

Relationship between allergic status and specificity of IgG antibody to inhaled allergens: the grass pollen model

A. MICHILS*, I. VERVIER*, G. CHOUFANI†, B. GOSSART‡ and J. DUCHATEAU‡

*Chest Department, Cliniques Universitaires de Bruxelles, Erasme Hospital, †Otolaryngology Unit, Head and Neck Surgery Department, Cliniques Universitaires de Bruxelles, Erasme Hospital and ‡Immunology Departments, Université Libre de Bruxelles, St Pierre and Brugmann Hospitals, Brussels, Belgium

Summary

Background We have previously reported that IgG antibodies from healthy individuals and patients suffering from non-seasonal mite allergy bind to different sets of epitopes on Der p 1, allowing almost complete discrimination of the populations.

Objectives To confirm this observation in a seasonal allergy model where a clear relationship between allergic symptoms and exposure to the offending agent is established. To investigate whether the pattern of modified specificity is related to the differences in IgG subclass hierarchy usually exhibited by nonallergic and allergic populations.

Methods The capacity of individual sera from patients allergic to grass pollen and healthy individuals, including grass pollen-sensitized subjects, to prevent the binding of pooled IgG, IgG1, and IgG4 fractions from grass pollen-allergic patients and healthy individuals to solid-phase bound grass pollen antigen was evaluated in enzyme-linked immunosorbent assay (ELISA) using streptavidin-biotin technology. Specificity controls were performed using sera from patients allergic to cat dander and house dust mite.

Results The capacity of sera to prevent the antigen binding of allergic IgG averaged $84 \pm 5\%$ for allergic sera and $53 \pm 6\%$ for healthy sera ($P < 0.001$ by one-way ANOVA). Conversely, using the antigen-binding capacity of healthy control IgG as reference, percentage inhibitions averaged $46 \pm 9\%$ in grass pollen-allergic subjects compared with $80 \pm 4\%$, $82 \pm 2\%$ in healthy individuals, and mite- and cat-allergic patients, respectively, resulting in two well-separated populations ($P < 0.0001$ by one-way ANOVA). Similar results were found regardless of whether pooled IgG1 or IgG4 were used.

Conclusion Together with previous data, our results define a new type of humoral signature in the immune response to inhaled allergens. Allergic and healthy status differ not only in the presence or absence of specific IgE antibody but also in the preferential expression of distinct IgG specificities that are better correlated with clinical manifestations and are unrelated to subclass distribution.

Keywords: epitope specificity, grass pollen allergy, IgG antibody, IgG subclass

Clinical and Experimental Allergy, Vol. 29, pp. 832–839. Submitted 3 June 1998; revised 23 September 1998; accepted 26 October 1998.

Introduction

Immune responsiveness to inhaled allergens is not restricted to atopic subjects [1–4]. While elevation of serum IgE antibodies towards environmental allergens is a distinctive

feature of atopy, allergen-specific IgG antibodies are detectable in the serum of both atopic and nonatopic subjects [5–12]. However, in the mite-allergy model, we have recently shown that the dominant specificity of IgG antibody to Der p 1, one major house dust allergen, is different in mite allergic and healthy individuals, allowing almost complete qualitative discrimination between the two populations [13]. However, clear identification of the offending agent is sometimes

Correspondence: A. Michils, Chest Department, Erasme University Hospital, 808 Route de Lennik, 1070 Brussels, Belgium.

difficult in non-seasonal allergies such as mite allergy. Therefore, it was decided to confirm our data in a seasonal-allergy model in which the relationship between allergic symptoms and allergen exposure is clearly established. For that purpose, we compared the specificity of serum IgG antibodies raised to the grass pollen allergen from *Lolium perenne* in patients allergic to grasses and in healthy individuals, using a competitive enzyme-linked immunosorbent assay (ELISA). Mite- and cat-allergic patients were also included in some experiments to further confirm the allergenic specificity of the described disturbance. The final aim of this study was to determine whether differences in IgG antibody specificity were linked to the differences in IgG subclass distribution previously reported between healthy individuals and patients allergic to grass pollen [6,7,11,12].

Materials

Serum samples were collected from:

- 61 patients (mean age $\pm$ SD: 32 ± 7 years; 34 females and 27 males) allergic to grass pollen who had never received immunotherapy. All had a history of seasonal asthma or rhinitis and gave positive responses to grass pollen extract (Bencard, Rixensart, Belgium) in a skin prick test.
 - 62 healthy individuals (blood donors, mean age 38 ± 9 years; 38 females and 24 males) with no history of allergic disease.
- Two additional control groups were selected:
- 35 mite-allergic patients (mean age 41 ± 9 years; 14 females and 21 males) selected for our previous study [13] on the basis of a suggestive clinical history and a positive skin prick test recorded against *Dermatophagoides pteronyssinus* extract (DPT, Bencard).
 - 20 patients allergic to cat dander (mean age 26 ± 7 years; 16 females and 10 males) with a suggestive history and a positive skin prick test for cat extract (Bencard).
- All sera were stored in aliquots at -20°C until use.

Methods

Specific IgE antibodies towards *Lolium perenne*

The presence of IgE antibodies against *Lolium perenne* was screened for all sera using a conventional radioallergosorbent test system (RAST) with ^{125}I -labelled antihuman, IgE antibodies (Cap System; Pharmacia, Uppsala, Sweden).

Antigen

Lolium perenne extract was purchased from Allergon (Angelholm, Sweden). A 1% w/v suspension of grass pollen extract in trisphosphate-buffered saline (TBS;

pH 7.0) was incubated overnight at 4°C with constant shaking. The mixture was then centrifuged for 10 min at 2300 g. The supernatant underwent several filtration procedures (cut-off 10 kDa Millipore, Brussels, Belgium) to get rid of most contaminating traces of peptides possibly contained in the extract. Sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the extract revealed the presence of several protein fractions with molecular weights from 12 to 97 kDa.

IgG titres

Specific IgG retention was evaluated by a solid-phase enzyme-linked immunosorbent assay (ELISA): 96-well polystyrene microplates (Nunc-Immunoplates; Gibco BRL, Merelbeke, Belgium) were passively coated with the antigen extract ($10\text{ }\mu\text{g/mL}$ in phosphate-buffered saline (PBS), 10 mmol/L, pH 7.2) and additional non-specific binding sites were blocked with PBS containing 1% w/v gelatine (Hemacel; Hoechst, Belgium); after 30 min incubation with ethylenediamine-tetraacetic acid (EDTA; 10 mmol/L, pH 7.2), $100\text{ }\mu\text{L}$ of each serum, diluted 1:25 in PBS containing 0.05% v/v Tween 20 and 0.1% w/v gelatine, was added to separate wells containing the antigen, and incubated overnight at room temperature; after washing with the PBS-Tween buffer, $100\text{ }\mu\text{L}$ of a solution of peroxidase-conjugated rabbit antihuman IgG (Dako, Glostrup, Denmark; 0.1% v/v in PBS-Tween) was added and incubated for 2 h at 37°C ; after a further wash, the substrate solution (0.15% v/v H₂O₂, 0.3% w/v o-phenylenediamine in 0.2 mol/L citric acid, and 0.4 mol/L sodium phosphate) was added ($100\text{ }\mu\text{L/well}$); the enzyme reaction was stopped by the addition to each well of $100\text{ }\mu\text{L}$ of 2 mol/L hydrochloric acid. The binding activity of each serum's antibodies was determined by reading the absorbance (optical density [OD]) at 492 nm on a microtiter plate reader (Dynatech MR 5000 Dynatech Laboratories, Chantilly VA, USA). Antibody titres were expressed in arbitrary units according to a calibration curve made with serial dilutions of hyper-immune pooled sera. This serum pool was constituted with 12 sera from allergic patients, exhibiting high OD values in preliminary experiments. The procedure was repeated twice for each serum. Non-specific binding was evaluated in parallel by performing the same assay with microplates previously coated with blocking buffer only and no antigen. The standard curve showed OD values ranging from 0.2 to 1.8 with a non-specific background of 0.15.

Competitive inhibition tests

IgG antibody purification and biotin labelling: Purification. IgG fractions were separated from grass pollen-allergic and healthy control pooled sera ($n=12$) by affinity

chromatography on recombinant protein G sepharose [14], after depletion of IgG rheumatoid factor by adsorption on sheep red cells sensitized with rabbit haemolysins. The purified IgG fraction was then dialysed against carbonate buffer (50 mmol/L, pH 9.6) to eliminate the glycine buffer (10 mmol/L, pH 2.0) used for elution during the purification process. The concentration of the IgG antibody solutions was evaluated by spectrophotometry.

IgG1 and IgG4 antibodies were then separated from pooled IgG fractions by chromatography on sheep antihuman IgG1 or IgG4 antibody (Behring, Marburg, Germany) sepharose (CL4B column), followed by a further dialysis against a carbonate buffer (50 mmol/L, pH 9.6). Control experiments indicated that purified IgG1 and IgG4 fractions were not contaminated with the other subclass: IgG1 and IgG4 fractions, diluted 1:10–1:1280 in the described carbonate buffer, were adsorbed passively on 96-well polystyrene microplates and residual adsorption capacities were blocked with gelatine (Hemacel, 1% v/v). After washing in PBS-Tween, 100 µL of a solution of mouse antihuman IgG1 or IgG4 (Bioyeda, Israel) diluted 1:1000 in PBS-Tween was added to each well. Plates were incubated for 2 h at 37 °C and washed again. Afterwards, 100 µL of a solution of goat antimouse IgG, conjugated to peroxidase (Chemicon, Temecula, CA, USA) diluted 1:1000 in PBS-Tween, was added and the plates were incubated for a further 2 h at 37 °C. Detection of binding activity was then performed as described above.

Biotinylation of IgG fractions. The pooled IgG, IgG1, and IgG4 fractions were incubated for 4 h at room temperature with 250 µg biotin (NHS-LC-Biotin, Pierce, PolyLab, Antwerpen, Belgium) per mg IgG antibody in sodium bicarbonate buffer (50 mmol/L, pH 8.5). Unreacted biotin was then removed by dialysis against PBS (10 mmol/L, pH 7.2). Biotinylated pooled IgG fractions were stored at 4 °C until use.

Competition between biotinylated pooled IgG fractions and individual sera for binding to solid-phase bound antigen. Microplates (96-well polystyrene) were passively coated with the antigen extract (1 µg/mL in PBS, 10 mmol/L, pH 7.2) and additional non-specific binding was prevented by saturation with PBS containing 1% w/v gelatine; 100 µL of solutions containing individual sera (diluted 1:100) and biotinylated pooled IgG fraction, diluted 8 µg/mL and 10 µg/mL for allergic and healthy control fractions, respectively, in PBS-Tween containing EDTA (10 mmol/L final concentration) and gelatine (0.1% w/v), was transferred, after mixing, to coated plates and incubated overnight at room temperature; after the samples had been washed three times with PBS-Tween buffer, 100 µL of a solution of peroxidase-conjugated polystreptavidin (Sigma; 0.05% v/v in PBS-Tween) was

added and incubated for 3 h at 37 °C. After an additional washing step, the binding activity was determined as described above. The binding activity (OD) displayed by the biotinylated pooled IgG fraction alone, served as the reference value. The results are expressed as the percentage reduction of this (OD) value when individual sera are added to the biotinylated pooled IgG fractions. The percentage binding inhibition was calculated as follows:

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\text{OD}_S - \text{OD}_{NS}}{\text{OD}_R - \text{OD}_{NS}} \right)$$

where OD_S, OD_R, and OD_{NS} represent, respectively, the optical density in the presence of serum, in the absence of serum (reference value) and corresponding to non-specific binding. Non-specific binding was evaluated in parallel by performing the same assay with microplates previously coated with blocking buffer only and no antigen. The blank was evaluated using coated plates and peroxidase-conjugated polystreptavidin only. Results showed a similar trend using different conditions for the inhibition assays (antigen coating, biotinylated IgG antibody concentrations and serum dilutions) but the most appropriate conditions were selected for the final experiments. In these conditions, the OD of the reference healthy control and allergic IgG were 1.286 and 1.730 with OD of the background being 0.120 and 0.180, respectively.

Competition between biotinylated pooled-IgG1 and IgG4 fractions and pooled sera. Similar competition experiments were performed with biotinylated pooled IgG1 and IgG4 fractions from each group (diluted 1:40 in the dilution buffer described above), and serial dilutions (1:20–1:640) of pooled sera from grass pollen-allergic patients and healthy individuals. Results are expressed as binding inhibition curves.

Specificity controls

Antigen specificity The capacities of sera from mite-allergic patients, healthy controls, and grass pollen-allergic patients to prevent the binding of a biotinylated pooled IgG fraction from mite allergic sera to Der pt extract (Bencard, Rixensart, Belgium) were compared in a similar competitive ELISA.

Population specificity The capacity of sera from grass pollen-allergic patients to prevent the binding of a biotinylated-pooled IgG fraction from healthy controls to *Lolium perenne* extract was compared with that of sera from mite and cat allergic patients, and healthy controls in a similar test.

Statistics

The data were entered onto a PC and analysed with the aid

of the SPSS statistical package. Group comparisons were made by parametric tests where appropriate (one-way analysis of variance with Scheffe's correction for multiple comparisons) and intragroup paired data were compared with paired *t*-test.

The Spearman's correlation coefficients were calculated and tested for statistical significance to evaluate the relationships between clinical variables and serum titres or inhibition capacities.

Results

Specific IgE towards *Lolium perenne*

All grass pollen-allergic patients exhibited class 2 to class 4 positive RAST against *Lolium perenne* extract. Six healthy individuals also showed class 1 to class 3 positive RAST to *Lolium perenne* determinants low values relative to that of the allergic population ($P < 0.05$).

IgG binding to grass pollen extract

Figure 1 shows the frequency histograms of specific IgG antibody titres, after natural logarithmic transformation, for the grass pollen-allergic and healthy populations. The figure shows distinct, but overlapping distributions with that of the allergic group slightly to the right, reflecting the higher IgG levels exhibited by this group ($P < 0.01$). The influence of age and sex on the antibody titres was excluded ($P > 0.1$). Similarly, the level of IgE antibodies in the two populations, as measured by conventional RAST, had no influence on the IgG levels ($P > 0.1$).

Inhibition of the binding of biotinylated pooled IgG fractions

Figures 2(a) and (b) show the frequency histograms of

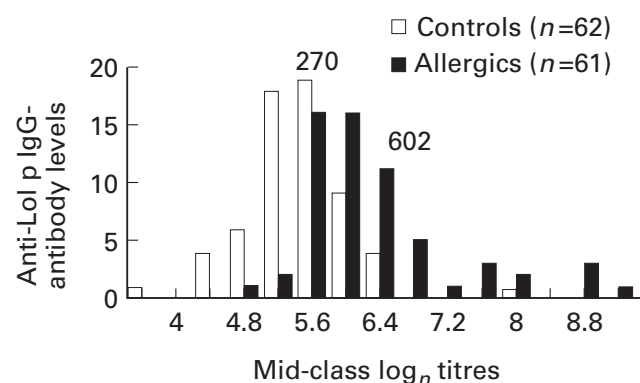


Fig. 1. Frequency histograms of anti-*Lolium perenne* IgG titres, after natural logarithmic transformation, for grass pollen-allergic and healthy control populations.

percentage binding inhibitions obtained with individual sera from the study groups.

Using the antigen-binding capacity of the biotinylated pooled IgG fraction from allergic sera as the reference (Fig. 2a), we observed two clearly separated distributions: grass pollen-allergic sera, with percentage inhibitions ($\pm$ SD) averaging $84 \pm 5\%$; and healthy control whether or not they exhibit specific IgE sera, with percentage inhibitions averaging $53 \pm 6\%$, respectively ($P < 0.0001$). In Fig. 2(b), using the antigen-binding capacity of the biotinylated pooled IgG fraction from healthy control sera as reference, percentage inhibition averaged $46 \pm 9\%$ in grass pollen-allergic subjects, compared with 80 ± 4 , 82 ± 4 , and $82 \pm 2\%$ in healthy individuals, and mite- and cat-allergic patients, respectively, resulting in two well-separated populations ($P < 0.0001$). The six healthy individuals with grass-pollen IgE antibodies display inhibition values similar to that of their own clinical group ($80 \pm 3\%$, $P > 0.1$). The distributions of serum inhibition capacities from healthy controls, mite- and cat-allergic patients were similar ($P > 0.1$).

Clinical variables such as age, sex, and the type of

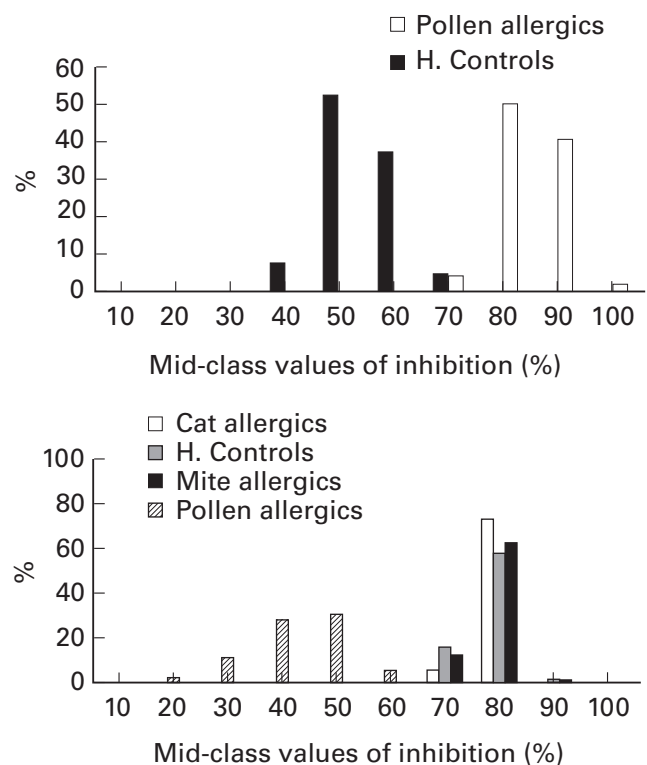


Fig. 2. Distribution of the percentage inhibitions of (a) allergic and (b) control, pooled IgG binding to *Lolium perenne* (pollen) extract by individual sera from (a) grass pollen-allergic and healthy control groups, and (b) grass pollen, cat, and mite allergic and healthy control groups.

reaction did not influence the capacity of sera to inhibit the antigen binding of pooled IgG fractions ($P > 0.1$). The levels of specific IgG as determined in the described ELISA and of specific IgE as measured by conventional RAST were not correlated with the inhibition capacities of the sera ($P > 0.1$).

Antigen specificity control

When using the Der pt binding capacity of the biotinylated pooled IgG fraction from mite-allergic patients as reference (Fig. 3), we observed a clearly separate distribution for mite allergic sera with percentage inhibitions averaging $81 \pm 7\%$. Sera from the other groups exhibited percentage inhibitions averaging $35 \pm 16\%$, significantly different from those of the mite-allergic group ($P < 0.0001$). No significant difference was observed in the behaviour of healthy control and grass pollen-allergic sera with percentage inhibitions averaging 33 ± 20 and $36 \pm 10\%$, respectively ($P > 0.1$).

Inhibition of the binding of biotinylated pooled IgG1 and IgG4 fractions by pooled sera

Figures 4(a) and (b) again show that the antigen binding of each pooled IgG fraction, whatever the subclass (IgG1 or IgG4), is best inhibited by the pooled sera from its own clinical group. Pooled sera from the other clinical group showed lower levels of inhibition and these levels decreased more sharply with increasing dilution.

Effect of immunotherapy: preliminary results

Figure 5 shows the development of inhibition capacity in six grass pollen-allergic sera after 1 year of successful, conventional immunotherapy with grass pollen extract (Stallergen, France). The capacity of sera to prevent the binding to grass pollen extract of the pooled allergic IgG changed with the

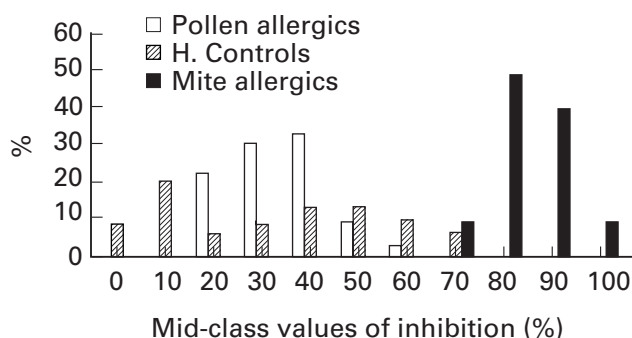


Fig. 3. Distribution of the percentage inhibitions of mite-allergic, pooled IgG binding to Der pt extract by individual sera from mite allergic, grass pollen allergic, and healthy control subjects.

mean percentage inhibition decreasing from 71 ± 7 to $49 \pm 5\%$ after 1 year of treatment ($P < 0.001$) while that from a group ($n = 10$) of healthy control sera averaged $46 \pm 6\%$ in this experiment.

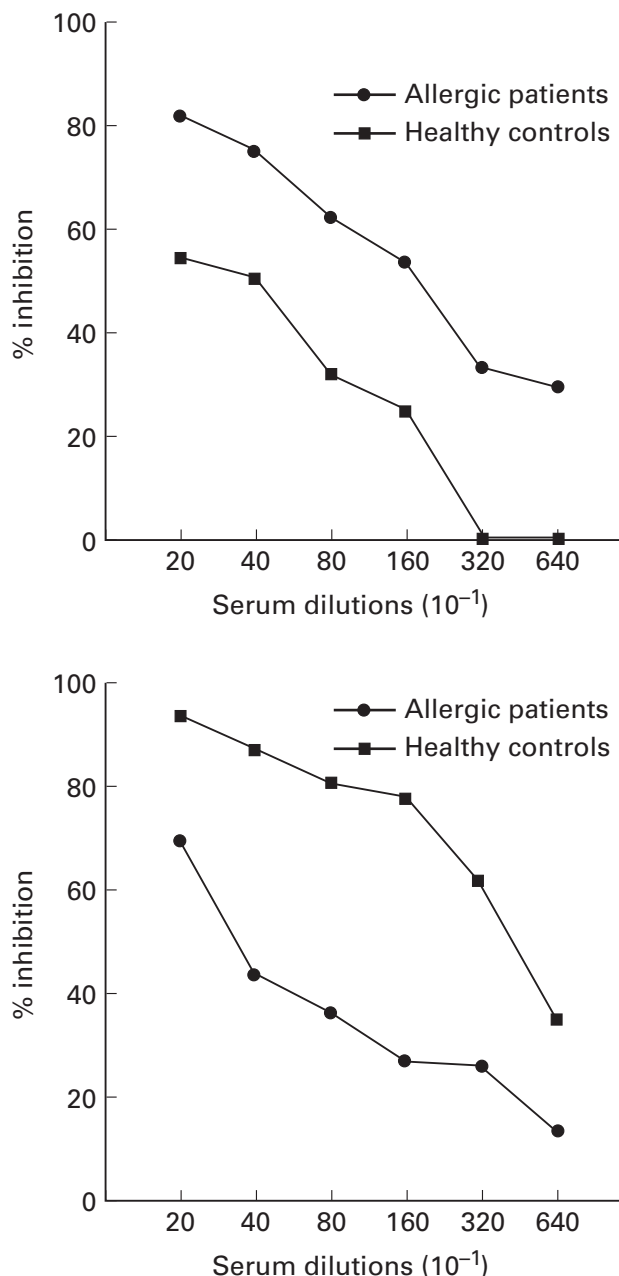


Fig. 4. Inhibition of the binding to *Lolium perenne* (pollen) extract of pooled (a) IgG1 from the grass pollen-allergic sample group and (b) IgG4 from the control group by pooled sera from allergic patients and healthy individuals.

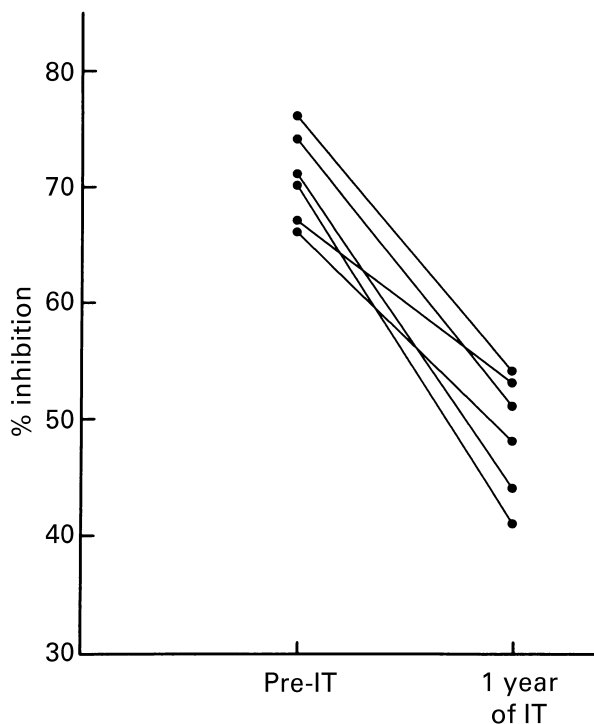


Fig. 5. Percentage inhibition of allergic, pooled IgG binding to *Lolium perenne* extract by individual sera from allergic patients ($n=6$) before and after one year of immunotherapy.

Discussion

While the presence of IgE antibodies towards inhaled allergens is a distinctive feature of atopic patients, specific IgG antibodies can be detected in the serum of both atopic and nonatopic subjects [5–12]. Although atopic patients usually exhibit higher IgG levels than healthy individuals, in this study the titre distributions showed a rather large overlap precluding the delineating of a discriminate cut-off value. Nevertheless, the IgG subclass distribution is quite different in the two populations with IgG1 predominating in normal sera, and IgG4 in allergic sera [6,7,11,12]. Furthermore, the present data show that the IgG response from allergic patients towards grass pollen of *Lolium perenne* differs from that of healthy individuals in demonstrating a distinct pattern of specificity.

The difference in IgG specificity was demonstrated by direct competition for binding to the antigen between pooled IgG, representing a library of the IgG epitopes recognized by each reference group, and individual sera, allowing recognition of a dominant specificity. In this test, the binding to *Lolium perenne* extract of the biotinylated IgG fraction from grass pollen-allergic patients was more strongly inhibited by allergic sera than by healthy control sera. At first glance, this is not surprising as sera from

allergic patients display greater levels of specific IgG antibody. However, in a similar way, binding by the biotinylated pooled IgG fraction from healthy individuals was prevented to a greater extent by healthy control sera than by allergic sera in spite of the higher antibody titres in the allergic group. It is a paradoxical situation if the antibodies are competing directly for binding to the same antigens. A difference in antibody avidity [15] is also unlikely to explain the differences observed in the inhibition capacities of the various sera. Indeed, allergic sera, containing antibody with a higher binding affinity [15], would exert a stronger inhibition than control sera in all situations including in competition experiments performed with biotinylated pooled IgG fraction from healthy individuals, which was not the case. Therefore, if not related to antibody titre or affinity, the differences in inhibition capacities must reflect antibody populations with different specificities, in particular IgG antibodies since the competition experiments were performed with IgG fractions. The contribution of other antibody isotypes to the inhibition capacity of sera is a possibility which remains to be investigated. Nevertheless, the homogeneity of our results does already suggest a shared specificity between the different antibody isotypes of each clinical group. Involvement of anti-IgE IgG antibodies [16–19] in the competition is unlikely since the test conditions (low antigen-coating and serum concentrations) prevented significant IgE binding. Differences in antibody specificity have already been suggested by Batard and colleagues [11] who showed that human plasma selected on the basis of high vs low level of Dactylis pollen-specific IgG4, exhibited different binding patterns as demonstrated by isoelectric focusing (IEF) immunosorbent analysis. In our study, however, not only does the methodology differ notably (direct competition between antibodies) but also the clinical basis on which the patients were selected i.e. the presence/absence of allergic symptoms (rhinitis and/or asthma) exclusively related to grass pollen exposure. Indeed, it seems that allergy-related symptoms correlate best with the modified profile of specificity since IgG antibodies from asymptomatic, grass-sensitized subjects, who exhibit IgE antibodies towards grass pollen, retain the antigenic specificity of their clinically defined group (i.e. healthy individuals).

The modified pattern of specificity appears to be antigen specific since grass pollen-allergic sera prevent the binding of pooled IgG fraction from mite-allergic patients to Der pt extract in exactly the same way as healthy control sera. Conversely, sera from mite- and cat-allergic patients did not differ from healthy control sera in their capacity to inhibit the binding of biotinylated pooled IgG fractions from healthy subjects to grass pollen extract. This change in antibody specificity can not therefore be considered to be a general characteristic of sera from allergic patients but seems instead to be exclusively related to the particular

antigen responsible for development of the symptomatic, allergic response. The current data confirm those reported for mite-allergic patients despite the use of a crude antigen extract contrasting with purified Der p 1 used in the previous work [13]. This suggests a rather striking disturbance as the large number of allergens/antigens and thus epitopes could have masked the differences.

As mentioned above, the IgG subclass distribution is also quite different in allergic and nonallergic populations: IgG detected in healthy sera are mainly restricted to the IgG1 subclass while IgG4 subclass antibodies predominate in allergic sera [6,7,11,12]. It is therefore tempting to speculate that there is a direct correlation between IgG in subclass hierarchy and antibody specificity. However, our results do not support this hypothesis since both IgG1 and IgG4 subclass antibodies express to a similar degree the distinct specificity of their clinically defined group.

It has been shown that various T-cell populations may determine the antibody isotype generated [20] i.e. although the TH1/TH2 paradigm has not yet been fully demonstrated in humans [21,22] it has been proposed that TH2 CD4⁺ T-cell subsets, dominating in atopic subjects, drive B-cell production of antigen-specific IgE and IgG antibodies while CD4 T cells from nonatopic subjects, exhibiting the TH1 phenotype, direct B cells to produce IgG but not IgE antibodies. It is presently unknown whether this dichotomy in T-cell regulation is linked to the different antigenic specificities of IgG antibodies described here.

Finally, although only a few cases have been studied, preliminary results obtained with sera from patients successfully treated by specific immunotherapy show that IgG specificity returns to that expressed by healthy individuals when patients become asymptomatic. This again, suggests a relationship between actual clinical status and IgG antibody specificity confirming previous data documented in hymenoptera venom [23,24] and cow's milk [25] allergy models. A change in IgE specificity has also been documented during grass pollen immunotherapy [22]. Furthermore, these early results emphasize that, even when established, antigenic immunodominance of IgG antibody is not a permanent state and can be reversed by intervention on the immune system, for example, through the injection large amounts of antigens [23–25].

In conclusion, this study confirms, in a model where the relationship between symptoms and allergen exposure is well defined, that allergic and clinically unreactive states towards inhaled allergens are associated with different patterns of IgG antibody specificity which are unrelated to subclass expression. Further studies must now be performed to identify the responsible mechanism and the possible relationship between the specificity and functional activity of IgG antibodies in human allergic diseases as has already been shown in several other disease models [26–30].

Acknowledgements

The authors thank A. Van Muylem for help during the study, and P. Ioannidis and C. Piesen for typing the manuscript.

A. Michils is supported by an Avis Grant from the Fondation Erasme.

References

- 1 Halvorsen R, Bosnes V, Thorsby E. T cell responses to a Dermatophagoides farinae allergen preparation in allergics and healthy controls. *Int Arch Allergy Appl Immunol* 1986; 80:62–9.
- 2 Cavaillon JM, Fitting C, Guinnepain MT, Rassemont R, David B. Lymphocyte proliferative response to the purified Dermatophagoides farinae major allergen in untreated and hyposensitized atopic patients. *Allergy* 1988; 43:146–51.
- 3 Kimura JY, Ohta N, Ishi A, Nagano T, Usui M. Functional characterization of lymphocyte response to fractionated house dust mite antigens (Dermatophagoides pteronyssinus) in atopic and non-atopic individuals. *Immunology* 1990; 70:385–90.
- 4 Upham JW, Holt BJ, Baron-Hay MJ et al. Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995; 25:634–42.
- 5 Delespesse G, Debisschop MI, Flament J. Measurement of IgG antibodies to house dust mite and grass pollen by a solid phase radioimmunoassay. *Clin Allergy* 1979; 9:503–14.
- 6 Hammerstrom L, Smith CIE. Immunoglobulin subclass distribution of specific antibodies in allergic patients. *Allergy* 1987; 42:529–34.
- 7 Kemeny DM, Urbanek R, Ewan P et al. 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. *Clin Exp Allergy* 1989; 19:545–9.
- 8 Mariani F, Price JF, Kemeny DM. The IgG subclass antibody responses to an inhalant antigen (Dermatophagoides pteronyssinus) during the first year of life: evidence for early stimulation of the immune system following natural exposure. *Clin Exp Allergy* 1992; 22:29–33.
- 9 Sallusto F, Quintieri F, Pugliese O, Reale G, Pini C, DiFelice G. T-cell and antibody response to Parietaria judaica allergenic fractions in atopic and nonatopic individuals. *Allergy* 1993; 48:37–44.
- 10 Saint Rémy JMR, Lebrun PM, Lebecque SJ, Masson PL. Human immune response to allergens of house dust mite, Dermatophagoides pteronyssinus. *Allergy* 1988; 43:338–47.
- 11 Batard T, Basuyaux B, Lambin P et al. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma. I. Specialization of certain classes and sub-classes in the immune response. *Int Arch Allergy Immunol* 1993; 100:68–78.
- 12 Batard T, Basuyaux B, Laroze A et al. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma. II. Quantification of the IgE, IgM, IgA class and the IgG sub-class antibodies. *Int Arch Allergy Immunol* 1993; 102:279–87.
- 13 Duchateau J, Michils A, Michel O, Baras L. Mite allergy is associated with a specific profile of IgG epitopes recognized on

- antigen p1 of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1997; 27:296–305.
- 14 Bjorck L, Krowall L. Purification and source properties of streptococcal protein G. A novel IgG binding reagent. *J Immunol* 1984; 133:969–74.
- 15 de Boluda L, la Cuadra B, Benens L. Binding affinities of allergens from pollen, mites and house dust for specific IgG sub-class antibodies. *Allergy* 1996; 51:706–11.
- 16 Williams RC, Griffiths RW, Emmons JD, Field JD. Naturally occurring human antibodies with specificity for gamma-E. *J Clin Invest* 1972; 51:955–1003.
- 17 Stevens WJ, Bridts CH. IgG-containing and IgE-containing circulating immune complexes in patients with asthma and rhinitis. *J Allergy Clin Immunol* 1984; 73:276–84.
- 18 Inganas M, Johansson SGO, Bennich H. Anti-IgE antibodies in human serum: occurrence and specificity. *Int Arch Allergy Appl Immunol* 1981; 51:955–63.
- 19 Lichtenstein LM, Kagey-Sobotka A, White JM, Hamilton RG. Anti-human IgG causes basophil histamine release by acting on IgG-IgE complexes bound to IgE receptors. *J Immunol* 1992; 148:3929–36.
- 20 Romagnani S. Induction of Th1 and Th2 response: a key role for natural immune response. *Immunol Today* 1992; 13:379–81.
- 21 Allen JE, Maizels RM. Th1–Th2: reliable paradigm or dangerous dogma? *Immunol Today* 1997; 18:387–92.
- 22 Umetsu DT, De Kruffy R. Th1 and Th2, CD4+ cells in human allergic diseases. *J Allergy Clin Immunol* 1997; 100:1–6.
- 23 Michils A, Ledent C, Mairesse M, Gossart B, Duchateau J. Wasp venom immunotherapy changes IgG antibody specificity. *Clin Exp Allergy* 1997; 27:1036–42.
- 24 Michils A, Mairesse M, Ledent C, Baldassarre S, Gossart B, Duchateau J. Modified antigenic reactivity of anti-PLA2 IgG antibodies in patients allergic to bee venom: conversion with immunotherapy and relation to sub-class expression. *J Allergy Clin Immunol* 1998; 102:118–26.
- 25 Duchateau J, Michils A, Lambert JP, Gossart B, Casimir G. Anti-betalactoglobulin IgG antibodies bind to unusual epitopes when children are allergic to cow's milk. *Clin Exp Allergy* 1998; 28:824–33.
- 26 Van Ree R, Van Lecuwen WA, Dieges PH et al. Measurement of IgE and IgG antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. *Clin Exp Allergy* 1997; 27:68–74.
- 27 Kolbe L, Heusser CH, Kolsch E. Isotype associated recognition of allergen epitopes and its modulation by antigen dose. *Immunology* 1995; 84:285–9.
- 28 Ranpach B, Schmidt MA. Elucidation of linear epitopes of pertussis toxin using overlapping decapeptides: identification of a human B cell determinant in the S1 subunit indicative of acute infections. *Microb Pathog* 1994; 17:213–26.
- 29 Lundkuist A, Bjorsten S, Niklasson B, Ahlborg N. Mapping of B cell determinants in the nucleopeptide protein of Puumala virus: definition of epitopes specific for acute immunoglobulin B recognition in humans. *Clin Diag Lab Immunol* 1995; 2:82–6.
- 30 Gilleland LB, Gilleland HE Jr. Synthetic peptides representing two protective linear B cell epitopes of outer membrane protein F of *Pseudomonas aeruginosa* elicit whole cell reactive antibodies that are functionally pseudomonal specific. *Infect Immun* 1995; 63:2347–51.