

Identification and quantification of mite allergens

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Allergens from the house dust mite are ubiquitous in house dust. Exposure to these allergens can cause acute symptoms in patients who are sensitized to house-dust mite and have asthma or other allergic diseases, such as rhinitis or atopic dermatitis. It is not clear how much mite-allergen exposure is a risk factor for the development of sensitization or for triggering acute symptoms. Therefore, it is important to be able to quantify accurately the amount of mite allergen present in the indoor environment in order to carry out studies to identify risk factors for exposure, to investigate exposure-response effects, and to monitor the progress of allergen-reducing regimens.

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Discussions regarding the indoor environment generally start with the observations that we spend more time indoors in our homes than outside (1); that the home environment can contain many different pollutants, both biological and chemical; and that there is growing concern about the indoor environment and its relationship to human health. There is evidence that the prevalence of symptomatic asthma is increasing (2, 3), and this may be due to an increase in atopy, which is a known risk factor for asthma. Atopy is in turn dependent on exposure to allergens in order for sensitization and allergic disease to occur. Although there is some evidence that house dust mite levels have remained constant over time (4), it is possible that the home environment which we find comfortable, and have developed to be energy efficient, may result in higher concentrations of indoor allergens, particularly mite allergens (5).

In the UK and most of central Europe, the most important indoor allergen is Der p 1, from the house dust mite *Dermatophagoides pteronyssinus* (6), which is found in the faecal particle of the house dust mite (7). Skin test results from a random survey of 1050 adults living in East Anglia (UK) showed that approximately 30% of the population is sensitized to *D. pteronyssinus* (8). The European Community Respiratory Health Survey (9), an international study of asthma prevalence and risk factors for asthma, collected information on

specific IgE to common allergens in over 13 000 adults living in 37 centres in 16 countries, and found a median prevalence of 20.3% (range 6.7-35.1%) for sensitization to *D. pteronyssinus* (10).

Sampling and assay methodology

The most common way of determining exposure to an allergen is to sample the environment. In homes, samples can be collected from the air or from settled dust. Dust samples are relatively easy to obtain and are useful in large epidemiological studies or for monitoring allergen avoidance regimens, whereas air samples require more complicated equipment and depend on the allergen being present in the air. Recommended methods for sampling house dust mite allergen are described in the Report of a Second International Workshop on Dust Mite Allergens and Asthma (11).

Dust sampling

Studies of the distribution of allergen in houses have shown that the house dust mite allergen is principally found in the mattress, bedroom floor, and living room floor dust. Dust samples can be collected in several different ways, but some of the methods are difficult to standardize and carry out reproducibly, and there can be substantial variations in measured levels of allergens in different

locations (12). Most methods collect dust by filtration into a receptacle in the hose of a vacuum cleaner or pump. The dust collection device developed by ALK Laboratories (13) is easy to use and is the best known of the commercially available dust collectors. It consists of a nozzle which is attached to the vacuum cleaner hose in place of the conventional brush, and the dust is collected onto filter paper in a plastic dish, for which a lid is provided. The sealed "cassette" can then be stored until assayed or sent by mail to a laboratory for testing.

The collection efficiency of different filters is usually limited by the smallest pore size (7 μm in the ALK device) that can be used while sampling with a conventional vacuum cleaner, as the air resistance increases with the amount of dust collected (which can be gram quantities). Small hand-held machines (less than 800 W) with low flow rates are therefore unlikely to work well with these devices. After collection from a specified area, usually 1 m^2 , for 2 min, the dust is removed from the filter paper and weighed, and an extract (usually 5%) is made in an aqueous buffer, which can then be assayed for allergen content.

Air sampling

Air sampling for house dust mite allergens is less frequently carried out than dust sampling, probably because of the cost of equipment and time involved for air sampling, which can be restrictive in larger studies. The faecal pellets, which have been shown to be the source of allergen, are 10–40 μm in aerodynamic diameter and settle quickly from the air (7). Therefore, air sampling may be useful only during periods of domestic activity such as house cleaning, and it is likely that measuring the dust reservoir from which airborne mite allergens are derived is the most accurate and consistent measure currently possible.

Studies from Japan suggest that smaller particles of Der p 1 may exist in very low concentrations (14). Researchers using a low-noise air sampler in the bedroom during the night and a sensitive radioimmunoassay measured airborne levels of Der 1 (Der p 1 plus Der f 1 from *D. farinae*) of 0.22 ng m^{-3} (15). Further studies on airborne mite allergen are required using characterized size-selective samplers, such as those developed by Mark et al. (16) and by Griffiths & Boysan (17). In a crude method of air sampling for determining cumulative airborne Der p 1, developed by Tovey and described by Platts-Mills & de Weck (18), a Petri dish coated with agar is exposed in a room for a period of time, usually 2 weeks, after which the

agar surface is washed with buffer and the extract is assayed.

Allergen assays

Until about 10 years ago, assessment of allergen exposure was possible only by counting mites in dust samples. However, this does not give a quantitative estimate of the amount of allergen present in the environment. One of the first techniques used to measure allergens was inhibition radioimmunoassay. This is a competitive radioimmunoassay which measures the antigenic activity in an unknown extract by the degree to which it inhibits binding of antibodies to the same antigen immobilized on a solid phase. This technique has some limitations: units are arbitrary and the assay is dependent on a supply of sensitized serum, preferably containing IgE antibodies only to the allergen in question, but it can still be useful in investigations of putative allergens, such as in the occupational setting.

The production of monoclonal antibodies to house dust mite allergens in 1987 enabled the development of sensitive and specific immunoassays. Selected specific clones remain homogeneous and can be produced in large quantities over indefinite periods. Enzyme-linked immunosorbent assays (ELISAs) were developed for Der p 1 and Der f 1 (19) and Der 2. The assays currently available measure one allergen at a time. However, multiple allergens are present in mites, and for the purpose of assessing exposure to mites, it is assumed that the ratio of Der p 1 to all the other mite allergens remains constant. Because the assays are also specific for one species of mite, infestations by other mites, e.g., storage mites, which could still be a risk factor for symptoms, need to be considered.

The monoclonal assays, as described by Chapman (20), are usually of a "sandwich" design, in which a plastic microtitre plate is coated with a monoclonal "capture" antibody. Unknown extracts and known standards are added to the plate, and binding is detected with a second monoclonal antibody, directed to a different epitope on the allergen. The second monoclonal is biotinylated, which allows for amplification and detection using streptavidin-peroxidase. Colorimetric substances can then be used for quantification. Alternatively, a semiquantitative colorimetric assay (Acarex test) that detects guanine, another excretory product of house dust mites, can be used, and it appears to correlate well with mite levels (21). It is not specific for Der p 1 and gives results only in terms of "low", "medium", or "high", but it can be useful for screening homes for allergen and can be used by

patients themselves. In the near future, rapid diagnostic tests (dipsticks) that can be used at home by patients will be available for house dust mite allergens (22). These use monoclonal antibodies bound to nitrocellulose membrane, they are specific for the allergen being tested, and they have lower detection limits than the ELISA (2.5 ng ml⁻¹ for Der 2).

Discussion

There has been some debate about which units should be used to express the allergen content of dust. Most published data have expressed results as concentrations ($\mu\text{g g}^{-1}$). This is most suitable for epidemiological studies, as it is independent of the sampling techniques, i.e., neither the type of vacuum cleaner used, nor the sampling time or area sampled affect the concentration, and it is not affected by domestic activities such as recent vacuuming. Moreover, using units of concentration overcomes the substantial inaccuracies which may occur at different stages of collecting and sieving of dust. However, it is possible that the amount of allergen in a given area ($\mu\text{g m}^{-2}$) is more representative of total allergen exposure and thus may be more closely related to the amount of allergens that can become airborne. It has been recommended that $\mu\text{g m}^{-2}$ should be used when assessing allergen levels after allergen-avoidance measures (23), but, in any case, there appears to be a good correlation between results reported as concentration or per unit area (24).

Indoor allergen exposure is an important risk factor for sensitization and allergic diseases, such as asthma and rhinitis. Since it has become possible to measure concentrations of common allergens in indoor environments, interest has focused on the effects of environmental factors on allergen exposure and symptoms. From the epidemiological data obtained on allergen exposure, it has been possible to propose risk levels for sensitization and symptoms for some indoor allergens. A concentration of 2 $\mu\text{g g}^{-1}$ Der p 1 (and Der f 1 where relevant) is thought to be a risk factor for sensitization and symptoms, and a concentration of 10 $\mu\text{g g}^{-1}$ is a risk factor for acute attacks of asthma in sensitized subjects (11). These "risk levels" were reviewed at a third WHO international workshop in June 1995, and although the value of 2 $\mu\text{g g}^{-1}$ as a risk level for sensitization was considered to be too high (25), there is some epidemiological evidence for an increased risk of asthma symptoms in children whose mattress concentrations of Der p 1 were above 10 $\mu\text{g g}^{-1}$ (26). However, the concept of "risk levels" is contentious, and more studies are

required to investigate allergen-exposure relationships. A better understanding of allergen exposure and the distribution of allergen in the indoor environment is essential in the design and implementation of allergen-avoidance techniques. It is also likely that awareness of allergen exposure by both patients and physicians could lead to a decrease in morbidity and health-care costs due to allergic disease. Further research to assess allergen exposure, particularly airborne allergen and household characteristics associated with high levels of indoor allergens, will help to achieve these objectives.

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