

Original article

IgE and IgG antihouse dust mite specificities in allergic disease

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Background: There are few studies that quantitatively compare IgE and IgG antibody binding to the major and minor house dust mite allergens.

Objective: To measure the IgE and IgG antibody specificities produced by adults and children, including children admitted to an emergency department for asthma.

Methods: Antibodies were measured by solid-phase microtiter assays.

Results: Children recruited from the emergency department had similar titers and patterns of IgE antibody binding compared with children without acute disease. Der p 1 and 2 bound 50% to 65% of the IgE antibody, and most of the remaining binding was to Der p 4, 5, and 7. Der p 3, 8, 10, and 20 induced low titers. The pattern was similar across a wide range of antihouse dust mite titers. IgG₁ and IgG₄ antibodies predominantly bound the major and midrange allergens and were mainly found in children with allergy. Children recruited in the emergency department had lower titers.

Conclusion: The same IgE antibody-binding pattern and predominant contribution of Der p 1 and 2 was found across a wide range of total IgE antibody titers and for children admitted to an emergency department. IgG₁ and IgG₄ antibodies bound to the more allergenic specificities and were largely found in children with allergy. The IgG antibody titers were lower in sera from children admitted to the emergency department for asthma exacerbations.

Clinical implications: Der p 1 and 2 and possibly Der p 4, 5, and 7 provide a formulation suitable for immunotherapy and diagnosis. Low IgG antibodies were a feature of acute disease. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: House dust mite allergens, IgE, IgG₄, IgG₁, asthma

Abbreviations used

DELFA: Dissociation-enhanced lanthanide
fluoroimmunoassay

HDM: House dust mite

SOS: Sum of the specificities

Sensitization to house dust mite (HDM) allergens is prevalent (20% to 30%) and is an independent risk factor for the development of asthma in children.¹⁻⁴ Several new strategies such as the administration of peptides and recombinant and genetically modified allergens are being explored for their use in new more effective types of immunotherapy. Many of these will need to use a defined formulation of allergens, as would strategies that use a balanced and standardized formulation of the significant allergens. For these purposes, it is essential to ascertain a more precise perspective of the size and nature of the responses to the different house dust mite allergens. In this study, we have measured the IgE and IgG responses to a panel of 9 HDM allergens.

IgE antibody to HDM allergens induces early allergen-specific mast cell degranulation and contributes to the late-phase reactions by chronic tissue damage via the downstream effect of mast cell mediators⁵ and by facilitating allergen presentation to T cells.⁶ Currently, 20 HDM allergens have been identified from pyroglyphid and glycyphagid mites, with 13 described for the most abundant HDM, *Dermatophagoides pteronyssinus*.⁷ Der p 1 and Der p 2 are known to be major allergens, but a quantitative comparison with the IgE binding of other allergens has not been made, despite reports that mite extracts depleted of the major allergens can elicit asthmatic responses.^{8,9}

Anti-Der p 1 IgG antibody is almost exclusively produced by subjects with allergy.¹⁰ IgG could therefore be considered to be a marker of allergenicity but conceivably could also regulate allergic responses by blocking mast cell degranulation or IgE antibody-facilitated allergen presentation. In contrast with Der p 1, the major cat and mouse allergens induce IgG₄ in the absence of IgE in subjects without allergy in what has been proposed as high-dose immune deviation, a phenomenon that could mediate or be a marker of protection from disease.¹¹ As

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Supported by the National Health and Medical Research Council of Australia. Disclosure of potential conflict of interest: W. R. Thomas has a patent on house dust mite allergens assigned to the Telethon Institute for Child Health Research. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 13, 2006; revised March 31, 2006; accepted for publication April 2, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.04.001

TABLE I. Patient characteristics*

	Hospitalized subjects
Subjects, n	126
Sex, % male/female	34.1/65.9
Age, y	7.21 $\pm$ 3.68
Asthma severity†	9.31 $\pm$ 2.35
FEV ₁ , % predicted‡	67.4 $\pm$ 20.0

*Unless specified, data presented as means $\pm$ SDs.

†Asthma severity score: sum of 1 to 3 for oxygen saturation, respiratory rate, auscultation, retractions, and dyspnea (mild = 5-7, moderate = 8-11, severe = 12-15). Scoring system adapted from a National Institutes of Health score (National Institutes of Health publication #91-3042).

‡n = 20: Children younger than 5 to 6 years old or who required ongoing treatment were unable to participate.

well as measuring IgE to the panel of allergens, IgG subclasses have been measured to investigate whether they are related to allergenicity and the possibility that minor allergens induce deviated responses.

METHODS

Subjects

Plasma or sera from a collection of nonhospitalized subjects and from a collection of hospitalized subjects were examined. The nonhospitalized subjects with allergy were selected from local community-based populations and reported symptoms of asthma or allergic rhinitis. The hospitalized subjects were children presenting to the hospital with an acute asthma exacerbation and were either successfully treated in the emergency department or admitted into hospital for further treatment. The nonhospitalized subjects consisted of 77 subjects allergic to mite (39 children, 38 adults) and 33 subjects without allergy (7 children, 26 adults) subjects with an age range of 0.9 to 55.3 years. The hospitalized subjects consisted of 105 children allergic to mite and 21 children without allergy with an age range of 2.1 to 16.0 years. Other patient characteristics for the hospitalized subjects are listed in Table I. IgE to *D pteronyssinus* mite extract was measured by using the CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden) performed by the Immunology Department at Princess Margaret Hospital (Subiaco, Australia). The CAP score is measured in kU/L where 1 kU/L is equal to 2.24 ng/mL. The Princess Margaret Hospital Human Ethics Committee gave approval for the procedures in this study.

Allergens

The allergens selected were natural allergens or recombinant allergens considered to contain most of the IgE epitopes of the naturally occurring allergens. Natural Der p 1 was purified from spent mite medium by antibody affinity chromatography. Natural Der p 3 was purified according to Heymann et al.¹² and then further substrate affinity purified with benzamidine Sepharose 6B (Amersham Pharmacia Biotech, Uppsala, Sweden). Natural Der p 4 was similarly purified by substrate affinity with β -cyclodextrin Sepharose 6B (Amersham). Recombinant Der p 2.0101, Der p 5, Der p 7, Der p 8, Der p 10, and Der p 20 were produced as recombinant polypeptides with a N-terminal hexa-histidine tag. The recombinant allergens were purified by Ni-NTA (Qiagen, Hilden, Germany) and by high-resolution size exclusion chromatography.

Quantification of antibody binding

IgE, IgG₁, and IgG₄ dissociation-enhanced lanthanide fluoroimmunoassay (DELFA; Wallac, Turku, Finland) assays were used to

quantify specific antibody binding to individual allergens. The quantities of IgE, IgG₁, and IgG₄ were interpolated from a standard curve created with human/mouse anti-Der p 2 chimeric antibodies (Indoor Biotechnologies Ltd, Cardiff, United Kingdom) by the procedures of Schuurman et al.¹³

The IgE DELFIA assay is described in detail elsewhere.¹⁴ The IgG DELFIA assays were performed with minor modifications. The plates were blocked with 3% human serum albumin (Sigma, St Louis, Mo.) in 0.5% BSA (CSL, Melbourne, Australia), Tris-HCl, 0.05% Tween 20 buffer and the allergens diluted to 100 ng/mL in DELFIA assay buffer (Wallac). The chimeric standards were either the anti-Der p 2 chimeric IgG₁ (diluted 2.5-fold from 0.52 to 5000 ng/mL) or the anti-Der p 2 chimeric IgG₄ (0.52 to 5000 ng/mL). The biotinylated mouse-antihuman IgG₁ or IgG₄ mAb (BD Pharmingen) was diluted 1:2000 in assay buffer (Wallac). The lower limit of detection was approximately 10 ng/mL for IgG₁ and 1 ng/mL for IgG₄. The sensitivity and specificity of the IgG assays were determined by comparing the titration of the IgG₁ and IgG₄ chimeric anti-Der p 2 antibodies with titrations of patient sera as described by Schuurman et al.¹³ and by the reactivity to human myeloma IgG₁ and IgG₄ proteins (Bioscience International, Saco, Maine). The IgG binding could also be inhibited by the specific allergens. The isotype specificity was demonstrated by comparing the IgG₁ and IgG₄ assays measured with both IgG₁ and IgG₄ anti-Der p 2 antibodies (only the relevant reactivities were found).

Statistical analysis

Correlations between the antibody responses were analysed by Spearman correlation. Differences in the level of antibody binding by selected groups were compared with either the paired or independent Student *t* test (antibody levels were analyzed after log-transformations to normalize the data). All analyses were performed by using the SPSS 6.1 statistical software (Chicago, Ill).

RESULTS

IgE binding: Nonhospitalized subjects

In nonhospitalized subjects, the most prevalent IgE responses were found to Der p 1 (77%), Der p 2, and Der p 4 (74%; Table II). Der p 5, 7, and 20 had a midrange prevalence of 40%, Der p 8 and 10 had a prevalence of 25%, and Der p 3 bound appreciably lower at 9%. More marked differences were evident when the quantitative binding was considered. Der p 1 and 2 were the highest IgE binding allergens with an average of 36 and 50 ng/mL. Der p 4 was the next highest with 20 ng/mL, followed by Der p 5 and 7 at 15 and 16 ng/mL. The values for Der p 3, 8, 10, and 20 were quantitatively small with averages of 0.8, 2, 4, and 5 ng/mL.

The summation of the IgE binding (ng/mL) to each of the 9 allergens for each subject provides a measure of their total IgE binding to the specificities, the sum of the specificities (SOS). This value had a strong correlation with the CAP score for all of the groups with mite allergy. Fig 1 shows the correlation for all of the subjects with mite allergy combined. The defining contribution of the group 1 and 2 allergens to the level of the HDM titer is shown by the strong correlation between SOS and the sum of the Der p 1 and 2 titers across the entire range of reactivity (Fig 2). When the IgE binding to individual allergens of each donor was calculated as a percent of the SOS, the Der p 1 and Der p 2 accounted for an average of 21 and 27%, and the Der p 4, 5, and 7 were 15.1%, 6.8%, and 7.1% (Table II).

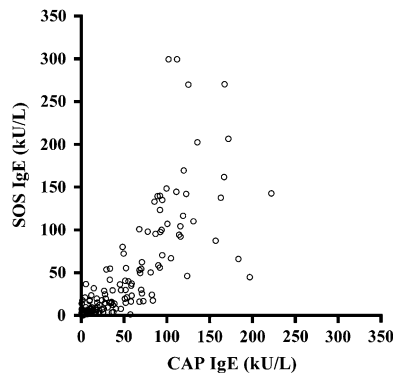
TABLE II. Summary of IgE binding†

	Der p 1	Der p 2	Der p 3	Der p 4	Der p 5	Der p 7	Der p 8	Der p 10	Der p 20
Nonhospitalized with allergy (n = 77)									
Mean IgE ng/mL	36.1*	50.1*	0.79	20.0*	16.2*	15.0*	2.12	4.62	5.24
	(20.8-51.4)	(29.2-70.9)	(0.24-1.33)	(12.5-27.6)	(5.60-26.8)	(5.29-24.8)	(0.59-3.66)	(5.60-26.8)	(3.01-7.46)
Prevalence‡	76.6	74.0	9.09	74.0	39.0	44.2	22.1	28.6	44.2
Mean % SOS	21.9	27.0	0.90	15.1	6.84	7.17	3.20	4.35	13.4
	(17.0-26.9)	(21.9-32.2)	(0.30-1.49)	(11.4-18.8)	(4.04-9.63)	(4.36-9.68)	(1.16-5.24)	(2.72-5.98)	(8.54-18.4)
Hospitalized with allergy (n = 105)									
Mean IgE ng/mL	66.6*	65.2*	1.16	9.42*	14.0*	12.4*	1.98	0.81	3.05*
	(41.2-91.9)	(39.2-91.2)	(0.71-1.61)	(4.20-14.6)	(3.14-24.9)	(2.13-22.7)	(0.17-3.79)	(0.40-1.21)	(0.54-5.55)
Prevalence	74.3	61.9	14.3	43.8	29.5	30.5	9.52	8.57	15.2
Mean % SOS	34.5	30.7	2.31	4.06	6.65	9.65	0.59	2.36	6.64
	(29.0-40.0)	(25.1-36.4)	(1.44-3.17)	(2.63-5.48)	(4.06-9.23)	(6.10-13.2)	(0.24-0.94)	(0.80-3.91)	(2.95-10.3)

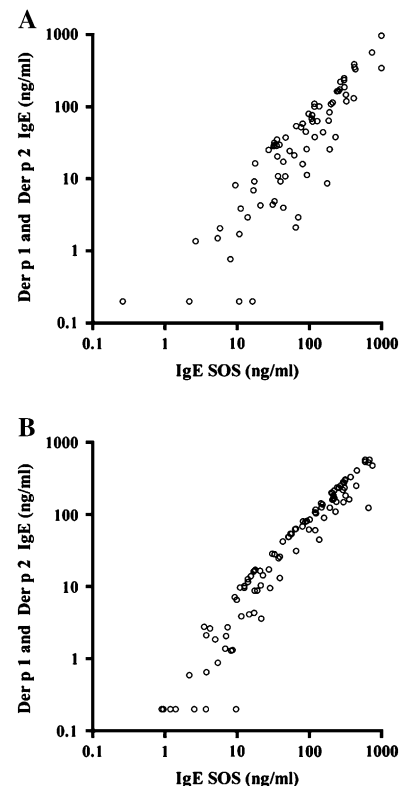
* $P < .001$, between-group (allergic/nonallergic) comparisons.

†Percent prevalence on the basis of the cutoff level of 2.24 ng/mL (equivalent to 1 kU/L).

‡The 95% CIs are in parentheses.

**FIG 1.** Linear correlation of IgE binding between the conventional CAP measurement to HDM extracts and the sum of the 9 individual allergen specificities (SOS). The results show individual values for all of the nonhospitalized and hospitalized subjects with mite allergy ($r = 0.8631$; $P < .001$). The SOS score was converted into kU/L by dividing the ng/mL value by 2.24 (ie, 1 kU/L = 2.2 ng/mL).

The Der p 3, 8, and 10 percentages were low. The anti-Der p 20 had a relatively high average value (13%) mainly because of responses in sera with very low overall titers. The SOS percentage, however, depends on both the binding to the allergen in question and the IgE binding to the other allergens. To consider the IgE binding of the different allergens relative to the major group 1 and 2 allergens, the IgE titer (ng/mL) was plotted against the sum of the Der p 1 and 2 values calculated for each serum. For the midrange allergens, Der p 4, 5, and 7, the correlation to the Der p 1 plus 2 binding was strong, showing that the percent of IgE binding was similar in sera with high or low binding to Der p 1 and 2 (Fig 3). The slopes of the relationships (β value) show that the contribution to the IgE binding for the midrange allergens was typically 10% to 15% of the binding to Der p 1 and 2. There was only very occasional strong IgE binding (to Der p 4) found when the IgE titers to Der p 1 and 2 were less than 10 ng/mL. The IgE binding to the Der p 3, 8, 10, and 20 allergens (which were typically low) did not have a linear

**FIG 2.** Correlation of IgE binding (ng/mL) between the SOS and the major Der p 1 and 2 allergens for each nonhospitalized ($r = 0.9002$; $P < .001$; A) and hospitalized ($r = 0.9721$; $P < .001$; B) mite-allergic donor.

correlation to Der p 1 plus 2 binding (data not shown). The few sera with high binding to either the group 3, 8, 10, or 20 allergens also had high titers to Der p 1 and 2.

IgE binding: Nonhospitalized children and adults

When stratified into children and adults (Fig 4), the sera from the nonhospitalized subjects showed children

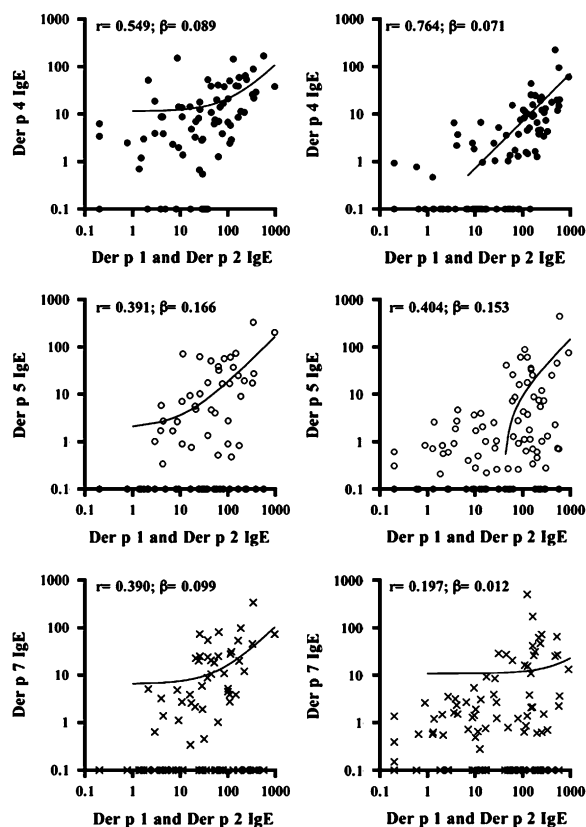


FIG 3. Correlation of IgE binding (ng/mL) between Der p 1 and Der p 2 and the midrange allergens Der p 4, 5, and 7 for the nonhospitalized (left panels) and hospitalized (right panels) subjects. The slope ($\beta = y/x - \alpha$) shows the relationship in IgE binding between the major and the midrange allergens. The correlation of binding to Der p 1 and 2 was statistically significant ($P < .001$ except for Der p 7 for the hospitalized subjects, where $P < .05$).

had a higher overall response to all of the allergens, particularly for Der p 1 and 2 ($P < .001$). However, the proportion of IgE binding to each allergen was the same as that found for the adults, except for Der p 20, for which the IgE titer was similar in absolute terms to the titer of the children, but low compared with the major allergens. The average IgE binding in the sera of children was 58 and 81 ng/mL for Der p 1 and 2.

IgE binding: Hospitalized children

The children recruited during an acute asthma exacerbation had similar IgE binding levels to the nonhospitalized subjects, with 67 and 65 ng/mL of anti-Der p 1 and 2, respectively and 9 to 14 ng/mL of the midrange allergens Der p 4, 5, and 7 (Table II; Fig 4). IgE binding to the other allergens was low. When each allergen was considered as a function of the binding to the major Der p 1 and 2 allergens (Fig 3), the same strong association was found for Der p 4 and 5, and a lesser one for Der p 7. The slopes of Der p 4 and 5 remained about 0.1 and 0.15, whereas the Der p 7 decreased to 0.012. Inspection of the graph shows that there were no large responses to Der p 4, 5, or 7 in the absence of large responses to Der

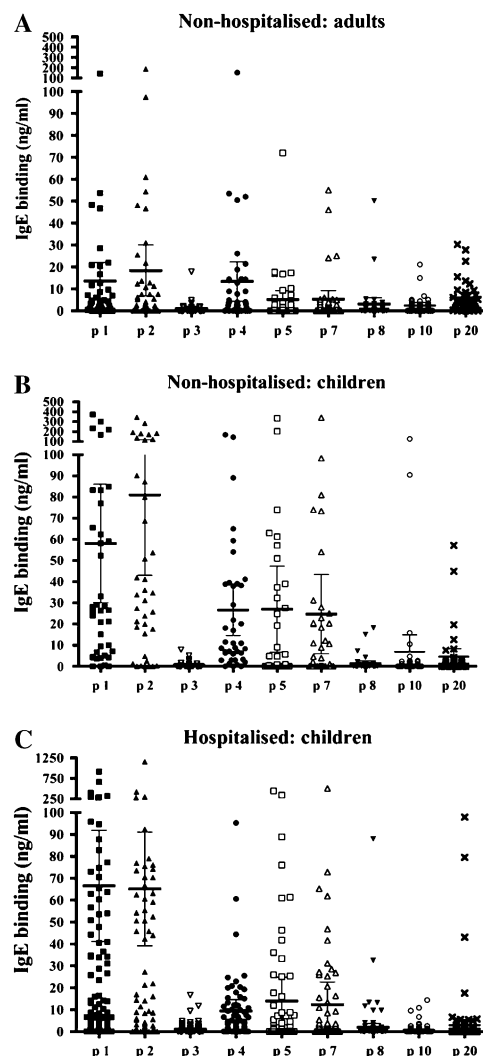


FIG 4. Quantification of IgE binding (ng/mL) to the mite allergens for the allergic nonhospitalized adults ($n = 38$; A) and children ($n = 39$; B) and for children hospitalized with acute asthma ($n = 105$; C). The mean and 95% CI are indicated.

p 1 and 2. Similarly, the highest responses to Der p 3, 8, 10, and 20 were typically in sera with much higher IgE binding to Der p 1 and 2 (data not shown). There were a few sera that showed IgE binding to Der p 20 without binding to Der p 1 and 2, but the titers were less than 10 ng/mL.

Although the IgE binding to Der p 20 was allergen-specific and the highest binding was found in the subjects with allergy, there was a high prevalence (48%) of low-titer IgE binding (6.5 ng/mL) in the subjects without allergy (data not shown).

IgG₄ binding

The IgG₄ binding in the nonhospitalized children showed a similar pattern to the IgE binding, although not all sera with IgE antibody had IgG₄. Sera from subjects without allergy rarely had IgG₄ to any allergen. Der p 1 and Der p 2 were the major binders of IgG₄ and bound at a prevalence of 72% at an average of 521 and 645 ng/mL

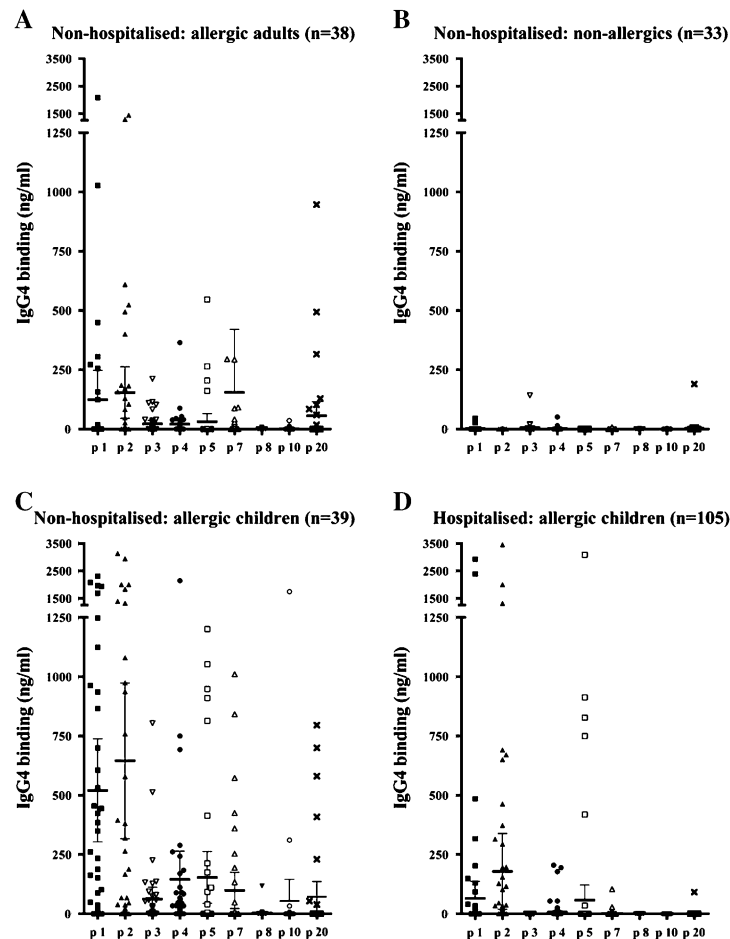


FIG 5. IgG₄ binding for the nonhospitalized adults with allergy (A), subjects without allergy (B), children with allergy (C), and hospitalized children with allergy (D). The mean and 95% CI are indicated.

(Fig 5). However, there was no correlation in binding between IgG₄ and IgE, and the slopes for Der p 1 and 2 were negative ($\beta = -0.557$ and -0.558). The midrange allergens Der p 4, 5, and 7 showed the next highest IgG₄ binding (100-150 ng/mL). The IgG₄ binding in the non-hospitalized adults was less prevalent and lower than that found for the children. The prevalence for Der p 1 and 2 was 29% and 45%, and the titers were 124 and 154 ng/mL ($P < .001$). There was little IgG₄ binding to the other allergens in the adults with or without allergy. In comparison with the nonhospitalized children, the sera from children from the emergency department showed much lower IgG₄ antibody responses ($P < .001$). The prevalence of IgG₄ to Der p 1 and 2 was 14% and 25% with an average of 65 and 179 ng/mL.

IgG₁ binding

The IgG₁ antibody was also examined, with similar results (data not shown in detail). It bound Der p 1 and 2 in 69% and 51% of sera from nonhospitalized children with allergy (at an average of 14,596 and 5131 ng/mL). Only 7% of the sera from the emergency department patients had detectable IgG₁ anti-Der p 1 and 2 antibodies ($P < .001$).

IgG₁ antibodies to Der p 1 and 2 were detected in 18% of adults at low titers (2008 and 100 ng/mL), which were lower than the titers for the nonhospitalized children ($P < .001$). The prevalence of binding to Der p 4 was high (76% to 97%) for all of the groups with allergy, but there was little or no binding to the other allergens. The children and adults without allergy rarely had IgG₁ binding to Der p 1 and 2 or to the other allergens, but had low levels of binding to Der p 4 at a prevalence of 45%.

DISCUSSION

These quantitative antibody studies with a panel of 9 HDM allergens showed that the Der p 1 and 2 allergens accounted for half the IgE response in hospitalized and nonhospitalized subjects and in low and high responders. Children had higher responses than adults in both the hospitalized and nonhospitalized population, but children admitted to hospital with acute asthma had greatly decreased IgG antibodies. The same pattern of IgE binding to the different allergens was found for the hospitalized children and nonhospitalized children, with little

difference in the levels of IgE binding. Importantly, the children with asthma requiring admission to an emergency department did not have IgE binding to a more complex range of allergens.

The combined IgE binding calculated from the individual binding to the 9 HDM allergen specificities (SOS) correlated well with the CAP measurement to HDM extracts. This is in accord with studies that have shown that the allergens used in the panel could absorb out the IgE antibody binding reactivity of almost every allergen specificity of HDM extracts as demonstrated by 2-dimensional electrophoresis.¹⁵ The use of a panel in contrast to extracts allows the binding to each specificity to be measured in standard and optimal conditions, especially with respect to the concentration of allergen.

Der p 1 and Der p 2 accounted for an average of 65% of the IgE binding specificities in the hospitalized children with allergy and 50% to 55% of the binding in the nonhospitalized children and adults with allergy. The next most important allergens were Der p 4, 5, and 7, with considerably smaller contributions from Der p 3, 8, 10, and 20. When considered as a fraction of the total response, each of the midrange allergens typically contributed an average of 10%. They were the highest specificities for 25% of the nonhospitalized subjects and 13% of the hospitalized patients, but these subjects still had high responses to either Der p 1 or 2. The average values of IgE for Der p 4, 5, and 7 were 15 to 20 ng/mL, compared with 36 and 50 ng/mL for Der p 1 and 2. The IgE binding to cat and cockroach allergens, however, has an average titer for the Fel d 1 and Bla g 5 of only 15 ng/mL and 17 ng/mL, so the binding to the midrange HDM allergens could be just as important.^{16,17} The results indicate that a combination of the major and midrange allergens would be an effective formulation for immunotherapy, but it might also be expected that the large responses to Der p 1 and 2 could regulate the entire allergen response. The low values for the tropomyosin allergen Der p 10 were expected from previous studies in a European population,¹⁸ but high values have been found in African and Asian populations, suggesting that other sources of allergen could induce responses to this highly conserved allergen. The relative importance of the group 3 allergens has been controversial. It is clear that Der p 3 is poorly allergenic. Der p 20 had a negative correlation with Der p 1 and 2 but correlated well with Der p 10. It could account for as much as 100% of the SOS of a few subjects who were weakly allergic, showing a low sum of the specificities (below 10 ng/mL).

Previous reports have shown the IgG₄ anti-Der p 1 is found only in sera from individuals with mite allergy, although studies with solid-phase assays have reported the presence of IgG₄ against Der p 2 in nonallergic sera.¹⁹ The results here show that the IgG₄ antibody is both limited to the sera from allergic subjects and limited to the major allergens or the responders to the midrange allergens. Similar results were found for the IgG₁ antibodies, so IgG antibody in general is a marker of allergenicity of an allergen. The high IgG₁ shows a T_H1 component of

allergen sensitization, as also indicated by the T_H1 cytokine responses to HDM allergens in T-cell studies.²⁰⁻²² The less important allergens did not induce IgG₄, so for HDM allergy, allergenicity correlates with immunogenicity rather than a response deviated away from IgG. Mite allergy is therefore different from cat allergy, in which T_H2-dependent IgG₄ is frequently observed in the absence of IgE, and this was found for all allergens.²³

The lower and less prevalent IgG antibody response found in the hospitalized children are of interest. IgG is known to block mast cell degranulation and IgE-mediated allergen presentation. Competition assays (not shown) have demonstrated that the strength of IgG₁ and IgG₄ antibody binding is similar or higher than that found for IgE, so it could successfully compete with IgE for the allergen and the FcεRI receptor on mast cells. An alternative or adjunct to a protective hypothesis is that, as shown by the lower responses of adults in this study, the IgG is transient, and continual responses to HDM allergens favor IgE. This would be consistent with the aged response of adults, and the low IgG in the emergency department population would represent a more rapid progression to down-regulation in highly allergic people.

In summary, the antibody studies using a 9-allergen panel for *D pteronyssinus* have demonstrated the predominance of the Der p 1 and 2 allergen specificities in subjects with allergy, including children hospitalized for acute asthma. The midrange allergens Der p 4, 5, and 7 bound IgE antibody at titers that were typically 10% of the major allergens. Both IgG₁ and IgG₄ antibodies were found and were shown to be largely restricted to the major and midrange allergens in the nonhospitalized children. The paucity of IgG in children admitted to an emergency department for asthma reveals an interesting avenue for further research.

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