

Prevalence of IgG Anti-Der p 2 Antibodies in Children from High and Low Antigen Exposure Groups: Relationship of IgG and Subclass Antibody Responses to Exposure and Allergic Symptoms

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Serum IgG PAN, IgG1-4, and IgE antibodies (Ab) specific for the house dust mite *Dermatophagoides* antigen (Ag) Der p 2 were measured by enzyme-linked immunosorbent assay (ELISA) in two groups of school children, age 12–14, exposed to high (Charlottesville, VA) and low (Los Alamos, NM) mite Ag levels in their environment. More than 90% of the children were found to have Der p 2-specific IgG antibodies, although the levels of both IgG PAN and IgG 1-4 Ab were substantially higher in the high exposure group ($P = 0.001$). In addition, there was considerable overlap between these two groups in all Ab measurements. The presence of IgG anti-Der p 2 Ab in the Los Alamos children was unexpected and suggests continuing Ag exposure, although the source of such exposure is not apparent. There is no correlation between RAST (+) and RAST (–) subjects with respect to the level of IgG Ab measured by ELISA. These results suggest that the IgG Ab response to house dust mite has been underestimated in the RAST negative population. Two twin pairs were included in this study and the divergent and varied Ab responses in these individuals indicate that factors in addition to environmental exposure and genetic susceptibility require further investigation and provide evidence for the complexity of the pathogenesis of the allergic response. © 1998 Academic Press

Key Words: IgG antibody; allergen; enzyme immunoassay.

INTRODUCTION

Antigens from the house dust mite species *Dermatophagoides* are potent immunogens and have been shown to elicit IgE and IgG antibody (Ab) and specific T cell responses in house dust mite allergic individuals. The humoral and cellular immune responses to these antigens can now be studied in detail as eight house dust mite allergens have been purified, biochemically

characterized, cloned, and sequenced (1, 2). The Group 2 *Dermatophagoides* allergens (Der p 2 and Der f 2) are soluble, 14-kDa nonglycosylated proteins and share 85% amino acid sequence homology. Eighty to 90% of house dust mite allergic individuals have specific IgE and IgG Ab to the Group 2 proteins (3). Antibody responses are directed by cytokines and surface signals provided by allergen-specific T cells. T cells from atopic as well as nonatopic subjects have been shown to respond to Der p 2, suggesting that an immune response to these environmental antigens is generated regardless of atopic state (4–7). Clonal analysis of T cells from atopic and nonatopic donors reveals predominately the TH2 subset, that is, these clones produced IL-4 and little or no IFN- γ (8, 9). Other studies using population responses demonstrate that all T cell phenotypes can be recovered and the levels of IL-4, IL-5, and IFN- γ are unique to each Der p 2-specific T cell line and do not correlate with asthma, rhinitis, or atopic dermatitis (10). A recent study by Upham *et al.* using serum free media demonstrated proliferative responses to Der p 2 in 95% of the subjects tested, and the magnitude of these responses was not different between atopic and nonatopic individuals. These authors suggest that qualitative rather than quantitative differences in this T cell response distinguish the atopic state (4).

Many investigators have documented the IgG component of the immune response to inhalant as well as food allergens (11–20). The role of IgG PAN and IgG1-4 Ab response to house dust mite allergens in the pathogenesis of asthma and atopic dermatitis is poorly understood. Studies by Tame *et al.* and Oshika *et al.* examined the IgG Ab responses to Der p 2 in mite allergic and nonallergic subjects using enzyme immunoassays (21, 22). Measuring Ab to native Der p 2, Oshika *et al.* found approximately twofold higher IgG PAN and IgG1-4 Ab levels in patients with bronchial asthma compared to nonallergic subjects (21). Tame *et al.*, using a recombinant Der p 2 fusion protein, found higher levels of IgG4 Ab, but not IgG PAN or IgG1-3 Ab, in allergic children compared to controls (22).

To address these discrepancies and to further inves-

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tigate the prevalence and magnitude of IgG PAN and IgG1-4 Ab responses to the mite allergen Der p 2, we compared Der p 2-specific antibody levels among two closely age-matched populations: middle school children from an area of high dust mite exposure (Charlottesville, VA) and middle school children from an area of low house dust mite exposure (Los Alamos, NM). The results show that IgG, IgG1, and IgG4 Ab responses to Der p 2 occur in >90% of the children from the high exposure group, both RAST (+) and RAST (-), as well as in the majority of children from the low exposure group. The magnitude of these responses is significantly greater in the high exposure group compared to the low exposure group. These results suggest that previous studies have underestimated the IgG Ab response to mite allergens, especially in the RAST (-), house dust mite-exposed, population. The persistence of the IgG Ab response in the low exposure group suggests that even low levels of Ag can stimulate an immune response.

MATERIALS AND METHODS

Subjects

The sera used in this study were obtained from patients, age 12–14 years, enrolled in separate studies that have been reported previously (23–26). From the subjects enrolled in the Henley Middle School study in central Virginia (high exposure group), 47 sera were available: 16 sera from subjects that were skin test positive to *Dermatophagoides* extract, 31 sera from skin test-negative subjects. From the subjects enrolled in the Los Alamos, New Mexico school study (low exposure group), 29 sera were studied: 13 sera from subjects skin test positive to *Dermatophagoides* extract, 16 sera from skin test-negative subjects. None of the subjects were receiving immunotherapy at the time serum was collected. Serum RAST to *Dermatophagoides* was determined as previously described (27). Among the children enrolled in the Los Alamos, New Mexico study, 10 of 111 subjects had positive RAST to *Dermatophagoides pteronyssinus* extract, using ≥ 20 U/ml as a cutoff. Using a lower threshold, a total of 41 subjects had detectable IgE Ab (range, 2–505 U/ml; mean RAST score, 43 U/ml). The range of RAST values among the children in the Charlottesville, Virginia study was 2–1440 U/ml, mean = 344 U/ml. The assay limit of detection for IgE Ab to *D. pteronyssinus* extract was 2 U/ml, and this value was selected as the lower limit for RAST (+) classification. Selected sera were reevaluated by ELISA for IgE Ab to Der p 2 and the results were in good agreement with the RAST values (data not shown).

Natural and Recombinant Der p 2

Natural Der p 2 was isolated by monoclonal antibody (α DpX) affinity chromatography, followed by gel-filtration

chromatography, from saline-solubilized spent mite culture media, kindly provided by Dr. Larry Arlian, Wright State University, Ohio. The recombinant Der p 2 used in this study was produced from a bacterial expression system, developed in our laboratory, based on the pET vector from Novagen (28). Recombinant material was purified as described above and protein purity of the natural and recombinant Der p 2 was assessed by SDS-PAGE using silver nitrate staining. Protein concentration was determined by Bradford assay or by absorbance at 280 nm using the calculated extinction coefficient of 0.7. Purified Der p 2 and rDer p 2 were stored in PBS at -20°C .

Antibodies

Goat anti-human IgG PAN (Fc specific) was purchased from Zymed Laboratories, Inc. (South San Francisco, CA) and used at 5 $\mu\text{g}/\text{ml}$. The human anti-IgG subclass antibodies were generated from the cell lines HP6002 (anti-IgG2), HP6047 (anti-IgG3), and HP6025 (anti-IgG4), purchased from ATCC, and were affinity purified from ascites fluid (29, 30). The monoclonal antibody from cell line HP6069 (anti-IgG1) was purchased from Zymed Laboratories, Inc. The secondary antibodies used in these studies were goat anti-mouse IgG and goat anti-human IgE, conjugated to alkaline phosphatase (Southern Biotechnologies). The secondary antibodies were absorbed against human and bovine serum before use. The myeloma proteins used for subclass specificity assays were a gift from Alan Soloman, University of Tennessee, Knoxville.

Immunoassays

Determination of specificity of subclass Ab. Immulon IV microtiter plates (Dynatec) were coated with subclass-specific mAb at 5–20 $\mu\text{g}/\text{ml}$, 50 $\mu\text{l}/\text{well}$ in carbonate buffer, pH 9.6, overnight at 4°C . Plates were washed with PBS containing 0.05% Tween 20 (PBS-T) and unreacted sites were blocked with 200 $\mu\text{l}/\text{well}$ of PBS-T, 5% BSA for 2 h at room temperature. Plates were washed and subsequently incubated with increasing concentrations (0.001–1 $\mu\text{g}/\text{ml}$ in PBS-T, 1% BSA) of biotinylated IgG subclass myeloma reference proteins. The assay was developed by incubating the plates with streptavidin-alkaline phosphatase conjugate for 2 h at room temperature and then adding the substrate *p*-nitrophenyl phosphate (pNPP). The results were evaluated at an OD of 405 nm using a Titertek multiscan plate reader.

Der p 2-Specific IgG PAN and IgG1-4 Subclass ELISA

Immulon IV microtiter plates (Dynatec) were coated with rDer p 2 (50 $\mu\text{l}/\text{well}$ of 5 $\mu\text{g}/\text{ml}$ solution) diluted in carbonate buffer, pH 9.6. Plates were incubated over-

night at 4°C and then washed four times with PBS-T. Wells were filled with wash buffer and left at room temperature for 10 min and decanted, and then nonreacted sites were blocked by the addition of 200 μ l/well of PBS-T containing 5% BSA for 2 h at 37°C. After blocking, the wells were washed four times with PBS-T, filled with PBS-T after the last wash, and left at room temperature for 10 min and then decanted. Serum was added (50 μ l/well), diluted in PBS-T containing 1% BSA, and plates were incubated for 2 h at 37°C. Typically, serum was diluted 10-fold through 1/1000 for IgG PAN, IgG1, IgG2, IgG3, and IgG4 Ab assays. After incubation with serum samples, plates were washed four times, left to sit in PBS-T for 10 min at room temperature, and then processed with anti-subclass mAb followed by goat anti-mouse IgG-alkaline phosphatase-conjugated secondary antibody. The reaction was developed with substrate and evaluated as described above. Results were expressed as arbitrary units per milliliter of serum (units/ml) and calculated from a control curve from a serum (subject M.S.) that was designated as containing 1000 units/ml of IgE anti-Der p 2 Ab, 1000 units/ml of IgG PAN anti-Der p 2 Ab, and 1000 units/ml of IgG1-4 anti-Der p 2 Ab. Microtiter wells coated with control antigens (PBS/BSA diluent and recombinant staphylococcal nuclease) were processed as above to assess nonspecific binding.

Competitive Inhibition ELISA

The antigen specificity of the IgG PAN and IgG1-4 assays was determined using a competitive inhibition assay. Increasing concentrations of Der p 2, rDer p 2, or tetanus toxoid (negative control) were mixed with a predetermined serum dilution to yield a final Ag concentration of 0.0001–100 μ g/ml. The Ag-serum mixtures were incubated overnight at 4°C and then pipetted in duplicate into a microtiter plate previously coated with rDer p 2. The assay was processed as described above and the results were expressed as percentages inhibition of the reaction in the absence of inhibitor Ag (i.e., PBS/BSA diluent only).

Antibody Production from Lymphocytes Stimulated in Vitro with IL-4 and Anti-CD40

B cells were purified from the peripheral blood of atopic and nonatopic donors as previously described (31–33). Briefly, PBMC from 100 ml of heparinized whole blood were isolated over Ficoll–Hypaque. T cells were depleted by E-rosetting and the B cells were cultured at 2×10^5 cells/ml in complete RPMI (10% FCS) at 37°C in 5% CO₂ for 12 days. Cultures were prepared in triplicate in 96-well plates in the presence or absence of IL-4 (100 U/ml, Hoffman–La Roche) and the anti-CD40 mAb 626.4 (10 μ g/ml) (31). After 12 days, culture supernatants were harvested and the presence of IgE, IgG, and IgG4 Ab was determined by ELISA essentially

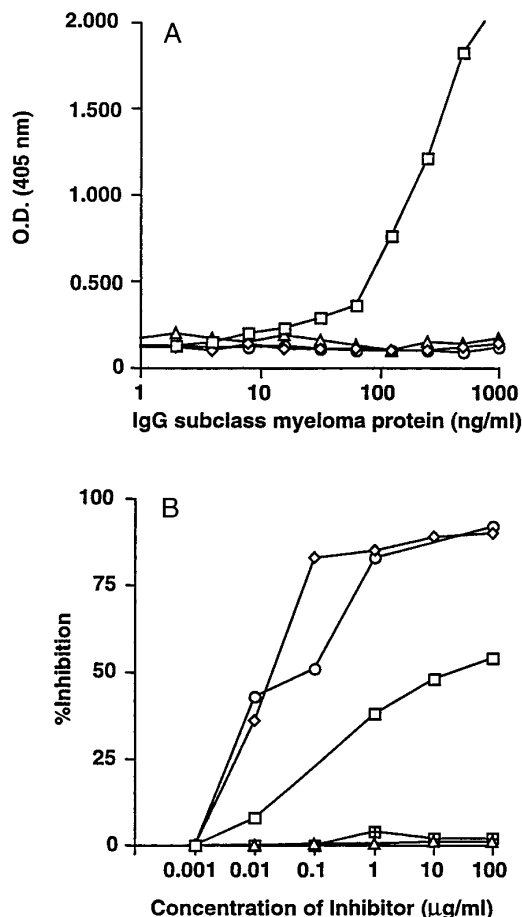


FIG. 1. Immunoglobulin subclass and antigen specificity of ELISA. A shows the results of IgG1 subclass myeloma proteins (□) binding to a microtiter plate coated with the anti-IgG1 monoclonal antibody HP6069. Myeloma proteins of IgG2 (◇), IgG3 (○), and IgG4 (△) subclass failed to bind HP6069-coated plates. In B, increasing concentrations of Der p 2 inhibit the binding of serum from patient VP to a Der p 2-coated microtiter plate. The assay was developed with a secondary monoclonal antibody specific for IgG1 (◇), IgG4 (○), or a polyclonal anti-IgGPAN (□). Tetanus toxoid, used as a negative control, failed to inhibit IgG1 (△) or IgG4 (□) binding to Der p 2.

as described above. Parallel cultures were established using 5×10^5 /ml PBMC in the presence or absence of IL-4 and mAb 626.4 as described above.

RESULTS

Specificity of the Human IgG1-4 ELISA and the ELISA for Der p 2-Specific Ab

The specificity of the mAb to human IgG subclasses used in this study was determined. The ability of biotinylated subclass myeloma proteins to bind to subclass-specific mAb-coated microtiter plates was measured by ELISA. Figure 1A shows the binding curve for IgG myeloma proteins on an anti-IgG1 mAb HP6069-coated plate. Only the IgG1 subclass myeloma proteins

bound in a dose-dependent manner in this assay. No cross-reactivity was seen with the other subclass myeloma proteins. The IgG2, IgG3, and IgG4 subclass mAb showed complete specificity for the appropriate subclass myeloma protein (data not shown).

The antigen specificity of the ELISA for IgG PAN, IgG1, and IgG4 anti-Der p 2 was demonstrated by competitive inhibition with free Der p 2. Representative results from one of the eight sera tested are shown in Fig. 1B. The IgG, IgG1, and IgG4 binding could be inhibited in a dose-dependent manner using soluble Der p 2 or rDer p 2, and all three assays gave parallel inhibition curves. The polyclonal anti-IgG PAN gave overlapping dose-dependent binding curves with the monoclonal antibody HP6017 (anti-IgG PAN) in this system (data not shown). Similar inhibition was also seen in the IgG2 and IgG3 systems (data not shown). Tetanus toxoid and PBS/BSA diluent, used as control antigens, showed no inhibition. The results demonstrate that the assay system was specific for IgG subclass and for the antigen Der p 2.

IgG PAN and IgG1-4 Titers to Der p 2

Figure 2 shows representative results from one of four experiments measuring IgG PAN, IgG1, and IgG4 antibody to Der p 2. Similar results were obtained for IgG2 and IgG3 subclass antibodies (data not shown). The results are presented as units/ml of Der p 2-specific antibody, calculated from a control curve plotted from OD_{405 nm} values obtained using a serum (subject M.S.) arbitrarily assigned 1000 units/ml of Der p 2-specific IgG PAN and IgG1-4. The OD_{405 nm} on PBS/BSA-coated wells was 0.1–0.14 for each assay.

Der p 2-specific IgG PAN Ab was detected in both RAST (+) and RAST (–) subjects from the high exposure group and no significant difference in the mean values was obtained for IgG levels (Fig. 2A). The RAST (+) individuals had slightly higher IgG1 Ab to Der p 2 compared to the RAST (–) individuals: The mean IgG1 level was 736 units/ml in the RAST (+) group vs 716 units/ml in the RAST (–) group, but this difference was not statistically significant (Fig. 2B). The mean IgG4 level was 1283 units/ml for the RAST (+) group vs 869 units/ml for the RAST (–) group; however, this was not a significant difference ($P = 0.08$, Fig. 2C). In each case there is an extensive overlap between the two populations.

In the low exposure population (Fig. 3), the values were significantly lower than the high exposure group, $P < 0.001$: for example, the range of values for IgG PAN Ab was 170–12,500 units/ml in the low exposure group vs 170–29,000 in the high exposure group. IgG PAN Ab to Der p 2 was measured in 19/28 subjects in the low exposure group and IgG1 in 18/28 subjects (Figs. 3A and 3B, respectively). The values for IgG4 Ab were <30 units/ml in the majority of low exposure

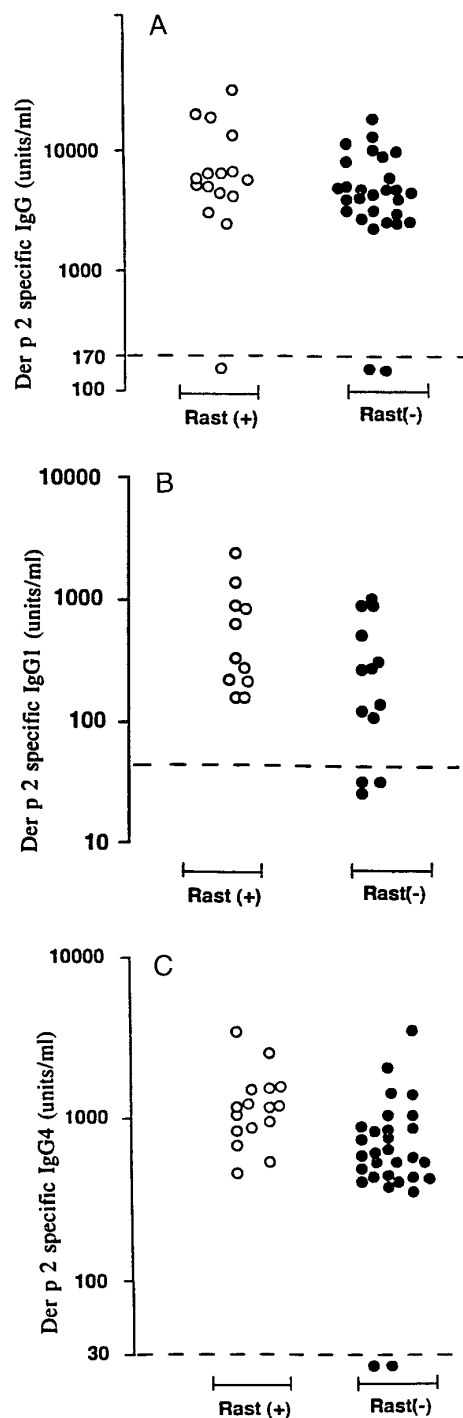


FIG. 2. Serum IgG and subclass Ab levels in middle school children from an environment of high house dust mite exposure. Serum Ab levels were measured by ELISA as described under Materials and Methods and as illustrated in Fig. 1B. RAST (+) subjects are shown by open circles and RAST (–) subjects are shown by closed circles. A shows results of Der p 2-specific IgG PAN; B shows IgG1 Ab; and C shows IgG4 Ab. The dashed line indicates the sensitivity of each assay.

subjects (Fig. 3C). There was no significant difference in mean values between the RAST (+) and RAST (–) groups for IgG PAN and IgG1 Ab. IgG4 Ab was mea-

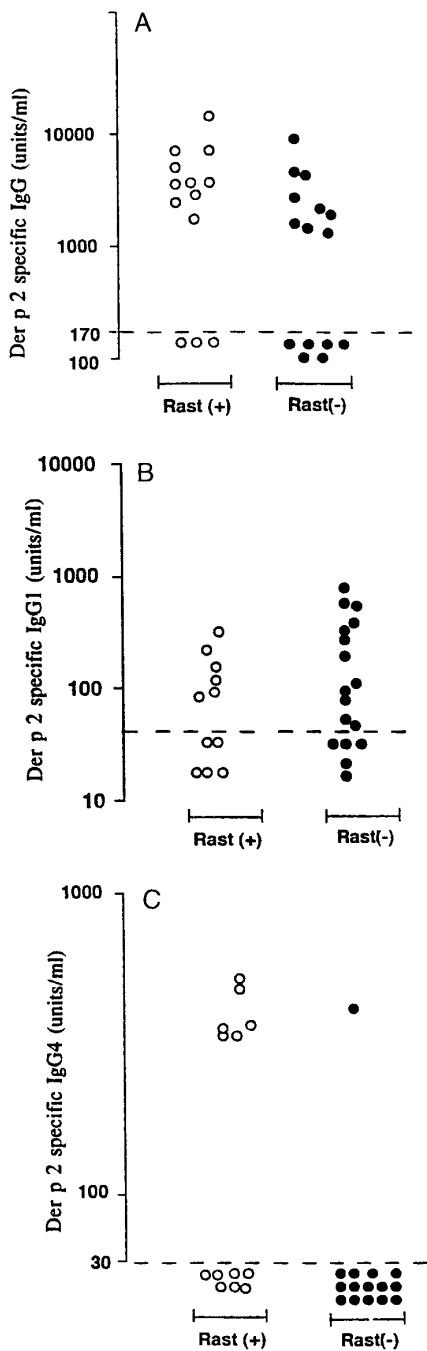


FIG. 3. Serum IgG and subclass Ab levels in middle school children from an environment of low house dust mite exposure. Serum Ab levels were measured as described for Fig. 2.

sured in 6/13 in the RAST (+) group and only 1/15 in the RAST (–) group (Fig. 3C).

The levels of IgG, IgG1, IgG4, and IgE Ab specific for Der p 2 of selected subjects are shown in Table 1. There was no correlation between IgG level and positive RAST score. It is of special interest to note the divergence of the responses to Der p 2 in subjects H23 and H24. They are fraternal twins who have the same

exposure to environmental allergens. Yet, one is atopic while the other is not. H33 and H34 are identical twins. Their levels of Ab specific for Der p 2 have significant differences. It appears that H34 has much higher IgG and IgE Ab. The data on these twins, while anecdotal, illustrate that both genetic and environmental factors interplay in allergic responses.

Taken together, the results shown in Figs. 2 and 3 and Table 1 suggest that the majority of individuals make IgG PAN and IgG1-4 subclass Ab responses to Der p 2, regardless of their atopic state. As would be expected, those individuals exposed to higher environmental levels of house dust mite allergen have higher Der p 2-specific IgG antibody levels. In addition, only those subjects exposed to high levels of allergen had readily measurable Der p 2-specific IgG4 Ab.

In Vitro Ab Production by Lymphocytes from Atopic and Nonatopic Donors

The serum IgG4 antibody levels in RAST (–) subjects shown in Fig. 2C suggest that levels of IgE Ab and IgG4 Ab are not correlated. To investigate the ability of B cells to produce these antibody isotypes, short-term lymphocyte cultures were established in the presence or absence of IL-4 and anti-CD40 mAb. Table 2 shows results from representative experiments of two subjects. PBMC from Subject A [RAST (–)] could be induced to produce low levels of both IgE Ab and IgG4 Ab when cultured in the presence of IL-4 and anti-CD40 mAb, while under the same culture conditions partially purified B cells produced IgG4 Ab but no IgE Ab. PBMC from Subject B [RAST (+)] produced similar levels of IgE Ab when cultured in the presence of IL-

TABLE 1
Antibody Levels in Subjects from Environments with High and Low Dust Mite Levels

<i>D.pt.</i> RAST (U/ml)		Der p 2-specific antibody (units/ml) ^a			
		IgE	IgG	IgG1	IgG4
High exposure subjects					
H23	480	2,150	2,200	248	500
H24	<2	<16	1,550	33	<30
H33	185	450	2,050	180	360
H34	820	2,375	7,000	832	1,250
Low exposure subjects					
LA109	16	412	12,500	112	320
LA20	<2	<16	7,800	400	430
LA39	58	210	6,900	25	<30
LA23	<2	<16	4,150	64	<30
LA24	<2	<16	2,600	128	<30

^a Der p 2-specific Ab was measured by ELISA as described under Materials and Methods.

TABLE 2

Antibody Production by Lymphocytes Stimulated with Anti-CD40 mAb and IL-4

	IgE (ng/ml)		IgG4 (ng/ml)	
	–	+	–	+
Anti-CD40 ^a				
Subject A				
I. Medium	<0.6	<0.6	<2.6	<2.6
IL-4	1.2	63.0	<2.6	24.0
II. Medium	<8	<8	<40	<40
IL-4	<8	<8	<40	1750
Subject B				
I. Medium	<1.2	<1.2	<20	<20
IL-4	<1.2	70.0	<20	138
II. Medium	<2.0	<2.0	<12	18
IL-4	<2.0	269	21	1150

^a The anti-CD40 mAb 626.4 was added to 5×10^5 /ml PBMC (I) or 2×10^5 /ml B cells (II), in the presence or absence of 100 U/ml of IL-4. Culture supernatants were assayed by ELISA after 12 days.

4 and anti-CD40 mAb and higher levels of IgG4 Ab compared to Subject A. Addition of Der p 2 to the cultures did not alter the production of IgE Ab or IgG4 Ab (data not shown). These results demonstrate that production of IgE Ab and IgG4 Ab is not always coupled and support the finding of serum IgG4 Ab found in the RAST (–) subjects.

DISCUSSION

The present study reports Der p 2-specific IgG and subclass Ab responses in two populations of middle school children, ages 12–14. These two study groups were recruited for an epidemiological study reported by Sporik *et al.* and Squillace *et al.* (23, 26). Previous studies have established that a level of $>2 \mu\text{g}$ of the Group 1 allergen Der p 1 per gram of house dust is correlated with the risk of asthma (27, 34). Ninety-four percent of the homes of the school children in Charlottesville, Virginia were found to have Der p 1 allergen levels above $2 \mu\text{g/g}$, while only 4% of the homes of children in Los Alamos, New Mexico had Der p 1 levels above $2 \mu\text{g/g}$. Previous studies showed a good quantitative correlation between Der p 1 and Group 2 allergen levels in house dust; thus, we assume that these two study populations are representative of high and low exposure to Der p 2 (35). Der p 2 was chosen for the development of the subclass-specific ELISA because of the availability of recombinant Der p 2 that shows comparable immunoreactivity to the natural allergen (28).

In keeping with this level of exposure to dust mites, only 9% of the children in the Los Alamos study had positive RAST to house dust mite extract. In contrast, 34% of the children enrolled in the Charlottesville, Virginia study were RAST (+) to mite extract (23, 26). In this study, we report that Der p 2-specific IgG Ab could

be detected in $>90\%$ of the subjects in the high exposure group and there was no significant difference in IgG Ab levels between RAST (+) and RAST (–) subjects. However, there was a trend toward higher IgG1 and IgG4 levels, although this difference did not reach statistical significance. For all Ab measurements, there was a large overlap between the two groups. As would be expected, the IgG PAN and subclass Ab levels among the low exposure subjects were significantly lower than those of the high exposure subjects, whether comparing RAST (+) or (–) or the combined groups.

The presence of IgG anti-Der p 2 Ab in the low exposure group would indicate continuing Ag stimulation in these children. It may reflect allergen exposure of these subjects outside the home or may be due to exposure to cross-reacting allergens from other mite species such as storage mites. Few studies have addressed mite fauna of house dust in arid regions; however, among the Group 2 homologues identified to date, the proteins show extensive immunologic cross-reactivity (Smith and Chapman, 1996, unpublished observations).

Previous studies demonstrated IgG Ab in only 30% of nonatopic subjects and some studies failed to detect an IgG Ab response (36–38). This may be due to differences in the assay technique; RIA such as that used by Chapman and Platts-Mills measures high-affinity interactions, whereas the ELISA used in this study is able to detect lower affinity Ab (36). The high antigen density of solid-phase assays may reveal these low-affinity interactions that are not detected at the low antigen concentrations used in liquid-phase assays. Our results suggest that previous reports underestimated the IgG Ab response in RAST (–) subjects, in keeping with the findings of Upham *et al.* and O'Hehir *et al.* who showed Der p 2-specific T cell responses in nonatopic subjects (4, 6).

Age-related influences on the Ab response may also contribute to the different findings of other investigators. Children in the early teen years show peak total IgE and IgG levels, and the 12- to 14-year old middle school children in this study fall directly within this age group (39, 40). Rawle *et al.* documented a decrease in mite-specific IgG and IgE antibody with age and it will be of interest to follow this study population prospectively (41).

This study demonstrates that IgG Ab to a major house dust mite allergen can be detected in the majority of subjects in a closely age- and exposure-matched group, and this IgG Ab response is not correlated with RAST score. In addition, the results demonstrate an IgG4 Ab response in RAST (–), exposed individuals, suggesting that additional factors other than the TH2 cytokines also participate in the development of an allergen-specific IgE Ab response. van Milligen *et al.* have demonstrated a difference in epitope specificity between IgG4 and IgE Ab to the major cat allergen Fel d 1 and suggest that these Ab responses arise from

distinct B cell populations (42). Using the assays developed here, studies of the fine specificity of IgG Ab responses to well-defined allergens such as Der p 2 in RAST(+) and RAST(−) individuals will help elucidate the relationship of this response with other factors that influence the outcome of allergen exposure including the role of antigen-presenting cells, Fc-receptors, and T cell subsets. At another level, we have observed several individuals who have strong Der p 2-specific IgE and IgG4 Ab levels without any allergic symptoms. This observation indicates that end-organ resistance may be responsible for the lack of clinical symptoms. End-organ resistance has been demonstrated in the NOD mouse model of diabetes mellitus (M. McDuffie, personal communication) and this factor may play a dominant role in the development of allergic disease. In this regard, the role of Fcε and Fcε receptor polymorphisms should be considered (43).

The data on the two twin pairs who have exposure to high levels of dust mite allergens, although anecdotal, are of special interest. One of the fraternal twins was atopic, with positive skin test and RAST score, while the other was nonallergic, with no evidence of a Der p 2-specific IgE Ab response. Recently, it has been reported that infants less than 1 year of age are able to mount an IgG1 response to dust mites while they lack an IgG4 response due to the immaturity of the human immune system (17, 40, 41). In this population, some children will develop an allergic response to dust mites, while others will not despite their continuing exposure to high levels of allergen.

Each of the identical twins had positive skin tests and RAST scores to dust mite. However, only one (H34) had allergic symptoms. He had much higher levels of Der p 2-specific IgE and IgG4 Ab, suggesting that subtle differences in environmental exposure among these two individuals may be responsible for the differing levels of Der p 2 Ab. An additional factor that deserves further investigation is the role of respiratory tract infection as a secondary component in determining the manifestation of allergic disease. Studies on further twin pairs using the assays developed here could provide additional insights into the factors that determine the development of allergic disease.

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