

# Mite allergy is associated with a specific profile of IgG epitopes recognized on antigen p1 of *Dermatophagoides pteronyssinus*

J. DUCHATEAU\*, A. MICHILS†, O. MICHEL‡ and L. BARAS§

\*Immunology Department, Centres Hospitalo Universitaires Brugmann et St Pierre, †Chest Department, CUB Hopital Erasme, ‡Chest Department, CHU St Pierre, and §Dermatology Department, CUB Hopital Erasme, Brussels, Belgium

## Summary

**Background** Immune response to inhaled antigens differs in allergic patients and healthy individuals, mostly in the quality of T cell help provided (i.e. Th2 or Th1 dominant subset). However, while different in many functional aspects, both groups of T cells shared the capacity to support the synthesis of antigen-specific immunoglobulin G (IgG) antibodies detected in different amounts in the serum of atopic and healthy individuals.

**Objective** The present study investigates whether these IgG responses display similar or different epitopic dominance in the mite sensitization model.

**Methods** Antibody specificity was evaluated by comparing the IgG binding to native Der p1 (nDer p1) and its products of pepsin hydrolysis (dDer p1) in 56 mite-allergic patients and 148 healthy individuals, including 24 mite-sensitized individuals in a solid enzyme linked immunosorbent assay (ELISA). Antibody specificity was also studied in competitive ELISA using streptavidin biotin technology.

**Results** Mite-allergic sera showed a higher degree of binding to nDer p1 than to dDer p1, whereas control sera and mite-sensitized sera bound at a similar level to the two forms of the antigen. Allergic sera and control sera, including mite-sensitized sera, showed distinct capacities to prevent the binding to nDer p1 of pooled IgG from each group as well as murine monoclonal antibodies specific to Der p1.

**Conclusion** The IgG response to Der p1 of mite-allergic patients differs from that of healthy controls and mite-sensitized subjects, not only in its increased titres but also in its consistent pattern of modified specificity, displaying a marked preference for conformational epitopes. Cross-competition experiments confirm the clinically associated, restricted specificity, allowing almost complete discrimination between groups, particularly between mite-sensitized and mite-allergic subjects, which is currently impossible with routinely available assays.

**Keywords:** Der p1, epitopes, IgG antibody, mite allergy

*Clinical and Experimental Allergy*, Vol. 27, pp. 296–305. Submitted 21 June 1996; revised 20 August 1996; accepted 27 August 1996.

## Introduction

House dust mite (HDM) antigens are important indoor aeroallergens causing diseases such as rhinitis, asthma and

dermatitis in as much as 10 % of the population, i.e. mite-allergic patients [1].

However, immune responsiveness to such environmental allergens is not a unique feature of allergic subjects whose immune response is different from that of non-atopic individuals, principally in the nature of the dominant memory T cell subsets. Briefly, Th2 CD4<sup>+</sup> T cell subsets

Correspondence: Jean Duchateau, MD, PhD, Immunology Department, CHU Brugmann, Place Van Gehuchten 4, 1020 Brussels, Belgium.

**Table 1.** Study population characteristics

|                   | Healthy controls | Mite allergic patients |            |
|-------------------|------------------|------------------------|------------|
|                   |                  | Asthma                 | Rhinitis   |
| <i>n</i>          | 148              | 35                     | 21         |
| Age $\pm$ SD (yr) | 41 $\pm$ 9       | 33 $\pm$ 8             | 36 $\pm$ 8 |
| Sex (M/F)         | 88/66            | 21/14                  | 11/10      |
| RAST Der pt +/-   | 24/124           | 35/0                   | 21/0       |

dominate in atopic subjects and drive B cell production of antigen specific immunoglobulin E (IgE) as well as immunoglobulin G (IgG) antibodies. In contrast, CD4<sup>+</sup> T cells from non-atopic individuals exhibit the Th1 functional phenotype, supporting B cells to produce antigen specific IgG but not IgE antibodies [2–5].

The present study investigated whether these humoral responses, distinguishable in allergic patients by the production of specific IgE antibodies, also display differences in the epitopic dominance of the antibodies.

Therefore, we compared the epitope specificity of IgG antibodies directed towards antigen p1 of *Dermatophagoides pteronyssinus*, one of the major house dust mite antigens, in mite-allergic patients and healthy individuals. IgG rather than IgE response was investigated because, as mentioned above, the former can be detected in the serum of almost all subjects [6].

## Material

Serum samples were collected from:

**1** Fifty-six patients suffering from asthma ( $n = 35$ ) or rhinitis ( $n = 21$ ) related to house dust mite allergy. All patients had an isolated positive skin prick test for *Dermatophagoides pteronyssinus* (Der pt) extract and a clinical history highly suggestive of allergy to house dust mites. No treatment had been administered at the time of the serum's collection.

**2** One hundred and forty-eight healthy individuals (blood donors) with no complaint of allergic disease.

The population characteristics are summarized in Table 1.

## Method

### Specific IgE antibodies towards Der pt

The presence of IgE antibodies against Der pt was screened for all sera using a conventional radioallergosorbent test system (RAST) with I<sup>125</sup> labelled anti-human IgE antibodies (Pharmacia-Cap System).

### IgG binding to native and denatured Der p1

#### Allergen preparations

**Native protein:** Der p1 antigen was kindly supplied by Bencard<sup>®</sup> (Rixensart, Belgium) as a lyophilised powder, displaying one major band (24 K daltons (Kd)) in sodium dodecyl-sulphate polyacrylamide gel electrophoresis. Filtration procedures (cut-off 10 Kd, Millipore, Brussels, Belgium) were repeated in order to get rid of most contaminating traces of peptides possibly contained in the antigen extract.

**Digested protein:** 5 mg of native protein (1 mg/ml in TRIS-HCL: 10 mM, pH 2.0) were added to insoluble pepsin, bound to 300 mg of 4% cross-linked beaded agarose (activity: 170 units/mg dry agarose; Sigma<sup>®</sup>, Dorset, England) which had been washed twice with distilled water and then twice with the above described TRIS-HCL buffer. The digestion was carried out at 40°C for 3 h. The reaction was then stopped by centrifugation (5000 rpm for 10 min) which separated the solid phase insoluble enzyme from the protein solution. The pH of the supernatant was raised to 7.0 by the addition of a neutralizing buffer (TRIS-HCL: 100 mM, pH 9.6). A filtration procedure (cut-off 10 Kd, Millipore<sup>®</sup>) was then performed several times to remove most contaminating traces of the native protein.

The concentration of the collected solution was evaluated by spectrophotometry and adjusted in line with the concentration of the native protein solution used in parallel for the coating procedure.

#### Specific IgG antibodies towards the native protein (nDer p1) and its products of digestion (dDer p1)

Specific IgG retentions of nDer p1 and dDer p1 were evaluated in parallel by a solid phase enzyme-linked immunosorbent assay (ELISA): 96-well polystyrene microplates (Nunc-Immunoplates, Gibco, Merelbeke, Belgium) were passively coated with nDer p1 and dDer p1 (5 µg/ml in phosphate buffered saline (PBS: 10 mM, pH 7.2) containing 0.05% v/v Tween 20 (PBS-Tween)) and additional non-specific binding was prevented by saturation with gelatin (Hemacel<sup>®</sup>, 3% v/v; Hoechst, Belgium); after 30 min incubation with ethylene diamine tetraacetic acid (EDTA: 10 mM, pH 7.2), 100 µl of each serum, diluted 1:25 (similar results were obtained with different serum dilutions from 1:12.5 to 1:100) in the above described PBS-Tween buffer were added to separate wells containing the different antigens and incubated overnight at room temperature; after washing with the PBS-Tween buffer, 100 µl of a solution of peroxidase conjugated protein A (Sigma; 0.1% v/v in PBS) was added and incubated for 2 h at 40°C; after a further washing the substrate solution (0.15 % v/v H<sub>2</sub>O<sub>2</sub>, 0.3% w/v o-phenylenediamine in 0.2 M citric acid and 0.4 M sodium phosphate) was then added (100 µl/well); the enzyme reaction

was stopped by the addition in each well of 100 µl of 2 M hydrochloric acid. The binding activity of each serum's antibodies was determined by reading the absorbance at 492 nm on a microtitre plate reader (Dynatech MR 5000, Dynatech Laboratories, Chantilly, VA). Antibody titres were expressed in arbitrary units according to a calibration curve made with serial dilutions of hyperimmune pooled sera.

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 gelatin (Hemacel<sup>®</sup>, 3% v/v) only and no antigen. It must be emphasized that all assay conditions were identical, allowing comparison of the results.

#### Specificity controls

As a specificity control, we also compared, for our two groups of subjects, the specific IgG retention of native and similarly denaturated forms of the grass pollen antigen, *Antoxantum odoratum* (Allergon<sup>®</sup>-Engelholm-Sweden) [6]. These experiments were performed with sera from 32 patients with mite allergy and 32 healthy control subjects, as described for Der p1.

#### Competitive inhibition tests

##### IgG antibody purification and biotin labelling

**Purification:** IgG fractions were separated from either healthy control or mite allergic pooled sera ( $n = 12$ ) by affinity chromatography on protein G sepharose [6], after depletion of IgG rheumatoid factor by adsorption on sheep red cells sensitized with rabbit hemolysins. The purified IgG fraction was then dialysed against carbonate buffer (50 mM, pH 9.5) to eliminate the glycine buffer (10 mM, pH 2.0) used for elution during the purification process. The concentration of the IgG antibody solutions was evaluated by spectrophotometry.

**Biotinylation of IgG fractions and mouse monoclonal antibodies (mAb) specific for Der p1:** Six mouse mAb specific for Der p1 (IgG class) were kindly provided by Professor G. Delespesse from Hôpital Notre Dame, Montreal, Canada. They were designated as mAb 1 to 6.

The pooled IgG fractions and mAb were incubated for 4 h at room temperature with biotin (125 µg/mg of IgG antibody, in the described carbonate buffer). Excess biotin was then eliminated by further dialysis procedures against PBS (10 mM, pH 7.2).

##### Competition between biotinylated pooled IgG fractions and individual sera for binding to solid phase bound nDer p1

Ninety-six-well polystyrene microplates were passively coated with nDer p1 (0.3 µg/ml in PBS-Tween buffer, pH 7.2) and additional non-specific binding was prevented

by saturation with gelatin (Hemacel<sup>®</sup>, 3% v/v); 100 µl of solutions containing individual sera and biotinylated pooled IgG fraction, diluted, respectively, 1:100 and 1:200 in PBS-Tween, containing EDTA (10 mM) and gelatin (Hemacel<sup>®</sup>, 0.1% v/v), were transferred, after vigorous 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 (0.1% v/v in PBS-Tween) was added and incubated for 3 h at 40°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 of binding inhibition was calculated as follows:

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

where OD<sub>S</sub>, OD<sub>R</sub> and OD<sub>NS</sub> 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 gelatin (Hemacel<sup>®</sup>, 3% v/v) only and no antigen. The blank was evaluated using coated plates and peroxidase conjugated polystreptavidin. Appropriate conditions for the inhibition assays (antigen coating, biotinylated IgG antibody and serum dilutions) were determined in preliminary experiments.

**Specificity controls:** As a specificity control, the capacities of sera from grass pollen-allergic patients ( $n = 16$ ), healthy controls and mite allergic patients to prevent the binding of a biotinylated pooled IgG fraction, purified from healthy control sera ( $n = 12$ ), to *Lolium perenne* extract, were compared, in the same competitive ELISA.

##### Competition between biotinylated monoclonal antibodies and individual sera for binding to nDer p1

Competition for binding to nDer p1, between biotinylated mAb and individual sera, was performed as described above under pre-determined optimal conditions: wells were passively coated with nDer p1 (5 µg/ml in PBS-Tween buffer, pH 7.2) and monoclonal antibodies and human sera were diluted, respectively, 1:400 and 1:100 in the PBS-Tween buffer, containing EDTA (10 mM), gelatin (Hemacel<sup>®</sup>, 0.1% v/v) and mouse serum (1:100, final concentration), which was added to reduce interactions between human anti-mouse antibodies and murine mAb. The binding activity displayed by the mAb alone served as a reference value. Results are expressed as the percentage reduction

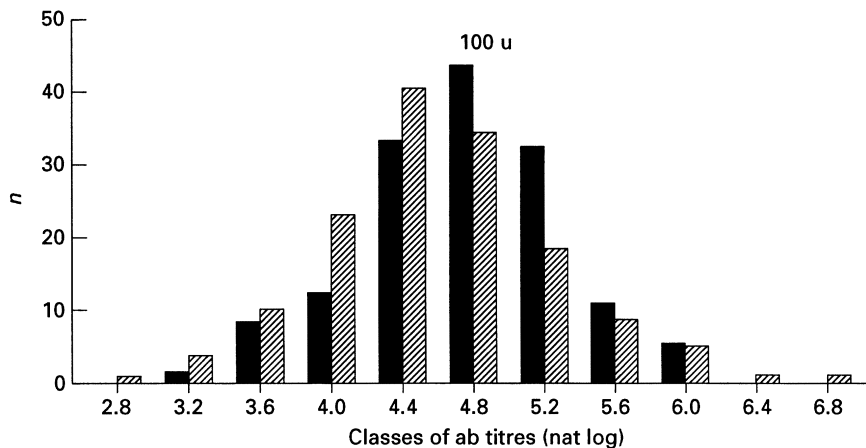


**Fig. 1.** Frequency histograms of anti-nDer p1 ■ and anti-dDer p1 ▨ IgG antibody titres, after natural logarithmic transformation, for the healthy control subjects.

of this value when individual human sera are added to biotinylated mAb and are calculated as described above.

## Results

### *Specific IgE towards Der pt*

All mite-allergic patients exhibited class 2 to class 4 positive RAST against DPT extract. Twenty-four healthy individuals also showed class 1 to class 2 positive RAST to DPT determinants.

### *IgG binding to dDer p1 and nDer p1*

Figures 1 and 2 show the frequency histograms of IgG antibody titres, after natural logarithmic transformation, for the two populations with regard to nDer p1 and dDer p1. Median titre values expressed in arbitrary units have been included. Figure 1 shows similar distributions, with no

significant difference between the two antigens ( $P > 0.1$ , one way ANOVA), in the healthy control population. In Figure 2, which presents the same titre class distribution for the mite-allergic population, the histograms are clearly separated with only a marginal overlap ( $P < 0.001$  by one way ANOVA). Indeed, the two most common classes in each distribution are separated from each other by 1.6 natural log units, indicating that the respective antibody titre values dDer p1:nDer p1 are in a ratio of 1:4.9.

These figures also document higher antibody levels towards the native protein in mite-allergic patients compared with healthy individuals (mean ln antibody titres  $5.13 \pm 0.65$  versus  $4.75 \pm 0.56$ ,  $P < 0.001$  by one way ANOVA).

Considering now the distribution of ratios (Figure 3) between the ODs measured for dDer p1 and nDer p1, respectively, for each serum (thus combining two objective measurements of OD and eliminating any potential bias introduced by referring to a calibration curve),

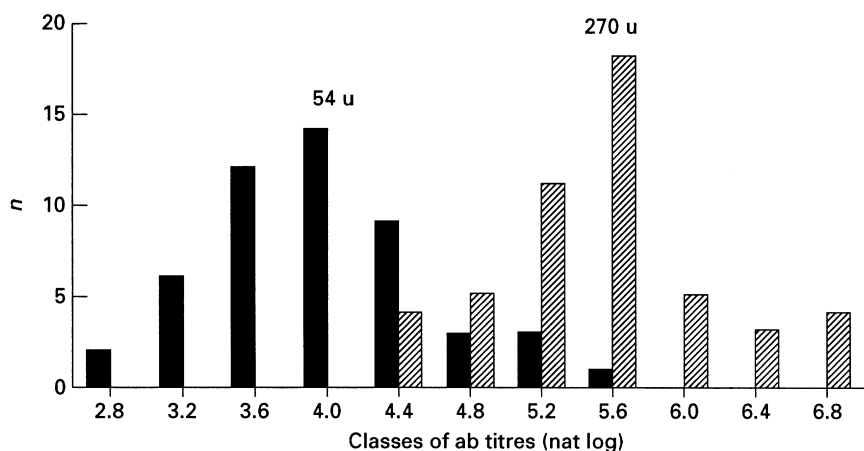
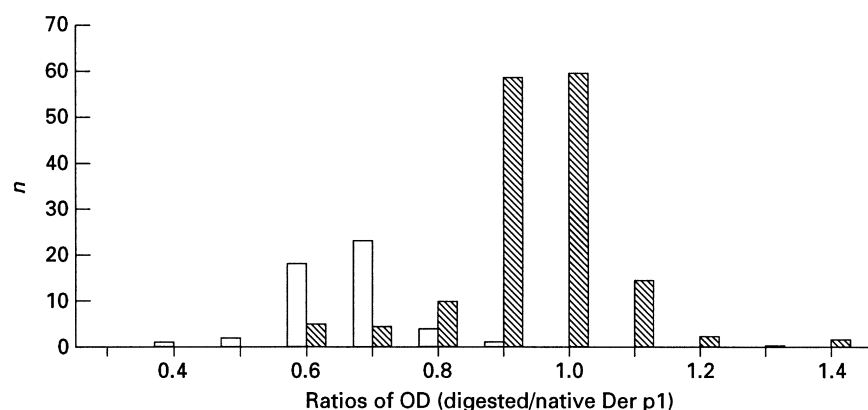


**Fig. 2.** Frequency histograms of anti-nDer p1 ▨ and anti-dDer p1 ■ IgG antibody titres, after natural logarithmic transformation, for the mite-allergic patients.

**Fig. 3.** Frequency histograms of the ratios between optical density measured for digested and native Der p1 for each population. □ = allergics ( $n = 50$ ); ▨ = controls ( $n = 148$ ).



the sera can be separated into two categories: sera from mite-allergic patients with a mean ratio ( $\pm$  SD) of  $0.66 \pm 0.12$  and sera from healthy control subjects with a mean ratio ( $\pm$  SD) of  $0.95 \pm 0.09$  ( $P < 0.0001$ , one way ANOVA).

The influence of age, sex and smoking habits on the defined ratio (OD dDer p1: OD nDer p1) was excluded ( $P > 0.1$  by Student's *t*-test). The presence of anti-Der p1 IgE antibodies was detected in the serum of 24 healthy individuals. These subjects were not different from those displaying no specific IgE and their mean ratios were similar:  $0.96 \pm 0.13$  versus  $0.95 \pm 0.09$  ( $P = 0.98$  by Student's *t*-test).

The distributions of ratios between ODs measured on the denaturated and native forms of grass pollen extract are similar for each population, with a mean ratio of about 0.9 ( $P > 0.1$ , one way ANOVA, data not shown).

#### Binding inhibition of biotinylated pooled IgG fractions

Figures 4 and 5 show the frequency histograms of percentage binding inhibition obtained with individual sera from each group.

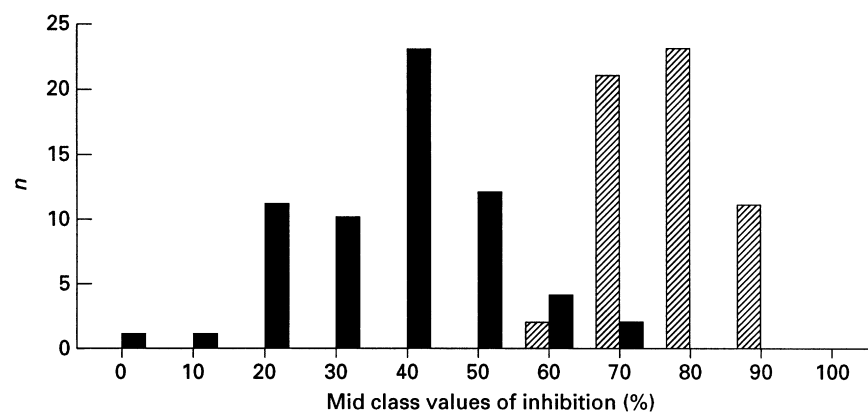
Using the nDer p1 binding capacity of the biotinylated

pooled IgG fraction from allergic sera as the reference (Figure 4), we observed clearly separate distributions: allergic sera with percentage inhibitions averaging  $77 \pm 8$  and healthy control sera with percentage inhibitions averaging  $38 \pm 13$  ( $P < 0.0001$ , one way ANOVA). In Figure 5, using the nDer p1 binding capacity of the biotinylated pooled IgG fraction from healthy control sera as reference, the percentage inhibition averaged  $81 \pm 6$  in healthy controls subjects and  $44 \pm 12$  among allergic patients, with well separated distributions ( $P < 0.0001$ , one way ANOVA).

When using the grass pollen binding capacity of the biotinylated pooled IgG fraction from healthy controls as reference (Figure 6), the binding capacity of sera from healthy controls and mite-allergic patients averaged  $61 \pm 9\%$  and  $63 \pm 6\%$  respectively, whereas that of sera from grass pollen-allergic patients averaged  $18 \pm 13\%$  ( $P < 0.0001$  by one way analysis of variance, reaching the significant level of  $P < 0.05$  after Scheffe's correction for multiple comparison).

Clinical variables such as age, sex and smoking habits did not influence the capacity of sera to inhibit the binding of pooled IgG fractions to nDer p1 ( $P > 0.1$  by Student's

**Fig. 4.** Distribution of the percentage inhibitions of mite allergic pooled IgG binding to nDer p1 by individual sera. ▨ = allergics ( $n = 56$ ); ■ = controls ( $n = 64$ ).



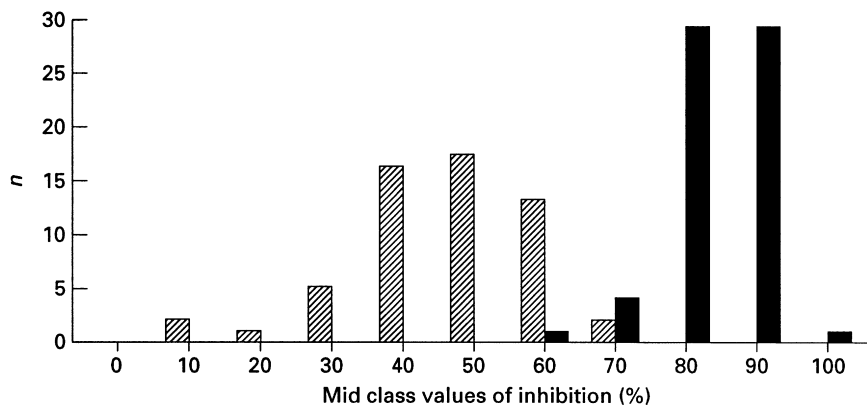


**Fig. 5.** Distribution of the percentage inhibitions of healthy control pooled IgG binding to nDer p1 by individual sera. ▨ = allergics ( $n = 56$ ); ■ = controls ( $n = 64$ ).

*t*-test). The presence of anti Der pt IgE antibodies had again no influence on the inhibition capacities of control sera ( $P > 0.1$  by Student's *t*-test).

#### *Binding inhibition of biotinylated mouse monoclonal antibodies specific for Der p1*

The results obtained with just two of the six mAb, designated 5 and 6, are presented here to illustrate two different situations. Firstly, when the nDer p1 binding capacity of biotinylated mAb 5 is used as reference (Figure 7), the percentage inhibition values showed distinct distributions, with a mean value of  $40 \pm 12\%$  for allergic sera and  $28 \pm 10\%$  for control sera ( $P < 0.001$  by one way ANOVA).

Using the nDer p1 binding capacity of biotinylated mAb 6 as a reference (Figure 8), percentage inhibitions again showed clearly separated distributions with an average value of  $34 \pm 13\%$  for mite-allergic sera and  $76 \pm 5\%$  for healthy control sera ( $P < 0.0001$  by one way ANOVA).

The inhibition capacity was not influenced by age, sex and smoking habits ( $P > 0.1$  by Student's *t*-test), nor by the presence of anti Der pt IgE antibodies in the case of control sera ( $P > 0.1$  by Student's *t*-test).

#### **Discussion**

These results document a significant increase of specific IgG titres in mite-allergic patients compared with healthy individuals even though the titres distributions show a rather large overlap precluding to delineate a discriminant cut-off value. However, the most striking feature is the recognition of different epitopes of the protein by the two groups of sera. Der p1 is a 222 amino acid globular glycoprotein bearing several epitopes, as determined by murine monoclonal antibodies and human polyclonal IgE and IgG antibodies and by studies using synthetic or recombinant peptides [8–11]. It is generally accepted that most B cell epitopes on such antigens are discontinuous (conformationally dependent) [12–14] even though antibody binding to short linear peptides, located on exposed surfaces of Der p1 (continuous epitopes), has also been detected [11,15,16].

In the present study, by comparing antibody binding to the native antigen with antibody binding to the enzymatically denatured antigen [17], we document discriminant binding consistently related to the clinical status of the tested subjects. Indeed, enzymatic digestion of native Der p1 is supposed to modify its immunoreactivity by destroying, theoretically, all discontinuous epitopes and some continuous ones and unfolding some determinants

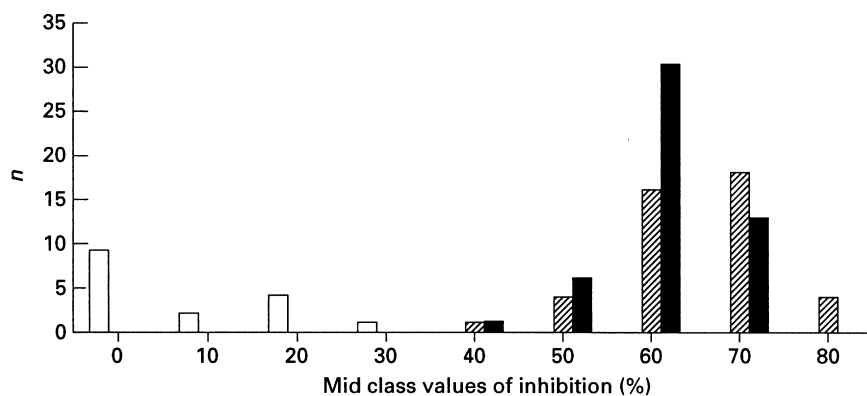


**Fig. 6.** Distribution of the percentage inhibitions of healthy control pooled IgG binding to the grass pollen antigen by individual sera. □ = G1 pollen allergics ( $n = 16$ ); ▨ = Der p1 allergics ( $n = 43$ ); ■ = controls ( $n = 50$ ).

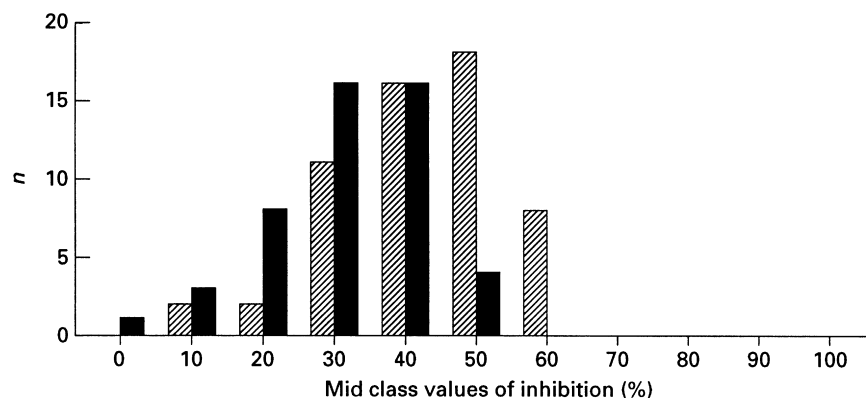


**Fig. 7.** Distribution of the percentage inhibitions of mAb 5 binding to nDer p1 by individual sera. ▨ = allergics ( $n = 56$ ); ■ = controls ( $n = 48$ ).

that may be either hidden (cryptic epitopes) or adjacent to each other in the native molecule, with possible steric interference due to the molecule's spatial structure [12–14]. Thus, serum IgG from healthy individuals which show similar binding levels to the two forms of the protein, seem to be preferentially directed towards epitopes present on both antigens, that is, continuous epitopes unaffected by pepsin digestion.

In contrast, mite-allergic serum IgG exhibit a higher degree of binding to the native form of the antigen and appear to be preferentially directed towards conformational epitopes or continuous epitopes profoundly affected by the proteolysis. These latter results are consistent with those of recent studies indicating that significant conformational structure of group I mite allergens is required for IgE binding [18–19] and indirectly confirm the similar pattern of IgE and IgG binding to Der p1 epitopes already documented [11–18].

The specificity of these observations is supported by the results documented here for the grass pollen, *Antoxantum odoratum*, which retains its antigenic reactivity to the IgG from most sera of each group after its denaturation.

One may emphasize that protein A used for antibody binding detection in the solid phase ELISA also binds to

other serum antibody isotypes [20]. However, it has been shown that the IgG response to such environmental allergens is quantitatively by far more important than other classes' responses [21], so that the signal obtained in the present experiment may be considered as reflecting mainly the IgG binding. Moreover, the competitive inhibition tests used to further demonstrate the differences in antibody specificity were performed with exclusively pure IgG fractions obtained by affinity chromatography on recombinant protein G.

In these experiments, the binding of the mite-allergic biotinylated IgG fraction to nDer p1 was more inhibited by allergic sera than by healthy control sera. This is not surprising as allergic sera display greater amounts of IgG antibodies towards nDer p1. Surprisingly, however, the binding of the biotinylated pooled IgG fraction from healthy control individuals was prevented to a greater extent by healthy control sera than by mite allergic sera despite higher antibody titres in the latter. It is a paradoxical situation when considering direct competition for binding to the same epitopes.

Therefore, if not related to antibody titres, the differences in the inhibition capacities of each group of sera must reflect antibody populations with different specificities, although

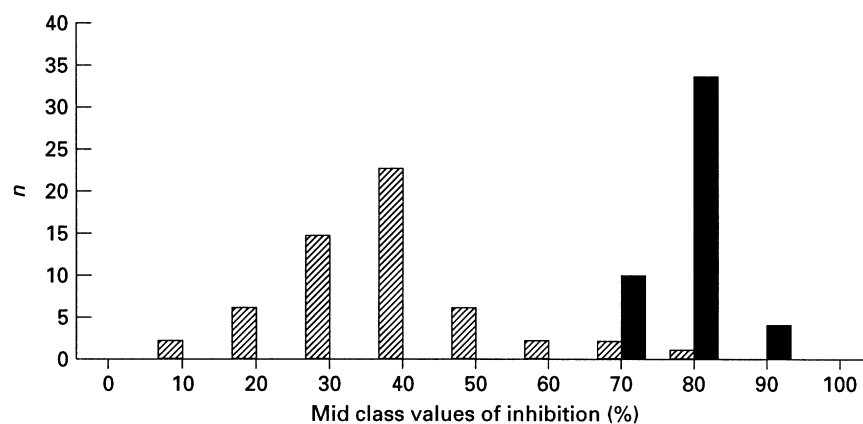


**Fig. 8.** Distribution of the percentage inhibitions of mAb 6 binding to nDer p1 by individual sera. ▨ = allergics ( $n = 56$ ); ■ = controls ( $n = 48$ ).

autologous anti-idiotypic antibodies documented in the serum of DPT allergic patients [22], could have interfered in the competitive inhibition experiments. However, this possibility was virtually excluded as the inhibition capacities of sera from mite-allergic patients or healthy individuals were not influenced by the addition of pooled sera from healthy controls or mite-allergic patients, respectively, after depletion of anti Der pt idiotype activity by exhaustive adsorption on a Der pt sepharose CLB<sub>4</sub> column ( $P=0.69$  by Student's *t*-test for paired data (data not shown)). Moreover, the use of monoclonal antibodies in competitive ELISA, confirms the clinically restricted specificity at the level of the epitope.

Two situations are presented to illustrate the concept. The binding of mAb 5 to nDer p1 was greatly reduced by mite-allergic sera and hardly inhibited by healthy control sera. Conversely, healthy control sera prevent the binding of mAb 6 to nDer p1 much more than mite-allergic sera. This means that the epitopic specificities of mAb 5 and mAb 6 are differently represented in the polyclonal responses of mite-allergic patients and healthy control subjects. These experiments, however, do not address the issue of the relative importance of each specificity in the polyclonal responses of the study populations. Together these results indicate that the immunodominant epitopes for the IgG responses to Der p1 are at least partially distinct in mite-allergic patients and healthy individuals. This distinction appears antigen specific as mite-allergic sera prevent the binding of a pooled IgG fraction from healthy control subjects to *Antoxantum odoratum* exactly as healthy control sera do.

The surprising but striking influence of clinical status on the choice of epitopes recognized as dominant somewhat challenges the usual view that the immunodominance of B epitopes is mostly dependent upon the molecular architecture of the antigens [23–25], even though the importance of host characteristics has been highlighted [26]. In our work, it seems rather to be the allergy-related symptoms, rhinitis or asthma, which correlate best with the modified profile of epitopic dominance. Indeed, IgG antibodies from non-symptomatic mite-sensitized subjects who already exhibit specific IgE antibodies towards Der pt, and thus also probably towards Der p1 [27], retain the epitope specificity of their group [i.e. the healthy control group].

The relationship between symptoms and the epitopic immunodominance of antibodies will need further investigation in models which allow the establishment of more reliable discrimination between a state of sensitization and actual allergic status such as in grass pollen allergy, allergy to house pets or food allergy where seasonal variations, eviction or diet, by avoiding the offending allergen, allow clear identification of the responsible agent which is sometimes difficult in perennial allergies such as mite allergy. The functional implication, if any, of modified

antibody immunodominance on the expression of allergic symptoms is unknown. If present, it would indicate a similar change in IgE epitope specificity which is suggested by studies mentioned above [18–19] even though different IgE and IgG<sub>4</sub> antibody specificities have been recently documented in the Der p2 model [28]. From a theoretical point of view, antibody with a preferential specificity for conformationally dependent epitopes is expected to act more efficiently in triggering an immediate reaction after deposition of native antigens on mucosal membranes.

Recently, associations between epitope specificity and the functional activity of antibodies has been reported in several other models: specificity indicative of acute infection in anti-viral and anti-microbial responses [29–30], specificity associated with protective activity in pseudomonal infection [31] or a blocking effect in an animal model of bee venom sensitization [32]. One should emphasize that, in each of these models, a higher antigenic load seems to play a key role in determining the immunodominance of one or other epitope. Thus, higher levels of mite exposure which have an influence on the symptoms in mite-sensitive patients [33–34] could be implicated in the preferential selection of particular B cell clones.

In conclusion, our data indicate that mite allergy is associated with a specific profile of IgG epitopes recognized on Der p1. As far as we are aware, this is the first study reporting such an association between allergic status and the relative immunodominance of antigenic determinants. The observed homogenous behaviour of the groups is somewhat unexpected as other exhaustive studies investigating the immune response to epitopes of Der p1 have, in contrast, documented heterogeneity at the individual level. The mechanism which determines this change, possibly related to altered antigen processing, is presently unknown. Be that as it may, these findings could have implications in the biological diagnosis of allergy, and especially in distinguishing between a sensitized state and symptomatic allergic status, which is rather difficult with available routine assays even though cut-off values for specific IgE antibody levels allowing such a discrimination have recently been proposed [35].

### Acknowledgements

This work has been supported by an Interbuilt grant from the Fondation Erasme. The authors thank Béatrice Gossart for help during this study and Christine Piesen for typing the manuscript.

### References

- 1 Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease and environmental control. *J Allergy Clin Immunol* 1987; 80:755–72.

- 2 Kemeny D, Urbanek R, Ewan P. 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.
- 3 Devey ME, Beckman S, Kemeny DM. The functional affinity of antibodies of different IgG subclones to dietary antigens. *Clin Exp Immunol* 1993; 94(1):117–21.
- 4 MacMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I restricted CD8<sup>+</sup> T cell mediate but MHC class II restricted CD4<sup>+</sup> T cell dependent immune duration resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993; 178:889–99.
- 5 Holt PG. Immunoprophylaxis of atopy: light at the end of the tunnel. *Immunol Today* 1994; 15(10):484–9.
- 6 Johansson SGO, Donnaens A, Lilja G. The relevance of anti-food antibodies for the diagnosis of food allergy. *Ann Allergy* 1984; 53:665–72.
- 7 Björck L, Kronvall G. Purification and some properties of streptococcal protein G: a novel IgG binding reagent. *J Immunol* 1984; 133:969–74.
- 8 Chapman MD, Hayman PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens. Der p1 and Der f1 from mites of the genus *Dermatophagoïdes*. *J Immunol* 1987; 139: 1479–84.
- 9 Lind P, Hansen OC, Horn N. The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, Der p1, of *Dermatophagoïdes pteronyssinus*: relative binding site location and species specificity studied by solid phase inhibition. *J Immunol* 1988; 140:4256–62.
- 10 Stewart GA, Armstrong L, Krska K et al. Epitope mapping analysis of the major mite allergens using synthetic peptides. In: Sehon AH, Kraft D, Kunkel G, eds. *Epitopes of atopic allergens*. Brussels: UCB Institute of Allergy, 1990:41–7.
- 11 Greene WK, Cyster JG, Chua KY, O'Brien RM, Thomas WR. IgE and IgG binding peptides expressed from random fragments of cDNA encoding the major house dust mite allergen, Der p1. *J Immunol* 1991; 147(4):3763–73.
- 12 Van Regenmoortel MHV. Which structural features of antigens do antibodies recognize? In: Sercarz EE, Berzofsky JA, eds. *Immunogenicity of protein antigens: repertoire and regulation*. Boca-Raton, Florida: CRC Press, 1987:21–8.
- 13 Arnon R, Van Regenmoortel MHV. Structural basis of antigenic specificity and design of new vaccines. *Faseb* 1992; 6:3265–74.
- 14 Vailes LD, Li Y, Bao Y. Fine specificity of B cell epitopes on *Felis domesticus* allergen I (Fel dI): effect of reduction, alkylation or deglycoxylation on Fel dI structure and antibody binding. *J Allergy Clin Immunol* 1994; 93:22–33.
- 15 Greene WK, Chua KY, Stewart GA, Thomas WR. Antigenic analysis of group I dust mite allergens using random fragments of Der p1 expressed by recombinant lituaries. *Int Arch Allergy Appl Immunol* 1990; 92:30–8.
- 16 Greene WK, Thomas WR. IgE binding structure of the major house dust mite allergen Der p1. *Mol Immunol* 1992; 29: 257–66.
- 17 Yuan J, Low PS. Epitope mapping by a method that required no aminoacid sequence information. *Anal Biochem* 1992; 205:179–82.
- 18 Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD. B cell epitopes on group I and II mite allergens. *J Immunol* 1990; 144:1353–61.
- 19 Collins SP, Ball G, Vonarx E et al. Absence of continuous epitopes in the house dust mite major allergens Der p1 from *Dermatophagoïdes pteronyssinus* and Der f1 from *Dermatophagoïdes farinae*. *Clin Exp Allergy* 1996; 26:36–42.
- 20 Peng Z, Becker AB, Simons FE. Binding properties of protein A and protein G for human IgE. *Int Arch All Immunol* 1994; 104(2):204–6.
- 21 Platts Mills TAE. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979; 122:2218–25.
- 22 Saint-Remy JMR, Lebecque S, Lebrun PM, Jacquemin MG. Human immune response to allergens of house dust mite, *Dermatophagoïdes pteronyssinus*. IV. Occurrence of natural autologous anti-idiotypic antibodies. *Eur J Immunol* 1988; 18:83–7.
- 23 Sercarz EE. The architectonics of immune dominance: the aleatory effects of molecular position on the choice of antigenic determinants. *Chem Immunol* 1989; 46:169–85.
- 24 Ju ST, Nonogaki T, Bernatowicz MS, Matsueda GR. The B immune response to an idio-type-inducing peptide epitope can be inhibited by immunodominance of a neighbouring epitope. *J Immunol* 1993; 150(7):2641–7.
- 25 Kotera Y, Fontenot JD, Pecher G, Metzgard RS, Finn OJ. Humoral immunity against a tandem repeat epitope of human mucin MUC-1 in sera from breast, pancreatic and colon cancer patients. *Cancer Res* 1994; 54:2856–60.
- 26 Berzofsky JA. Intrinsic and extrinsic factors in protein antigenic structure. *Science* 1985; 229:932–5.
- 27 Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoïdes pteronyssinus*. Antigen p1. *J Immunol* 1980; 125(2):587–92.
- 28 Kobayashi I, Sakiyama Y, Tame A, Kobayashi K, Matsumoto S. IgE and IgG<sub>4</sub> antibodies from patients with mite allergy recognize different epitopes of *Dermatophagoïdes pteronyssinus* group II antigen (Der p2). *J Allergy Clin Immunol* 1996; 97:638–45.
- 29 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(4):213–26.
- 30 Lundkuist A, Björsten S, Niklasson B, Ahlberg 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(1):82–6.
- 31 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(6):2347–51.
- 32 Kolbe L, Heusser CH, Kolsch E. Isotype associated recognition of allergen epitopes and its modulation by antigen dose. *Immunology* 1995; 84:285–9.

- 33 Vervloet D, Charpin D, Haddi E. Medication requirements and house dust mite exposure in mite sensitive asthmatics. *Allergy* 1991; 46:554–8.
- 34 de Blay F, Pauli G, Velten M, Bessot JC. Influence of mite exposure on symptoms of mite sensitive patients with asthma. *J Allergy Clin Immunol* 1994; 93:136–8.
- 35 Pastorello EA, Incorvaia C, Ortolani C et al. Studies on the relationship between the level of specific IgE antibodies and the clinical expression of allergy: I. Definition of levels distinguishing patients with symptomatic from patients with asymptomatic allergy to common aeroallergens. *J Allergy Clin Immunol* 1995; 96:580–7.