

Evaluation of household dust mite exposure and levels of specific IgE and IgG antibodies in asthmatic patients enrolled in a trial of immunotherapy

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Background: Monitoring the response to immunotherapy entails understanding exposure to relevant allergens. For the major indoor allergens, this requires sampling of dust from the patient's house. The objectives of this study were to measure indoor allergen levels during a controlled trial of dust mite immunotherapy for asthma and to relate these results to serum antibody levels.

Methods: Eighty-eight asthmatic patients with mite allergy from seven geographic areas in the United States were enrolled in and completed a course of immunotherapy with *Dermatophagoides extract* or placebo control. Sensitization was evaluated by quantitative measurements of IgG and IgE antibodies. Dust samples were assayed for group I mite (*Der p 1* and *Der f 1*), cat (*Fel d 1*), and cockroach (*Bla g 1*) allergens by monoclonal antibody-based ELISA.

Results: Over the 4 years of the study, each of the houses had at least one sample that contained more than 2 µg of group I mite allergen per gram of dust. Mean mite allergen levels, however, varied over a wide range, from 0.2 µg/gm or less to more than 50 µg/gm. IgE antibodies to mite were present in sera from 78% of the patients, whereas IgE antibodies to cat and cockroach allergens were found in sera from 34% and 11% of patients, respectively. Sixty-four percent of the patients had exposure and sensitization to mite, whereas the comparable figure for each of the other allergens was 5%.

Conclusions: Examination of the results suggested that allergen exposure, relative to a trial of immunotherapy, could be expressed as (1) the maximum level found in the house, (2) the percentage of sites having greater than 2 µg/gm, or (3) the mean value at the site with the maximum level. This report provides a background for evaluating the clinical results of immunotherapy in these patients and a model for the way in which sensitization and exposure should be monitored in studies of this kind. (*J Allergy Clin Immunol* 1996;97:1071-8.)

Key words: Immunotherapy, asthma, exposure, sensitization, skin testing, dust mites

Asthma is characterized as: (1) inflammation of the airways, (2) airway obstruction that is reversible either spontaneously or with treatment, and

(3) increased airway responsiveness to nonspecific stimuli.¹⁻³ Furthermore, epidemiologic studies have shown that sensitization to indoor allergens is an important risk factor for the occurrence of acute attacks of asthma.⁴⁻⁶ To better understand this relationship, accurate measurements of aller-

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Supported by grants AI-20565, AI-21984, AI-21987, AI-21991, MOI-RR-00073, and 1U01 AI-34607 from the National Institutes of Health.

Received for publication Nov. 23, 1994; revised June 7, 1995, accepted for publication June 29, 1995.

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0091-6749/96 \$5.00 + 0 1/1/67407

Abbreviations used

NIBSC: National Institute for Biological Standards and Control
RU: RAST units

gen exposure and specific IgE antibodies are necessary. Several recent studies have shown that many of the patients with allergy who are first seen with asthma are exposed to relevant indoor allergens.⁶⁻⁸ The development of specific monoclonal antibody-based immunoassays has greatly simplified the assessment of exposure to indoor allergens.⁹ Immunotherapy has been widely used for the management of allergic conditions since its first reported use in 1911, although the exact mechanism of immunotherapy is not clear.^{10, 11} Early reports in the United States by Spain and Cooke¹² suggested that immunotherapy was more effective for asthma than for allergic rhinitis. Several controlled studies have shown the effectiveness of immunotherapy in treating patients with seasonal allergic rhinitis and seasonal asthma; however, the efficacy of immunotherapy in treating patients with perennial asthma remains controversial.¹³⁻¹⁵

In 1987 a double-blind, placebo-controlled study was initiated to investigate the effectiveness of immunotherapy in asthmatic patients with mite allergy living in seven different geographic locations across the United States. We have previously reported the results of microscopic examination of mites in dust from patients' houses, which demonstrated regional differences in recovery of mites and species identified.¹⁶ This article reports the results of measuring serum IgE antibodies and the levels of allergens in houses. The results provide important information for evaluation of the study's clinical results and also demonstrate the importance of using a separate monitoring laboratory for multicenter studies of this kind.

METHODS**Subjects**

In this study, 88 male and female patients between the ages of 16 and 60 years completed a course of immunotherapy (i.e., at least 20 injections of mite allergen extract or placebo). Approval to conduct this study was obtained from the human investigation committee of each center. The seven centers and one subcenter included the University of Cincinnati Medical Center;

University of Texas Medical Branch, Galveston; University of Tennessee, Memphis; Tulane Medical School, New Orleans; The School of Medicine, East Carolina University, Greenville; Southern California Permanente Medical Group, San Diego; University of California, Los Angeles Medical Center, and University of California, San Diego. All patients had at least a 1-year history of reversible airway obstruction and a positive skin test response to dust mite. Patients who had a baseline FEV₁ of greater than or equal to 70% of predicted value were included in the study if they showed a greater than or equal to 20% decrease in FEV₁ after methacholine challenge. If baseline FEV₁ was less than 70%, then patients had to demonstrate an increase in FEV₁ of greater than or equal to 15% after treatment with bronchodilator.

Patients who were regularly exposed to animals to which they were allergic and patients who were receiving immunotherapy in the 3 years before the start of the study were excluded. Blood and dust samples were collected from patients and their homes at the time of recruitment, during the initial observation period, and during the course of immunotherapy (totaling roughly 5 years). In addition to the dust samples taken during the recruitment and observation periods, sampling was done approximately five times during the course of the study. Selected homes were sampled up to an additional four times during the first year. During the remaining years, sampling was done at the time of year when mite allergen levels were highest for that geographic area, as determined during the observation period.

***Dermatophagoides pteronyssinus* and *D. farinae* extracts**

A 50% mixture of glycerinated *D. pteronyssinus* and *D. farinae* extracts was provided by Berkeley Biologicals (Berkeley, Calif.) in amounts needed to complete the entire study. The glycerinated extracts for immunotherapy and skin testing were analyzed for both group I (24 kd) and group II (15 kd) mite allergen content by two-site monoclonal antibody-based ELISA.^{17, 18} The mean group I concentration was 37.5 µg/ml compared with 26.2 µg/ml of group II mite allergen.

Total IgE, specific IgE, and specific IgG antibodies

Total serum IgE was measured by a monoclonal antibody-based ELISA.^{5, 6, 19} Monoclonal anti-IgE (CIA/E/7.12 and CIA/E/4.15) was kindly provided by Dr. Andrew Saxon of the University of California, Los Angeles. IgE results were expressed as international units per milliliter (~2.4 ng/ml IgE) substandardized against National Institutes of Health IgE Standard (900 IU/ml). Specific IgE antibodies to five common inhalant allergens were measured by quantitative RAST as described previously.⁵ The allergen extracts (Miles Inc., Hollister-Stier Laboratories, Spokane, Wash.) included *D. pteronyssinus*, *D. farinae*, cat hair epithelium, short

ragweed, ryegrass, and cockroach mix (Oriental, German, American). Cyanogen bromide-activated disks were coupled to allergen extracts ($\sim 10 \mu\text{g}$ protein/disk). RAST assays were quantitated by using a control curve of *D. farinae* disks coated with twofold serial dilutions of a mite allergic serum pool (UVA 87/01), substandardized against an international reference serum (National Institute for Biological Standards and Control [NIBSC] number 82/528).¹⁸ Specific IgE RAST results, expressed as units per milliliter, showed the UVA serum pool contained 1000 RAST units (RU)/ml of IgE antibody to *D. farinae*. One unit is approximately 0.1 ng/ml IgE.⁵ Total IgE and specific IgE antibodies were assayed in first samples only, with the exception of *D. pteronyssinus* and *D. farinae*, which were assayed in all serum samples. Mite-specific IgG antibodies to group I mite allergens were measured with radiolabeled Der f 1 in an antigen-binding assay with a standard serum substandardized from the NIBSC serum pool 82/528 as the positive control.^{18, 20, 21}

Allergen levels

Dust samples were collected from the homes of all patients enrolled in the study. The methods of collection were similar to those previously described.^{16, 22, 23} Samples were obtained from mattresses, bedroom floors beside beds, and family room couches or carpets. Patients were instructed not to change cleaning methods during the study. After collection, dust was stored at 4° C. Dust was sieved by using a 0.3 mm mesh screen, and 100 mg of fine dust was extracted overnight at 4° C in 2 ml of borate-buffered saline solution containing 0.1% Tween-20, pH 8. Extracts were then centrifuged, and supernatants were stored at -70° C until they were assayed for allergen content. Allergen content for group I *Dermatophagoides* allergens (Der p 1 and Der f 1); cat allergen, *Felis domesticus* (Fel d 1); and cockroach, *Blattella germanica* (Bla g 1) were measured with a monoclonal antibody-based ELISA.^{23, 24} References with known amounts of Der p 1, Der f 1 (NIBSC 82/518), and Fel d 1 (UVA 91/01, substandardized from Office of Biologicals and Research Reagents branch of Food and Drug Administration, Cat E3) were used; and results were expressed as micrograms per gram of dust.²⁵ The Bla g 1 assay was quantitated by using a German cockroach reference (UVA 89/01), and results were expressed as units per gram of dust. Group I mite allergens (Der p 1 and Der f 1) were measured in all dust samples. Cat and cockroach allergens were measured only in the first sample obtained from each patient's house.

Statistical analysis

Statistical analysis was done by using simple linear regression, Pearson correlation coefficient, and Student's *t* test. Levels with probabilities less than 5% were considered significant ($p < 0.05$).

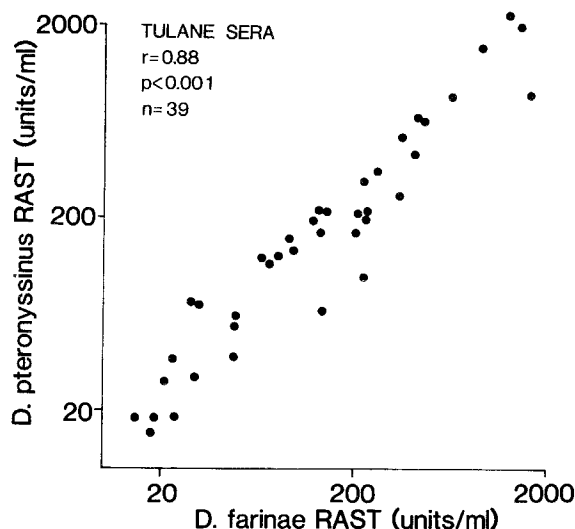


FIG. 1. Correlation between IgE antibody to *D. pteronyssinus* and IgE antibody to *D. farinae* in sera from 39 patients enrolled in Tulane. Values are the mean of three separate determinations. These results include sera from patients who were subsequently excluded from the study.

RESULTS

Total IgE and specific IgE antibodies

Total serum IgE level, expressed as the geometric mean, was 157 IU/ml for the active treatment group compared with 135 IU/ml for the placebo group. Sixty-three percent (54 of 86) of the patients had a total IgE value greater than 100 IU/ml. Specific IgE antibodies to dust mites, *D. pteronyssinus* and *D. farinae*, were assayed in sera from patients in both active treatment and placebo groups by means of RAST. Results obtained showed that 36 of 46 of the patients receiving active treatment had high levels of mite antibody, of whom 25 had levels of 200 U/ml or greater. Among patients with high levels of IgE antibody to mite, 16 of 51 had total IgE levels greater than 400 IU/ml. No significant difference in specific IgE antibody level was seen between active treatment and placebo groups, either before or after the study; however, there was a significant increase in IgE antibody during the study in the active treatment group ($p = 0.02$, *t* test). For the entire group, significant antibody levels (≥ 40 RU/ml) for ryegrass and ragweed were seen in 27% and 17%, respectively. Similar positive RAST results were obtained for cat in 33% and cockroach in 10% of the sera. There was a close correlation between IgE antibody to the two mite species in patients in the active treatment and placebo groups ($r = 0.80$, $p < 0.001$ and $r = 0.80$, $p < 0.001$, respectively). In the early phase of the study, sera from Tulane were

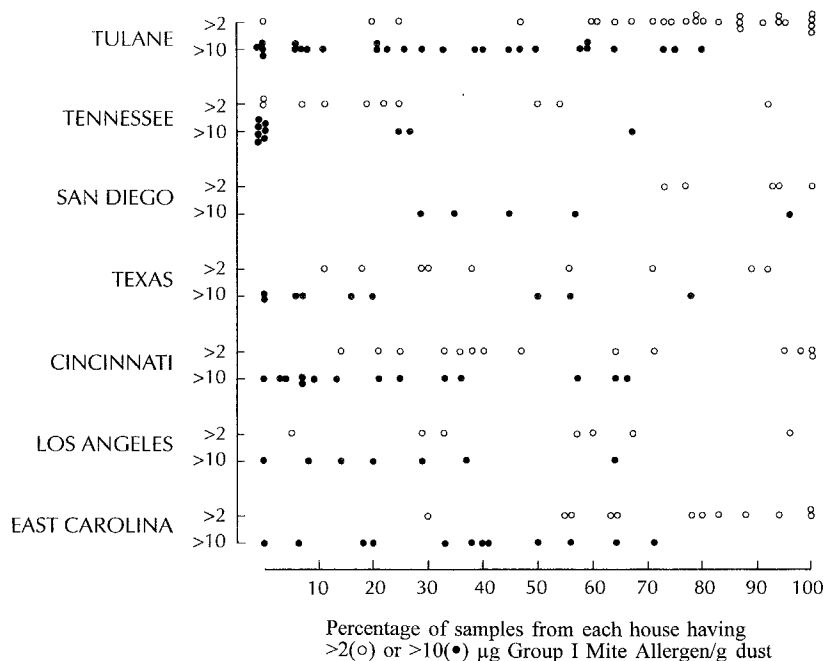


FIG. 2. For each house, the percentage of all samples with more than 2 µg (○) or more than 10 µg (●) of group I mite allergen/per gram of dust is shown. (Thus each house is shown twice.) The number of samples analyzed for each house varied from 18 to 41.

assayed on three separate occasions, and the correlation between *D. farinae* and *D. pteronyssinus* IgE antibodies is shown (Fig. 1). Measurement of IgG antibodies to Der f 1 with an antigen-binding assay confirmed the presence of these antibodies in almost all the sera with IgE antibody. Furthermore, the quantity of IgG antibody was significantly correlated with that of IgE antibody.

Indoor allergen exposure

Among patients who completed the study, 98% had at least one dust sample containing 2 µg or more of group I mite allergen per gram of dust, and 80% had at least one sample containing 10 µg or more per gram of dust.^{9, 25} There were no significant differences between the allergen levels in the houses of patients in the active treatment group compared with those in the placebo group ($p = 0.43$, chi square test). When the percentage of samples from each house that contained more than 2 or more than 10 µg of mite allergen per gram of dust is examined more closely, a wide range of values is shown (Fig. 2). In some houses 70% of samples had more than 10 µg, whereas in others less than 10% of samples had more than 2 µg. In keeping with previous studies, in 76% of the houses the highest level of mite allergen was found in the bedroom dust sample (mattress, bedroom carpet or floor) (Fig.

3).^{7, 8, 25, 26} In Fig. 3 the results are expressed as the geometric mean value for the site in each house with the highest level. These results illustrate the wide range of natural exposure to mite allergen (i.e., from <0.2 µg/gm to >50 µg/gm). Specific examples of the changes that occurred are shown in the sequential dust data from three patients from Cincinnati (Fig. 4). Der p 1 was the predominant allergen in 51% of the homes, especially those in Louisiana, Texas, and San Diego. In contrast, Der f 1 was predominant in 45% of the homes, especially those in Ohio and Tennessee. In 4% of the homes neither Der p 1 nor Der f 1 was dominant, but equal amounts of both allergens were present (Table I). Our results are in agreement with previously reported data that *D. farinae* and *D. pteronyssinus* are the predominant mite species found in the United States and that their relative prevalence is variable, with more *D. pteronyssinus* recovered in southern states that are continuously warm and humid and more *D. farinae* recovered in central and northern states. Indeed, the distribution of Der f 1 and Der p 1 seen in Table I correlated ($r = 0.8$, $p < 0.05$ and $r = 0.9$, $p < 0.05$, respectively) with the identified numbers of *D. farinae* and *D. pteronyssinus* mites reported previously.¹⁶ For the other indoor allergens tested, 24 of 80 houses were found to have significant levels of Fel d 1 (≥ 8 µg/gm dust) and 20 of 80 with significant levels of Bla

TABLE I. Predominant mite allergen as assessed by ELISA on house dust extracts in different geographic areas

	<i>n</i>	Dominant mite allergen		
		Der f 1	Der p 1	Der f 1 equals Der p 1*
Tulane	27	7 (26%)	19 (70%)	1 (4%)
Tennessee	10	9 (90%)	1 (10%)	0 (0%)
San Diego	5	0 (0%)	5 (100%)	0 (0%)
Texas	9	1 (11%)	8 (89%)	0 (0%)
Cincinnati	14	12 (86%)	2 (14%)	0 (0%)
Los Angeles	7	3 (43%)	4 (57%)	0 (0%)
E. Carolina	12	6 (50%)	4 (33%)	2 (17%)
Totals	84	38 (45%)	43 (51%)	3 (4%)

The dominant mite allergen in each house was that present at the highest concentration in the majority of samples obtained from that house.

*Houses in which an equal number of samples had Der p 1 or Der f 1 at the highest level.

g 1 (≥ 2 U/gm dust) (Table II). Overall, 37 of 80 houses had significant levels of an allergen other than dust mites.

Sensitization and exposure

The criteria for entry into this study were positive skin test responses to dust mite with negative or weakly positive skin test responses to other common indoor allergens. The initial serum sample from each patient was assayed for IgE antibodies to mite, cat, cockroach, and ryegrass and ragweed pollen. The results show that many of the patients had positive RAST responses to cat or cockroach (Table II). Furthermore, four of the patients had both IgE antibody and exposure to cat allergen, whereas an additional four patients had both sensitization and exposure to cockroach. Comparable values for dust mite were 52 of 87 patients who had 40 U or more of IgE antibody and were exposed to 10 μ g or more of group I mite allergen (Table II).

DISCUSSION

Evaluation of the response of patients with allergy to either pharmacotherapy or immunotherapy requires that the treated and control groups be exposed to similar quantities of allergen. For patients allergic to pollen this is usually achieved by performing the study during a pollen season within a defined geographic area. This is not the situation for the major indoor allergens, because it is not possible to assume that exposure is the same, or even similar, in houses within any one geographic

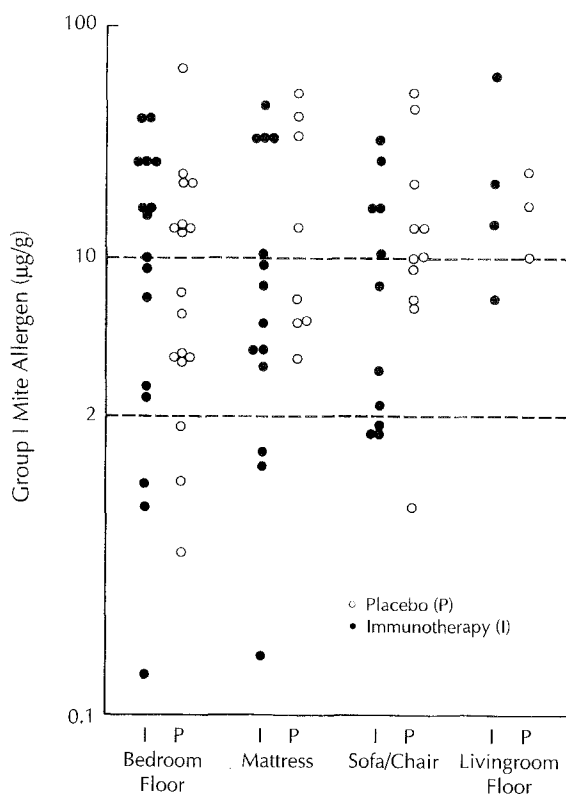


FIG. 3. Geometric mean value of mite allergen calculated for the site in the house with the highest mean value. In seven houses the site with the highest value was in the living room, whereas in 32 houses the bedroom floor had the highest mean level. The highest geometric mean value varied from 0.2 μ g/gm to 83 μ g/gm; however, no significant difference was observed between the houses of patients receiving placebo (○) and those of patients receiving immunotherapy (●).

area. Thus measurement of exposure during a study is essential for proper assessment of the response to treatment. Because exposure and symptoms are usually perennial in nature, it is also necessary to evaluate the presence of sensitivity to other perennial allergens. This report details measurements of serum antibodies and indoor allergens for asthmatic patients enrolled in a multicenter trial of immunotherapy. The results make it clear that there are not only differences between houses within one area but that there are also regional differences and variations over time. Furthermore, it became clear that, despite denials by the patients, several were exposed and sensitized to indoor allergens other than dust mite.

To assess the relevance of indoor allergen exposure and symptoms, it is necessary to relate the values to some standard. This point is important when examining the houses of control and treated

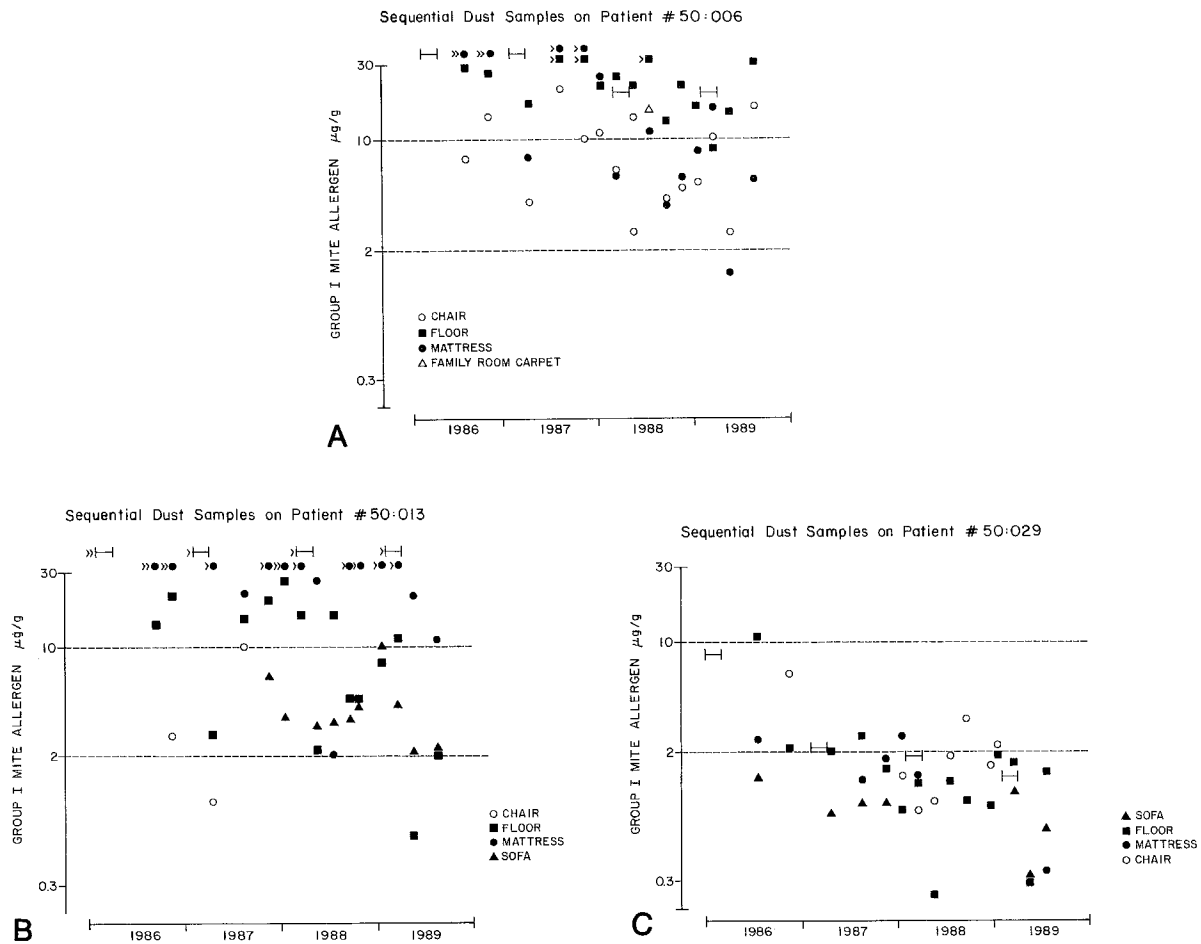


FIG. 4. Results for dust samples collected from three houses over a 4-year period show the variation within a house. Mean values are shown for each year (—). Only a minority of houses was studied in this detail. Each panel (A, B, and C) gives the center number (50), followed by the patient number.

TABLE II. Exposure and sensitivity to mite, cat, and cockroach

	Mite					Cat				Cockroach			
	Exposure*		RAST & exposure†			Exposure*		RAST & exposure‡		Exposure*		RAST & exposure‡	
	≥10	≥2	RAST†	≥10	≥2	Exposure*	RAST†	≥8	≥1	Exposure*	RAST†	≥8	≥1
Tulane	23/27	27/27	25/28	20	24	10/24	8/26	2	4	10/24	5/26	2	
Tennessee	3/10	8/10	5/10	1	4	2/10	2/9	0	2	4/10	1/9	1	
San Diego	5/5	5/5	5/5	5	5	1/4	3/5	1	1	1/4	0/5	0	
Texas	7/9	9/9	9/10	6	8	4/9	0/9	0	0	4/9	2/9	1	
Cincinnati	13/14	14/14	12/15	10	11	4/14	7/15	1	5	1/14	0/15	0	
Los Angeles	6/7	7/7	5/7	4	5	1/7	5/7	0	2	0/7	0/7	0	
E. Carolina	11/12	12/12	7/12	6	7	2/12	3/11	0	1	0/12	1/11	0	
Totals	68/84	82/84	68/87	52	64	24/80	28/82	4	15	20/80	9/82	4	

*Exposure is defined as micrograms of group I mite allergen, micrograms of Fel d 1, or units of Bla g 1 per gram of dust.

†Sensitivity is defined as ≥40 RU.

‡Sensitization plus exposure is expressed as the number with ≥40 RU and exposure to relevant allergen.

patients with similar levels of exposure. For example, although it would be relatively simple to find patients allergic to mites in Denver, Colorado, very few houses have significant numbers of mites as judged by the standards of other areas. The primary thresholds used here were 2 μg and 10 μg of group I mite allergen per gram of dust. The evidence for threshold levels comes from several different countries (e.g., France, Germany, United Kingdom, Australia, Denmark, United States) and has been discussed in detail.^{4, 7, 9, 25-28} The evidence, however, is primarily about the level of mite allergen necessary to give rise to sensitization among genetically atopic children.⁷ The exposure levels relevant to symptoms of asthma are not well defined and 10 μg of group I allergen/gm is best seen as a level at which most patients allergic to mites will have symptoms. Perhaps the clearest evidence comes from experiments in which reduction of exposure either in the home^{29, 30} or in a hospital^{31, 32} has been associated with significant reduction in symptoms and bronchial hyperreactivity.

The analysis of allergens in house dust was carried out after randomization of the patients and was not part of the entry criteria for the study. As with dust mite, we have proposed specific levels of other allergens that should be regarded as relevant (i.e., 2 U of Bla g 1 or 8 μg of Fel d 1). Even when different allergens are measured in absolute units, it is not clear that 10 μg of Fel d 1 is equivalent to 10 μg of group I mite allergen. The form in which these two allergens become airborne is very different. Ideally, we would make measurements of airborne exposure, but with current technology that approach would only be possible in a few houses.

In relating sensitization and exposure it is tempting to assume that there is some inverse relationship between the quantitative level of IgE antibody and the level of allergen necessary to cause symptoms of asthma. A relationship of this kind has been reported for ragweed-induced hay fever; however, it is likely that the relationship of allergen exposure to bronchial inflammation and symptoms is more complex. We have chosen to analyze results by using 40 RU of IgE antibody (~ 4 ng of IgE antibody). The patients enrolled in this study had positive skin test and bronchial provocation test responses to mite allergen (data not shown); we are not suggesting that negative RAST results demonstrate that the patients were not allergic to mites. The *in vitro* serum results, however, make it easier to compare the groups and also to relate the sensitivity of these patients with those reported in

other studies. By evaluating exposure and sensitization, it is then possible to identify patients with high levels of sensitization and exposure. We suggest that the response to immunotherapy should be analyzed, taking into account exposure and the level of IgE antibody. In particular, it is essential to determine whether immunotherapy is more or less effective when given to patients with concurrent exposure to relevant allergens.

In a study in which multiple measurements have been made over 4 years complete data (Fig. 3) may be very instructive. It is difficult, however, to see how these results could be analyzed statistically, relative to the results of immunotherapy. We have suggested that the data could be analyzed in three different ways. The simplest would be to identify the highest level observed in each house. This would, however, exaggerate the importance of sites with great seasonal variation. The next approach would be to identify the percentage of samples assayed that contained 2 μg or more per gram of dust (or ≥ 10 $\mu\text{g}/(\text{m})$) (Fig. 2). The third approach is to identify the site in each house with the highest levels and to present the mean level for that site. This third approach (Fig. 3) is in line with previous reports in which the highest level in the house is taken as the index of exposure but also allows inclusion of multiple measurements.

In conclusion, the results reported here illustrate very well the problems of carrying out controlled trials in patients with perennial asthma. We believe it is essential to have both sensitization and exposure monitored in a central laboratory. Furthermore, it is clear that these measurements should not be restricted to the specific allergen that is the focus of the study.

We thank Nancy Malone for assistance with the manuscript.

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