

Airborne dust mite allergens: Comparison of group II allergens with group I mite allergen and cat-allergen *Fel d 1*

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*The form in which allergens become airborne is important because it may influence both symptoms caused by allergen exposure and methods used to reduce exposure. The group I allergens from dust mites only become airborne during disturbance and fall rapidly, which is in keeping with their being carried on fecal pellets. Their mean size is ~20 µm in diameter. By contrast, the cat-allergen *Fel d 1* is airborne on particles varying from >10 to <2 µm in diameter, some of which remain airborne even without disturbance. A second group of mite allergens, molecular weight 14,000, are equally important and are associated predominantly with mite bodies. With a monoclonal antibody-based assay and a cascade impactor, we have investigated the form in which group II mite allergens become airborne. The results reveal that these allergens only become airborne during disturbance and that they fall within 15 minutes. However, the mean size of the particles carrying group II allergens appears to be slightly smaller than the mean size of particles carrying group I allergens. In addition, the quantities of group II allergen becoming airborne during disturbance (mean, 26 ng/m³) could not be explained by the quantity found in fecal particles. Thus, group II mite allergens become airborne in a form quite distinct from cat allergens and very similar to group I mite allergens; however, it appears unlikely that fecal particles are the main form in which group II allergens become airborne. (J ALLERGY CLIN IMMUNOL 1991;88:919-26.)*

Key words: Mite allergens, airborne allergens, cat allergens, group II mite allergen

Sensitization to allergens produced by house dust mites of the genus *Dermatophagoides* has been associated with symptoms of asthma and rhinitis in many parts of the world.^{1,2} Three species, *D. pteronyssinus*, *D. farinae*, and *D. microceras*, are recognized as important sources of allergen in house dust.^{3,4} A first group of major mite allergens has been isolated, which

Abbreviations used

MW: Molecular weight
MAb: Monoclonal antibody
BSA: Bovine serum albumin
PBS: Phosphate-buffered saline
T: Tween

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includes *Der p 1*, *Der f 1*, and *Der m 1*.^{5,6} These allergens have the same MW (24,000) and are structurally homologous.⁷ Furthermore, the cDNA and sequence analyses suggest that group I allergens are thiol proteases.⁸ These proteins are heat labile and pH sensitive⁹ and are predominantly excreted in mite feces.¹⁰ Although the group I proteins reveal extensive cross-reactivity in allergic individuals, MAbs produced against these allergens are species specific.⁷ Lind¹¹ initially described the partial purification of a second *D. pteronyssinus* allergen, antigen *Dp X*. At the same time Holck et al.¹² and Yasueda et al.¹³ separately reported the purification of a major allergen

TABLE I. Levels of dust mite and cat allergen in dust obtained from the houses used for airborne studies

	<i>Der p</i> I (μ g/gm)	<i>Der f</i> I (μ g/gm)	Group II (μ g/gm)	Ratio group I to group II	<i>Fel d</i> I (μ g/gm)
M. Ga.	24	16.5	33	1.2	1000
L. Bl.	12	11.2	9	2.5	2.4*
A. M. Wi.	26	10	30	1.2	5600
F. Bl.	3.6	8.5	10	1.2	16*
A. Sm.	7	0.37	9	0.8	134
T. Ju.	14	7.2	11	1.9	1400
A. Ho.	1.8	27.5	14	2	1250

*Houses without a cat.

from *D. farinae* with an MW of 14,000 to 15,000 daltons. With MAbs, we purified *Der f* II, and from the amino acid sequences, demonstrated that *Der p* II, *Der f* II, and *Der m* II could be recognized as a second group (group II) of major mite allergens.¹⁴ In contrast, with the group I allergens, group II proteins are heat stable and pH resistant, and their physiologic function is unknown. Studies of the group II allergens have demonstrated that both murine and human antibodies recognize common epitopes on *Der p* II and *Der f* II. The cDNA cloning and sequence analysis of *Der p* II demonstrated that it is a 129 residue protein of MW 14,131 with no glycosylation sites and confirmed that there is no sequence homology with other proteins.¹⁵

Previous experiments demonstrated that group I mite allergens were airborne only during disturbance and were carried on particles $\geq 10 \mu$ m in diameter, which fall rapidly, in keeping with their large size.¹⁶ In contrast, cat allergen (*Fel d* I) remained airborne in undisturbed conditions and was associated with a large range of particle sizes, including particles $< 2.5 \mu$ m.¹⁷ We report in our study measurements of the airborne concentrations and particle-size distribution of group II mite allergens and comparison of these findings with group I and *Fel d* I in seven houses. The present results suggest that airborne group I and group II allergens were both associated with large particles but that group II allergens were associated with smaller particles than group I allergens. Neither type of mite protein was measurable under undisturbed conditions. The results raise questions about the nature of the particles carrying group II allergens. The results also demonstrate that both group I and group II mite allergens become airborne in a different form from that of cat allergen and demonstrate strikingly different settling characteristics. Furthermore, the results strengthen the view that strategies for controlling dust

mite allergens must be different from those for cat allergens.

MATERIAL AND METHODS

Two-site MAb-based assays for *Fel d* I, group I, and Group II mite allergens

The assays used here to measure group I mite allergen (*Der p* I and *Der f* I) and *Fel d* I were two-site MAb-based ELISA.¹⁸ Immulon II flat-bottom ELISA plates (Dynatech, Alexandria, Va.) were coated with 10 μ g/ml of either 6F9 (anti-FdIB MAb) or 5H8 anti-*Der p* I MAb, or 6A8 anti-*Der f* I MAb in 0.05 mol/L of carbonate-bicarbonate buffer, pH 9.6, overnight at 4° C. The plates were washed twice with PBS-T and blocked for 1 hour with 1% BSA-PBS-T (assay buffer).

After additional washes, 100 μ l of cat-allergen standard (The Office of Biologic Resources and Reagents, Food and Drug Administration cat E₅ standard contains 10.5 U of *Fel d* I per milliliter, 1 U equals 4 μ g of *Fel d* I), diluted in a range of 0.16 to 84 ng/ml or 100 μ l of *Der p* I (University of Virginia No. 87/03) or *Der f* I (University of Virginia No. 87/02) standard solutions diluted in a range of 0.05 to 250 ng/ml or 100 μ l eluate from the agarose, was added to the wells. These mite standards were substandardized from the World Health Organization standard, National Institute for Biological Standards and Control, 82-518. After several washes, 100 μ l of biotinylated MAb was added, either 3E4 anti-Fd1A MAb or 4C1 anti-*Der p* I and *Der f* I MAb. After additional washes, 100 μ l of streptavidin peroxidase (0.25 μ g/ml) (Sigma Chemical Co., St. Louis, Mo.) was added and incubated for 30 minutes. The assay was developed with 100 μ l of 0.01 mol/L of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonic acid) (A1888, Sigma Chemical Co.) in 0.07 mol/L of citrate phosphate. As reported previously, group II allergen was measured with a two-site monoclonal immunoassay.¹⁴ An anti-*Der f* II MAb 7A1 was coupled to CNB4-activated cellulose disks and incubated for 4 hours with 50 μ l of samples or serial dilutions of a standard. After additional washings, a ¹²⁵I-labeled anti-*Der p* II MAb CLB Dp X was added and incubated overnight at room temperature. After 10 washes,

TABLE II. Airborne *Fel d* I, group I, and group II mite allergens in the houses in undisturbed conditions*

Stages	Particle size (μm)	Mean <i>Fel d</i> I (ng/m^3)† (n = 8)	Range	%‡	Group II (ng/m^3) (n = 27)	Group I (ng/m^3) (n = 17)
1	6->20	1.78	<0.2-4.07	26	<0.3	<0.2
2	2-15	7.14	<0.2-30.5	34	<0.3	<0.2
3	1-5	2.5	<0.2-9.16	18	<0.3	<0.2
4 plus	<2.5	3.04	<0.2-3.8	22	<0.3	<0.2
Final filter						
Total		14.7	1-51.2		<0.3	<0.2
Parallel filter		17.4	2.8-51.6		<0.3	<0.2

*In four of the seven houses, airborne *Fel d* I, group I, and group II mite allergens were assayed during the same air sampling.†Particle size ranges are ranges listed by the manufacturer as described previously.^{16,17}

‡Airborne cat-allergen levels were sampled for 45 minutes in four houses.

§Values for percent on stage are mean values.

||Sampling for group I and group II mite allergens was performed for 20 and 45 minutes in seven houses and for 2 hours in one house.

the radioactivity was counted in a gamma counter (4/200, Micromedex System, Inc., Horsham, Pa.).

Air sampling

Air sampling was performed with a Cassella Mark II cascade impactor (Cassella, London, England). The four stages of the cascade impactor were loaded with 2.5 cm glass disks (T13 206, Cassella) coated with 5% agarose-sorbitol gel (5 gm of agarose [MCB AX 05 17-3] and 50 gm of D-sorbitol, S 1876, Sigma Chemical Co., in 100 ml of borate-buffered saline, pH 8.0). A glass fiber filter was run in parallel at the same flow rate to collect total airborne particles. The cascade impactor and the parallel filter were connected via a flow meter (British Oxygen Co., Boreham Wood, U.K.) to a vacuum pump. Air was sampled for periods of up to 2 hours at flow rates of between 18 to 19 L/min. The agarose-sorbitol gel was removed and eluted in 0.5 ml of BSA-PBS-T overnight at 4° C. As in our previous article,¹⁷ the results for the fourth stage of the cascade impactor were combined with the results of the final filter and expressed as particles <2.5 μm in diameter. The eluate from the glass-fiber filters was collected in 1 ml of BSA-PBS-T by compressing the filters in a 3 ml plastic syringe.

Design of experiments

Seven houses with high levels of group I and group II mite allergen in the carpet and furniture dust were selected. Five of these houses also had high levels of *Fel d* I in the dust (Table I). House dust was obtained with a hand-held vacuum cleaner, sieved, and extracted as previously described.¹⁹ Air was sampled for 20 minutes to 2 hours to assess concentration and particle-size distribution of airborne allergen in the room in which the dust contained the highest concentration of allergens. In five houses, air sampling was performed in the living room, and in the two other houses, in a bedroom. In the houses with a cat, airborne

measurements were performed in absence of the cat(s). All the living rooms were carpeted and contained a sofa. One bedroom had a carpet, the other bedroom had a wooden floor. The age of the houses varied from 150 to 10 years of age with an average of 42 years.

RESULTS

Airborne cat and mite allergens in undisturbed rooms

Under undisturbed conditions, air was sampled in houses in which high levels of cat and mite allergen were found in the dust (Table I). A mean value of 17.4 ng/m^3 of airborne *Fel d* I was measured. Sampling was performed with both a filter paper disk (parallel filter) and a cascade impactor. The results are listed as the quantity of allergen on the stages of the cascade impactor, and the equivalent diameters listed are the published values for this apparatus as described previously.^{16,17} In keeping with previous results, 22% of the particles carrying airborne cat allergen had an equivalent diameter of <2.5 μm , and a considerable variation in the concentration and the distribution of particle size of airborne *Fel d* I from house to house was observed. In none of the houses was group I or group II mite allergen measurable in the air, even with sampling times up to 2 hours (Table II).

Airborne mite and cat allergen during and after disturbance

Air sampling was performed during artificial disturbance with a vacuum cleaner without a filter at 1.5 m from the intake of the cascade impactor. Under these conditions, both group I (mean, 68 ng/m^3) and group II (mean, 25 ng/m^3) allergens were measurable

TABLE III. Airborne *Fel d* I, group I, and group II mite allergens in seven houses during disturbance*

Stages	Size (μm)	Airborne allergen in houses during disturbance				Nebulizer output			
		Mean group I (ng/m ³)	%†	Mean group II (ng/m ³)	%†	Mean <i>Fel d</i> I (ng/m ³)	%†	Mean group II‡ (ng/m ³)	%
1	6->20	37 (16/16)§	78	17 (18/18)	62	71 (8/8)	47	38	3
2	2-15	7.5 (13/16)	17.5	4.5 (17/18)	20.7	36 (8/8)	25	78	11
3	1-5	<1.0 (2/16)	<1.1	3.4 (2/18)	11.3	15 (8/8)	11	107	21
4 plus final filter	<2.5	<0.9 (2/16)	<2.4	0.9 (2/18)	<5.1	29 (8/8)	16	338	64
Total		52		26		172		527	
Parallel filter		68		25.5		212		—	

*Air was sampled during 20 minutes of disturbance; a vacuum cleaner (Shop-Vac Corp., Williamsport, Pa.) without a filter was used to clean the carpet, staying at least 1.5 m away from the sampler.

†Values are mean percent of group I allergen found on each stage of the cascade impactor.

‡Nebulizer contained 5 μg of group II mite allergen in 1 ml of saline with a nebulizer and a model 5610D pump (DeVilbiss Co.) ($n = 2$).

§Values in parenthesis indicate the proportion of experiments in which allergen was detected on that stage.

||The cat allergen measured on stage IV and the final filter was significantly different from the levels of either group I or group II allergen ($p < 0.01$) measured on the same stage and the final filter.

in all seven houses. Airborne group I and group II allergens were predominately associated with large particles, 96% and 83%, respectively (Table III). However, group II allergens appeared to be associated with smaller particles than particles of group I (Table III; Fig. 1). In most experiments, no mite allergen was detected on stage IV or the final filter. The total airborne *Fel d* I during disturbance was very high (i.e., 212 ng/m³). In contrast to mite, a significant proportion of this allergen (mean, 29 ng/m³) was associated with particles <2.5 μm (Table III; Fig. 1).

To assess the function of the cascade impactor with another form of small particles, we sampled nebulized group II mite allergens with a disposable nebulizer that is in use both for therapy and challenge procedures at the University of Virginia Hospital. A mean value of 3% of nebulized group II allergen was associated with particles collected on the first stage, and 64% was collected on the stage IV and final filter (Table III). These results were in keeping with the predicted size of droplets produced by a DeVilbiss (DeVilbiss Co., Somerset, Pa.) nebulizer, that is, ~0.5 to 5 μm in diameter and are very similar to droplets previously reported for nebulized cat and group I mite allergens.^{16, 17}

The falling properties of airborne mite and cat allergen were dramatically different (Table IV). Measurements were performed in parallel 20 minutes after the disturbance. At that time, no detectable group I

and group II allergens were airborne. In contrast, 32 ng/m³ of *Fel d* I was airborne 20 minutes after disturbance, corresponding with 15% remaining.

Measurements of *Der f* I and *Der f* II in fecal pellets isolated from a culture of *D. farinae*

When *D. farinae* fecal particles were isolated (60 to 400 in separate experiments) and eluted in saline, >90% of the measurable *Der f* I and *Der f* II eluted within 2 minutes (data not presented). High levels of *Der f* I were measured in mite feces, mean value, 0.07 ng per particle (mean value from four experiments). By contrast, the amount of *Der f* II was very low, mean value, 0.0035 ng per particle (mean value from four experiments). Comparison of these values suggests a ratio of group I to group II allergens of 20:1; however, when the ratio was calculated for each experiment, the mean value was 32:1. These values should be compared to the ratio of group I to group II in dust, mean value, 1.5:1 (Table I) or airborne, mean value, 2:1 (Table III). The results suggest that the group II allergen present in feces cannot explain the levels of group II allergen found either in floor dust or airborne during disturbance.

DISCUSSION

The structural and biochemical properties of the group II mite allergens are clearly different from the

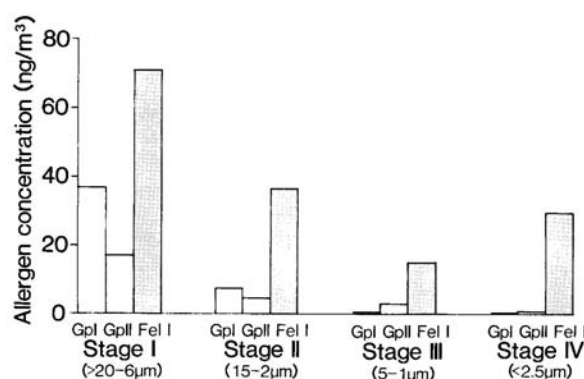


FIG. 1. Airborne mite- and cat-allergen levels on cascade-impactor stages during disturbance. Results are illustrated for the concentration in nanograms per cubic meter of Group I (*Gp I*) and group II (*Gp II*) mite allergens as well as cat allergen *Fel d I* (*Fel I*) on the stages of the cascade impactor. Values are the mean levels for 30-minute sampling (15 minutes of disturbance and 15 minutes after disturbance) in seven houses. Results illustrate that both group I and group II mite allergens are reduced relative to *Fel d I* in the smaller particles sizes. In addition, there was a significant increase in group II relative to group I when levels in stage III were compared with stage I.

TABLE IV. Total levels of mite and cat allergen before, during, and after disturbance

	Before disturbance (20-45 min)	During disturbance (15-20 min)	After disturbance (20 min)	% Remaining airborne after disturbance
Group I	<0.2 (n = 16)	68 (n = 16)	<0.2 (n = 4)	<0.3
Group II	<0.3 (n = 25)	25.5 (n = 18)	<0.3 (n = 4)	<1
<i>Fel d I</i>	17.02 (n = 8)	212.2 (n = 8)	32.27 (n = 4)	15

Values are from assays of parallel filter in nanograms per cubic meters. Sampling times from 15 to 45 minutes.

group I allergens.^{9, 14, 15} In keeping with this finding, there is no evidence for cross-reactivity between the two groups either with human sera or MAb. Most mite-allergic patients (>90%) reveal positive immediate skin tests and make IgG and IgE antibodies to either the group I or the group II allergens or both. The comparison reported in this study between airborne group I and group II mite allergen demonstrated that these two groups of proteins behaved aerodynamically very similarly. Airborne allergens were measured in parallel during the same air sampling. Our study demonstrated that group I and group II allergens were airborne only during disturbance and fell rapidly after disturbance. Previous experiments with group I allergens from our laboratory have demonstrated identical results.^{16, 20} Sakaguchi et al.,²¹ measuring allergen *Der f II*, which is now known as *Der f II*, reported similar results. Other studies with

different methods of measurements also found that airborne mite allergens fell more rapidly than airborne cat allergen.²² The investigators from Japan also reported very low concentrations of airborne group I and group II in rooms in which people "had an ordinary life," and they found that the ratio of group I to group II in the air during disturbance was 2.5:1.²¹ The techniques used for disturbance of dust continue to be a major problem in research on airborne allergens. Currently, there is no convincing way of standardizing disturbance because vacuum cleaner performance is very difficult to define. The procedure used here is clearly very vigorous, and the comparison between the different allergens during disturbance is of more interest than the total quantities. Indeed, the difficulty in obtaining measurable quantities of mite allergen airborne in a room might suggest that natural exposure occurs with the patient close to the source,

that is, with pillows, bedding, and sofas more than carpets.

We found 32 times more group I than group II in mite feces. Comparing this ratio to the ratio of group I to group II allergens in the air (i.e., 1.5:1; $n = 16$) or to the same ratio in the dust (2:1; $n = 7$), it was clear that the quantity of group II allergen detected airborne could not be explained solely by fecal pellets. Group II mite allergens are found in relatively higher amounts in mite bodies.²³ We assume that during a vigorous disturbance, airborne group II allergen is associated both with fecal particles and mite-body particles. Presumably, the mite-body parts contain more group II than group I allergen. The lower relative levels of group I allergen in dust and airborne particles could also be explained by the greater stability of group II allergens.⁹ However, group I allergens have not been demonstrated to be unstable in dust, and it is not clear how long mite allergens stay in a carpet before being removed by regular cleaning.

When airborne group I and group II mite allergens were compared with airborne cat allergen, a dramatic difference in airborne behavior was demonstrated. Mite-allergic patients are usually unaware of the relationship between house dust and their asthma symptoms. By contrast, cat-allergic patients often develop symptoms within minutes of entering a house in which a cat lives. Previous studies on domestic houses had demonstrated that cat allergen could become airborne on particles that had an aerodynamic equivalent diameter of $<2.5 \mu\text{m}$. Indeed, in some houses, the levels of *Fel d 1* associated with small particles were comparable to the quantities previously reported to produce acute airway obstruction in provocation experiments. From published studies, it is possible to estimate that bronchial provocation of cat-allergic patients requires between 8 to 80 ng of *Fel d 1* inhaled during 2 minutes.^{24, 25} In the present experiments, 127 ng/ m^3 of airborne *Fel d 1* associated with particles $<2.5 \mu\text{m}$ was measured during disturbance in one house. We estimate that it would take 6 minutes to inhale 8 ng of *Fel d 1* in a room with 127 ng/ m^3 of airborne *Fel d 1*. By contrast, during the same air sampling, the quantity of group I or group II allergens associated with small particles was $<1 \text{ ng}/\text{m}^3$.

When airborne group II allergen levels airborne in these houses were compared with nebulized extract of this allergen, a dramatic difference was also demonstrated. During the artificial disturbance used in our experiments (i.e., vacuuming with a modified vacuum cleaner), 83% of airborne group II was associated with large particles, whereas $\leq 5\%$ was apparently present on particles $<2.5 \mu\text{m}$. By contrast, when the output of a nebulizer is sampled, a reverse proportion was

observed, 14% of group II allergen was carried on large particles ($10 \mu\text{m}$) and 64% on particles $<2.5 \mu\text{m}$. These results suggest that for both group I and group II mite allergens, natural exposure is not similar to the procedure used for experimental bronchial provocation. However, bronchial-challenge test with nebulized cat extract appears to be a relevant model of the rapid bronchospasm that can occur after a natural exposure to airborne cat allergen. Much of the mite allergen is present on particles $>10 \mu\text{m}$ in diameter, which are often described as "nonrespirable." However, the actual data on inhalation of particles demonstrate that as much as 5% to 10% of these "large" particles will enter the lung.^{26, 27} The term "nonrespirable" should be considered to refer to the terminal bronchi and alveoli. We believe that the entry of a smaller number of large particles carrying high concentrations of group I or group II mite allergens will cause local foci of inflammation, which accumulate to contribute to overall bronchial reactivity. Thus, it appears that exposure to either group I or group II mite allergen may be in a form that can progressively contribute to bronchial reactivity without the patient being aware of acute bronchospasm at the time of exposure.^{28, 28a, 29} Although we refer to the sizes of these particles in micrometers of diameter, the two procedures used, that is, settling and the cascade impactor, measure the terminal velocity of the particles. The estimates of the diameters assume that the particles are approximately spherical and that they have a density close to that of water. If group II allergens are carried on fragments of mite cuticle, then they could be physically larger than the estimates. Similarly, the particles carrying airborne cat allergen, which have not yet been defined, may be flakes that are larger but behave as smaller particles, both on settling in a cascade impactor and in a liquid impinger.¹⁷ Differences of this kind could alter the impact of particles on the lung but would probably not alter the conclusions about appropriate techniques for reducing exposure to these allergens.

A major test of the relevance of airborne measurements of allergen will be the ability to design successful strategies to reduce exposure and disease. Experimental studies have demonstrated that airborne cat allergen can be dramatically reduced by a combination of washing the cat, reducing furnishings, vacuum cleaning, and air filtration.³⁰ Controlling airborne mite exposure appears to be different. Mite allergen is only airborne in very limited quantities before disturbance and falls relatively rapidly afterward; therefore, avoidance measures should primarily be directed at the source. Several previous studies in which bedrooms have been the focus of efforts to reduce

mite-allergen exposure have produced successful results.³¹⁻³⁴ The reduction of exposure can be achieved either by use of physical measures or acaricides, although it is clear that additional work is necessary on the techniques for applying acaricides to carpets and sofas. A recent study is encouraging that has confirmed that simple measures, such as covering mattresses, pillows, and bedding, combined with removing or treating bedroom carpets, can be effective in reducing both the symptoms of asthma and nonspecific bronchial reactivity.³⁵

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