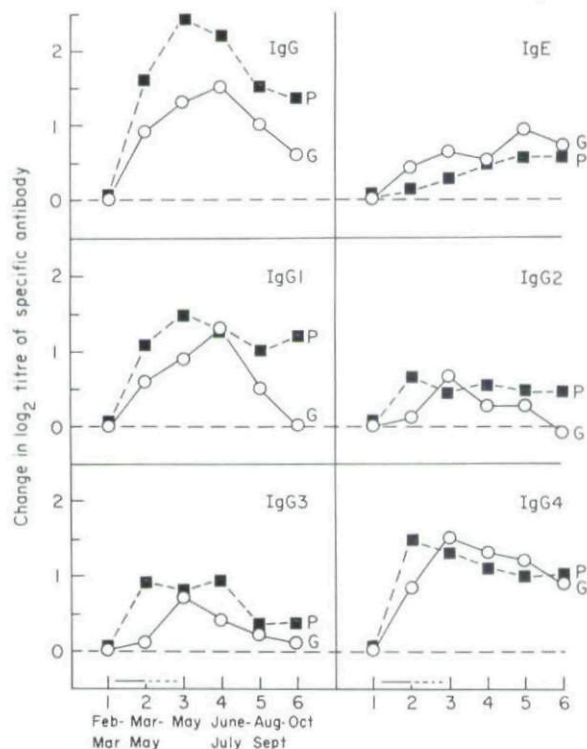


Fig. 3. The changes in the mean titres of specific antibodies to grass pollen allergens from the levels in serum sample 1 (taken before the start of hyposensitization) in ten patients with good or very good clinical responses (○—○ G) and in nine patients with poor clinical responses (■—■ P). (—, Period of hyposensitization therapy.)

hyposensitization reaching peak values by about the middle of the pollen season. Specific IgE antibody titres showed a more gradual rise reaching a peak at the end of the pollen season.

Antibodies to grass pollen allergens were detected in all the IgG subclasses. In serum sample 1 (taken before hyposensitization) IgG antibodies were mainly IgG1. After treatment the mean IgG1 antibody titre increased reaching a peak in June–July and there was also a marked increase in the mean IgG4 antibody titre which reached a peak in May. Less pronounced increases in titre were seen for IgG2 and IgG3 antibodies and these had returned to their original values by October, while titres of IgG1 and IgG4 antibodies remained elevated. There was no statistically significant difference between the mean antibody titres for IgG2, IgG3 and IgG4 in serum sample 1, but in all the subsequent samples the mean titres of IgG1 and IgG4 antibodies were significantly greater than those of IgG2 and IgG3 antibodies ($P < 0.05$).

Attempted correlation with clinical response

Whilst recognizing the heterogeneous nature of the mixed grass pollen allergen preparation used for coating the red cells in the RCLAAR we considered it worthwhile to attempt to correlate the antibody responses with the clinical responses to hyposensitization. Figure 2 shows a comparison between the titres of specific antibody to grass

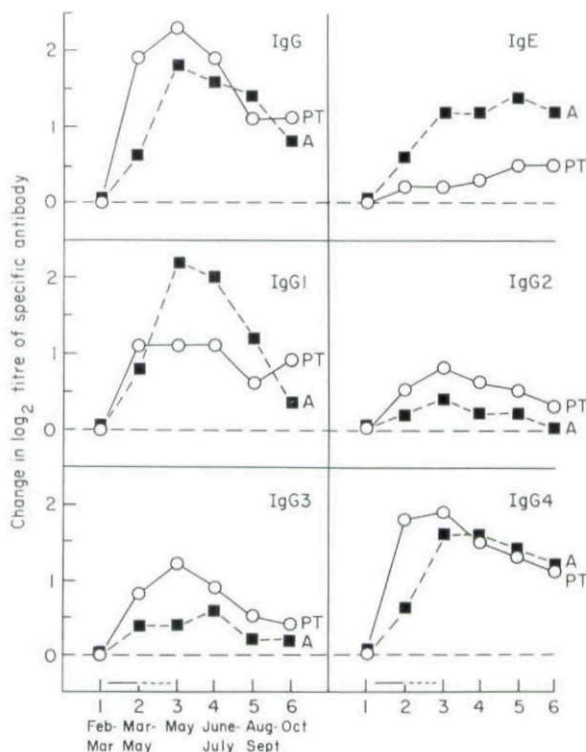


Fig. 4. The changes in the mean titres of specific antibodies to grass pollen allergens from the levels in serum sample 1 (taken before the start of hyposensitization) in ten patients receiving pollens tyrosine (○—○ PT) and in five patients receiving Alavac-P (■—■ A). (—, Period of hyposensitization therapy.)

pollen allergens in the two clinical response groups ('good' and 'very good' responses were considered together). It may be seen that the magnitude of increase in mean antibody titres for IgE, total IgG and the IgG subclasses was rather similar in both groups.

However, it was noticeable that the starting titre for IgE antibodies in serum sample 1 (taken before the start of hyposensitization) was much lower in the 'good' clinical response group when compared to the titre in the 'poor' clinical response group while the initial IgG antibody titres tended to be higher in the 'good' clinical response group. In Fig. 3 the actual changes in mean antibody titres from the levels in serum sample 1 are compared for the two response groups. There was no significant difference between the changes in antibody titre for the two response groups except for IgG1 in serum sample 6 which had returned to its starting level in patients with clinical improvement but remained elevated in the 'poor' response group.

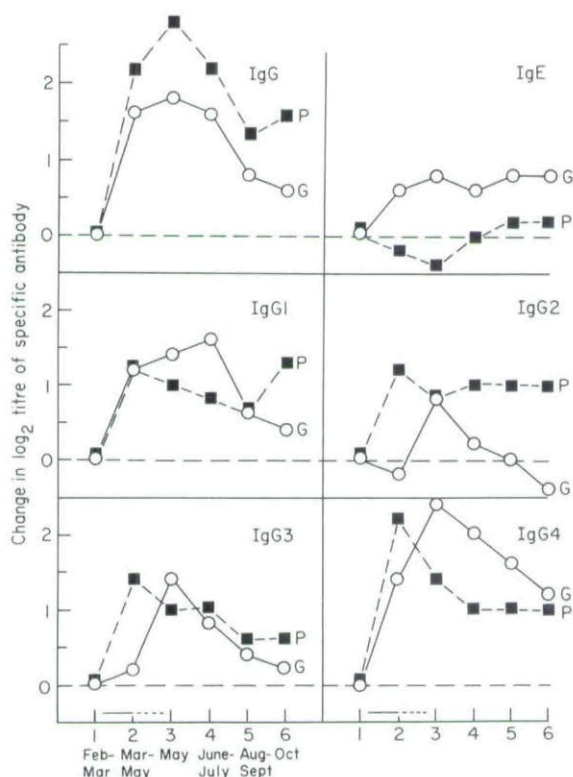


Fig. 5. The changes in the mean titres of specific antibodies to grass pollen allergens from the levels in serum sample 1 (taken before the start of hyposensitization) in ten patients receiving pollen-tyrosine: five with good or very good clinical responses ($\circ$ — $\circ$ G) and five with poor clinical responses ($\blacksquare$ — $\blacksquare$ P). (—, Period of hyposensitization therapy.)

Influence of hyposensitization therapy

It might be expected that the type of hyposensitization therapy may affect the antibody response to grass pollen allergens. Therefore in Fig. 4 the antibody responses are compared for the two main treatment groups (pollen-tyrosine and Alavac-P). The IgE antibody response was significantly greater in patients receiving Alavac-P in serum samples 3, 4 and 5 ($P < 0.05$) while patients receiving pollen-tyrosine showed significantly greater responses in serum sample 2 for total IgG antibodies ($P < 0.01$) and IgG4 antibodies ($P < 0.05$). As the hyposensitization therapy did affect the antibody response, particularly for IgE, the antibody responses were compared for the two clinical response groups in patients receiving only pollen-tyrosine (Fig. 5). In these patients there appeared to be a correlation between an increase in mean IgE antibody titre and clinical improvement and this was significant in serum samples 2 ($P < 0.05$) and 3 ($P < 0.05$). Clinical improvement also appeared to correlate with a decrease in mean IgG2 antibody titres and this was significant in serum samples 2 ($P < 0.01$), 5 ($P < 0.05$) and 6 ($P < 0.005$), a decrease in mean IgG and IgG1 antibody titres in serum sample 6 ($P < 0.05$) and an increase in mean IgG4 antibodies in serum samples 3 and 4 ($P < 0.05$).

However, in the smaller number of patients receiving Alavac-P there was no similar correlation between clinical improvement and an increase in IgE antibody titres or a

change in IgG antibodies. The IgE antibody response tended to be low in all patients receiving gluteraldehyde-treated pollen extracts regardless of clinical response.

Discussion

Various coupling methods such as photo-oxidation, bis-diazotized benzidine and toluene-2,4-diisocyanate have not proved successful in coupling grass pollen extracts with red cell antibodies to provide a suitable reagent for use in the RCLAAR (Hunter & Coombs, 1969). However, the new coupling procedure of Wilson & Devey (1973) was found to give a greatly improved antibody-coupled pollen reagent which allowed measurement of specific antibodies in the RCLAAR. The general sensitivity of the test remained low but this was probably because the grass pollen extract used was a crude mixture from several different grasses whereas the antibodies in the sera of the patients were specifically directed to perhaps only a few allergenic components of only some grass pollens.

IgG and IgE antibodies to a mixed grass pollen extract were measured by the RCLAAR in a series of serum samples from hay fever patients who had received hyposensitization therapy. Titres of specific IgG and IgE antibodies were shown to increase after treatment above the seasonal rise normally seen in untreated hay fever patients in agreement with the findings of Johansson *et al.* (1974). In serum samples taken before the hyposensitization treatment most of the IgG antibody was IgG1 which might be expected as this subclass makes up about 70% of the total IgG. However, after treatment there was a marked increase in the titres of specific IgG4 antibodies which approximately equalled the IgG1 antibody response. This was surprising as IgG4 makes up only about 3% of the total IgG and suggests that there was a selective stimulation of IgG4 antibody production by grass pollen allergens in response to hyposensitization. As discussed previously (Devey & Panzani, 1975) the RCLAAR is only a semi-quantitative test but it was felt that a meaningful comparison could be made for antibody titres in the IgG subclasses as these antiglobulin sera were used at dilutions at which they were approximately equipotent.

When antibody responses to hyposensitization were compared with clinical responses in all the patients there appeared to be little difference between the two clinical groups. Although it may have been significant that patients subsequently showing clinical improvement started off with higher titres of IgG antibodies and lower titres of IgE antibodies as compared with those that did not show clinical improvement. The type of hyposensitization therapy given did, however, influence the antibody response and patients who had been given alum precipitated pollen extracts (Alavac-P) developed significantly higher titres of specific IgE antibodies whereas patients who had received pollen-tyrosine tended to have higher titres of IgG antibodies. In patients who only received pollen-tyrosine clinical improvement did appear to correlate with increased titres of IgE antibodies and, rather surprisingly, with decreased titres of IgG2 antibodies. However, similar correlations could not be made for patients receiving Alavac-P. These results should be interpreted with great caution. It is possible that most of the patients were hypersensitive to a very limited number of components of the heterogenous preparation used in this study both for hyposensitization and for antibody measurements. This grass pollen extract was prepared from a mixture of twelve different grasses and therefore it was quite possible that many of these allergenic components were not adequately represented on the red cells in the RCLAAR. Until it is possible to monitor the patient's antibody response to very pure

allergen preparations definite conclusions about the relationship between antibody responses and clinical responses should not be made.

The significance of enhanced antibody production in the IgG4 subclass is difficult to interpret. It may have been simply a selective response to the particular antigen or route of inoculation. On the basis of previous findings (Devey & Panzani, 1975) it was considered unlikely that IgG4 antibodies to grass pollen were the short-term skin sensitizing IgG antibodies first described by Parish (1970) although passive cutaneous anaphylaxis tests in primate skin were not performed with these sera. The finding of increased IgG4 antibodies in hay fever patients undergoing hyposensitization together with previous findings of elevated IgG4 antibodies in castor bean hypersensitivity (Devey & Panzani, 1975) provide grounds for speculation that IgG4 plays a regulatory role in hypersensitivity. However, it was disappointing that IgG4 antibodies to grass pollen allergens did not show a more dramatic correlation with clinical response, although in patients receiving pollen-tyrosine they were significantly increased in the May and June/July serum samples in patients showing clinical improvement.

These results may be regarded as providing further evidence against the hypothesis that effective hyposensitization depends upon eliciting specific 'blocking antibodies' which combine with free antigen in serum and thereby lower the antigen concentration below that necessary to initiate a hypersensitivity reaction. This of course does not exclude the possible importance of secretory IgA antibodies which may block at the site of antigen entry. Serum antibodies produced during hyposensitization might more effectively inhibit a hypersensitivity reaction by blocking the uptake of IgE antibodies onto mast cells and basophils. Perhaps this might be mediated by a particular IgG subclass which is able to combine with the same receptors as IgE, or ones closely adjacent, and which either is not able to combine with antigen or, if this occurs, is not able to trigger the release of pharmacological mediators. In support of this it has been reported that an IgG4 myeloma protein will block passive sensitization by IgE antibodies in baboon skin (Stanworth & Smith, 1973). Another possible mechanism by which IgG may control the IgE response is by feed-back inhibition through T cells. It has been shown that IgG antibodies induced by hyposensitization can depress the *in vitro* response to grass pollen of lymphocytes from untreated hay fever patients (Romagnani, Biliotti & Ricci, 1975). In rats and rabbits soluble T cell factors have been shown to control the synthesis of IgE antibodies but the suppressor components do not appear to be immunoglobulin in nature (Okumura & Tada, 1974; Kishimoto & Ishizaka, 1974). Thus it would seem unlikely that any of the IgG subclasses exert a suppressor effect directly on IgE-B cells.

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