

## The subclass of IgG antibody in allergic disease: II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals

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### Summary

In an attempt to understand the role of the different IgG subclasses in allergic disease, we have studied the subclass of IgG antibody to dust mite (HDM) and grass pollen (GP) produced as a result of natural exposure. Studies were carried out on 40 atopic children and 100 atopic adults who had never received immunotherapy. Thirty-two non-atopic adult controls were also studied. The specificity of the assay for IgG antibodies to dust mite was confirmed by inhibition with the homologous extract but not mite culture medium or fetal calf serum. IgG<sub>1</sub> antibodies to HDM could be detected in most atopics (94%) and non-atopics (97%), and similar results were obtained for GP (81% and 100%, respectively). IgG<sub>4</sub> antibodies to HDM were detected in more atopics (66%) than non-atopics (53%) and the difference was more marked for GP (72% vs. 19%). While the levels of IgG<sub>1</sub> antibodies were not significantly different in the two groups, the levels of IgG<sub>4</sub> antibodies were much lower in the non-atopics ( $P < 0.001$ , Mann-Whitney *U*-test). These data show that all subjects were capable of recognizing and mounting an IgG<sub>1</sub> antibody response to these inhaled antigens. Atopic individuals differed from normal subjects in the frequency with which they made IgG<sub>4</sub> antibodies in response to natural exposure to both dust mites and pollen.

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### Introduction

The different IgG subclasses [1-3] arose in humans as the result of duplication of the immunoglobulin G gene [4]. This is believed to have occurred early in human evolution at around the time that mammals diversified and the fact that the variants have been conserved implies some selective advantage in possessing the different immunoglobulin subclasses.

Much remains to be discovered about the advantages of producing one subclass over another and the factors which regulate this. The ability to mount an immune

response to inhaled allergens is generally regarded as a characteristic of the atopic state [5]. However, recent reports have shown that T cells from both atopic and non-atopic individuals can proliferate in response to these allergens [6], and studies in rats have shown that low IgE responder strains (analogous to non-atopic humans) can produce antigen-specific T cells in response to nanogram quantities of inhaled ovalbumin [7]. It appears that antigen can penetrate the respiratory tract mucosa more easily than the skin or the gut [8].

In previous studies we have shown that antibody responses to some soluble antigens, such as bee venom, ovalbumin, casein and horse  $\gamma$ -globulin, are mainly restricted to IgG subclasses 1 and 4 [9]. The predominance of one or other of these subclasses depends on the degree

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of exposure to antigen [10,11]. After repeated exposure to foods, and after repeated immunization during insect venom immunotherapy, the IgG<sub>4</sub> antibody response is the most persistent [12,13]. As the pattern of response has been shown to vary with different antigens [9] the aim of this study was to determine the normal immune response to inhaled allergens in atopic and non-atopic subjects.

## Subjects and methods

### Subjects

Serum samples were obtained from 20 children studied in Freiberg who were allergic to dust mite and from 20 who were allergic to grass pollen. Serum samples from 80 adults allergic to dust mite and 41 to grass pollen who had never received immunotherapy were collected from patients attending the allergy clinic at St Mary's Hospital. On skin-prick testing (Bencard) all had positive responses to the relevant allergens with weals at least 3 mm greater than the negative control. All had a history of asthma and or rhinitis. The non-atopic control subjects were 32 patients attending a Medical out-patient clinic at Guy's Hospital and healthy departmental staff. All had negative skin-prick tests to a panel of common allergens including grass pollen, dust mite and cat epithelium, and in addition had a negative (<1+) Phadebas and Phadiatop serum radio-allergosorbent test (RAST).

### Reagents

Immuno-1 microtitre plates (Nunc) were purchased from Gibco Ltd (Uxbridge, U.K.). Horse serum was purchased from Sera Lab Ltd (Crawley, U.K.). Tween 20 and *p*-nitrophenyl phosphate were purchased from Sigma Ltd (Poole, U.K.). All other reagents were purchased from BDH Ltd (Poole, U.K.).

### Antigens and antisera

The Timothy grass pollen extract used was a generous gift from Mr B. Overell and Mr D. Hordle of Beechams Pharmaceuticals, Epsom Down, U.K. The dust mite allergen was a kind gift from Dr H. Maasch, formerly of Allergopharma, Reinbeck, FRG. The dust mite culture medium was an ox liver extract, purchased from Oxoid Ltd, Basingstoke, U.K. Monoclonal antibody to human IgG and IgG subclasses 1-4 were kindly donated by Dr J. Lowe (University of Birmingham, U.K.), and supplies were subsequently purchased from Oxoid Ltd (U.K.). The clones used were 8a4 (IgG), NL16 (IgG<sub>1</sub>), GOM 2 (IgG<sub>2</sub>), Zg4 (IgG<sub>3</sub>) and RJ4 (IgG<sub>4</sub>). Affinity purified horseradish peroxidase-conjugated rabbit anti-mouse IgG (HRP anti-MIG), and alkaline phosphatase-conjugated rabbit anti-mouse IgG (AP anti-MIG) were purchased from Sigma Ltd (Poole, U.K.) and stored at 4°C.

## ELISA

IgG and IgG subclass antibodies were measured by ELISA as described previously [14]. Dust mite or grass pollen diluted to 1 µg/ml in pH 9.6 carbonate/bicarbonate buffer was used to coat the microtitre plates (Nunc immuno-I) at 4°C overnight. All incubations used 50 µl at 4°C, and between each incubation there were three washes with phosphate-buffered saline (PBS, pH 7.4, 0.1 M containing 0.05% Tween 20). The assay diluent was PBS containing 0.5% Tween 20 and 0.5% normal horse serum. The test sample (1/100 and 1/1000) was added and after 2 hr the plates were washed and monoclonal anti-IgG or IgG<sub>1-4</sub> was added (1/1000 dilution). After 1 hr AP-anti-MIG was added in a dilution of 1/500, and after a further 1 hr incubation and wash, the substrate *p*-nitrophenyl phosphate (1 mg/ml in pH 9.8 di-ethanolamine buffer) was added. After 1.5 hr at 37°C the colour reaction was stopped by the addition of 50 µl of 3 M sodium hydroxide. The optical density was read in a Titretek multiskan plate reader (Flow Labs, Rickmansworth, U.K.) at 405 nm. Results were expressed as arbitrary units ml<sup>-1</sup> by reference to an internal standard serum, as described elsewhere [14]. The interassay coefficient of variation was 7.5% for the grass pollen assay and 9% for the dust mite assay.

### Inhibition ELISA

To test the specificity of the assays, free antigen was pre-incubated with the sample (diluted 1/100) for 1 hr at room temperature, added to the microtitre plate and the assay completed as above. All inhibition assays used the pan IgG subclass-reactive monoclonal antibody 8a4. Results are expressed as percentage inhibition by reference to microtitre wells in which no free antigen was included.

### Statistical tests

Comparison between groups was by Mann-Whitney *U*-test where the results were not normally distributed and by Student's *t*-test where they were. All tests were performed on a BBC model B computer (Acorn Ltd, U.K.) using a BSTAT+ statistics program (Finersoft © 1984, Guy's Hospital, London, U.K.).

## Results

### Specificity of the assays

The specificity of the assays was determined by inhibition with free antigen. Similar results were observed for both antigens with binding of IgG antibody inhibited in a dose-dependent manner only with the homologous antigen (Fig. 1). The possibility existed that the high levels of

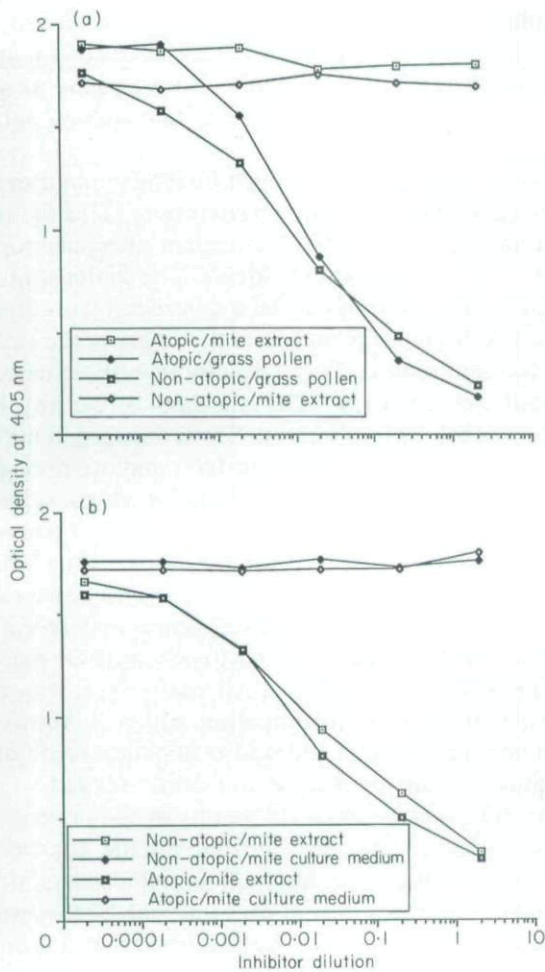


**Fig. 1.** The specificity of the assays for IgG<sub>1</sub> antibodies to (a) grass pollen and (b) dust mite was demonstrated by inhibition of ELISA. With the homologous but not the heterologous antigen the concentration of inhibitor started at 10 mg/ml.

antibody binding to the dust mite-coated microtitre plates could be due to the presence of proteins derived from the ox liver mite culture medium. However, it was possible to inhibit binding with free mite allergen but not with an extract of mite culture medium alone or fetal calf serum. Specificity was tested in this way using 32 sera from non-atopic adults whose sera were pre-incubated with 100 µg of dust mite extract, 10% foetal calf serum, or 100 µg/ml dust mite growth medium. The results (Table 1) show that only the dust mite extract inhibits binding. Some mite extracts contain traces of human protein but this did not appear to cause difficulties in the assays used, as binding of anti-human IgG to the dust mite-coated plate was minimal.

**Table 1.** Inhibition of IgG antibody binding to dust mite-coated microtitre plates. All samples were diluted 1/100. With 100 µg dust mite allergen there was nearly complete inhibition but with fetal calf serum and dust mite culture extract there was rarely inhibition and in many cases enhanced binding, possibly due to the formation of immunoglobulin aggregates

| Subject | Percentage inhibition |                     |                  |
|---------|-----------------------|---------------------|------------------|
|         | Dust mite extract     | Mite culture medium | Fetal calf serum |
| 1       | 87.9                  | 13.4                | 13.7             |
| 2       | 84.4                  | 15.1                | -0.69            |
| 3       | 81.8                  | 2.5                 | -2.5             |
| 4       | 79.1                  | -2.2                | -3.4             |
| 5       | 79.4                  | 4.0                 | -14.9            |
| 6       | 80.8                  | 7.5                 | -10.0            |
| 7       | 90.6                  | 6.8                 | -7.2             |
| 8       | 79.8                  | -2.2                | 25.6             |
| 9       | 72.8                  | 18.2                | 4.7              |
| 10      | 70.8                  | 11.5                | -37.3            |
| 11      | 86.6                  | 25.8                | -21.6            |
| 12      | 85.5                  | 5.3                 | -42.3            |
| 13      | 84.4                  | 8.5                 | -28.4            |
| 14      | 81.2                  | -23.4               | -37.9            |
| 15      | 75.5                  | 30.3                | 1.7              |
| 16      | 76.0                  | -5.5                | -17.2            |
| 17      | 72.6                  | -5.3                | -30.0            |
| 18      | 73.5                  | -11.6               | -38.2            |
| 19      | 79.0                  | -19.2               | -44.5            |
| 20      | 31.9                  | 5.3                 | -37.3            |
| 21      | 75.8                  | -20.9               | 5.22             |
| 22      | 75.4                  | -24.4               | -12.7            |
| 23      | 85.2                  | -28.1               | -6.5             |
| 24      | 43.8                  | -6.4                | -13.7            |
| 25      | 86.2                  | 15.5                | -26.9            |
| 26      | 86.1                  | 5.7                 | -13.7            |
| 27      | 84.8                  | 17.8                | 10.7             |
| 28      | 87.3                  | -1.8                | 0                |
| 29      | 70.4                  | -88.5               | -8.6             |
| 30      | 80.7                  | 36.9                | -1.6             |
| 31      | 60.0                  | -83.5               | -6.5             |
| 32      | 71.4                  | -20.5               | -14.6            |
| Mean    | 76.9                  | 3.4                 | -14              |

#### *IgG subclass antibodies to dust mite and grass pollen in atopic and non-atopic individuals*

Using the IgG subclass ELISA, antibodies were measured in sera from dust mite-allergic children (20) and adults (80) and from 32 non-atopic controls. IgG<sub>2</sub> and IgG<sub>3</sub> antibody was detected in only two children (not shown). The majority of the IgG antibody (Fig. 2) was in subclass

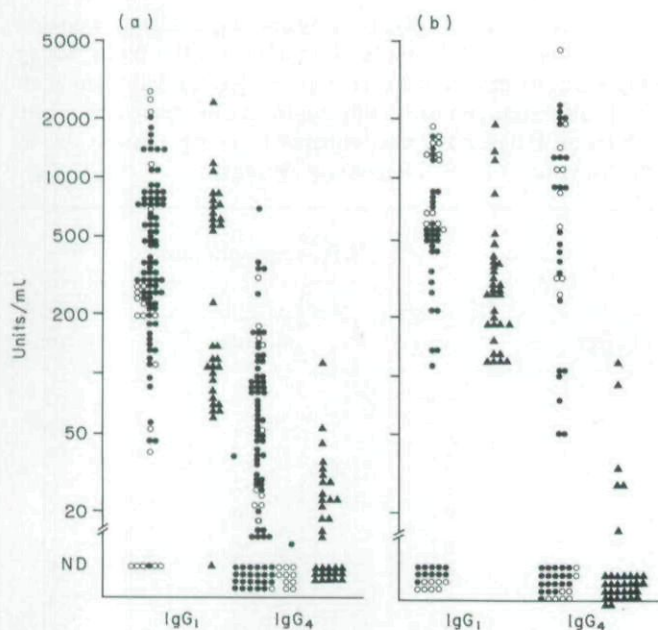


**Fig. 2.** IgG<sub>1</sub> and IgG<sub>4</sub> antibodies to (a) dust mite and (b) grass pollen in atopic children (○) and adults (●), and in non-atopic controls (▲). There was no difference in the frequency or quantity of IgG<sub>1</sub> antibodies to either antigen in the atopic or non-atopic groups, however, there was less IgG<sub>4</sub> antibody in the non-atopic group.

1 ( $P < 0.001$ ; Mann-Whitney  $U$ -test). Both mite-allergic and non-atopic individuals possessed similar concentrations of IgG<sub>1</sub> antibodies but the level of IgG<sub>4</sub> antibody was lower in the non-atopic subjects ( $P < 0.01$ ; Mann-Whitney  $U$ -test). The grass pollen-allergic group produced IgG<sub>1</sub> and IgG<sub>4</sub> antibodies to grass pollen at similar concentrations. In contrast, the non-atopic subjects had IgG<sub>1</sub> antibodies to grass pollen in all cases at concentrations similar to those seen in grass pollen-allergic subjects, but IgG<sub>4</sub> antibodies to grass pollen could be seen in only 6/32 of the group and at a much lower level ( $P < 0.0001$ ; Mann-Whitney  $U$ -test). No difference was observed between atopic children and adults.

## Discussion

There are a number of functional differences between the different human IgG subclasses. While IgG<sub>1</sub> and IgG<sub>3</sub> antibodies fix complement [15], IgG<sub>2</sub> and IgG<sub>4</sub> do not. The function of these antibodies that do not fix complement is unclear, but they may have advantages in preventing bacteria and viral adherence [16] or by having the ability to react with antigen without forming potentially damaging immune complexes [17–22]. Previous studies have shown that repeated exposure to food antigens or repeated antigenic stimulation during allergen

immunotherapy often results in an IgG<sub>4</sub>-restricted response [6–9]. In studying the IgG subclass response to the inhalant allergens of grass pollen and dust mite, we have tried to define the type of response that follows natural exposure to their antigens.

When radiolabelled antigen-binding immunoassay techniques were used, our own laboratory [23] and others [11] found IgG antibodies to inhalant allergens in only 20–30% of non-atopic individuals. The findings of our present study show this to be a considerable underestimate. The discrepancy may lie in the ability of the ELISA to detect antibodies of lower affinity than those detected by radioimmunoassay. The subclass to which these antibodies belong is of interest. Those detected in normal subjects are largely restricted to IgG<sub>1</sub> and are present at similar concentrations to those found in atopic subjects, but it is the level of IgG<sub>4</sub> antibody which seems to distinguish the non-atopic from the atopic. If the level of IgG<sub>4</sub> antibody represents a response to repeated exposure [10–13] it is possible that these differences may reflect the extent to which antigens are able to penetrate the mucous membrane in atopic subjects. Alternatively, it may be a function of airways inflammation which is known to affect immune function and studies of patients with other inflammatory airway diseases are clearly needed.

The difference between the results of the present and previous studies may well be due to the contrasting technique of antigen-binding radioimmunoassays, which use nanogram quantities of antigen, and ELISA which uses microgram amounts. The likelihood that this difference is the result of methodology is supported further by the finding that IgG<sub>4</sub> antibody produced early in the immune response is of low affinity [24,25], while IgG<sub>1</sub> and IgG<sub>4</sub> antibody produced after prolonged exposure to antigen has a higher affinity [personal communication: Dr M. Devey, London School of Hygiene and Tropical Medicine, U.K.] than IgG<sub>1</sub> antibody to the same antigen. While the significance of this change in affinity has yet to be demonstrated these data show that normal as well as atopic individuals do make IgG antibodies to inhaled grass pollen and dust mite, and that most of this antibody is restricted to IgG subclass 1.

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