

Detection of inhaled Der p 1

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Summary

Background Measurement of personal exposure to Der p 1 aeroallergen has previously been limited by the low quantity of material collected by sampling systems and the assay sensitivity. This has meant that exposure could only be detected if long sampling periods were used or reservoir dust was artificially disturbed. We have developed a sampling method to sample true personal exposure and combined it with a novel method which is sensitive enough to measure allergen exposure over shorter time frames.

Objective To describe normal domestic exposure to dust mite allergen during a range of activities in houses in Sydney, Australia.

Methods Inhaled particles containing mite allergen Der p 1 were collected using a nasal air sampler which impacts particles ($> \approx 5 \mu\text{m}$) onto a protein-binding membrane coated with a thin, porous, adhesive film. The allergen is bound to the membrane in the immediate vicinity of the particle and detected by immunostaining with monoclonal antibodies specific for Der p 1. In addition, samples were collected using a standard Institute of Occupational Medicine (IOM) personal air sampler and the amount of eluted Der p 1 was assayed by ELISA.

Results The median number (range) of inhaled particles containing Der p 1 collected in each 10-min sampling period was: dust raising 5 (2–10); lying in bed, 0 (0–2); sitting on the bed, 1 (0–2); walking around the bedroom, 0 (0–2). This represented 0–5.1% of all particles captured. The Der p 1 concentration of floor and bed dust was 19.4 and 55.1 $\mu\text{g/g}$, respectively. The standard IOM personal sampler and ELISA were unable to detect Der p 1 for any of the activities performed.

Conclusions We were able to count individual allergen-carrying particles inhaled over short time periods, during different domestic exposure situations. This will offer new insight into several aspects of personal allergen exposure.

Keywords: aeroallergen, Der p 1, dust mite, immunodetection, monoclonal antibodies, personal exposure

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Introduction

Exposure to aeroallergens derived from house dust mite, cat, dog, fungi and cockroach results in sensitization, which is a strong risk factor for the development of asthma in susceptible atopic individuals (see review [1]). The quantity of these allergens which become airborne during normal activities is difficult to measure reliably [2–5]. Current air sampling techniques do not attempt to measure normal

personal exposures since they require: either high and constant flow rates [6,7]; massive disturbance of reservoirs [8,9]; or prolonged sampling periods [10] to collect sufficient allergen to measure. In view of this, most investigators have relied on the surrogate marker of reservoir allergen concentration as an index of exposure [1,11–14]. We have developed a small, discrete nasal air sampler which is worn inside the nasal vestibule and collects inhaled particles by impaction [15]. Airborne particles are impacted onto a collection plate with the normal breathing cycle. This should provide a more accurate measure of natural personal exposure. In addition, we have developed

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a sensitive method for the detection of allergen and its inhaled source [16]. Allergen is eluted from the particles collected by the nasal air sampler and is bound to a protein-binding membrane in a concentrated halo around the particle, and can be detected by immunostaining using allergen-specific monoclonal antibodies. The combination of the sampler and the immunodetection system would be suitable for clinical studies of personal exposure to a wide range of aeroallergens.

To describe normal domestic exposure and demonstrate the utility of this system, the nasal air sampler and the sensitive immunodetection method have been used to detect airborne house dust mite allergen. The number of inhaled particles containing Der p 1 were recorded for a range of activities performed in homes and in a low allergen room. A standard personal IOM (Institute of Occupational Medicine) [17] air sampler was run at a flow rate of 2 L/min in parallel to provide a comparison with the nasal sampler.

Materials and methods

Activities

Samples were collected by two different people, each in a different home. Person A performed six different activities in home A. The activities were: (1) lying in bed; (2) sitting on the bed; (3) walking around the centre of the bedroom; (4) dust raising — brushing the carpet with a wire brush every 30 s; (5) sitting in an uncarpeted laboratory tea room; and (6) sitting in an uncarpeted laboratory tea room and wearing a sweatshirt that was known to contain high levels of Der p 1. The first four activities were performed in a carpeted bedroom. Each activity was performed five times in succession, in the same room on the same day. For all activities, samples were taken while exclusively nose breathing (mouth closed). Person B performed six replicates of activity 1 and four replicates of activity 4 in home B.

Airborne allergen exposure during each activity was assayed using four different collection and immunodetection techniques. In addition, reservoir dust samples were obtained and assayed for Der p 1 content.

Intranasal air sampling

Intranasal air sampling was performed using small samplers worn just inside each nostril (Fig. 1) which collect most particles $>5 \mu\text{m}$ in diameter by impaction on a sample collection plate [15]. Sampling periods were for 10 min each and five consecutive replicates of each activity were separately sampled. A 10-min sampling period was chosen to demonstrate the sensitivity of the detection system, although the intranasal air samplers can be worn comfortably for several hours. Two different assays were

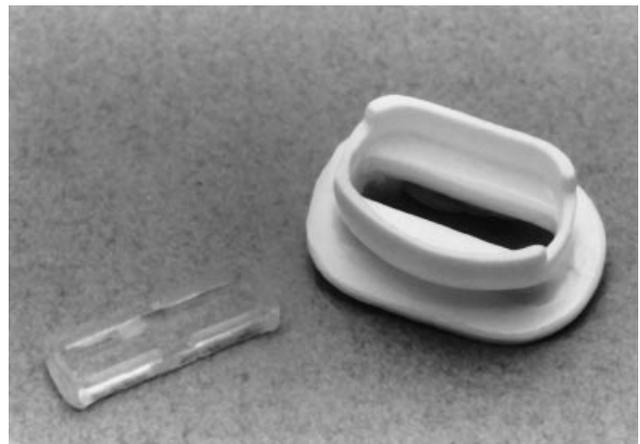


Fig. 1. The intranasal air sampler and collection plate.

performed on intranasal samples collected in parallel (one per nostril) and samplers were rotated between nostrils after 5 min to compensate for possible differences in air flow between nostrils. For each pair of nasal samples, one sample was directly immunostained to detect particles containing Der p 1, while the Der p 1 collected on the other sampler was assayed by ELISA. ELISA was chosen for comparison since this is the technique most widely used for measurement of Der p 1.

Assay: direct immunostaining of particles carrying Der p 1

Particles were directly impacted onto an allergen-binding membrane mounted in the sampler. Prior to collection, the membrane (0.22 μm NitroBind nitrocellulose transfer membrane, Micron Separations Inc., MA, USA) had been incubated in a mite-specific monoclonal antibody (5H8, 10 $\mu\text{g}/\text{mL}$ for ~ 3 h, Indoor Biotechnologies Inc., Charlottesville VA, USA). After this, membranes were coated with a thin film of agarose containing a humectant and an aqueous-based adhesive (proprietary system, Institute of Respiratory Medicine, Sydney, Australia). After collection of particles, the membranes were stored at 4°C for 12 h to allow allergens to elute from the particles and bind to the capture antibody on the membrane in close proximity to the particle. The membranes were then coated with a second layer of agarose to retain the particles in proximity to their eluted allergen. Membranes were blocked in 5% skim milk in PBS for 1 h and then incubated with a biotinylated second monoclonal antibody (4C1, Indoor Biotechnologies Inc.) for 3 h, washed three times in PBS/Tween, followed by incubation with ExtrAvidin® alkaline phosphatase conjugate (Sigma Aldrich Chemical Co., Sydney, Australia) for 2 h, followed by three washes and development of colour with BCIP/NBT (Sigma Aldrich Chemical Co.). Particles

containing Der p 1 were identified by the presence of a halo of stain around the particle. These particles were counted and the halos and particles were sized using a calibrated eye piece graticule under transmission microscopy at 100 \times and 200 \times magnification. The particle sizes presented are an average of their diameter, calculated as the square root of their length by width measurements.

Assay: ELISA assay of samples collected on intranasal sampler

Particles were directly impacted onto membranes which did not bind protein and these were mounted in the nasal sampler. These membranes were low protein-binding 0.22 μm hydrophilic Durapore (GVWP047, Millipore, Bedford, MA, USA) previously blocked with 3% BSA in PBS/Tween. These membranes were also coated with an agarose film containing a humectant and an aqueous adhesive prior to sampling. After sampling, the membranes were eluted in 500 μL of elution buffer (1% BSA in PBS/Tween) at 4°C overnight. After brief centrifugation, the neat supernatants were analysed for Der p 1 content using a conventional ELISA [18]. The detection limit was 0.98 ng Der p 1/mL.

Institute of Occupational Medicine (IOM) samples and ELISA

At the same time as the nasal samples were taken, a standard IOM personal sampler operating at a flow rate of 2 L/min was attached to the subject's collar [17]. The personal sampler contained a 25-mm diameter low protein-binding membrane (0.8 μm mixed cellulose AA type; Millipore) for the first three replicates of each activity for persons A and B. Allergens were eluted from the membrane and assayed neat for Der p 1 as described for the nasal samples.

IOM samples and direct immunostaining of particles carrying Der p 1

For the remaining two replicates of each activity, a PVDF membrane (Polyscreen, NEN Research Products, Boston, MA, USA) was placed in person A's IOM personal sampler and the flow rate adjusted to 2 L/min. After sampling, the membrane was covered with an adhesive tape, briefly wetted in absolute ethanol, followed by water and then PBS and left at 4°C overnight in PBS to allow elution of protein from the particles onto the PVDF membrane. Membranes were blocked in 5% skim milk in PBS and then directly immunostained using an anti-Der p 1 monoclonal antibody (5H8, Indoor Biotechnologies Inc.) followed by an antimouse alkaline phosphatase conjugate (Sigma) and development of colour using BCIP/NBT

(Sigma). Positively stained particles and halos were visualized through the adhesive tape on the membrane and were sized and counted as described above.

Reservoir sampling

Reservoir dust samples were taken from the bedroom carpet, bedding and sweatshirt using a Sanyo 5C-21R (850 Watt; Sydney, Australia) vacuum cleaner with modifications to hold two tapered nylon bags, mounted in series with decreasing pore size (coarse filter: 0.6 mm and fine filter: 25 μm). One square metre of carpet in the centre of the room was vacuumed for 1 min, the bedding (upper bedding 1 min, pillow 30 s and lower bedding 30 s), clothing and tea room floor were all vacuumed for 2 min. The fine dust was weighed and assayed for Der p 1 by ELISA [18].

Subjects

The nasal air samplers were worn by two healthy female volunteers (LP [subject A] — Home A and low allergen room; SD [subject B] — Home B). Mean nasal inspiratory flow rates were determined for each subject, with the nasal samplers in place, by breathing through a close fitting nasal mask attached to a pneumotachometer. Subject A breathed at 10 breaths/min with a mean inspiratory flow of 12 L/min and subject B breathed 20 breaths/min at 17 L/min. From an analysis of this data, this represented 63 and 105 L of air, respectively, at a flow of ≥ 10 L/min over the 10-min period. The studies were approved by the University of Sydney Ethics Committee. The use of the nasal sampling device was exempt from the Clinical Trials Notification Scheme of the Therapeutic Goods Administration of Australia.

Results

Inhaled particles containing Der p 1 were identifiable by immunostaining during all activities in at least one of the five replicates (Fig. 2). The number of inhaled particles containing Der p 1 collected in a single nostril over a 10-min period (equivalent to 5 min of total exposure) was low (Fig. 3). There was no evidence of filter overloading on any of the samples. The greatest number of Der p 1-containing particles was inhaled by Subject A during dust raising (median 5, range 2–10). Fewer particles were detected whilst lying in bed (median 0, range 0–2), sitting on the bed (median 1, range 0–2) and while walking around the centre of the bedroom (median 0, range 0–2). Subject B inhaled a similar number of Der p 1 particles while dust raising (median 4, range 2–6), but their highest exposure was for two of six replicates collected while lying in bed (median 3, range 1–9). The Der p 1 concentration

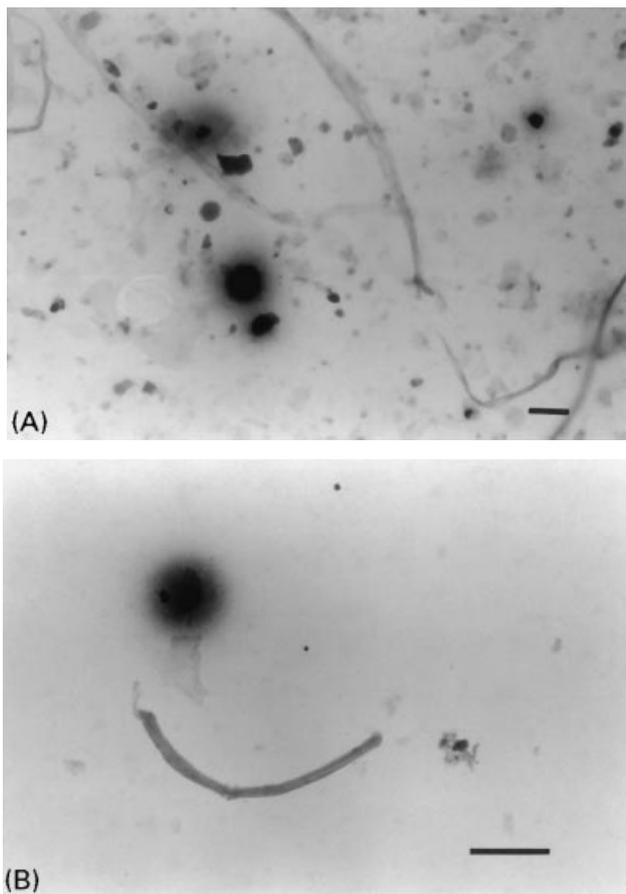


Fig. 2. Particles captured by the nasal air sampler, immunostained with Der p 1-specific monoclonal antibodies. (a) Dust raising sample showing four positively stained particles, bar is 50 μm . (b) Low allergen room sample with one particle containing Der p 1, bar is 110 μm .

in dust collected from the carpet and beds of the two houses studied were: House A, 19.4 and 55.1 $\mu\text{g/g}$; and House B 71 and 531 μg Der p 1/g sieved dust, respectively. In samples collected while sitting in an uncarpeted 'low allergen' room with floor Der p 1 concentrations of 0.2 μg Der p 1/g sieved dust, inhaled samples showed similar low or undetectable numbers of Der p 1-containing particles even while wearing a sweatshirt containing high (65 $\mu\text{g/g}$ sieved dust) levels of Der p 1. For Subject A, the percentage of all particles captured by the nasal sampler which contained detectable Der p 1 varied from a low of 0.5% (walking, and while sitting in the 'low allergen' room) to a high of 5.1% (lying in bed).

Der p 1 was contained on a variety of particles, and not exclusively on mite faecal pellets as previously observed [19]. Particles that contained Der p 1 ranged in largest diameter from 5 to 162 μm (Fig. 4), although particles less than 5 μm were difficult to enumerate. The mean sizes of

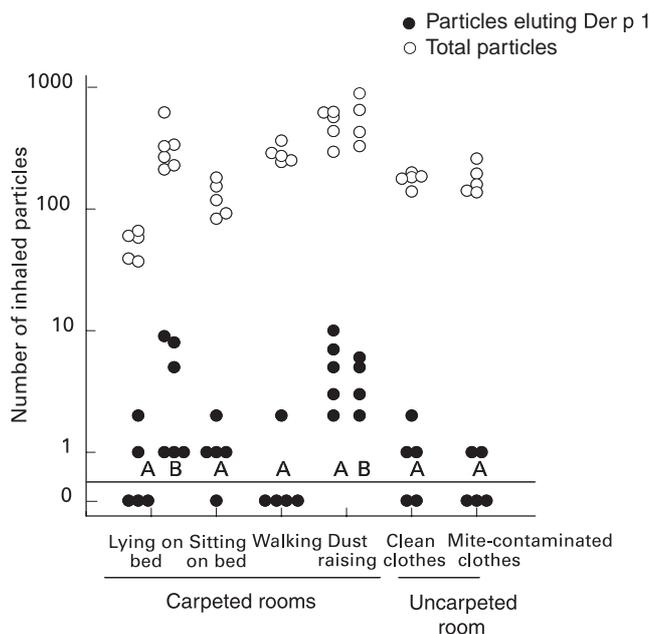


Fig. 3. Inhaled particles (per nostril) containing mite allergen and total number of particles captured. A and B represent the two houses sampled.

particles containing Der p 1 collected on the intranasal and IOM samplers were 49 and 27 μm , respectively. Of the Der p 1-containing particles collected by the intranasal and IOM samplers, 11 and 48%, respectively, were $\leq 10 \mu\text{m}$ in diameter.

The IOM sampler combined with the particle immunostaining technique provided an absolute quantification of ambient mite particles per volume of air as a contrast.

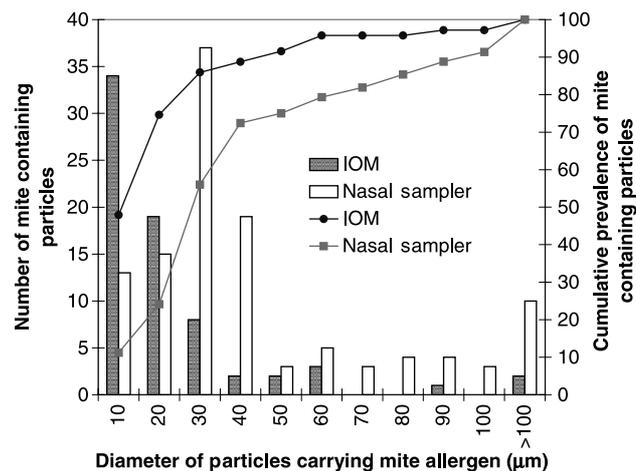


Fig. 4 Size distribution, and cumulative frequency plots of particle size of mite allergen-containing particles captured by the nasal sampler and the IOM sampler.

Using the IOM sampler, Subject A collected eight and 22 Der p 1 particles whilst lying in bed (equivalent to 400 and 1100 Der p 1 particles/m³) using a flow rate of 2 L/min for 10 min. During dust raising, the number of Der p 1 particles collected was 18 and 12 (900 and 600/m³).

In contrast with the results from direct immunostaining, the concentration of Der p 1 in eluted samples obtained from either the IOM or nasal air samplers, when both were fitted with non-protein-binding membranes, was below the detection limit of the traditional ELISA for all activities, including dust raising.

Discussion

We have been able to detect single airborne particles inhaled over short-sampling periods containing mite allergen in an undisturbed bedroom. In 10-min sampling periods we were able to detect inhaled particles containing Der p 1 in 18 of 31 samples taken from undisturbed environments and nine of nine taken during dust raising. This level of sensitivity has never been previously reported. Indeed there is controversy if airborne mite allergen can be detected using conventional assays and short sampling periods without disturbing the dust reservoir [3]. Most studies of undisturbed air use sampling times ranging from several hours, up to 7 days [4,7,10,20]. We choose a very short sampling time of 10 min as this is a period which enables individual activities to be examined while demonstrating the sensitivity of the immunodetection system.

The immunodetection system described is modified from the immunodiffusion method of Tovey *et al.* [21] and the press blot methods of Schumacher *et al.* [22] and Takahashi *et al.* [23]. We have developed simpler methods of particle and allergen capture. The previously described system [19], which permanently maintains the association between the particle and eluted allergen, used PVDF as the protein-binding membrane to non-specifically capture allergens eluted from particles. This system is a variant which uses a specific capture antibody on a nitrocellulose membrane to capture the allergen which is then detected with a labelled second antibody. Such a system potentially has the advantages of providing a highly specific detection system and may be useful where the allergen does not bind strongly to the membrane.

The two methods of air sampling, the novel nasal air sampler and the Institute of Occupational Medicine sampling head, each provide different information. The nasal air sampler provides an estimate of what would have been inhaled. These impaction filters work by accelerating particles, such that they are deposited by their inertia onto a collection surface [24]. An impactor has a characteristic particle cutoff for any given flow rate, particles smaller than this remain with the air stream and are not collected.

We have previously shown that the nasal air sampler has good capture efficiency for particles >10 µm during normal respiration [15]. The ability of both sampling systems to collect particles which contain allergen from the air was apparent, with the IOM sampler collecting a greater number of the smaller particles (<10 µm) while the nasal sampler collected more particles ≥ 30 µm. This may reflect a combination of factors including sampler efficiency and the different effects of low constant flow (IOM) and the fluctuating flow (with higher velocities) of natural inhalation.

Personal aeroallergen exposure is complex and likely to be influenced by the level of reservoir allergens, the nature of the allergen source, proximity to the source, level of dust disturbance, level of ventilation and indoor humidity, the size of particles carrying the allergen and the rate of breathing of the individual. It is apparent from our studies that allergen exposure over short periods of time varies markedly and is clearly influenced by the level of dust disturbance. For both subjects the maximum number of particles and Der p 1-containing particles inhaled was during dust raising. There was a 3.5-fold difference in reservoir Der p 1, between the bedroom carpets of subjects A and B (19.4 vs 71 µg/g, respectively), surprisingly dust raising on these carpets resulted in the inhalation of a similar number of particles that contained Der p 1. Similarly, despite large differences in the Der p 1 concentration in the bedding of subjects A and B (55 vs 531 µg/g, respectively) there were not large differences in the number of Der p 1-containing particles inhaled. These anomalies are further compounded by the fact that subject B has a higher inspiratory flow rate than subject A. Our data supports previous studies that have suggested personal aeroallergen exposure fluctuates massively with time, ventilation and activity [10]. The data we have presented also substantiates reports that reservoir concentration at sites are only weakly associated with personal exposure to aeroallergens [5,25].

In comparison with our previous studies of domestic exposure to cat allergen [26], there was approximately fivefold less mite allergen than cat allergen when dust raising/cat grooming and a 20-fold difference in the amounts inhaled from clothing. There was also much greater variation in inhaled Der p 1 between replicates. Repeatability studies on inhaled cat allergen in the same room using eight samples collected per sampling for 6 consecutive weeks show sample-to-sample coefficient of variations of 56–11% [27].

From our results it is apparent that 10-min sampling periods may be too short for detection of inhaled mite allergen in many environments, this is in contrast with our findings on inhaled cat allergen [26]. Further studies should use longer sampling periods, such as 20 min for multiple samples. The size of the impaction surface in the

filter appears adequate for longer sampling periods in most domestic studies since filter overloading was not observed on any of the samples in this study. Other studies have included 30-min and 1-h samples where no overloading was encountered.

One of the limitations of the intranasal sampler is the inability to capture very small particles. It could be expected that for particles $>10\mu\text{m}$ the nasal samplers effectively capture what is actually inhaled. What they miss is the smaller particles which should be efficiently captured by the IOM filter-type samples. We do not know how much allergen is missed, however, if the total amount of allergen is considered (i.e. larger particles carry more allergen than smaller particles) then intranasal sampling underestimates total allergen exposure by a small percentage. We are currently developing image analysis programmes to quantify halo density and relate this to amounts of allergen.

Conclusion

Intranasal air sampling is a valuable technique for the measurement of personal allergen exposure. Combining intranasal sampling with direct immunostaining of inhaled particles provides a highly sensitive method for determining personal allergen exposure. The results from the application of these techniques bring us closer to modelling true aeroallergen exposure to mite and other aeroallergens. This raises several questions. What is the pattern of this exposure over time, and, knowing that this fluctuates with time for an individual, can measurements be made which are relevant to clinical outcomes, such as sensitization and acute and chronic asthma symptoms? Such intranasal sampling/direct immunostaining provides a tool to explore these questions.

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