

High mite-allergen exposure increases the risk of sensitization in atopic children and young adults

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Concentrations of major allergens of Dermatophagoides pteronyssinus (Dpt) and D. farinae (Df), Der p I and Der f I, were determined in 183 dust samples of mattresses of 133 atopic and 50 nonatopic children by a sandwich-type ELISA. Atopic children and young adults living in houses with high levels of Der p I and Der f I (≥ 2000 ng/gm of dust) were found to have significantly higher serum IgE levels to Dpt and Df ($p < 0.0001$) compared to patients with low mite-allergen exposure. Washed leukocytes of 55 atopic children and 14 control subjects were investigated for in vitro histamine release to serial dilutions of Der p I; 86% of highly exposed ($\geq 10,000$ ng/gm) children demonstrated positive histamine release in response to Der p I compared to 17% in the group with very low exposure (< 400 ng/gm). There was a positive correlation between basophil sensitivity ($r_s = 0.6$; $p < 0.0001$) and reactivity ($r_s = 0.54$; $p < 0.0001$) to Der p I and mite-allergen exposure. The relative risk for sensitization in the highly exposed group versus the group with very low exposure was sevenfold to 32-fold increased. We conclude that high concentrations of mite allergen (≥ 2000 ng/gm) increase the risk of specific sensitization in atopic children and young adults and thus may facilitate allergic airway disease. (J ALLERGY CLIN IMMUNOL 1989;84:718-25.)

Hypersensitivity reactions to allergenic proteins from house dust mites play an important role in various atopic diseases in childhood^{1, 2} and in adulthood.^{3, 4} *DpT* and *Df*, found worldwide in human dwellings,^{5-7, 4, 8, 9} are of major significance as allergen sources.^{9, 10} The major allergens of these species, group I (*Der p I* and *Der f I*) and group II (*Der p II* and *Der f II*),¹¹⁻¹⁶ as well as other allergens,^{17, 18} have been biochemically characterized and purified. At least for the group I allergens, heat-labile glycoproteins of a molecular weight of approximately 24 kd, mite feces appear to be the major source of allergen.¹⁹ Group I allergens of *Dpt* and *Df* have close physicochemical and immunochemical homologies.^{15, 17}

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Abbreviations used

<i>Der p I</i> :	Antigen P_1 of <i>Dermatophagoides pteronyssinus</i>
<i>Der f I</i> :	Antigen F_1 of <i>Dermatophagoides farinae</i>
<i>Dpt</i> :	<i>Dermatophagoides pteronyssinus</i>
<i>Df</i> :	<i>Dermatophagoides farinae</i>
<i>Der m I</i> :	Antigen M_1 of <i>Dermatophagoides microceras</i>
<i>Dm</i> :	<i>Dermatophagoides microceras</i>
HR:	Histamine release
BHR ₃₀ :	Antigen concentration in micrograms per milliliter required to release 30% histamine (basophil sensitivity)
BHR ₂₀ :	Antigen concentration in micrograms per milliliter required to release 20% histamine
PIPES:	Piperazine- <i>N,N'</i> -bis-2-ethanesulfonic acid

Species-specific and cross-reacting epitopes have been recognized by monoclonal^{20, 21} and absorbed rabbit antibodies,¹⁵ and it has become possible to quantify mite allergens in the environment.^{22, 23}

Although several studies on an assumed relationship between sensitization to mite allergen and mite-

allergen exposure were performed,¹⁻³ there have been only indirect assessments about threshold concentrations necessary to sensitize an atopic individual because these data are based on mite counts that do not necessarily correlate precisely with the amount of allergens.²⁴

In our study we demonstrated a dose-response relationship between mite-allergen exposure and the risk of the individual sensitization by comparing the amount of *Der p* 1 and *Der f* 1 in mattress dust samples and immunologic in vitro findings.

We demonstrated that there is a threshold concentration of dust mite allergen that significantly increases the risk of sensitization in atopic children and young adults.

MATERIAL AND METHODS

Patients

We investigated 183 children (aged 1.5 to 25 years; mean age, 9.3 years), 133 atopic (mean age, 9.2 years) and 50 nonatopic individuals (mean age, 9.7 years), during 14 months. Atopy was defined as at least one of the clinical manifestations of either atopic eczema (according to the criteria of Rajka and Hanifin²⁵) and/or allergic airway disease (asthma or seasonal rhinitis) associated with a sensitivity to one or more allergen sources by skin prick test, as described by Malling,²⁶ and/or RAST; 99 of 133 atopic children had bronchial asthma, eight had bronchial asthma and allergic rhinitis, eight had bronchial asthma and atopic dermatitis, three had bronchial asthma, allergic rhinitis, and atopic dermatitis, eight had allergic rhinitis, six had atopic dermatitis, and one had urticaria. The 50 nonatopic control subjects consisted of children referred to our department with problems other than atopy.

For histamine release studies, 55/133 atopic children and 14/50 nonatopic children were selected. The patients did not receive glucocorticosteroids for 24 hours, theophylline for 36 hours, inhalation therapy with β_2 -sympathomimetics for 8 hours, and neither antihistamines nor ketotifen for 2 weeks. Patients were informed of the aim of the study and gave consent.

Dust collection and extraction and house dust-mite ELISA

All families were instructed to collect a dust sample with their own vacuum cleaner, installing a new dust bag, and vacuuming the child's mattress for 5 minutes. The dust bag was immediately placed into a plastic bag, mailed to our laboratory, and kept at 4° C until analysis. Dust extracts were produced by agitation for 3 hours at room temperature 1:5 (wt/vol) in 0.125 mol/L of ammonium hydrogen carbonate containing 0.1% sodium azide. After centrifugation, the supernatants were filtered through a 220 nm diameter filter (Millex GV, Millipore Ltd., Harrow, Middlesex, U.K.) and could be stored at -20° C. Precoated polystyrene microtiter trays and reagents for sandwich ELISA were obtained from ALK, Copenhagen, Denmark.

For assay of *Der p* 1, plates coated with monoclonal, noncross-reacting antibodies²¹ and horseradish peroxidase-conjugated, species-specific rabbit antibodies to *Der p* 1 were used. The *Der f* 1 assay was a combined determination of the two species, *Df* and *Dm* of the *Df* group. The rabbit antibodies used (coating as well as conjugated antibody) were anti-*Der f* 1, immunoabsorbed on a *Dpt*-Sephacrose column, and further affinity purified by acid elution from a *Der m* 1-Sepharose column. The assay was performed according to the method described in Lind.²³ In short, 100 μ l dust extracts and dilutions thereof were added to the precoated wells for overnight incubation, followed by repeated washings. After a 1-hour incubation with enzyme-conjugated antibody and repeated washings, substrate (hydrogen peroxide/orthophenyleneamine) was added to each well, and reaction was carried out for 15 minutes in darkness. Reading of the absorption at 492 nm (Titertek Multiskan MCC 340, Flow Laboratories, Rickmansworth, Hertfordshire, England) took place 5 minutes after addition of 1 mol/L of sulphuric acid. Standard response curves were constructed from reference antigen solutions of each species with major allergen concentrations in the range of 1.6 to 400 ng/ml (internal standards of ALK, the *Der p* 1 standard related to National Institute of Biological Standards and Control 82/518²⁷). Results were expressed as nanograms per gram of dust. Assay sensitivity was 5 ng/ml or 15 ng/gm of dust. Previous experiments demonstrated an interassay coefficient of variation of 17% to 25%, and an intra-assay coefficient of variation of 5% to 10%. All results were grouped according to the method of Platts-Mills et al.²²: class 1, <400 ng/gm of dust; class 2, ≥ 400 <2000 ng/gm; class 3, ≥ 2000 <10,000 ng/gm; class 4, $\geq 10,000$ ng/gm. The sum of *Der p* 1 plus *Der f* 1 was calculated for statistical evaluation.

The monoclonal antibody to *Der p* 1, Nhp5, is highly specific and has been thoroughly described.^{21,28} The affinity-purified, monospecific rabbit antibodies to *Der f* 1 do not cross-react with *Der p* 1, but react with *Der f* 1 and *Der m* 1 common epitopes. Both mites belong to the *Df* group and are closely related.^{15,23} House dust analysis demonstrated that *Dm* is of minor significance in the study area.

RAST

Pharmacia Phadebas RAST and Phadebas Prist were used in an enzyme immunoassay (Pharmacia, Uppsala, Sweden) to determine serum IgE to *Dpt* and *Df* and a panel of routinely tested allergens (pollen, molds, and animal dander) and total serum IgE.

Histamine release

Blood was collected in 50 ml plastic tubes containing 0.01 mol/L of ethylenediaminetetraacetic acid and sedimented for 90 minutes at room temperature with 2.5 ml of dextran (B. Braun Melsungen AG, Melsungen, West Germany; molecular weight of dextran, 60,000) and 3% glucose per 10 ml of blood. The upper layer was transferred to another plastic tube and centrifuged at 150 g for 8 minutes at 4° C. The pelleted cells were suspended in PIPES A containing 4 mmol/L of ethylenediaminetetraacetic acid

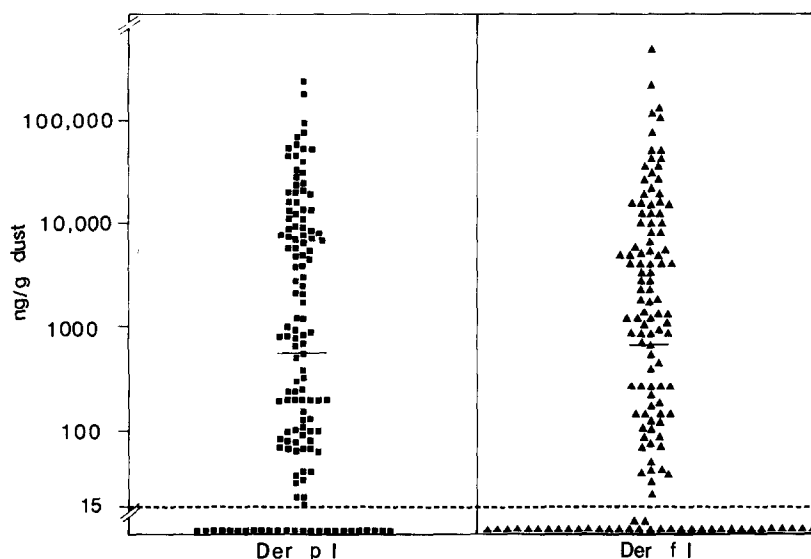


Fig. 1. *Der p I* and *Der f I* concentrations in dust samples from mattresses of atopic children and young adults living in West Berlin ($n = 133$).

centrifuged at 300 g for 8 minutes at 4° C. Cells were then washed in PIPES A. After centrifugation, the cells were resuspended in PIPES ACM buffer (2 mmol/L of CaCl_2 and 0.5 mmol/L of MgCl_2) and incubated with serial dilutions of purified *Der p I* (ALK) at concentrations from 10^{-6} to 1 $\mu\text{g/ml}$, and anti-IgE (anti-human IgE rabbit antibodies, Behring-Hoechst, Inc., Marburg, West Germany) at concentrations ranging from 3 to 2000 IU/ml. After 40 minutes, the tubes were centrifuged at 600 g, and supernatants were analyzed for histamine contents. HR reactions were assayed in duplicates with an automated fluorometric technique.²⁹ Results were expressed, as released histamine, as a percentage of total histamine. Basophil sensitivity is the antigen concentration in micrograms per milliliter required to release 30% (BHR_{30}) or 20% (BHR_{20}) histamine. Reactivity is the maximum HR. Diagnostic tests and mite-allergen analysis from dust samples were done in the same time period.

Statistics

Kruskal-Wallis test, Wilcoxon-Mann-Whitney U test, chi-square test, and Fisher's exact test, respectively, and Spearman rank-correlation coefficient were used. Statistical significance was assumed at $p < 0.05$. The relative risk of exposure for sensitization is expressed as an odds ratio with a 95% confidence interval.

RESULTS

The concentrations of mite allergen *Der p I* and *Der f I* in mattress dust samples demonstrated large variations, as well as in the total amount and in the distribution of *Dpt* and *Df*. The ranges for *Der p I* were between undetectable (<15 ng/gm of dust) and 210,000 ng/gm, and for *Der f I*, between undetectable and 500,000 ng/gm. Atopic and nonatopic subjects

were exposed to comparable allergen concentrations. For atopic children, the median value was 600 ng/gm of *Der p I* and 675 ng/gm of *Der f I* (Fig. 1), for nonatopic children, 400 ng/gm of *Der p I* and 450 ng/gm of *Der f I*. In only 12 dust samples (10 atopic and two nonatopic subjects) were we not able to detect either *Der p I* or *Der f I*. Fifteen mattresses contained *Der f I* only (11 atopic and four nonatopic subjects), whereas 24 samples had only *Der p I* (20 atopic and four nonatopic subjects). Although there was a similar prevalence of *Dpt* and *Df* in the houses participating in the study, in the individual homes in approximately 70%, one species was clearly dominating, that is, tenfold or more higher levels of one species than the other species. We were unable to find any correlation between *Der p I* and *Der f I* ($r_s = -0.06$).

In the atopic group, the median value for serum IgE antibodies to *Dpt* was 4.9 PRU/ml, and the median value for serum IgE antibodies to *Df* was 1.55 PRU/ml. There was a positive correlation between serum IgE antibody levels to *Dpt* and *Df* ($r_s = 0.9$; $p < 0.0001$) and between serum IgE antibody levels to *Dpt* and mite-allergen exposure (*Der p I* plus *Der f I*; $r_s = 0.46$; $p < 0.0001$).

Atopic children with significant (≥ 2000 ng/gm $<10,000$ ng/gm) or high ($\geq 10,000$ ng/gm) amounts of *Der p I* and the sum of *Der p I* plus *Der f I*, respectively (Fig. 2), were found to have significantly higher serum IgE antibodies to *Dpt* ($p < 0.005/p < 0.0001$, respectively) than patients with very low (<400 ng/gm) concentrations of *Der p I* plus *Der f I* (Table I). Similar results could be observed for serum IgE antibodies to *Df* and for *Der*

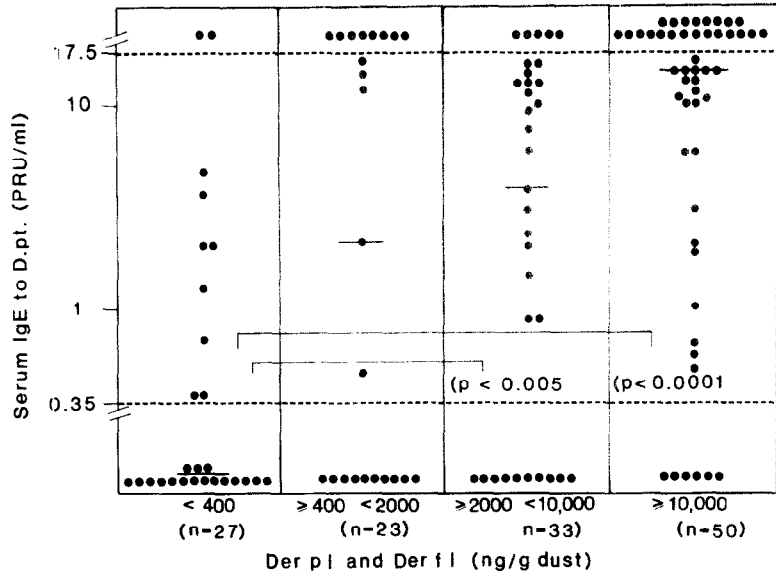


Fig. 2. Mite-allergen exposure (sum of *Der p I* and *Der f I*) and serum IgE antibody concentrations to *Dpt*.

TABLE I. *P* values for the difference of serum IgE antibodies to *Dpt* in atopic subjects between different mite-allergen exposure classes (sum of *Der p I* plus *Der f I*)*

<i>Der p I</i> plus <i>Der f I</i> (ng/gm of dust)	<i>Der p I</i> plus <i>Der f I</i> (ng/gm of dust)			
	≥400 <2000	≥2,000 <10,000	≥10,000	≥2000
<400	<i>p</i> < 0.05	<i>p</i> < 0.005	<i>p</i> < 0.0001	NC
≥400 <2000		NS	<i>p</i> < 0.05	NC
≥2,000 <10,000	NS		<i>p</i> < 0.005	NC
<2000	NC	NC	NC	<i>p</i> < 0.0005

NS, Not significant; NC, not calculated.

*Wilcoxon-Mann-Whitney U test.

p I allergen exposure alone and serum IgE to *Dpt* and *Df*, respectively.

No difference in median age could be found within the different dust-exposure classes and different RAST classes for IgE to *Dpt* and *Df*, indicating that the different IgE levels cannot be interpreted as a function of age.

Washed leukocytes from 55 atopic subjects and 14 nonatopic control subjects were incubated with serial dilutions of *Der p I* (10^{-6} to 1 $\mu\text{g/ml}$) and anti-IgE (3 to 200 IU/ml). Patients were divided into three groups: A+ (*n* = 35), atopic subjects with positive histamine release to *Der p I* (HR $\geq 10\%$); A- (*n* = 20), atopic subjects with negative HR to *Der p I*; and N (*n* = 14), nonatopic control subjects. No

difference in median age could be found among the three groups. In group A+, 33/35 patients and in group A-, 6/20 patients had detectable serum IgE to *Dpt*.

Group A+ demonstrated significantly higher levels of serum IgE to *Dpt* (and *Df*, respectively) than group A- (*p* < 0.0001). There was a correlation between BHR₃₀ to *Der p I* and IgE to *Dpt* and *Df* in group A+ (*r*_s = 0.4; *p* < 0.01).

The differences in exposure to *Der p I* and *Der f I* in the groups A+ and A- are illustrated in Fig. 3. Comparing the sum of *Der p I* plus *Der f I*, A+ reveals significantly higher levels of allergen exposure than A- (*p* < 0.0005). The median value is approximately 25-fold higher for A+ (12,600 ng/gm

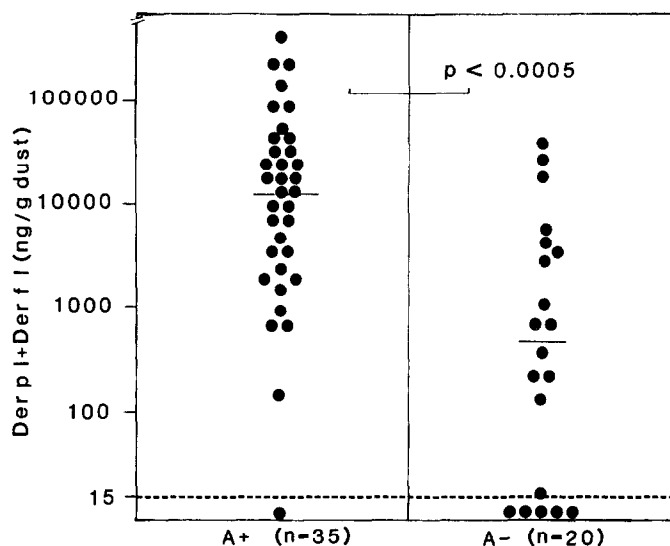


Fig. 3. Mite-allergen exposure (sum of *Der p I* and *Der f I*) in dust samples of mite-sensitive (A+) and nonmite-sensitive patients (A-). Sensitivity was defined by mite allergen-induced HR.

dust versus 450 ng/gm). The median value for all atopic children (A+ and A-) was 4100 ng/gm. *Der p I* exposure mainly causes the difference between group A+ and A- (median: A+, 5500 ng/gm; A-, 35 ng/gm). The difference in *Der f I* exposure was less impressive ($p < 0.05$; median: A+, 1500 ng/gm; A-, 435 ng/gm).

All three groups (A+, A-, and N) had comparable HR on incubation with anti-IgE (maximum HR for A+, 44%; for A-, 48%; and for N, 42% [median values]). There was no correlation between anti-IgE-induced HR and allergen exposure (sum of *Der p I* plus *Der f I*, $r_s = -0.17$; only *Der p I*, $r_s = -0.09$).

There was no significant difference in anti-IgE-induced HR between the group A+ with positive HR to *Der p I* and the group A- with negative HR to *Der p I* ($r_s = 0.1$) for BHR₃₀ to *Der p I* and BHR₂₀ to anti-IgE.

After the patients were grouped according to their *Der p I* and *Der f I* exposure into four dust classes, HR data revealed 86% of highly exposed children (sum of *Der p I* plus *Der f I* $\geq 10,000$ ng/gm of dust) demonstrated positive HR to *Der p I* compared to 17% in the group with very low exposure (sum of *Der p I* plus *Der f I* < 400 ng/gm of dust).

We evaluated the median dose-response relationship of HR with *Der p I* to compare basophil sensitivity (BHR₃₀) and reactivity (maximum HR) within the different allergen-exposure classes. Only 27/35 dose-response curves could be evaluated.

Among all atopic patients (A+ and A-) there was a positive correlation between basophil sensitivity to *Der p I* and allergen exposure to *Der p I* ($r_s = 0.71$;

$p < 0.0001$) and the sum of *Der p I* plus *Der f I*, respectively ($r_s = 0.6$; $p < 0.0001$). The median values for BHR₃₀ to *Der p I* in the dust class with very low exposure (< 400 ng/gm) were significantly higher ($> 1 \mu\text{g/ml}$) than for heavily exposed ($\geq 10,000$ ng/gm) children ($5.5 \times 10^{-4} \mu\text{g/ml}$; $p < 0.0005$) (Fig. 4). There was also a significant difference between dust class 1 plus 2 (< 2000 ng/g) and 3 plus 4 (≥ 2000 ng/gm; $p < 0.005$).

In group A+ (sensitized to *Der p I* in HR), the dose-response relationship between basophil sensitivity and allergen exposure was weaker ($r_s = 0.35$ and $p < 0.005$ for *Der p I* exposure; $r_s = 0.24$ and $p < 0.1$ for sum of *Der p I* plus *Der f I*). There was a significant difference between the different *Der p I* exposure classes (Kruskal-Wallis test, $p < 0.05$).

The maximum HR was found at *Der p I* concentrations of 10^{-2} to $3 \times 10^{-2} \mu\text{g/ml}$. In atopic children (groups A+ and A-), the median reactivity value for dust classes 1 plus 2 (sum of *Der p I* plus *Der f I*) was 0% ($n = 18$); for dust class 3, 28% ($n = 11$); and for dust class 4, 64% ($n = 18$) (Fig. 5) with a positive correlation between mite-allergen exposure and reactivity ($r_s = 0.54$; $p < 0.0001$). There was a significant difference ($p < 0.005$) between dust classes 1 plus 2 (< 2000 ng of *Der p I* plus *Der f I* per gram of dust) versus dust classes 3 plus 4 (≥ 2000 ng/gm) and between dust classes 1 and 4 ($p < 0.0005$). (Dust exposure class 3 and class 4 were not significantly different.)

Within group A+, median-dose response curves were similar for all mite allergen-exposure classes. No difference for maximum HR could be found among

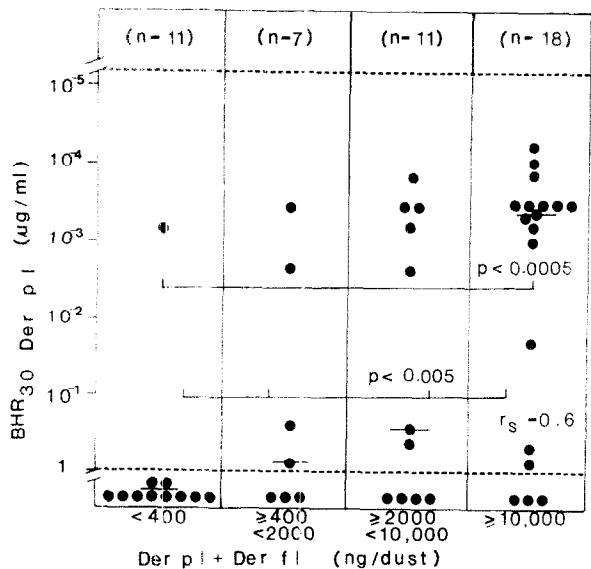


Fig. 4. Basophil sensitivity (BHR_{30}) to *Der p I* and mite-allergen exposure (*Der p I* and *Der f I*) in mattress-dust samples of atopic children and young adults.

the different mite allergen-exposure classes in this group. Maximum HR in dust class 1 plus 2 (sum of *Der p I* plus *Der f I*) was 60% ($n = 5$); in class 3, 66% ($n = 7$); and in class 4, 65% ($n = 15$).

From our data, a risk ratio considering the risk of exposure for the development of IgE antibodies and positive HR to *Der p I* in different mite allergen-exposure groups was calculated (Table II). Comparing atopic subjects with very low house dust-mite exposure with highly exposed atopic subjects, the risk is sevenfold to elevenfold higher for atopic subjects with significant and high mite-allergen exposure to develop serum IgE to house dust mites and 19- to 23-fold higher to release histamine from sensitized basophils.

DISCUSSION

The availability of purified environmental allergens and the production of noncross-reacting monoclonal or affinity-purified monospecific antibodies directed to these allergens as tools for research purposes are an important prerequisite for studies on the relationship between allergen exposure and human immune response.

We compared the parameters of sensitization (serum IgE and HR) and the sum of both major allergens, *Der p I* and *Der f I*, taking into account that a large percentage of human IgE to these mite allergens is directed against common epitopes, as has been demonstrated in cross-absorption studies.³⁰ However, *Dpt*-sensitized patients also produce IgE to species-specific epitopes.^{28, 30} In our study there may have been some

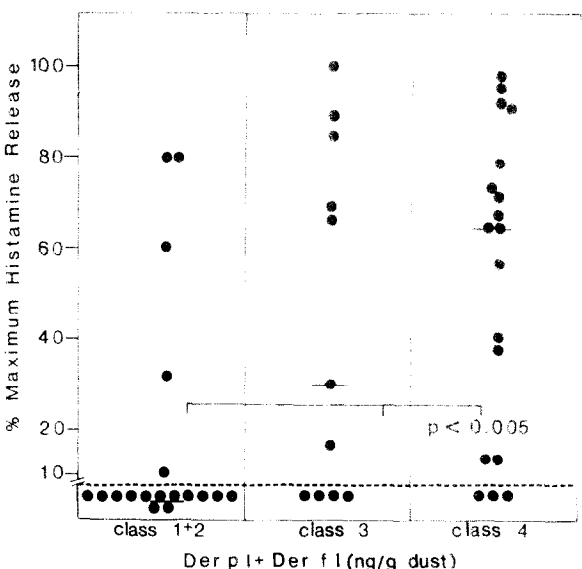


Fig. 5. Basophil reactivity (maximum HR) to *Der p I* and mite-allergen exposure in mattress-dust samples of atopic children and young adults (class 1 plus 2, <2000 ng/gm; class 3, ≥ 2000 ng/gm $<10,000$ ng/gm; class 4, $\geq 10,000$ ng/gm of *Der p I* plus *Der f I* per gram of dust).

TABLE II. Odds ratio and 95% confidence interval (in brackets) calculating the risk for the development of specific sensitization to mite allergen (serum IgE to *Dpt* and HR to *Der p I*)*

<i>Der p I</i> plus <i>Der f I</i> (ng/gm of dust)	Serum IgE to <i>Dpt</i>	Positive HR to <i>Der p I</i>
<400 versus $\geq 10,000$	11 [4, 34]	32 [5, 222]
<400 versus ≥ 2000	7 [3, 18]	19 [3, 105]
<2000 versus ≥ 2000	5 [2, 10]	5 [2, 27]

*Very low and low mite-allergen exposure compared to significant and high mite-allergen exposure.

patients with negative HR to *Der p I* who would have reacted to *Der f I* or other mite allergens. This might explain why the *Df* exposure is not so strongly related to specific sensitization in mite-allergic patients.

We collected mattress dust because it has been demonstrated to contain more mite allergen than carpets in our study (data not presented), which is in good accordance with a study by Platts-Mills et al.²⁴

We found *Der p I* and *Der f I* at similar con-

centrations in dust samples from Berlin. This indicates that *Df* is as important as *Dpt* in Berlin, in contrast to studies from Denmark,⁷ England,³ and West Germany³¹ where *Dpt* was more dominating. In most of the homes, we found one or the other species being almost exclusively prevalent. Other investigators reported similar findings,^{3, 32, 6} and *Df* prevalence was supposed to be related to long periods of dry weather, appearing to be less sensitive to humidity loss.⁶ Since the outdoor climate was the same for all homes in our study, there must be special indoor conditions that may favor the growth of one single species.

Dust samples were collected during more than 1 year. Dust sampling and testing (HR and RAST) were done in the same season. In 17 homes with high mite-allergen exposure ($\geq 10,000$ ng/gm), a second dust sample was taken in summer when the mite-allergen concentration should be lower, if seasonal variation occurs. In 10 homes the results were equivalent to the first measurement. Seven homes revealed slightly lower levels, but in five homes, the mite-allergen concentration remained in the same "exposure class," and two homes still had significant or high (≥ 2000 ng/gm) mite-allergen exposure; therefore, seasonal variation did not strongly influence our results.

This is in good accordance with a longitudinal study performed in Berlin³³ in which 50% of the houses investigated demonstrated constant mite-allergen exposure on mattresses during the year.

The results presented here demonstrate that there is a strong correlation between house dust mite-allergen exposure (*Der p* I or *Der f* I) and specific sensitization, that is, the presence of specific serum IgE to *Dpt* and *Df* or cell-bound IgE. Our data suggest a threshold concentration of ≥ 2000 ng/gm to be regarded as a high risk and a concentration $\geq 10,000$ ng/gm as a very high risk for mite sensitization in childhood. Allergen concentrations were similar for atopic and nonatopic patients, but within the group of atopic patients, 25-fold higher in mite-allergic compared to mite-nonallergic children. The risk for specific sensitization (induction of specific IgE antibodies in the serum or cell-bound IgE on basophils) for atopic individuals who are exposed to significant and high compared to very low mite-allergen concentrations is increased by the factor 7 to 32.

This finding confirms the results of Platts-Mills et al.²² who considered $\geq 10,000$ ng/gm as a high level that may facilitate asthmatic attacks in mite-allergic subjects with asthma. Rowntree et al.² found that the incidence of IgE and IgG and positive skin test to *Dpt* was increased in houses with >5000 ng/gm of *Der p* I per gram of dust in British school

children. Other investigators have demonstrated that high mite-allergen levels are associated with an increased prevalence of asthma.⁸

Korsgaard⁴ stated that levels >100 mites per gram of dust were a risk factor for mite-allergic patients with asthma, and he, as well as other investigators,⁵ found higher numbers of house dust mites in homes of mite-allergic compared to mite-nonallergic patients, whereas other investigators could not find this difference.^{3, 7, 24}

The influence of *Der p* I and *Der f* I exposure on specific immune response can be studied as a model for the relationship between environmental allergens and specific sensitization in individuals with atopic airway disease in general.

Our preliminary data already indicate a dose-response relationship between mite-allergen exposure and bronchial hyperreactivity.³⁴ It has also been demonstrated that mite-sensitive patients clinically improve if they move from a highly exposed environment to an area with low mite-allergen exposure.^{35, 36}

Procedures for allergen reduction either by chemicals (tannic acid or solidified benzyl benzoate) or bedding encasings may now be studied more scientifically than previously possible. According to our data, an allergen reduction of at least 80% to 90% in most houses to achieve allergen levels lower than the threshold concentration of 2000 ng/gm and to reduce the sensitization risk of an atopic individual should be demanded. Further studies are necessary to investigate whether the threshold concentration postulated in this study is similar for the development of atopic dermatitis that other investigators have already suggested.^{37, 38}

Finally, prospective studies are necessary to determine the significance of environmental allergens for the manifestation, severity, and clinical course of atopic disease in children with high and low genetic disposition for atopy.

REFERENCES

1. Smith TF, Kelly LB, Heyman PW, Wilkins SR, Platts-Mills TAE. Natural exposure and serum antibodies to house dust mite of mite-allergic children with asthma in Atlanta. *J ALLERGY CLIN IMMUNOL* 1985;76:782-8.
2. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalent allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727-35.
3. Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981;124:630-5.
4. Korsgaard J. Mite asthma and residency: a case-control study on the impact of exposure to house dust mites in dwellings. *Am Rev Respir Dis* 1983;128:231.
5. Ishii A, Takaoka M, Ichinoe M, Kabasawa Y, Ouchi T. Mite

- fauna and fungal flora in house dust from homes of asthmatic children. *Allergy* 1979;34:379-84.
6. Wharton GW. House dust mites. *J Med Entomol* 1976;12:557.
7. Schwartz B, Lind P, Lowenstein H. Level of indoor allergens in dust from homes of allergic and non-allergic individuals. *Int Arch Allergy Appl Immunol* 1987;82:447-9.
8. Dowse GK, Turner KJ, Stewart GA, Alpers MP, Woolcock AJ. The association between *Dermatophagoides* mites and increasing prevalence of asthma in village communities within the Papua New Guinea highlands. *J ALLERGY CLIN IMMUNOL* 1985;75:75.
9. van Bronswijk JEMH, Sinha RN. Pyroglyphid mites (Acari) and house dust allergy: a review. *J ALLERGY* 1971;47:31-52.
10. Voorhorst R, Spijkstra FThM, Varekamp H, Leupen MJ, Lykema AW. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces: identity with the house dust allergen. *J ALLERGY* 1967;39:325.
11. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P₁. *J Immunol* 1980;125:587.
12. Lind P. Purification and partial characterization of two major allergens from the house dust mite, *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753.
13. Holck A, Dale S, Sletten K. Purification and characterization of a major allergen from the house dust mite *Dermatophagoides farinae*. *Allergy* 1986;41:408-17.
14. Stewart GA. Isolation and characterization of the allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 1982;69:224.
15. Lind P. Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp 42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm 6). *Int Arch Allergy Appl Immunol* 1986;79:60-5.
16. Dandeu JP, Mao J, Lux M, Rabillon J, David B. Antigens and allergens in *Dermatophagoides farinae* mite. II. Purification of AG 11, a major allergen from *D. farinae*. *Immunology* 1982;46:679-87.
17. Le Mao J, Dandeu JP, Rabillon J, Lux M, David B. Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *J ALLERGY CLIN IMMUNOL* 1983;71:588.
18. Lind P, Lowenstein H. Identification of allergens in *Dermatophagoides pteronyssinus* mite-body extract by crossed radioimmuno-electrophoresis with 2 different antibody pools. *Scand J Immunol* 1983;17:263.
19. Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592-3.
20. Chapman MD, Heyman PW, Platts-Mills TAE. Epitope mapping of two major inhalent allergens, Der p I and Der f I, from mites of the genus *Dermatophagoides*. *J Immunol* 1987;39:1479-84.
21. Horn N, Lind P. Selection and characterization of monoclonal antibodies against a major allergen in *Dermatophagoides pteronyssinus*: species-specific and common epitopes in three *Dermatophagoides* species. *Int Arch Allergy Appl Immunol* 1987;83:404.
22. Platts-Mills TAE, Heyman PW, Chapman MD, Hayden ML, Wilkins SR. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P₁ equivalent in house dust and dust mite extracts. *J ALLERGY CLIN IMMUNOL* 1986;78:398-407.
23. Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae*, and *D. microceras*. *Allergy* 1986;41:442.
24. Platts-Mills TAE, Hayden ML, Chapman MD, Wilkins SR. Seasonal variation in dust mite and grass-pollen allergens in dust from houses of patients with asthma. *J ALLERGY CLIN IMMUNOL* 1987;79:781-91.
25. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Venereol* 1980;92 (suppl):44-7.
26. Malling HJ. Skin prick test testing and the use of histamine. *Allergy* 1984;596-601.
27. Ford AW, Seagroatt V, Platts-Mills TAE, Lowenstein H. A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J ALLERGY CLIN IMMUNOL* 1985;75:676-86.
28. Lind P, Hansen OC, Horn N. The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen Der p I of *Dermatophagoides pteronyssinus*: relative binding site locations and species specificity studied by solid-phase inhibition assay with radiolabeled antigen. *J Immunol* 1988;140(12):4256-62.
29. Siraganian RP. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383-94.
30. Heyman PW, Chapman MD, Platts-Mills TAE. Antigen Der f I from the dust mite *Dermatophagoides farinae*: structural comparison with Der p I from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 1986;137:2841.
31. Wahn U, Schweter C, Lind P, Lowenstein H. Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. *J ALLERGY CLIN IMMUNOL* 1988;360-70.
32. Arlian LG, Bernstein IL, Gallagher JS. The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. *J ALLERGY CLIN IMMUNOL* 1982;69:527-32.
33. Lau S, Weber A, Wahn U. Longitudinal study on house dust mite allergens. *N Engl J Allergy Proc* 1988;9:282.
34. Chur V, Falkenhörst G, Hermannsdoerfer P, Lay S, Wahn U. Studies on the influence of mite allergen exposure and the influence on sensitization and bronchial hyperreactivity of atopic children. *N Engl J Allergy Proc* 1988;9:295.
35. Platts-Mills TAE, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 1982;2:675-8.
36. Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust mite allergy: a controlled trial. *Pediatrics* 1983;71:418.
37. Mitchell EB, Crow J, Williams G, Platts-Mills TAE. Increase in skin mast cells following chronic house dust-mite exposure. *Br J Dermatol* 1986;114:65-73.
38. Norris PG, Schofield O, Camp RD. A study of the role of house dust mite in atopic dermatitis. *Br J Dermatol* 1988; (3):435-40.
39. Lind P, Ingemann L, Brouvez M. Demonstration of species-specific sensitization to major allergens of *Dermatophagoides* species by solid-phase absorption of human IgE antibodies. *Scand J Immunol* 1987;25:1.