Aerodynamic Properties of the Major Dog Allergen Can f 1: Distribution in Homes, Concentration, and Particle Size of Allergen in the Air

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Exposure and sensitization to dog allergen is a significant cause of asthma. In this study we investigated the distribution, aerodynamic characteristics, and particle-size distribution of the major dog allergen Can f 1. Dust samples were collected in 50 homes with a dog and 50 homes without dogs. Airborne Can f 1 concentration was measured in 28 homes with dogs and 36 homes without a dog. Particle-size distribution was determined by using 10 separate Andersen sampler measurements in a dog-handling facility, and in 10 homes with dogs, and by repeated measurements in a home with one dog. High levels of Can f 1 (> 10 μg/g) were found in dust in all but one home with a dog and in eight of 30 homes without dogs. Airborne Can f 1 levels varied greatly between the homes with dogs (range: 0.3 to 99 ng/m³). Low levels of airborne Can f 1 (range: 0.4 to 1.1 ng/m³) were detected in 11 of 36 homes without a dog. Can f 1 was predominantly associated with large particles collected on the first stage of the Andersen sampler (> 9 μm), which averaged 42 to 49% of the total allergen recovered in the dog-handling facility and in homes with dogs. Small particles (< 5 μm diameter) also carried Can f 1, and these particles comprised ~20% of the total airborne allergen load. There was an excellent concordance between the results obtained in different sampling areas, and between the total Can f 1 recovered on the Andersen sampler and on the parallel filter. In conclusion, airborne Can f 1 was detectable in undisturbed conditions in all homes with dogs and in almost one third of the homes without dogs. In houses with dogs, a significant proportion (~20%) of airborne Can f 1 was associated with small particles (< 5 μm diameter). Owing to their aerodynamic characteristics, these particles would be expected to remain airborne for a long period and, when inhaled, could penetrate into the lower airways and initiate asthma attacks. Custovic A, Green R, Fletcher A, Smith A, Pickering CA. Chapman MD, Woodcock A. Aerodynamic properties of the major dog allergen Can f 1: distribution in homes, concentration, and particle size of allergen in the air.

METHODS

Distribution and Airborne Levels of Can f 1 in Homes

Dust samples were collected by vacuuming a 1 m² area of mattress, living-room carpet, bedroom carpet, and upholstered furniture in 50 homes with a dog and 50 homes without a dog. The homes were located within a 10-mile radius of Wythenshawe Hospital, which is ~5 miles south of the center of Manchester, UK. The samples were collected with a Medix Due Sampler (Medivac Ltd., Wilmslow, UK) with an airflow rate 45 L/min, through a 355-μm-diameter mesh screen onto a 5-μm polyvinyl filter (Pallflex USA, Warrington, UK) that enabled collection of fine dust samples. Each sample was transferred into a preweighed petri dish, weighed, coded, and stored at 4°C until extraction. One hundred milligrams of fine dust was extracted with 2 mL of molar-buffered saline with 0.1% Tween 20 (BBS-T), pH 8.0. The extract was resuspended with a vortex mixer, and samples were rotated for 2 hr at room temperature before being centrifuged for 20 min at 2,500 rpm at 4°C. Supernatants were stored at −20°C prior to allergen analysis.

Airborne Can f 1 concentrations were measured in 28 homes with dogs and 16 homes without a dog. Air samples were collected in the absence of disturbance, using a fixed-location sampler and sampling volumes of 30-40 m³ of air. The sampling head was positioned in the middle of the living room at a height of 1.2 m.

Particle-size Distribution of Airborne Can f 1

Sampling techniques. Air sampling for particle-size distribution was done with an Andersen 2-stage ambient particle-sizer sampler Mark II (Gensho Anderson, Episcoach Div., Atlanta, GA). A low-volume pump (6 to 9 L/min; Medix-Dik, West Sussex, UK) sampled the air parallel to the Andersen sampler to collect total airborne particles. The samples were placed at a standardized monitoring location (center of the living room, 1.2 m above the floor).

The Andersen sampler is a multistage, multilfiber cascade impactor that comprises eight aluminum stages (17). The particle fractionation at different stages is as follows: preseparator and Stage 1 (0-10 μm); Stage 2: 0.7 to 5 μm; Stage 3: 5.9 to 7.5 μm; Stage 4: 7.5 to 10 μm; Stage 5: 10.0 to 15 μm; Stage 6: 15.0 to 25 μm; Stage 7: 25.0 to 40 μm; Stage 8: 40.0 to 60 μm. A continuous duty, carbon-vacuum pump that was attached to the sampler drew room air through the sampler at a constant airflow rate of 28.3 L/min (1 H/1/min). This flow minimizes particle bounce and fragmentation. The sampler collects particles according to their aerodynamic diameter.

Airborne particles were collected on 0.3-μm glass fiber filters (Whatman International Ltd., Maidstone, UK) placed into the insert of stainless-steel collection plates. At the end of a sampling period, the sampler was disassembled and the glass-fiber filters were placed in Petri dishes and kept at 4°C until extraction. The filters were cut into eight pieces, each placed into a 60-ml syringe. Three milliliters of 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) with 0.01% Tween 20 (1% BSA PBS-T) were added, and the samples were extracted at 4°C overnight. The extraction fluid was aspirated backward and forward several times through a three-way stopcock into a second syringe, transferred into a test tube, and centrifuged at 3,000 rpm for 30 min at 4°C. The supernatants were stored at −20°C. Filters from fixed-location samples were extracted in 1 ml 1% BSA PBS-T.

Experimental design. The study was designed to compare the levels of Can f 1 in the air over an 8-h period in a dog-handling facility and in homes with dogs. The sampling sites were as follows:

- The dog-handling facility was selected as a location likely to contain high levels of airborne allergens where repeated measurements could be performed. It comprised a domestic house together with various indoor units containing 100 kennels. A series of airborne measurements were made in a room in the house that formed part of the dog-handling facility.
- Three dogs (two Maltese, one Pekinese) were free to wander throughout the room, which was not carpeted and did not contain upholstered furniture. There was no artificial disturbance. Air samples were collected on 10 separate days. Fixed-location samplers (airflow rate 9 L/min) were used to measure the total airborne Can f 1 level at two different sites in the house; the room from which dogs were always excluded, and in a bedroom in the absence of the dogs.

Ten homes in which one or two dogs had been housed for at least 6 mo were selected to assess the particle-size distribution of airborne Can f 1 in houses containing dogs. An 8-h collection was made in each of the houses during the daytime hours. The dogs were free to wander throughout the house. The living room in one of the houses had a wooden floor and no upholstered furniture. All other living rooms were carpeted and contained a sofa. The age of the houses ranged from 20 to 30 yr.

One home with a dog was randomly selected to assess the reproducibility of the air-sampling measurements in a single house. Ten separate Andersen sampler collections were made overnight in the absence of artificial disturbance. The house was ~50-yr-old, and the living room was carpeted and contained a sofa.

Personal Sampler Measurements

To assess exposure to Can f 1 among individuals in close contact with dogs, personal breathing-zone air was sampled on 10 separate occasions, using 100,000 USP Class II ambulatory pumps with flow rates of 2 L/min by different individuals grooming the dogs. Samples were collected over a period of 1 to 2.5 h.

For all air sampling, allergens were collected on 25-mm-diameter filter papers, cut into circular pieces, and stored at −20°C until analysis. The filters were then analyzed by double-diffusion in gel (20) for the presence of Can f 1 antibodies.

Can f 1 Enzyme-linked Immunosorbent Assay

Can f 1 was measured with a two-site monoclonal antibody enzyme-linked immunosorbent assay (ELISA) using anti-Can f 1 Fab anti-Can f 1 Fab for antigen capture and polyclonal rabbit-anti-Can f 1 Fab for detection (8). Dust extracts were initially assayed at 5-, 25-, and 125-fold dilutions for homes without dogs, and at 100-, 500-, and 2,500-fold dilutions for homes with dogs. Airborne samples were assayed neat and at 2-, 4-, and 8-fold dilutions. For concentrations lying off the slope of the standard curve, the assays were repeated at an appropriate dilution.

The assay was quantitated with doubling dilutions of dog-allergen standard (VNAQ 94/02) from 1000 μg/mL to 1 μg/mL Can f 1. The VNAQ 94/02 standard contained 10,000 USP Can f 1/ml relative to the World Health Organization/International Union of Immunological Societies (WHO/IUIS) International Reference Preparation of dog hair and dander (Code NIBSC 84/885), which contains 100,000 USP/mg Can f 1. It has been estimated that 1 μg of this preparation is equivalent to 1 μg Can f 1, and this value was used to calculate the results (8).

RESULTS

Reservoir Levels of Can f 1 in Dust Samples from Homes

One hundred homes in Manchester, UK (50 with a dog and 50 without a dog) were visited and dust samples (from living-room carpet, upholstered furniture, bedroom carpet, and mattress) were assayed for Can f 1. High levels of Can f 1 (>10 μg/g dust) were found in all but one of the homes with a dog and in 16% (eight of 50) of the homes without a dog (Figure 1). In the homes with dogs, the highest levels of Can f 1 were found in living-room carpets (GM=340 μg/g; 95% CI: 279 to 500 μg/g), followed by the upholstered furniture (GM=293 μg/g; 95% CI: 204 to 422 μg/g). Bedroooms contained lower levels (carpet: GM=93 μg/g; 95% CI: 62 to 140 g; mattress: GM=67 μg/g; 95% CI: 47 to 97 μg/g). Can f 1 was readily detectable in homes without dogs, but the levels were 10 to 100-fold lower than in homes with dogs. The highest levels of Can f 1 in homes without a dog were found in the upholstered furniture from the living room area (GM: 2.3 μg/g; 95% CI: 1.5 to 3.6 μg/g), followed by the living-room carpet (GM: 1.4 μg/g; 95% CI: 0.9 to 2.2 μg/g). Bedroooms contained significantly lower levels than living rooms (carpet: GM: 0.3 μg/g; 95%
The concentration of Can f 1 in the air of 28 homes with dogs and 36 homes without a dog, in the absence of disturbance, is shown in Figure 2. Airborne Can f 1 was detected in all houses with dogs (the level in one of the homes being at the detection limit), at concentrations of 0.3 to 99 ng/m³. Low concentrations of Can f 1 (range: 0.4 to 1.1 ng/m³) were detected in 11 of 36 homes without a dog, and in the remaining 25 homes Can f 1 was not detectable (< 0.3 ng/m³). There was no correlation between airborne allergen and the levels in dust reservoirs.

Initial experiments to determine the aerodynamic particle size of Can f 1 were done in a dog-handling facility (kennels), where there was likely to be measurable Can f 1 in the air. Can f 1 was predominantly associated with large particles (> 9 μm), collected on the first stage of the Andersen sampler, which averaged < 49% of the total allergen recovered. In each of the 10 measurements performed, less than 20% of the total airborne Can f 1 was detected on the last five stages of the sampler, comprising particles < 4.7 μm diameter.

As shown in Figure 3, there was excellent concordance between the results of airborne Can f 1 measurements obtained in different sampling areas. The particle-size distribution was consistent despite considerable differences in the absolute allergen levels between the dog-handling facility and homes with dogs. The only difference was a slightly higher percentage of Can f 1 on smaller particles (< 4.7 μm) in the homes with dogs (mean ~ 20%) as compared with the dog-handling facility (mean ~ 2%). In a series of 10 homes housing dogs, the total airborne Can f 1 recovered from all stages of Andersen samplers ranged from 1.2 to 74.5 ng/m³. All houses contained Can f 1 carried on small particles (< 4.7 μm diameter), the proportion on these particles ranging from 7 to 34%. Analysis of Andersen-sampler measurements in the dog-handling facility and repeated measurements in the homes with a dog showed a very consistent pattern, both in terms of particle-size distribution and total allergen recovery (Figure 4). Mean levels of Can f 1 (ng/m³) recovered from different stages of Andersen samplers in three sampling areas are shown in Table 1. The airborne allergen was further measured on a parallel filter to confirm the levels obtained with the Andersen sampler. There was a good agreement between the total airborne Can f 1 recovered from the Andersen sampler (an aggregate of all stages) and on the parallel filter (Table 1).

Several further measurements were made in a house within the dog-handling facility. To investigate the effect of the presence of a dog on airborne Can f 1 levels, the allergen was measured in a room in this house adjacent to the room with three
TABLE 1

<table>
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<tr>
<th>Sample Size</th>
<th>Area</th>
<th>µm</th>
<th>µm</th>
<th>µm</th>
<th>µm</th>
<th>µm</th>
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</thead>
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<td>22</td>
<td>18</td>
<td>7</td>
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</tr>
<tr>
<td>10 homes</td>
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<td>7.9</td>
<td>10.5</td>
<td>14.7</td>
<td>22</td>
<td>2.1</td>
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<td>5.1</td>
<td>1.3</td>
<td>2.7</td>
<td>4.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Home with dog</td>
<td>3.6</td>
<td>2.7</td>
<td>6.6</td>
<td>3.0</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Home with one dog</td>
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<td>1.2</td>
<td>2.6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.2</td>
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</table>

*Results are expressed as mg Can f1/m³ (GM and 95% CI). Samples with Can f1 below the detection limit of the assay (0.2 ng/m³) were assigned the value of 0.2 ng/m³ for the analysis. Total airborne Can f1 levels (ng/m³) recovered from the Andersen sampler (an aggregate of all stages) and an on the personal filter (Can f1 and 99.5%) were as follows: 176 (21.5 to 334) and 55 (26 to 354) for the study in London, 7.3 (5.8 to 8.3) and 9.8 (7.0 to 10.6) in the dog-handling facility, 10 homes with dogs, and the home with one dog respectively.*

**DISCUSSION**

Our results for Can f1 levels in reservoir dust samples in Manchester, UK, are similar to the recent data reported by Ingrain and colleagues from Los Alamos (8). Furthermore, the results of the current study are in agreement with those reported by Wood and associates, which found that many homes nominally without a dog contained significant dog-allergen levels (18). The distribution of dog allergens in the dust differed between the homes with or without an animal. The highest concentrations of Can f1 in the homes without a dog were found in the upholstered furniture in the living room, supporting the view that allergen can be passively transferred into houses without dogs, probably on the owners’ clothing. Not surprisingly, in homes with dogs, the distribution of allergens reflected that of the animal; the highest levels were found in living-room carpets and the lowest in beds. Despite the importance of dog allergens in causing IgE antibody responses and asthma, little has been known about the distribution and aerodynamic characteristics of these proteins (5-8). This study is the first detailed investigation of the aerodynamic properties of dog allergen using Can f1 as a marker protein. The objective was to obtain data on the absolute quantities of airborne Can f1 and to establish the size of particles associated with the allergen. The results show that the majority of Can f1 (>95%) is carried on large particles >10 µm in diameter. However, >20% is carried on particles <4.7 µm in diameter. This is similar to cat allergen, since approximately 25% of airborne Fel d 1 was shown to be associated with small particles (<5 µm) (9). The physical properties of airborne particles, including their size, shape, and density, are important determinants of the sites of their deposition within the human respiratory tract (19, 20). Intrathoracic deposition of relatively large particles (>10 µm) is still controversial, whereas particles 2 to 5 µm in diameter can more readily penetrate into the lower airways (19). Although small particles may be more relevant in terms of acute symptoms, by virtue of their deposition in the lung, the relative roles of particles of different sizes and shapes is as yet undetermined. Large particles are likely to be effective in perpetuating the IgE response, thus possibly contributing to chronic inflammation.

We found a considerable difference in total airborne Can f1 between sampling areas, the mean levels in the dog kennel being ~30 times higher than those in the homes with dogs. Nonetheless, the particle-size distribution was consistent in all sites, and both total airborne Can f1 and the particle-size distribution remained relatively constant within a given indoor environment. Whatever the absolute quantity of dog allergen in the air, approximately 20% was associated with <5 µm particles. These particles would be expected to remain airborne for several hours, and when inhaled, to penetrate into the lung. In the absence of disturbance, airborne Can f1 was detected in all homes with an indoor dog, but the levels varied greatly between the homes. There are several possible explanations for this finding (e.g., differences in the air exchange rate between the houses, variability in the amount of Can f1 shed by different dogs). It is important to note that airborne Can f1 can be found in the absence of disturbance in houses that have never housed a dog, albeit in comparatively low concentrations. These results suggest that individuals living in homes without an animal can be exposed to low levels of Can f1 in their homes. Furthermore, exposure to the allergen of domestic pets can occur in schools, restaurants, cinemas, public transport, and even hospitals (21, 22). It is therefore possible that passive exposure could contribute to asthma symptoms in dog-allergic patients who have never lived in a home with a dog. It is not clear, and as yet there are no data on the dose of airborne allergen that causes sensitization in individuals at risk.

There are considerable differences between the airborne behavior of mite, cat, and dog allergens (8-15). Airborne Group 1 and Group 2 mite allergens can be detected only after vigorous disturbance, whereas airborne Fel d 1 and Can f1 can be readily measured in households without artificial disturbance (8, 9, 14, 15, 23, 24). These differences in the aerodynamic characteristics of different allergens could explain a substantial difference in the clinical presentation between mite-sensitive asthmatic individuals and those who are sensitized to pets. Mite-allergic patients are usually unaware of the relationship between exposure at home and asthma symptoms, and even a carefully taken history usually cannot unequivocally implicate mites as a cause of symptoms. The exposure to mite allergens is probably low grade and chronic, occurring predominantly overnight while the sub...
ject is in bed. On the other hand, cat- or dog-allergic patients often develop symptoms within minutes of entering a home with a pet, or simply by stroking an animal. This is consistent with a finding of airborne Fel d 1 and Can f 1 associated with small particles even in the absence of disturbance. Aerodynamic differences between mice and pet allergens have to be taken into account in assessing exposure. Although levels in settled dust are the best available index for mice allergens, airborne levels might be more suitable for defining exposure to Can f 1 and Fel d 1. Personal sampling while grooming dogs demonstrated extremely high exposure to airborne dog allergen, often above 1 µg Can f 1/m³. This suggests that a person in the close vicinity of a dog could inhale a large quantity of allergens, and is consistent with anecdotal reports of dog-allergic patients experiencing asthma symptoms after stroking a dog.

In conclusion, Can f 1 is readily detectable in the air of homes containing dogs, and provided an excellent marker of dog-allergen exposure. Most of the airborne allergen was associated with particles > 10 µm in diameter, but a significant proportion (~20%) was consistently associated with < 5 µm particles (irrespective of the absolute quantity of Can f 1 in the air). Our results suggest that it may be possible to use measurements of Can f 1 in the air as an alternative to reservoir dust measurements for assessing exposure to dog allergens. However, this will require further clinical studies to investigate the relationship between airborne allergen exposure, sensitization, and symptoms in dog-allergic patients. Additionally, knowledge of the aerodynamic properties of dog allergens should lead to greater understanding of the mechanisms of sensitization and factors that influence bronchial hyperreactivity in patients with asthma.

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References