

Human immune response to allergens of house dust mite, *Dermatophagoides pteronyssinus*

Isotypic analysis of antibodies in atopic and non-atopic subjects

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Specific antibodies isolated by immunoabsorption on four main insolubilized allergens from *Dermatophagoides pteronyssinus* (DPT) had the following isotypic distribution: in 16 atopic patients, 52% IgG, 40% IgM, 8% IgA, 0.1% IgE and, in 12 non-atopic individuals, 48% IgG, 46% IgM, 6% IgA, 0.03% IgE. The ratios between geometric means of antibody values in each class (atopic vs non-atopics) were 2.4 for IgG, 2.0 for IgM, 2.8 for IgA and 66.7 for IgE. The amount of anti-DPT antibodies in IgG subclasses did not follow the usual distribution of total IgG subclasses, i.e., IgG1 > IgG2 > IgG3 > IgG4. In atopics the order was IgG2 > IgG1 > IgG4 > IgG3 and in non-atopics, IgG4 > IgG1 = IgG2 > IgG3 although 6/12 of the latter had no detectable (< 0.5 µg/ml plasma) IgG4.

Key words: *D. pteronyssinus*; IgG subclass; immediate hypersensitivity; immunoglobulins.

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Atopic patients are known to produce in addition to specific IgE antibodies significant amounts of specific antibodies pertaining to IgG class (16), especially after immunotherapy (15), and to the IgA class (6). Little attention has apparently been paid to the IgM class. Data are also rare on the isotypic distribution of anti-allergen antibodies in non-atopic individuals. In the study of the immune response against allergens from *Dermatophagoides pteronyssinus* (DPT), we were able to isolate by immunoabsorption large amounts of anti-DPT antibodies. Because of the allergenic complexity of DPT (28), we deliberately chose to use a solution containing four main soluble proteins; this study should therefore be considered as an analysis of the isotypic distribution of antibodies against some of the DPT allergens and not as an analysis of DPT allergens.

In contrast to previous studies where specific antibodies were detected in the serum, we ana-

lysed the isotypic distribution of anti-DPT antibodies after their purification and a 30-fold increase in concentration as compared to the serum. We found that non-atopic as well as atopic subjects produce significant amounts of IgM antibodies as well as IgG and IgA antibodies and that within the IgG antibodies of both atopics and non-atopics IgG2 and IgG4 subclasses are primarily represented.

MATERIAL AND METHODS

Reagents

Carboxylated latex particles of 0.8 µm diameter (Estapor K150, lot 501P) were obtained from Rhône-Poulenc, Coubevoie, France; 1-ethyl-3(3-dimethyl-amino-propyl) carbodiimide-HCl from Sigma Chemicals, St. Louis, MO; Tween 20 from Technicon, Tarrytown, NY; bovine

serum albumin (BSA) from Calbiochem, La Jolla, CA; CH-Sepharose from Pharmacia Fine Chemicals, Uppsala, Sweden; fetal calf serum from Gibco Ltd., Paisly, Scotland, and anti-IgG1, -IgG2, -IgG3 or -IgG4 antisera from Central Laboratory Netherlands Red Cross, Amsterdam, The Netherlands.

Phosphate buffered saline (PBS) pH 7.4 contained 8 mM of phosphate and 9 g/l of NaCl and glycine buffered saline (GBS) 0.1 M pH 9.2, 0.1 M of glycine and 9 g/l of NaCl.

Patients

Sixteen patients (6 males and 10 females; average age 33 years, range 19–49) were selected on the basis of severe asthma primarily caused by sensitivity to DPT. They had high levels of anti-DPT IgE antibodies as demonstrated by intradermal tests (mean wheal diameter > 20 mm) and radioimmunoassay (RIA; see below) and most of them (14/16) had been desensitized to DPT in the past. The mean time since the last desensitization and the present study was 7 years and 2 months (range: 8 months to 20 years). Desensitization consisted of weekly injections of an increasing amount of aqueous house dust extract for 3 months followed by monthly injections of a maintenance dosage for an average of 2 years. The treatment resulted in a mild transient clinical improvement in a third of the patients but had no effect in the others. When plasma was collected the patients were taking no glucocorticosteroids. Twelve healthy volunteers (8 males and 4 females; average age 32 years, range 25–42), with no history of allergic reactions, negative skin prick tests, total serum IgE < 20 kU/l and no anti-DPT IgE antibodies as shown by radioallergosorbent assay (Phadebas RAST; Pharmacia) were included in this study.

Allergen preparation

DPT glycerinated extract from Bencard Ltd. (Epsom, Surrey, England) was extensively dialysed at 4°C against PBS containing 100 U aprotinin/ml as proteolysis inhibitor, concentrated by ultrafiltration (YM10, Amicon

Corp., Danvers, MA) and stored at -70°C until use. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a 12% gel of this extract followed by Coomassie brilliant blue staining showed 5 main protein bands of 15,000 to 90,000 molecular weight (MW). Higher MW proteins were also present as will be described in a separate paper (manuscript in preparation). Allergen Der p1 was identified in this preparation by cross-immuno-electrophoresis (courtesy of Dr. P. Lind, Copenhagen, Denmark) and gave repetitively two bands on the gel corresponding to 22,000 and 26,000 MW.

Antisera

Rabbit anti-human Fcε antisera were prepared by repeated fortnightly injections of 100 µg IgE (DES) (29) in complete Freund's adjuvant. The antisera were absorbed on human IgG, IgA, IgM, kappa and lambda chains coupled with carbodiimide to CH-Sepharose according to the manufacturer's recommendation, then on Fab-fragment from papain digest (12) of monoclonal IgE (DES). Anti-Fcε antibodies were purified by absorption on IgE(DES)-Sepharose conjugate followed by elution with 50 mM citric acid, pH 2.7, and labelled with ¹²⁵I by the chloramin-T method (19).

Immunosorbent

DPT was covalently coupled to carbodiimide-activated CH-Sepharose by using 1 mg allergen/ml of packed Sepharose according to manufacturer's instructions. After 20 h incubation at 21°C with continuous rotation, glycine was added to 1 M final concentration to saturate the excessive reactive groups. After 4 h incubation at 21°C the gel was washed successively with PBS, 50 mM citric acid, pH 2.7 and 3 M ammonium thiocyanate. Under these conditions of considerable excess of reactive groups vs the amount of ligand added, 85 to 92% of the initial material bound to the solid phase. The above was calculated by evaluating the agglutinating activity of unbound DPT towards latex particles coated with rabbit anti-DPT F(ab')₂ (21).

Preparation of specific antibodies

Human plasma (100 ml) collected by plasmapheresis was precipitated by addition of 1 vol. 0.2 M phosphate buffer, pH 8.0, containing 360 g Na_2SO_4 /l. After 4 h at 21°C the precipitates were spun at 8,000 g for 20 min, washed and resuspended in PBS containing 1 M NaCl. After extensive dialysis against PBS, IgM and IgG fractions were separated on a 6 × 90 cm Fractogel TSK HW-55F column (Merck, Darmstadt, FRG). After concentration to 25 ml by ultrafiltration and dialysis against PBS, the fractions were absorbed onto 5 ml of packed DPT-Sepharose in a 2 × 8 cm column at a flow rate of 15 ml/h. The gel was then washed successively with PBS, PBS containing 1 M NaCl and saline (9 g/l NaCl), each washing continued until the 280-nm absorbance decreased below 0.02. The absorbed material was eluted with 50 mM citric acid pH 2.7, immediately neutralized with tris (hydroxymethyl)-aminomethane (Tris), dialysed against PBS and concentrated to 3 ml by ultrafiltration.

Analysis of eluates

Albumin, haptoglobin, alpha-1-antitrypsin, complement C3 and transferrin were assayed in the eluates by immunonephelometry (AIP, Technicon), and IgG, IgA and IgM by RIA using anti-isotype antibodies coupled to polystyrene beads (courtesy of Dr. Delacroix (7)) or in some cases by radial immunodiffusion (RID). IgG subclasses were assayed by RID in agarose gel containing 2 to 5% polyethylene glycol and anti-IgG1, -IgG2, -IgG3 or -IgG4 antisera according to the manufacturer's recommendations, with a sensitivity of 50 µg/ml. Ultracentrifugation was carried out with a SW41-Ti rotor in a Spinco L2-65B ultracentrifuge (Beckman Instruments, Palo Alto, CA). The samples were diluted in 50 mM Tris-HCl buffer, pH 7.4, containing 9 g/l NaCl. Samples of 100 µl containing 15 to 20 µg Ig were centrifuged on a 5 to 30% linear sucrose gradient at 40,000 RPM for 16 h at 4°C. IgG or IgA was assayed by RIA in the 0.4 ml-fractions. For *in vivo* testing, the dilution of

allergen causing, after 15 min, a wheal of 2 cm diameter was tested again after incubation with the plasma eluate of the corresponding patient. IgE antibodies in the eluate had been inactivated by heating at 56°C for 1 h. In the absence of skin reaction, the amount of allergen was gradually increased up to 100 times the initial amount.

Total IgE assay

Polystyrene wells (Dynatech, Kloten, Switzerland) were coated with 100 µl of anti-human Fcε rabbit F(ab')₂ diluted to 10 µg/ml in 0.1 M GBS and incubated overnight at 21°C. The plates were washed 3 times with 9 g/l NaCl containing 0.1% Tween-20 and 3 times with NaCl alone. The samples (100 µl) diluted in PBS containing 10% fetal calf serum were applied and incubated for 20 h at 21°C. The plates were then washed as above and 100 µl of affinity-purified ¹²⁵I-labelled rabbit anti-human Fcε (100,000 cpm, ~ 10 ng protein) diluted in PBS containing fetal calf serum was added. After 20 h at 21°C, the plates were washed 5 times with NaCl-Tween before counting. This RIA had a detection limit of 20 picog. IgE/ml. Since the rabbit anti-human Fcε antiserum contained auto-antiidiotypic antibodies recognizing idiotopes on affinity-purified anti-human Fcε antibodies, the antiserum was absorbed on to purified antibodies coupled to Sepharose.

Assay of anti-DPT antibodies

Anti-DPT IgG were quantified by agglutination of DPT-coated latex. Carboxylated particles were activated by carbodiimide and DPT coupled as described (18). Samples (30 µl) diluted in GBS containing 0.1% BSA were incubated with DPT-latex (30 µl of a 0.5% suspension) for 10 min at 21°C before adding a 1/200 dilution (30 µl) of a serum with high rheumatoid factor titre. Such a serum is used to enhance the agglutination by IgG antibodies (23). The suspension was incubated for 60 min at 37°C while shaking constantly. The assay was calibrated by dilutions of the solution containing an amount of anti-DPT IgG determined

by immunoabsorption. It detected less than 0.5 µg/ml of anti-DPT IgG in purified anti-DPT IgG solutions, but only 5 µg/ml when the serum was used.

Anti-DPT IgM antibodies were assayed by direct agglutination of DPT-latex. A sample dilution (30 µl) was added to 30 µl of GBS-BSA and 30 µl of DPT-latex and incubated for 120 min at 37°C while shaking. Agglutination was measured by instrumental counting of the residual non-agglutinated particles in the IMPACT® system (ACADE Diagnostic Systems s.a., Brussels, Belgium) as described in (18). The lower limit of detection was 1 µg/ml. The absence of agglutination by non-specific IgG or IgM at a concentration 10,000 times higher than that of the affinity-purified antibodies demonstrated the specificity of these assays. Soluble DPT in µg quantities, but not unrelated allergen like timothy pollen, was found to inhibit the agglutination.

IgE anti-DPT antibodies were assayed by RIA using DPT-coated paper discs (0.6 cm diameter; 541 filter paper; Whatman Ltd., Maidstone, England) (5). An average of 7 µg of allergen was bound to each disc. After incubation of undiluted samples for 16 h at 21°C, the discs were washed 3 times with 9 g/l NaCl containing 0.05% Tween-20 and exposed to ¹²⁵I-rabbit anti-human Fcε antibodies (50 µl containing 5 ng and 50,000 cpm) diluted in PBS containing 30 g/l BSA. After a further overnight incubation at 21°C and 3 washes with NaCl-Tween, radioactivity was counted. The detection limit of this assay was 0.5 ng/ml.

Assay of anti-Ig autoantibodies

Anti-Fcγ, anti-Fcα and anti-Fcε antibodies were assayed by agglutination of Fcγ-, Fcα- or Fcε-coated latex, respectively. Fcγ, Fcα or Fcε prepared by papain digestion (26) were coupled to carbodiimide-activated latex particles as described (18). Anti-Ig IgM was quantified as above for anti-DPT antibodies. For the assay of anti-Ig IgG, the samples were previously digested with pepsin to generate F(ab')₂ (26). A dilution of the sample (30 µl) was incubated with the Fc-coated latex (30 µl) for 30 min at

37°C while shaking before the addition of an anti-Fcγ, -Fcα or -Fcμ IgM (30 µl). The degree of inhibition was converted into the quantity of anti-Ig IgG by reference to a calibration curve derived from a known amount of F(ab')₂ anti-Fcγ, -Fcα or -Fcμ antibodies.

Statistics

Correlation coefficients were calculated using the Spearman's rank correlation (*r_s*), and the differences between means using the Mann-Whitney U test.

RESULTS

Purification of specific antibodies

Ig from 16 sensitive patients and 12 non-atopics were separated after Na₂SO₄ precipitation into IgG and IgM by gel filtration. They were then passed separately through the DPT-Sepharose column and the absorbed material was eluted with citric acid. The effluent was checked for the presence of residual anti-DPT activity by particle-counting immunoassay for specific IgG and IgM antibodies. Specific IgE antibodies were estimated by RIA using allergen-coated paper discs. The effluent was passed through the immunosorbent until no specific antibodies could be detected. The total amount of antibodies eluted from the column was quantified by absorbance at 280 nm. The geometric mean was 4.45 mg/100 ml of plasma (range 2.44 to 10.77) for DPT-sensitive patients and 1.90 mg (range 1.14 to 4.97) for non-atopic individuals representing 43% of the atopics mean value.

At a total protein concentration of 1 mg/ml no albumin, haptoglobin, alpha-1-antitrypsin, complement C3 or transferrin were detected by immunonephelometry (sensitivity 1 µg/ml) indicating that contamination by one of these proteins was lower than 0.1%. Immunoelectrophoresis of the eluates with an antiserum to all major serum proteins failed to reveal any contaminant. The sum of the amount of IgG, IgA, IgM and IgE antibodies found in eluates by RIA exceeded only by 8% the values of total Ig

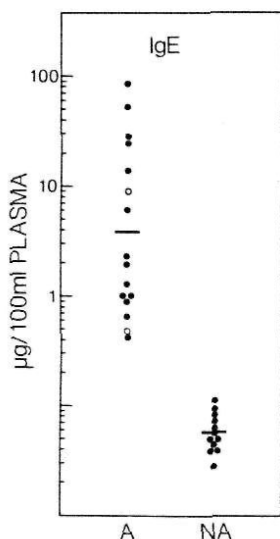


Fig. 1. Affinity-purified anti-DPT IgE antibodies quantified by RIA in 16 DPT-sensitive atopic patients and 12 non-atopic individuals. Open circles = undensitized patients; bars = geometric means. (A) atopics; (NA) non-atopics

estimated from absorbance at 280 nm ($E^{1\%}_{1\text{cm}} = 13.8$). We checked the eluates for anti-Ig autoantibodies. With particle-counting immunoassay using latex coated with Fc γ , Fc α or Fc ϵ we did not observe any significant agglutination. To detect a possible contamination of the eluates

by self-associated IgG-anti-IgG complexes, we ultracentrifuged 5 eluates on sucrose density gradient. A mean of 95.6% IgG sedimented in the 7S fraction, indicating that if IgG-anti-IgG complexes were present, they represented less than 5% of the material recovered in the eluate.

The antibodies recovered in the eluates of both atopic and non-atopic individuals retained their allergen-binding capacity. Indeed, the proportion of specific anti-DPT IgG and IgM in the eluates was estimated by agglutination of DPT-Lx; it represented 83 and 74% of eluted IgG and IgM antibodies, respectively. For specific anti-DPT IgE, the proportion reached 87% of the total IgE in the eluates. These figures probably reflect some denaturation of the specific antibodies due to their binding and elution from the immunoabsorbent, although the presence of non-specific antibodies in the eluates cannot be completely ruled out. Moreover, the purified antibodies were capable of neutralizing *in vivo* the wheal reaction produced by injection of the allergen. When mixed with the heated eluate, the allergen failed to cause any skin reaction even when the allergen dose was 100-fold higher than the reference value.

Isotype distribution of immunoglobulins in the eluates

The eluates of DPT-sensitive patients contained 70 times more specific IgE than normals (Fig. 1

Table 1
Concentration ($\mu\text{g}/100\text{ ml}$) of various classes of anti-DPT antibodies in plasma

		DPT-sensitive atopics (n = 16)	Non-atopics (n = 12)	P*
IgG	geom. mean	2158	882	< 0.001
	range	904-7651	396-1248	
	%	52	48	
IgA	geom. mean	336	118	< 0.01
	range	90-1463	56-382	
	%	8	6	
IgM	geom. mean	1676	843	< 0.005
	range	697-3495	482-2674	
	%	40	46	
IgE	geom. mean	3.8	0.057	< 0.0001
	range	0.44-88	0.029-0.116	
	%	0.1	0.003	

*Mann-Whitney U test

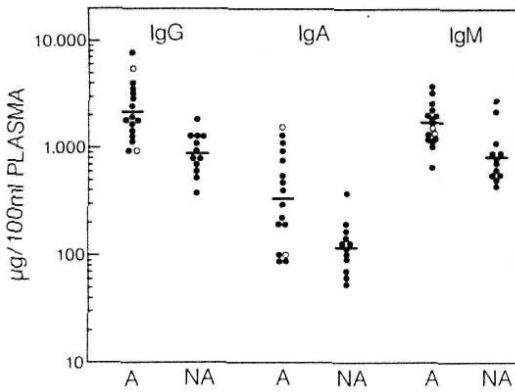


Fig. 2. Affinity-purified anti-DPT antibodies in 16 atopic patients highly sensitive to DPT and 12 non-atopic individuals assayed by RIA. Open circles = undesensitized patients; bars = geometric means. (A) atopics; (NA) non-atopics

and Table 1) but only 2 to 3 times more IgG, IgA and IgM (Fig. 2 and Table 1). In DPT-sensitive patients a positive correlation was found between IgG and IgA ($r_s = 0.83$; $P < 0.02$) and between IgA and IgE ($r_s = 0.62$; $P < 0.06$), whereas in the non-atopic group the correlation was restricted to IgG and IgA ($r_s = 0.55$; $P < 0.07$). In both groups no correlation

existed between IgG and IgE and between IgM and other Ig classes. Five eluates with a high content of IgA were ultracentrifuged on a sucrose density gradient. In addition to monomeric IgA, dimers (10S) accounting for 4 to 13% of total IgA were found in 4 eluates. This proportion of dimers is the same as that observed for total IgA in healthy individuals (7).

The IgG subclasses were assayed by RID in the 10-fold concentrated eluates (Table 2 and Fig. 3). In atopics IgG2 and IgG4 represented 50 and 10% of the anti-DPT IgG in the eluates, whereas the subclasses represented 28 and 3% of total IgG in the plasma. These differences were significant (Mann-Whitney U test; $P < 0.01$ and $P < 0.001$ for IgG2 and IgG4, respectively). Although none of the patients was found deficient in any IgG subclass, one patient had no detectable anti-DPT IgG1 and IgG3, another had no IgG1 and a third no IgG4. A significant correlation was found between IgG1 and IgG2 ($r_s = 0.93$; $P < 0.02$), IgG1 and IgG3 ($r_s = 0.85$; $P < 0.03$), and IgG2 and IgG3 ($r_s = 0.82$; $P < 0.03$). IgG4 was correlated with IgG2 ($r_s = 0.80$; $P < 0.03$) and with IgE ($r_s = 0.74$; $P < 0.04$).

In the non-atopic group where no deficiency

Table 2
Concentration ($\mu\text{g}/100 \text{ ml}$) of various IgG subclasses of anti-DPT antibodies in plasma

		DPT-sensitive atopics	Non-atopics	P^*
IgG1	geom. mean	1001	368	< 0.002
	range	427-3191	130-737	
	%	33	28	
	n	8	10	
IgG2	geom. mean	1535	345	< 0.002
	range	346-4312	40-690	
	%	50	26	
	n	10	11	
IgG3	geom. mean	205	162	NS
	range	102-414	107-249	
	%	7	12	
	n	9	12	
IgG4	geom. mean	308	458	NS
	range	142-776	269-781	
	%	10	34	
	n	9	6	

*Mann-Whitney U test

NS = not significant

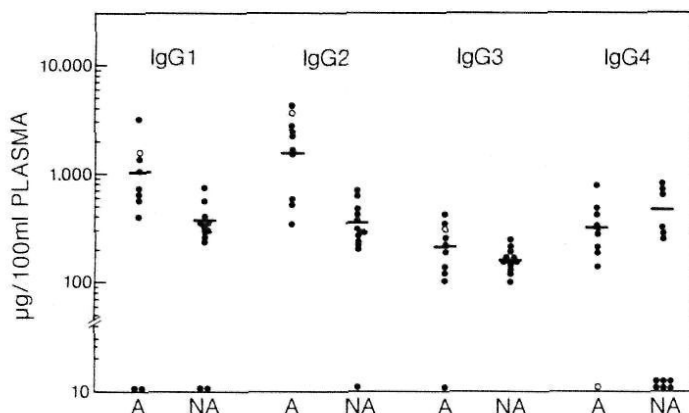


Fig. 3. IgG subclasses of anti-DPT antibodies assayed by RID. Open circles = undesensitized patients; bars = geometric means. (A) atopics; (NA) non-atopics

of IgG subclass was detected, one patient had no anti-DPT IgG2, two had no IgG1 and six had no IgG4 antibodies. Distribution of anti-DPT IgG subclasses was not significantly different from that of total IgG subclasses, with the exception of 6 subjects having anti-DPT IgG4 (Mann-Whitney U test; $P < 0.01$). No significant correlation was found either between subclasses, or between IgE and IgG subclasses.

DISCUSSION

The isotypic distribution of antibodies isolated by immunoabsorption can be determined reliably if one minimizes the non-specific protein binding to the immunosorbent. We believe this goal has been achieved here for the following reasons: (i) the eluates contained no other detectable proteins than immunoglobulins; (ii) the proportion of eluted antibodies exhibiting an antigen-binding capacity was high, especially for IgE; (iii) the isolated antibodies retained their capacity to neutralize the wheal reaction induced *in vivo* by injection of the allergen. For ethical reasons the *in vivo* experiment was carried out with the patient's own anti-DPT antibodies only and, consequently, antibodies of non-atopic individuals were not used.

The surprisingly high amounts of specific anti-DPT antibodies obtained by immunoabsorption suggest the recovery of lower affinity,

yet specific, antibodies. These are unlikely to be detected in conventional serum assays. Nevertheless, their antigen-binding capacity is progressively revealed by the removal of higher affinity antibodies as has been observed in this study. Although we did not estimate the amount of antibodies still bound after citric acid elution, washings with 3 M ammonium thiocyanate released proteins which represented less than 10% of the total protein recovered in the citric acid eluates; yet albumin was the main component detected in the thiocyanate washings. Since the majority of the patients had been desensitized in the past, immunotherapy could be responsible for the high antibody yield. However, the average amount of specific antibodies obtained by immunoabsorption of the immunoglobulins of 6 new undesensitized patients was 3.56 mg/100 ml of plasma, a value not significantly lower than the one obtained in this study.

Twelve non-atopic individuals in our study had specific antibodies against DPT pertaining to most Ig classes including IgE. The amount of IgE antibodies was 50 to 100 times smaller than in atopics but IgG, IgA and IgM antibodies were only 3 times less abundant. At first glance, these results are not in accordance with some of the earlier data (11). However, the use of affinity-purified antibodies has allowed a 30-fold concentration as compared to the serum,

which was the material used for specific antibody determinations in other studies. For instance, Chapman & Platts-Mills (6) reported that only 30 % of non-atopic individuals had specific IgG antibodies against antigen Der p1, one of the major DPT allergens. Differences in the assays might also be responsible for this lower proportion. The antigen-binding test of Platts-Mills et al. (20) is based on a double-antibody precipitation. Another possible explanation of the discrepancy is that non-atopics produce antibodies against DPT antigens other than Der p1. It is well documented that several allergens are present in DPT (13). However, when nitrocellulose membranes, on which DPT antigens had been transferred electrophoretically after their electrophoretic separation in SDS-PAGE, were incubated with purified antibodies from non-atopics and with ^{125}I -labelled IgG of a rabbit anti-Fc γ antiserum, it was demonstrated that most DPT fractions, including Der p1, had bound IgG antibodies (manuscript in preparation). We tried to detect anti-DPT IgG directly in the serum of non-atopic individuals and succeeded in 8 out of 12 sera only (data not shown). Therefore we assume that the lack of detection of specific anti-allergen IgG in the serum of non-atopic individuals is imputable to the use of serum instead of purified antibodies.

The presence of IgE antibodies in non-atopics with less than 20 kU/l of total IgE and a negative RAST for DPT could be surprising. However, the mean concentration of anti-DPT IgE antibodies in this group was 570 picog/ml or 0.24 kU/l, which is below the RAST sensitivity. The occurrence of IgM antibodies in non-atopics and atopics also deserves some comments. IgM antibodies represented up to 46 % of total anti-DPT antibodies. Since non-specific adsorption is unlikely, we believe that we have detected IgM antibodies, a finding which has not been reported to our knowledge, probably because we have separated IgM from IgG before absorption on the insolubilized DPT. In this way IgG antibodies could not "block" by competition the binding of IgM antibodies. It remains unclear why IgM antibodies persist at such high levels in atopics and non-atopics even after years of exposure. It

could by no means be related to the desensitization treatment followed by most of our patients since the interval between the last injection and the date of plasma collection was, on average, longer than 7 years.

Specific anti-DPT IgG4 and a significant increase of this subclass were found in all the patients and in half of the non-atopics. A positive correlation with specific IgE was demonstrated in the patients but not in the non-atopics. The involvement of IgG4 in atopy has been inferred from numerous but somewhat controversial studies since Stanworth reported on the inhibition of passive cutaneous anaphylaxis by IgG4 (25). Human IgG4 has been reported as having anaphylactic properties (8, 9), to be able to sensitize basophils (31) or not (30) and to act as "blocking" antibodies (10). Allotypic variations (14) of IgG4 could be responsible for these discrepancies as has been pointed out recently (2, 24). Elevation of IgG4 could result from prolonged immunization (1). In this respect our patients had a long history of asthma due to DPT hypersensitivity. Although half of the 12 non-atopics had no detectable IgG4 (i.e. < 0.5 $\mu\text{g/ml}$ plasma), the others had quite elevated values. Therefore a high titre of anti-allergen IgG4 cannot be restricted to the atopic status.

We found a high level of IgG2 mainly in atopics where it represented up to 50 % of anti-DPT antibodies, though without any significant correlation with IgE. IgG2 are produced preferentially against polysaccharides, as for example the outer membrane of some microorganisms (22), and a deficiency in IgG2 is regularly associated with increased susceptibility to infection with these organisms (4). A high level of IgG2 has also been found in bronchopulmonary aspergilloma (3). Thus, the prevalence of IgG2 may simply reflect the presence of polysaccharidic antigenic determinants mostly present within the higher MW proteins of DPT (27). It has, however, been reported that a human myeloma IgG2 was able to inhibit the IgE-mediated histamine release from basophils (17). The significance of this increase in anti-DPT IgG2 remains unexplained. Finally, it should be kept in mind that our data deal with *D. pteronyssinus*.

sinus hypersensitivity and should not be unduly extrapolated to other allergens.

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