

Personal exposure to house dust mite allergen in bed: nasal air sampling and reservoir allergen levels

R. B. Gore*, E. A. Hadi*, M. Craven*, F. I. Smillie*, T. J. O'Meara†, E. R. Tovey†, A. Woodcock* and A. Custovic*

*North West Lung Research Centre, Wythenshawe Hospital, Southmoor Road, Manchester, UK, †Institute of Respiratory Medicine, Blackburn Building, University of Sydney, NSW, Australia

Summary

Background Assessment of personal exposure to dust mite allergen has relied on proxy measures. Only recently has a means to directly measure inhaled allergen particle number become available (the intra-nasal air sampler).

Objective To quantify inspired dust mite group 1 and group 2 allergen-bearing particles in bed in undisturbed conditions prior to sleep by nasal air sampling and to investigate the relationship between inhaled particles and reservoir allergen levels.

Methods Twelve volunteers wore nasal samplers in bed for 6 evenings, nose-breathing in undisturbed conditions. Allergen-bearing particles ('halos') were detected by immunostaining for Der p 1, Der p 2, or Der p 1 and Der p 2 together, and counted by light microscopy. Count data were square root transformed for analysis of variance. Mattress dust samples were assayed for Der p 1 and Der p 2 concentrations.

Results Square root detransformed mean particle counts per 30-min sample were: Der p 1, 4.22; Der p 2, 5.9; Der p 1 + Der p 2, 4.87; and for all samples, 5.01, with no difference between the groups. With replicate samples, halo number correlated significantly with mattress allergen concentrations (Der p 1 $r = 0.80$, $P < 0.01$; Der p 2 $r = 0.68$, $P < 0.02$).

Conclusion Nasal air sampling can be used to quantify nocturnal Der p exposure in undisturbed conditions in an area with moderate exposure to mite allergen and can provide a direct measure of inhaled mite allergen. The choice of either Der p 1 or Der p 2 is appropriate for this purpose.

Keywords house dust mite, nasal air sampling, allergen exposure

Submitted 15 August 2001; revised 3 December 2001; accepted 28 January 2002

Introduction

Recently, a direct measurement of inhaled mite allergen has become available – the intra-nasal air sampler (NAS) [1]. The majority of Der p 1 and Der p 2 allergen is carried on particles $> 10 \mu\text{m}$ in diameter [2, 3] that are aerodynamically large. Thus, for exposure, close proximity to the mite allergen source is critical. Such proximity occurs during sleep.

Sakaguchi et al. [4] managed to measure airborne group I and II *Dermatophagoides* allergens during sleep using a low-flow air sampler collecting airborne dust on a filter membrane located 30 cm from the sleeper over several hours using radioimmunoassay. However, standard air sampling devices measuring airborne allergen in a set location and orientation will not accurately reflect the allergen inspired into the respiratory tract and the long sampling times required are cumbersome. The NAS has been shown to be sensitive enough to detect inhaled Der p 1-bearing particles after only 10 min of sampling while lying in bed in an area with very high reservoir allergen

levels [5]. However, NAS have not yet been used in areas with moderate mite exposure (e.g. the UK) and the relationship between this method and the gold standard of allergen levels in reservoir dust is unknown. Our aims were to:

- Determine the number of mite-bearing particles using NAS inspired with the head on the pillow in undisturbed conditions.
- Compare the number of Der p 1-bearing particles with Der p 2-bearing particles.
- Determine whether the number of detectable particles could be increased by staining for both allergens.
- Investigate the relationship between particle number and reservoir house dust allergen concentration for Der p 1 and Der p 2.

Methods

Experimental design

Twelve non-atopic adult volunteers wore NAS in bed in their own homes. There was a 15-min lag period after climbing into bed before sampling to allow dust particles to settle. Study participants were instructed to nose-breathe only, with the head resting on the pillow. Each nasal sampler was worn for

Correspondence: R. B. Gore, North West Lung Research Centre, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, UK. E-mail: rgore@fs1.with.man.ac.uk

30 min. Each individual took three samples per night for 6 nights, giving a total of 18 samples per person. Each group of three samplers from 1 night was randomly assigned for measurement of Der p 1, Der p 2, or both allergens. Across the 6 nights, therefore, there were six replicates per person for each antibody-staining condition.

Reservoir dust samples were collected from the bed [6] and assayed for Der p 1 and Der p 2 by enzyme-linked immunosorbent assay (ELISA) as previously described [7, 8].

Nasal air sampler processing

Nasal air samplers were processed using the HALOgen® (Inhalix Pty, IRM, Sydney, Australia) protocol [1, 5]. Briefly, adhesive tapes from the samplers were laminated with polyvinylidene difluoride, pre-wetted in 80% methanol, washed with deionized water and allergens eluted overnight in elution buffer at 4 °C in a 24-well plate. The primary and secondary antibodies and the positive control tapes were provided in the HALOgen® kits. Samples were probed with anti-Der p 1 antibody, anti-Der p 2, or a 1:1 mixture of the two. Each group of three samplers from 1 night was assayed for the three primary antibody conditions with the sample order randomized for processing. The primary antibody solutions were added (400 µL) and samples incubated for 3 h at room temperature (20 °C). Samples were then washed for 3 × 10 min with phosphate-buffered saline (PBS)–Tween 0.05%. The secondary alkaline phosphatase-conjugated antibody was then added (400 µL) with incubation at room temperature for 90 min and samples were then washed for a further 3 × 10 min in PBS–Tween 0.05%. All antibody incubations and washes were performed with gentle continuous agitation. Samples were developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium. Negative control samples were made by laminating unexposed adhesive tape in the laboratory. Developed samples were viewed by light microscopy (Olympus CH30 microscope × 40), and particles surrounded by a coloured 'halo' of eluted allergen were counted. These particles will be referred to as 'halos'. Halo count data were square root transformed [9] for analysis of variance (SPSS 9.0 statistical package; SPSS Inc., Chicago, IL, USA). Using these values, summary statistics were then calculated and the means and confidence intervals (95%) were obtained by squaring the square root transformed values. To assess the correlation between halo counts and reservoir house dust allergen concentrations, the median halo count of the six replicates for each antibody staining condition was determined and Spearman rank correlation calculated.

Results

The individual halo counts for each study participant are shown for anti-Der p 1 immunostained particles (Fig. 1). The summary statistics for the halo counts are shown (Table 1). Analysis of variance demonstrated that there was no significant difference between the halo counts when processed under the three different antibody conditions (d.f. = 2, $F = 2.362$, $P = 0.10$, Fig. 2). The day on which sampling took place was not related to particle count (d.f. = 5, $F = 1.572$, $P = 0.17$). There were significant correlations between halo count for each allergen (Der p 1 vs. Der p 2, $r = 0.818$, $P = 0.001$; Der p 1 vs. Der p 1 + Der p 2,

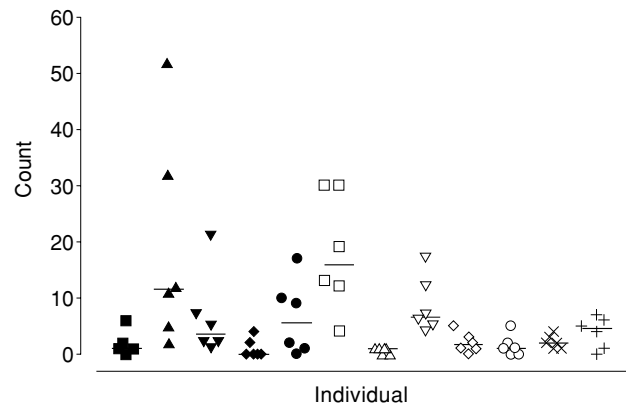


Fig. 1. The six replicate counts of samples immunostained for Der p 1 for each individual are shown. The bar represents the median.

Table 1. The summary statistics for the halo counts are shown for each antibody-staining condition

Antibody	Median	Min	Max	SRT mean*
Der p 1	2	0	52	4.22
Der p 2	4.5	0	52	5.99
Der p 1 + Der p 2	3	0	79	4.87
All samples	3	0	79	5.01

*SRT mean, square root detransformed mean.

$r = 0.625$, $P = 0.030$; Der p 2 vs. Der p 1 + Der p 2, $r = 0.877$, $P < 0.001$).

The median mattress dust allergen concentrations were: Der p 1, 1.28 µg/g; Der p 2, 0.45 µg/g dust. Four of the 12 mattress dust samples were below the limit of detection for Der p 1 and three were below for Der p 2. These values were treated as zero in statistical analyses.

The median of the six replicate samples for each volunteer correlated significantly with the reservoir mattress dust allergen concentration (Der p 1, $r = 0.80$, $P < 0.01$; Der p 2, $r = 0.68$, $P < 0.02$; Fig. 3).

Discussion

The current study has shown that the NAS is a sensitive tool for the direct quantification of inspired Der p 1 and Der p 2 allergen in sleep-simulated conditions in the UK using a convenient sampling time.

Previous studies using ELISA or radioimmunoassay have shown the relative airborne quantities of group 1:2 *Der-matophagoides* allergens to be in the order of 2.0:1–4.7:1 [10–13]. It may be expected therefore that the ratio of Der p 1- and Der p 2-eluting particles visualized by this technique would be within this range. Whichever mite allergen is detected on the greater number of particles by NAS will provide a more sensitive measure of exposure and will be of greater use in studies where different exposure conditions are compared. However, we have demonstrated no significant difference in the number of particles inspired bearing either Der p 1 or Der p 2 or with immunostaining for both allergens. This discrepancy between expected and actual particle count ratios for the two allergens may occur as a result of comparing two different methodologies, i.e. personal filter-based air samplers with the

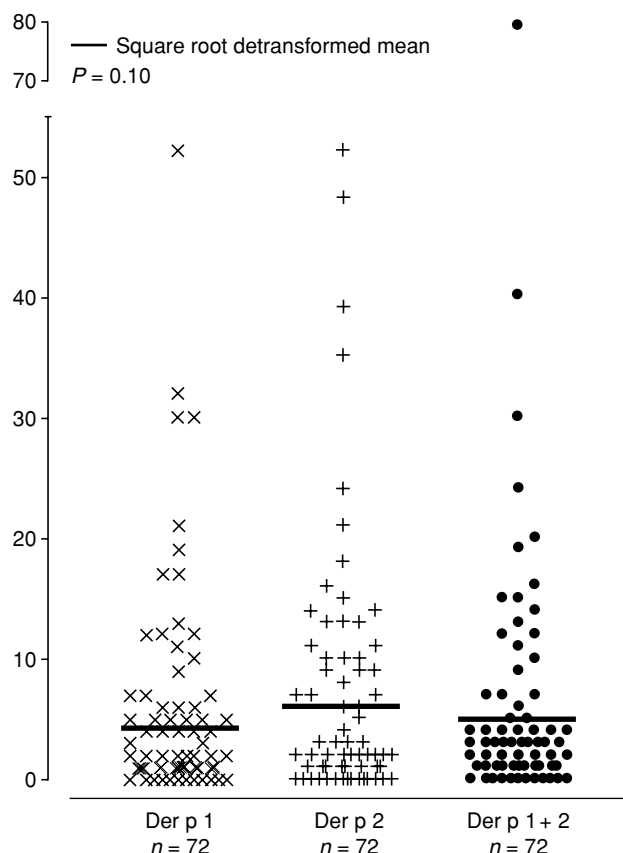


Fig. 2. Individual halo counts from intra-nasal air samplers are shown. Samples were immunostained for Der p 1, Der p 2 and Der p 1 and Der p 2 together. There were no significant differences between the number of particles detected for each allergen. The square root detransformed mean values are shown.

in vivo NAS. Alternatively, the data could suggest that Der p 1 and Der p 2 allergens are to some extent carried on the same particles [14] or that, on average, particles carry more allergen Der p 1 per particle than Der p 2. Dual staining on single particles for both allergens would help resolve this issue. For studies using the NAS, then either Der p 1 or Der p 2 can be chosen for immunodetection, and there is no merit in probing for both allergens simultaneously.

The median count of six replicate samples used in this study provided a measure of exposure for each allergen over 6 days and correlated with reservoir allergen levels in mattress dust. Assuming 6 h sleep, and the particle content of Der p 1 to be 0.1 ng/fecal pellet [15], our data suggest a nightly exposure to Der p 1 of approximately 6 ng and an annual exposure to Der p 1 of 2 µg/year during sleep. Using personal air samplers, Sakaguchi et al. [4] suggested a nightly exposure of merely 0.6 ng despite a respectable mean mattress dust Der p 1 concentration of 22.9 µg/g – some 20 times higher than in this study. This implies that the NAS may measure 9–10 times more allergen at the nose than personal filter-based sampling and that personal air samplers tend to underestimate true mite allergen exposure in these conditions.

The correlation between nasal air sampling halo count and reservoir mattress dust allergen concentration does not imply

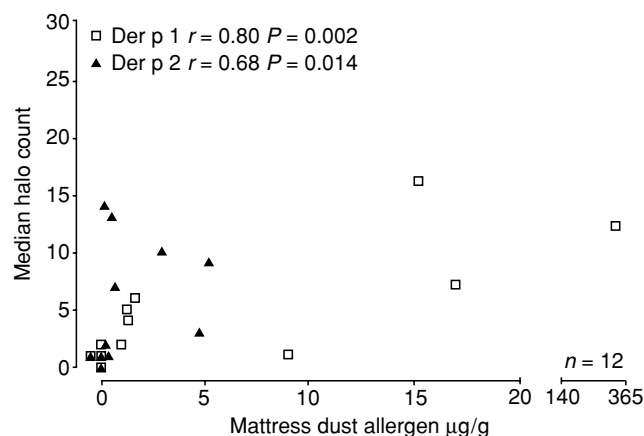


Fig. 3. The median of six replicate halo counts from each volunteer, immunostained for Der p 1 and Der p 2 are shown, together with the concentration of the allergens in dust collected from the mattress.

that one measurement technique validates the other, nor that one technique is superior. It would be a mistake to only compare the two techniques in terms of variability. The coefficient of variation for mattress dust allergen determination within homes is at least 50% [16]. Intra-nasal allergen exposure measured by NAS will vary as a complex function of activity level, distance of the nose from the source and inspiratory flow rate. However, the two techniques clearly each have a different applicability. Mattress reservoir dust is easy to sample and allergen determination is more practical on a large scale than using NAS, making dust assaying preferable for large-scale cohort studies. Yet, the NAS can measure inspired allergen exposure directly and avoids the criticism of being merely a proxy measure of exposure. It may be a more finely tuned tool for studies comparing different locations, levels of activity or evaluating allergen avoidance interventions using smaller numbers of people. The two techniques are therefore complementary and serve different purposes.

Studies of aeroallergen avoidance in the home are an exciting future application for the nasal air sampler. The assessment of mattress and bedding mite-impermeable covers and the assessment of interventions to reduce indoor pet allergens are possible applications. Current pet allergen interventions include pet removal, air filtration and vacuum cleaners with high efficiency air filters. In the working environment, latex exposure from gloves in the laboratory or clinical setting can be examined. Essentially, the only determining factors for the choice of application are the availability of the relevant monoclonal antibody and the aerodynamic properties of the aeroallergen itself. Fifty per cent of particles greater than 5 µm, and 100% of particles greater than 8 µm, impact on the nasal sampler tape [17]. This is in the range for the majority of aeroallergens, allowing many potential avoidance interventions to be investigated using this technique.

Acknowledgements

The authors would like to thank Dr Julie Morris and Mr Stephen Francis for statistical advice.

References

- 1 O'Meara TJ, De Lucca S, Sporik R, Graham A, Tovey ER. Detection of inhaled cat allergen. *Lancet* 1998; 351:1488–9.
- 2 Custovic A, Woodcock H, Craven M et al. Dust mite allergens are carried on not only large particles. *Pediatr Allergy Immunol* 1999; 10:258–60.
- 3 Platts-Mills TAE, Heymann PW, Longbottom JA, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986; 77:850–7.
- 4 Sakaguchi M, Inouye S, Yasueda H, Shida T. Concentration of airborne mite allergens (Der I and Der II) during sleep. *Allergy* 1992; 47:55–7.
- 5 De Lucca SD, Sporik R, O'Meara TJ, Tovey ER. Mite allergen (Der p 1) is not only carried on mite feces. *J Allergy Clin Immunol* 1999; 103:174–5.
- 6 Custovic A, Simpson BM, Simpson A et al. Manchester asthma and allergy study: low allergen environment can be achieved and maintained during pregnancy and in early life. *J Allergy Clin Immunol* 2000; 105:252–8.
- 7 Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f I. *J Immunol Meth* 1989; 118:27–35.
- 8 Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. Monoclonal antibodies to group II *Dermatophagoides* spp. allergens: murine immune response, epitope analysis and development of a two-site ELISA. *J Allergy Clin Immunol* 1994; 94:537–46.
- 9 Altman DG. *Practical Statistics for Medical Research*, 1st edn, Chapter 7. London: Chapman & Hall 1993, 143.
- 10 Sakaguchi M, Inouye S, Yasueda H, Irie T, Yoshizawa S, Shida T. Measurement of allergens associated with dust mite allergy. *Int Arch Allergy Clin Immunol* 1989; 90:190–3.
- 11 Sakaguchi M, Inouye S, Irie T et al. Airborne cat (Fel d 1), dog (Can f 1), and mite (Der I and Der II) allergen levels in the homes of Japan. *J Allergy Clin Immunol* 1993; 92:797–802.
- 12 De Blay F, Heymann PW, Chapman MD, Platts-Mills TAE. Airborne dust mite allergens. Comparison of group II antigens with group I mite allergen and cat-allergen Fel d 1. *J Allergy Clin Immunol* 1991; 88:919–26.
- 13 Swanson MC, Campbell AR, Klauck MJ, Reed CE. Correlations between levels of mite and cat allergens in settled and airborne dust. *J Allergy Clin Immunol* 1989; 83:776–83.
- 14 Park GM, Lee SM, Lee IY et al. Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2000; 30:1293–7.
- 15 Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592–3.
- 16 Simpson A, Hassall R, Custovic A, Woodcock A. Variability of house dust mite allergen levels within carpets. *Allergy* 1998; 53:602–7.
- 17 Graham JA, Pavlicek PK, Sercombe JK, Xavier ML, Tovey ER. The nasal air sampler: a device for sampling inhaled aeroallergens. *Ann Allergy Asthma Immunol* 2000; 84:599–604.