

Measurement of airborne mite allergen exposure in individual subjects

Masahiro Sakaguchi, PhD,^a Sakae Inouye, MD,^a Reiko Sasaki, MD,^a
Michiko Hashimoto, PhD,^b Chizuru Kobayashi, DVM,^b and
Hiroshi Yasueda, PhD^c Tokyo, Musashino, and Sagamihara, Japan

To evaluate the extent of personal exposure to airborne mite allergens, subjects were asked to carry a personal air sampler when in their houses. The level of Der 1 allergen trapped by the sampler was measured with a highly sensitive immunoassay. There were great variations in airborne Der 1 exposure in each subject. When used bedding was replaced with new allergen-free bedding, we detected a decrease in the allergen level. The use of new bedding seems to be an effective measure for reducing airborne mite allergen exposure. (J Allergy Clin Immunol 1996;97:1040-4.)

Key words: Airborne, allergen, bedding, Der 1, mite

Sensitivity to house dust allergens is common among patients with bronchial asthma and allergic rhinitis.¹ It has been well established that two species of mite, *Dermatophagoides pteronyssinus* and *D. farinae*, are important sources of house dust allergens.^{1,2} Immunoassays of Der 1 (Der f 1 and Der p 1), that is one of major allergens, are commonly used in studies on the level of mite allergens and mite avoidance measures.²

So far there is much information about airborne mite allergens; airborne allergen levels are very high in disturbed conditions such as bed making or vacuum cleaning done without a filter.²⁻⁵ However, the levels in undisturbed or calm conditions are very low or undetectable.^{2,3,5} We reported that a level of mite allergens during sleep was about 10-fold higher than during usual domestic life in the living rooms of the same houses.⁶ We also reported that dust specimens from bedding had more mite allergens per gram of dust than those from floor dust.⁵ Bedding is likely to be one of the major reservoirs of mite allergens.

In this study to assess the extent of natural exposure to mite allergens, the level of exposure of

individual inhabitants at home to Der 1 was measured by use of a personal air sampler and a highly sensitive immunoassay. Furthermore, to evaluate the effect of new bedding (mite allergen-free) on reducing the exposure to airborne mite allergens, exposure levels were compared before and after used bedding was replaced with new.

METHODS

Air sampling equipment

A small personal air sampler (MP-15CF; Shibata Scientific, Tokyo, Japan) is suitable for personal sampling of dust in the environment. The sampler (4.3 × 11.5 × 8.1 cm high and 500 gm in weight) with vertical inlet of the filter has a constant flow rate device and ensures a stable suction flow rate (1 L/min) for 26 hours, powered by batteries.

Japanese bedding

Japanese bedding⁶ (i.e., a futon) consists of two thick quilts made of cotton wool wadding with a cloth cover. It is placed directly on the floor, and one sleeps between the sheets. During the day, the quilts are folded and stored in a closet.

Sampling for airborne allergens

Air samplers were carried by six young adults (subjects A to F) living in Tokyo during November 1993. Subjects were selected without regard to allergy history. They attended universities or worked during the day. Air samples were collected four times, each for about 5 days (weekdays), while the subjects were at home. When they were away from home, the sampler was left behind with the switch off. On their return it was again carried. The noise level in this sampler is very low, so that it could be placed near the bedding during sleep. Two kinds of air

From ^aDepartment of Epidemiology, National Institute of Health, Tokyo; ^bDivision of Wild Animal Medicine, Nippon Veterinary and Animal Science University, Musashino; and ^cClinical Research Center for Allergy and Rheumatology, National Sagamihara Hospital, Sagamihara.

Received for publication Dec. 29, 1994; revised June 16, 1995; accepted for publication June 20, 1995.

Reprint requests: Masahiro Sakaguchi, PhD, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/67341

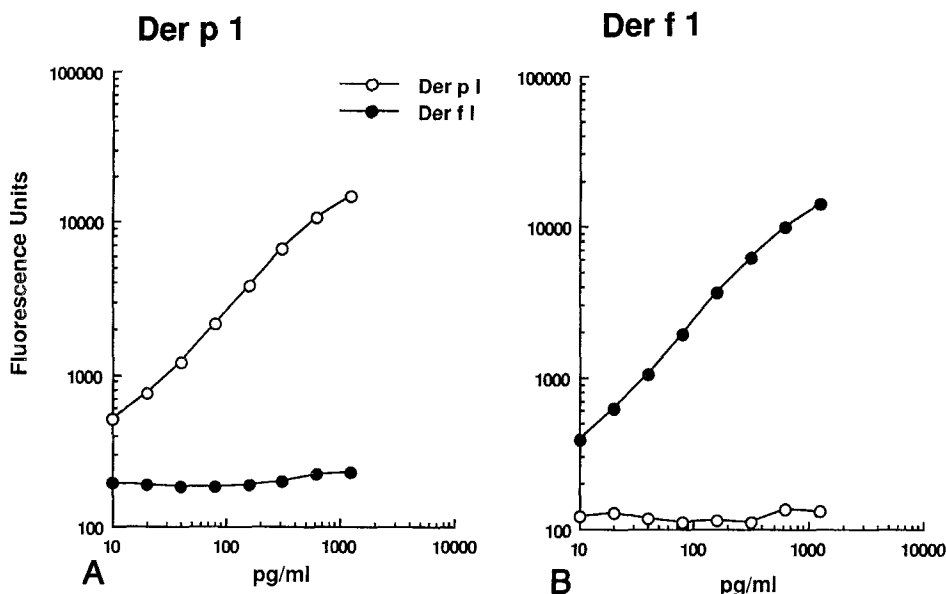


FIG. 1. Standard curve of Der p 1 (open circles) and Der f 1 (filled circles) in the ELISA for Der p 1 (A) and Der f 1 (B).

samples were collected: (1) when the subject used old bedding, which had been used for more than 1 year and (2) when the subject used new bedding, which was free of mite allergens (<2 ng/gm cotton). All airborne particles were collected on a 25 mm diameter fiberglass filter (AP 40; Millipore, Bedford, Mass.) in the sampler. The allergens collected on the filter were eluted overnight with 2 ml of phosphate-buffered saline containing 0.05% Tween-20 and 1% bovine serum albumin. After centrifugation, the supernatant was used as an airborne allergen sample.

Allergen in floor dust of the bedroom

Dust was collected from the floor in each bedroom, and allergens were extracted from the dust as previously described.⁵

Allergen in cotton of Japanese bedding

We collected a small amount of cotton (about 0.1 gm) with a pair of tweezers from eight points on one futon. The cotton was weighed and immersed overnight in 100 times quantity of phosphate-buffered saline containing 0.1% bovine serum albumin and 0.05% Tween-20. After centrifugation, the supernatant was stored at -30°C .

Reference preparation of *D. pteronyssinus* and *D. farinae* extracts

The reference preparations of the extract, 92-Dp for Der p 1 and 92-Df for Der f 1, were found to contain the same level of the Der 1 allergens, 10.1 $\mu\text{g/ml}$ Der p 1 and 10.0 $\mu\text{g/ml}$ Der f 1, respectively.⁷ In the assay system used in this study, the World Health Organization international standard for house dust mite (*D. pteronyssinus*) extract (82/518) was 4.4 μg per vial of Der p 1.⁸ The Center for Biologics Evaluation and Research stan-

dard mite extracts, E4 Dp and E5 Df, were 13.3 $\mu\text{g/ml}$ Der p 1 and 9.5 $\mu\text{g/ml}$ Der f 1, respectively.⁷ These extracts were kindly provided by Dr. Yuan L. DeVries of the Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Md.

Quantitation of mite allergens

Der p 1 and Der f 1 allergens were quantified by a sandwich ELISA^{8,9} with modifications. A white microplate (Microfluor W plate; Dynatech, Chantilly, Va.) was coated with either 1 $\mu\text{g/ml}$ monoclonal anti-Der p 1 or 1 $\mu\text{g/ml}$ anti-Der f 1 IgG⁷ for 3 hours at 37°C . The plate was emptied, then postcoated with 1% bovine serum albumin-phosphate-buffered saline at 37°C for 1 hour. After washing, several concentrations of standard allergens or diluted specimens were added to the wells, and the plate was incubated overnight at 4°C . The plate was washed, then 0.2 μg of biotinylated rabbit anti-Der p 1 and Der f 1 IgG⁸ were added as detectors. The plate was incubated for 1 hour at room temperature. After washing, β -D-galactosidase-conjugated streptavidin (Zymed Laboratories, San Francisco, Calif.) was added. The plate was incubated for 1 hour at room temperature. After final washing, 0.1 mmol/L 4-methylumbelliferyl- β -D-galactoside (Sigma Chemical Co., St. Louis, Mo.) was added to each well. The plate was incubated for 2 hours in a water bath at 37°C . The enzyme reaction was stopped with 0.1 mol/L glycine-NaOH (pH 10.2), and the fluorescence intensity was read as fluorescence units on a microplate fluorescence reader (Fluoroskan; Flow Laboratories, McLean, Va.).

The lowest allergen concentration detected was 10 pg/ml for both Der p 1 and Der f 1. This method was sensitive enough to detect airborne allergen levels of 6.7 pg/m^3 if the sampler was run for 50 hours.

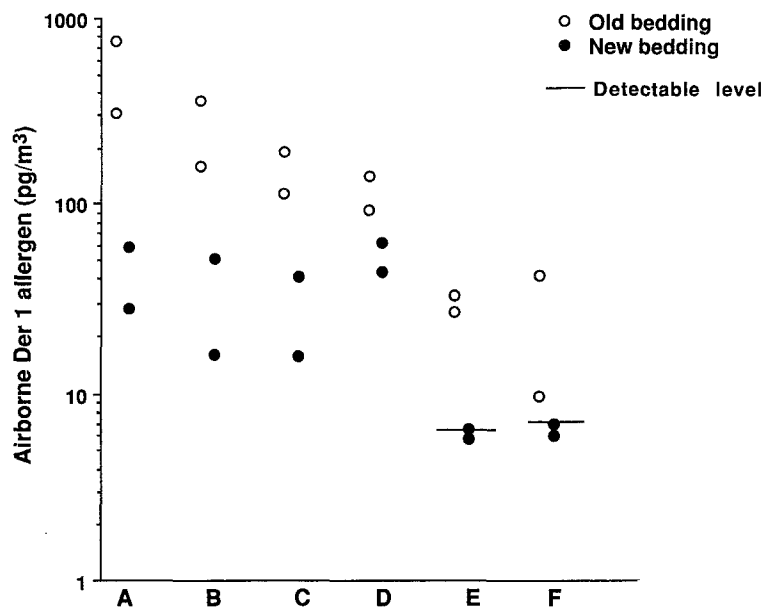


FIG. 2. Exposure to airborne mite allergen before and after change to new bedding. Mean air sampling times ($\pm$ SD) were: A, 43.5 ± 7.9 hours; B, 54.5 ± 8.2 hours; C, 57.1 ± 6.7 hours; D, 49.9 ± 4.4 hours; E, 61.4 ± 7.4 hours; F, 50.5 ± 3.0 hours.

RESULTS

Specificity and sensitivity of a fluorometric ELISA for Der 1 allergens

By using monoclonal antibodies to a species-specific determinant as the capture antibody, Der p 1 and Der f 1 were measured separately. Standard curves for Der p 1 and Der f 1 assay are shown in Fig. 1. Both assays were highly species-specific and very sensitive (10 pg/ml). The intraassay coefficients of variation ($n = 6$) for Der p 1 and Der f 1 were 4.6% and 3.4%, respectively. The interassay coefficients of variation ($n = 6$) for Der p 1 and Der f 1 were 6.3% and 6.5%, respectively.

Exposure to mite allergens in the home

The level of exposure to Der 1 allergen was measured before and after the bedding was changed (Fig. 2). Before changing to new bedding, we conducted duplicate air sampling. The mean levels of exposure to Der 1 ranged from 29.6 to 485 pg/m^3 (geometric mean value, 102 pg/m^3). After changing to new bedding, the mean levels of exposure to the mite allergen ranged from less than 6.00 to 52.2 pg/m^3 (geometric mean value, 19.9 pg/m^3). Airborne mite allergen levels in the rooms of subjects E and F were not detectable. In all subjects, the levels of exposure to airborne Der 1 decreased.

Floor dust allergen in the bedroom

The levels of Der 1 in floor dust in the subjects' bedrooms were measured before and after changing to new bedding (Fig. 3). Dust was collected three times for 2 weeks. The mean values of the allergen levels in floor dust before and after changing to new bedding were 10.4 and 11.3 $\mu\text{g}/\text{gm}$ fine dust, respectively, with no significant difference.

Mite allergen level in cotton of bedding

To evaluate the amount of Der 1 in bedding, we measured the level of mite allergens in the cotton of bedding (Fig. 4). The levels of Der 1 in the cotton of bedding that had been used for more than 1 year ranged from 26.6 to 437 ng/gm cotton (geometric mean value, 140 ng/gm cotton). The level of Der 1 in the cotton of new bedding was not detectable (<2 ng/gm cotton). Subject C's bedding was made of feathers, so we measured the level of the allergen from the bedding dust. The level of mite allergen in dust of subject C's bedding was very high (34.4 $\mu\text{g}/\text{gm}$ fine dust). In the other subjects' bedding the levels of mite allergen from the dust were very high. The levels of Der 1 ranged from 16.4 to 115 $\mu\text{g}/\text{gm}$ fine dust (geometric mean value, 38.0 $\mu\text{g}/\text{gm}$ fine dust) (data not shown). The level of Der 1 in the dust of new bedding was not detectable (<20 ng/gm fine dust).

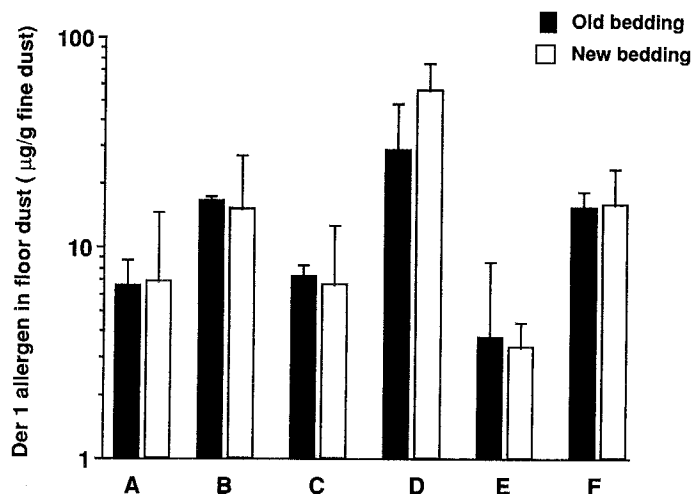


FIG. 3. Level of floor dust allergens in bedrooms before and after change to new bedding. Types of material on bedroom floors were: A, carpet; B, straw matting (tatami in Japanese); C, carpet; D, wood flooring; E, straw matting; F, carpet.

DISCUSSION

To determine the level of personal exposure to mite allergens in the home, a subject must carry an air sampler. The air sampler used in previous studies^{5,6} was too big ($24 \times 40 \times 32$ cm high) and heavy (weight of 9.5 kg) to carry. In this study we used a smaller and lighter-weight air sampler, which is readily available and can be used in any country because it is battery-powered.

Determination of the quantity of mite allergen collected by means of personal samplers operating at low flow rates (1 L/min) requires the use of highly sensitive assays capable of detecting exposure in the order of 10 pg/m^3 . We achieved this with a modification of our previously reported sandwich ELISA assay by using fluorometric detection. In previous studies,^{8,9} we used anti-Der f 1 and anti-Der p 1 polyclonal antibodies. In this study we used monoclonal antibodies as capture antibodies and polyclonal antibodies as detector antibodies to develop a more sensitive assay for Der p 1 and Der f 1. Of course, this assay has high species specificity.

This is the first report to estimate mite allergen exposure of individual persons living under normal circumstances. In our previous studies^{5,6} a sampler was placed on the floor of each room, and we measured mite allergens in the air of each room in the houses. In another study¹⁰ personal samplers were used for the collection of airborne allergens, but it seems that a sampling period of 3 hours was too short to evaluate the airborne mite allergen exposure of individual persons. In this preliminary

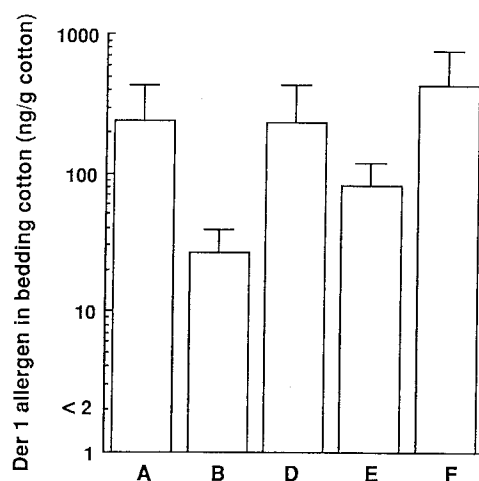


FIG. 4. Level of mite allergen in the cotton of used bedding.

study we collected the air for 5 days and measured the level of personal exposure to mite allergen. The mean level of mite allergen was 102 pg/m^3 . Great variations in airborne allergen levels were observed in individual subjects. There was no correlation between mite allergen levels in the air and those in bedding cotton, cotton dust, and floor dust. It was reported that there was no correlation between the level of Der p 1 in carpets and the level in the air.¹⁰ Probably the human activity in the house, which makes the allergens float into the air, varies with each person.

We evaluated the effect of new bedding on airborne mite allergens. When old bedding was

replaced with bedding free of mite allergens, the level of airborne Der 1 decreased. There was no change in mite allergen levels in floor dust before and after the bedding change. Further, there were high levels of mite allergen in the cotton of used bedding but no detectable mite allergen in the cotton of new bedding. From these results, it seems that the decrease in airborne mite allergen depends on the use of new bedding that is free of mite allergen.

The airborne mite allergen level was not completely abolished after bedding was changed. The source of the remaining airborne allergen remains unknown. At present, we speculate that the allergen in the floor dust was stirred up by bed making.

There have been many studies of house dust mite allergen control and avoidance.^{11, 12} The use of new bedding appears to be one of the most effective measures for reducing airborne mite allergen exposure.

We thank Mr. Tomohiro Suzuki and Miss Emiko Yama for their assistance with air and dust sampling. We also thank Dr. Hiroshi Nitta and Dr. Soichiro Sakata for advice about the air sampling.

REFERENCES

1. Platts-Mills TAE, Solomon WR. Aerobiology and inhalant allergens. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. *Allergy: principles and practice*. 4th ed. St. Louis: Mosby, 1993:469-528.
2. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 1992;89:1046-60.
3. Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981;124:630-5.
4. Swanson MC, Agarwal MK, Reed CE. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol* 1985;76:724-9.
5. Sakaguchi M, Inouye S, Yasueda H, Irie T, Yoshizawa S, Shida T. Measurement of allergens associated with dust mite allergy. II. Concentrations of airborne mite allergens (*Der I* and *Der II*) in the houses. *Int Arch Allergy Appl Immunol* 1989;90:190-3.
6. 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.
7. Yaseuda H, Saito A, Akiyama K, et al. Estimation of *Der p I* and *Der f I* quantities in the reference preparations of *Dermatophagoides* mite extracts. *Clin Exp Allergy* 1994;19:1030-5.
8. Yasueda H, Mita H, Yui Y, Shida T. Measurement of allergens associated with dust mite allergy. I. Development of sensitive radioimmunoassays for the two groups of *Dermatophagoides* mite allergens, *Der I* and *Der II*. *Int Arch Allergy Appl Immunol* 1989;90:182-9.
9. Sakaguchi M, Inouye S, Irie T, et al. Airborne cat (*Fel d I*), dog (*Can f I*), and mite (*Der I* and *Der II*) allergen levels in the homes of Japan. *J Allergy Clin Immunol* 1993;92:797-802.
10. Price JA, Pollock I, Little SA, Longbottom JL, Warner JO. Measurement of airborne mite antigen in homes of asthmatic children. *Lancet* 1990;336:895-7.
11. Tovey ER. Allergen exposure and control. *Exp Appl Acarol* 1992;16:181-202.
12. Colloff MJ, Ayres J, Carswell F, et al. The control of allergens of dust mites and domestic pets: a position paper. *Clin Exp Allergy* 1992;22:1-28.

Bound volumes available to subscribers

Bound volumes of *The Journal of Allergy and Clinical Immunology* are available to subscribers (only) for the 1996 issues from the Publisher, at a cost of \$80.50 for domestic, \$107.54 for Canadian, and \$100.50 for international subscribers for Vol. 97 (January-June) and Vol. 98 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.