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Relationship between mite, cat, and dog allergens in reservoir dust and ambient air

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Background: Standardized methods to measure allergen exposure are essential to assess the relationship between exposure, sensitization, and asthma. Most studies have measured allergen levels in reservoir dust, although air samples may be more representative as a measure of inhaled allergen. The aim of this study was to define the relationship between mite, cat, and dog allergen content in the reservoir dust and the levels in the ambient air.

Methods: Dust samples from the living-room floor (LF) and sofa (S) were collected in 127 homes: 62 without and 65 with pets (31 dogs, 34 cats). Air samples were taken in the same room, with a high-volume pump for 1 h (flow 60 l/min). Der p 1, Fel d 1, and Can f 1 were determined by mAb-based ELISA.

Results: Airborne Der p 1 was below the detection limit (0.8 ng/m³) in all homes, with reservoir levels (GM and range) being 1.14 µg/g (0.2–66) and 1.15 µg/g (0.2–127) in LF and S, respectively. Airborne Can f 1 was detected in 40/62 homes without pets (range 0.6–12.4 ng/m³) and in all homes with dogs (range 0.5–99 ng/m³). In the multiple linear regression analysis, Can f 1 level in the LF was an independent correlate of the airborne Can f 1 ($P=0.01$, homes with dogs; $P=0.04$, homes without dogs). Fel d 1 was detected in the air in 16/62 homes without pets (range 0.16–1.8 ng/m³) and in all homes with cats (range 0.4–22.3 ng/m³). Fel d 1 level in the LF was an independent correlate of the airborne Fel d 1 in homes without cats ($P=0.008$), but airborne levels in homes with cats did not correlate with reservoir levels.

Conclusions: The aerodynamics of each allergen must be taken into account when assessing exposure: while levels in reservoir dust are the best available index for mite allergens, airborne levels might be more suitable for defining exposure to pets. If

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air samples are difficult to obtain, levels of Can f 1 and Fel d 1 in the LF samples should be used as a surrogate measure of personal exposure.

Standardized methods for measuring personal exposure to indoor allergens are important for assessing the relationship between exposure and sensitization, and the degree of exposure associated with provocation of asthmatic symptoms. Over the past decade, immunoassays for measuring dust-mite, cat, dog, cockroach, rodent, and some fungal allergens have been developed. This technology has been used to compare allergen levels in reservoir dust samples from the homes of patients with asthma and other atopic disorders, to analyze the effect of various avoidance procedures on allergen levels, and to investigate the particle-size distribution of airborne allergens. These studies resulted in a better understanding of the dose-response relationship between IgE-mediated sensitization, asthma, and exposure to indoor allergens (1).

However, there is still a considerable debate as to what is the best indirect index of personal exposure, and whether exposure should be assessed by measuring settled dust from allergen reservoirs or by sampling ambient air (2). The aim of this study was to define the relationship between mite, cat, and dog allergen content in the reservoir dust and the levels in the ambient air.

Material and methods

Dust samples from the 1 m² of the living-room floor (LF) and sofa (S) were collected in 127 homes: 62 without and 65 with pets. Of the homes with pets, 31 contained dogs and 34 cats. Dust samples were collected with a Medivac dust sampler (Medivac Plc, Wilmslow, UK) with air-flow rate of 45 l/s through a 355- μ m diameter, stainless steel mesh (50 mm diameter) on to a 5-mm vinyl filter (56 mm diameter), thus enabling collection of samples of fine dust. The filters were supported in a plastic dust trap located behind the cleaner attachment (nozzle). A 1 m² area of carpet and upholstered furniture (sofa) were sampled for 2 min. An amount of 100 mg of fine dust was extracted with 2 ml borate-buffered saline with 0.1% Tween 20, pH 8.0. The dust was resuspended with a vortex mixer. Samples were then mixed end over end on an orbital rotator for 2 h at room temperature before being centrifuged for 20 min at 2500 RPM at 4°C. Supernatants were removed and stored at -40°C for future analysis of allergen content.

Air samples were collected in the same rooms before the collection of dust samples with a high-volume air sampler (Rotheroe-Mitchell, London, UK; air flow rate 60 l/min), situated 1 m above the floor, onto a 37-mm Whatman GFA micro-glass fibre filter (pore size 0.3 μ m). All rooms were carpeted and contained a sofa. The windows were closed during the sampling period, and no air-cleaning devices were present in any of the houses. The sampling was performed in the absence of any artificial disturbance. Each sampler had a flow meter indicator and a time counter. Flow rates were measured at the commencement of the sampling, and then at 10 min, 20 min, 30 min, and 1 h. The volume of the air sampled was calculated by multiplying a geometric mean of flow rate by the sampling time. Each filter was placed in a syringe, and 1 ml 1% bovine serum albumin in phosphate-buffered saline with 0.1% Tween 20 was added. After overnight extraction at 4°C, the extraction liquid was aspirated backward and forward several times through a three-way stop lock into a second syringe, and then transferred into a test tube and centrifuged at 3000 RPM for 30 min at 4°C. The supernatants were removed with a Pasteur pipette, coded, and stored at -40°C.

ELISA measurement of indoor allergens

Der p 1 and Fel d 1 were assayed with a two-site immunometric ELISA, as previously described (3, 4). The standard used to establish the control curve for Der p 1 assay (UVA 93/02) was considered to contain 2500 ng Der p 1/ml (relative to WHO/IUIS *Dermatophagoides pteronyssinus* standard NIBSC 82/518, which has been estimated to contain 12.5 μ g Der p 1 per ampoule). UVA 91/01 standard for Fel d 1 contained 2 units Fel d 1/ml (relative to CBER [Centre for Biological Evaluation and Research] Cat E5 standard containing 9.7 Units/ml; 1 Unit=4 μ g protein). Can f 1 was measured by a monoclonal/polyclonal ELISA using anti-Can f 1 mAb 6E9 for allergen capture and polyclonal rabbit anti-Can f 1 for detection. Dust extracts were initially assayed at 5-, 25-, and 125-fold dilution for homes without dogs, and at 100-, 500-, and 2500-fold dilutions for homes with dogs. The assay was quantitated by doubling dilutions of dog allergen standard (UVA 94/02) from 500 IU/ml to 1 IU/ml Can f 1. The UVA 94/02 (10000 IU Can f 1/ml) was substandardized against WHO/IUIS International Reference Preparation of dog hair and dander (NIBSC 84/685), which contains 100000 IU/ml Can f 1. One international unit is approximately 1 ng Can f 1 protein, and this value was used to calculate the results.

Table 1. Concentration of house-dust mite (Der p 1), cat (Fel d 1), and dog (Can f 1) allergens ($\mu\text{g/g}$; geometric means and 95% confidence intervals) in homes with and without pets

	With pets	Without pets
Der p 1 LF	1.1 (0.63–1.31)	1.45 (0.93–2.27)
Der p 1 S	0.96 (0.67–1.36)	1.39 (0.91–2.12)
	With dogs	Without dogs
Can f 1 LF	181.34 (102.02–322.33)	1.56 (1.17–2.08)
Can f 1 S	100.02 (42.01–238.07)	1.87 (1.37–2.57)
	With cats	Without cats
Fel d 1 LF	204.5 (108.54–385.3)	0.8 (0.61–1.06)
Fel d 1 S	208.89 (86.76–503.04)	1.36 (0.96–1.73)

LF: living room floor; S: sofa.

Air samples were assayed neat and at two-, four-, and eightfold dilutions. For concentrations lying off the linear portion of the standard curve, the assays were repeated at an appropriate dilution.

The allergen data were found to follow a log-normal distribution; the results are thus reported as geometric means (GM) and 95% confidence intervals (95% CI). Statistical significance was set at the conventional $<5\%$ level.

Results

Airborne Der p 1 was below the detection limit (0.8 ng/m^3) in all homes, with reservoir levels (GM and range) being $1.14 \mu\text{g/g}$ (0.2–66) and $1.15 \mu\text{g/g}$ (0.2–127) in LF and S, respectively. There was no difference in Der p 1 levels between homes with and without pets in any of the sampling sites (Table 1).

Airborne Can f 1 was detected in 40/62 homes without pets (range $0.6\text{--}12.4 \text{ ng/m}^3$) and in all homes with dogs (range $0.5\text{--}99 \text{ ng/m}^3$). High levels of Can f 1 ($>10 \mu\text{g/g}$ dust) were found in all homes with a dog and in 14% of homes without a dog. Can f 1 was readily detectable in homes without dogs, but the mean levels were 53-fold (95% CI 26–110) lower in upholstered furniture, and 116-fold (95% CI 64–210) lower in the living-room carpet than in houses with dogs (Table 1). In the multiple linear regression analysis, Can f 1 level in the LF, but not in the S, was an independent correlate of the airborne Can f 1 ($P=0.01$, homes with dogs; $P=0.04$, homes without dogs). When the analysis was conducted in the whole group, with the levels of mite and cat allergens being taken into account, Can f 1 level in

the LF remained the only significant correlate of the airborne Can f 1 ($P<0.001$).

In the homes without pets, there was a significant correlation between Fel d 1 and Can f 1 concentrations (LF: $r=0.36$, $P=0.004$; S: $r=0.46$, $P<0.0001$), but no correlation was found between mite-allergen levels and the concentrations of either Fel d 1 or Can f 1.

Fel d 1 was detected in the air in 16/62 homes without pets (range $0.16\text{--}1.8 \text{ ng/m}^3$) and in all homes with cats (range $0.4\text{--}22.3 \text{ ng/m}^3$). Fel d 1 was detectable in the dust from all homes without cats, but the levels were 153-fold (95% CI 71–331) lower in upholstered furniture and 254-fold (95% CI 141–456) lower in the LF than in houses with cats (Table 1). In the multiple linear regression analysis, the only independent correlate of the airborne Fel d 1 in all homes ($P=0.001$) and in homes without cats ($P=0.008$) was Fel d 1 concentration in LF. Airborne levels in homes with cats did not correlate with reservoir levels.

Discussion

It is important to define what constitutes clinically relevant personal exposure to allergens in the environment, as this can facilitate investigations of the quantitative relationship between exposure and allergic disease. Determining the quantity of inhaled allergen would be an ideal measure of personal exposure. Unfortunately, it is still technically very difficult to measure the amount of allergen inhaled by an individual. The aim of measuring allergen levels in homes is to determine the measure that will represent an “index of exposure” of person(s) living in that environment. Furthermore, in large epidemiologic studies, the technique of measuring exposure must be simple, reliable, and reproducible, and not time-consuming. To date, most studies have assessed allergen levels in several dust reservoirs (e.g., mattress, bedroom floor, living-room floor, upholstered furniture, and kitchen), without a clear indication of how the concentrations of allergens in different sites contribute to the personal exposure. In addition, it may be both time-consuming and expensive to collect all these samples when a large number of houses have to be visited.

It is also important to know where most personal exposure occurs. The pattern of exposure reflects the nature of allergens: in the case of mites, the highest levels of allergens are found in mattresses, and the exposure is low grade and chronic, occurring predominantly overnight in bed. In contrast, both in homes with and without pets, the highest levels of pet allergens in dust reservoirs and air are

found in the living-room, and it is here that most exposure may occur (5, 6).

Sampling of the ambient air is used in the assessment of occupational exposure and may be considered more representative as a measure of inhaled allergen than allergen levels in the reservoir dust (2). However, in most laboratories, airborne mite allergens are below the detection limit of the currently available assays if air samples are collected in undisturbed conditions. This was confirmed in the current study, as we failed to detect airborne Der p 1 in any of the air samples from 127 homes. This finding is in accord with the particle-size distribution of α Group 1 and α Group 2 allergens, which are predominantly associated with large particles ($>20\text{ }\mu\text{m}$) and fall rapidly after disturbance (7). We have previously found detectable airborne Der p 1 in homes only after vigorous disturbance (8).

Price et al. have reported a good correlation between sensitization and airborne levels of mite allergen (9). However, in this study, the reported levels of airborne Der p 1 were very low. Different investigators tried various methods to overcome the difficulties of airborne sampling. Sakaguchi et al. used a more sensitive radioimmunoassay for allergen measurement (10). The airborne levels of Der p 1 were very low (around 30 pg/m^3), but they increased about 1000-fold in disturbed conditions (bed-making). Furthermore, the levels in bed were approximately 10 times higher than those found during everyday life in the living-room (11).

Swanson et al. used an mAb-based ELISA for allergen determination, but increased the volume of air sampled to

288 m^3 by using high-volume samplers (200 l/min) operating for 24 h (12). Again, the concentration of airborne mite allergens was very low in the absence of disturbance ($20\text{--}30\text{ pg/m}^3$), but increased greatly in the bedroom during bed-making.

It is likely that pet allergens are being carried on clothing into homes without pets (13). Airborne Fel d 1 and Can f 1 were readily measured in houses without artificial disturbance, probably because a proportion of these allergens is associated with small particles ($<5\text{ }\mu\text{m}$ diameter) which easily become airborne, and may remain in the air for long periods of time (5, 6). Unfortunately, it is time-consuming to collect the air samples; moreover, the levels of cat and dog allergen are below the detection limit of the conventional assays in a proportion of homes without pets. The results of the current study indicate that the concentrations of cat and dog allergens in the LF may be used as a surrogate for the measurement of allergens in the ambient air.

The choice of whether to use reservoir dust or air samples should depend on the question the study addresses. Whichever method is used to monitor exposure, it has to be clinically valid, robust, reproducible, and reliable. When a detailed assessment of an individual's personal exposure is necessary, both air samples and settled dust samples from different reservoirs should be collected. However, in large epidemiologic studies, sampling settled dust is much more convenient than air sampling, and our data suggest that the concentration of mite allergens in the mattress and of pet allergens in the LF is an appropriate index of personal exposure.

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