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Abstract

The house dust mite (*Dermatophagoides pteronyssinus*) antigen and allergen contents were measured by enzyme-linked immunosorbent assay (ELISA) with enzyme-labelled anti-human IgE and anti-mite rabbit IgG antibodies. Antigen content was high in dust samples from homes of patients with allergy but not in samples from homes of patients with Kawasaki disease or of normal control subjects. Allergen content was high in dust samples from homes of Kawasaki disease patients. However, the values overlapped, and we considered these differences to be of little ecological significance, although the assay method itself is useful.

KEYWORDS: mite, dust, allergen, *Dermatophagoides*, mucocutaneous lymphnode syndrome

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Mite Antigen and Allergen Contents of House Dust Samples

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The house dust mite (*Dermatophagoides pteronyssinus*) antigen and allergen contents were measured by enzyme-linked immunosorbent assay (ELISA) with enzyme-labelled anti-human IgE and anti-mite rabbit IgG antibodies. Antigen content was high in dust samples from homes of patients with allergy but not in samples from homes of patients with Kawasaki disease or of normal control subjects. Allergen content was high in dust samples from homes of Kawasaki disease patients. However, the values overlapped, and we considered these differences to be of little ecological significance, although the assay method itself is useful.

Key words : mite, dust, allergen, *Dermatophagoides*, mucocutaneous lymphnode syndrome

More than 20 years have passed since the house dust mite of the genus *Dermatophagoides* was incriminated as the major cause of respiratory allergic diseases in Holland and Japan. Ecological studies employing laborious morphological identification methods have revealed detailed mite faunas in dust samples. Microscopic observation is accurate but requires time and experience to identify mite species in dust samples which already exceed 30 recorded species. Microscopic observation is not suitable when large numbers of samples are involved. However, the allergen content of dust samples from the environment of patients must be measured when patients are advised to avoid allergen exposure in daily life (1, 2). Environmental measurement is essential to the guidance of allergic pa-

tients. We attempted to measure mite allergen and antigen content by enzyme-linked immunosorbent assay (ELISA) as a specific and sensitive method.

House dust extract was prepared by mixing 1 g of dust sample in 10 ml of phosphate buffered saline (pH 7.0) containing 0.1 % Tween 20. After 30 min, the mixture was centrifuged at 3,000 rpm for 10 min, and the supernatant was used as the dust extract. The dust extract contained 1-3 mg protein/ml, and a 1:25 diluted solution was used in the present study.

The mite antigen content in the dust extracts was assayed by a sandwich method ELISA, using peroxidase-labelled anti-mite (*Dermatophagoides pteronyssinus*) rabbit antibody. Crude mite antigen was immunized with Freund's complete adjuvant in rabbits, and the IgG fraction was prepared by

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(NH₄)₂SO₄ precipitation. IgG was labelled with peroxidase by the glutaraldehyde method. First, anti-mite rabbit antibody was put in wells of a microtiter plate and left overnight at 4°C. After washing three times with 0.1% Tween 20-saline, the reaction with dust extract was performed at 37°C for 1 h. Then, peroxidase-labelled anti-mite IgG was added and left overnight at room temperature. The labelled antibody was used at 1:400 dilution, and the substrate was *o*-phenylenediamine which was prepared fresh everytime with addition of 25 μ l of 30% H₂O₂. The enzyme reaction was done in the dark at room temperature for 40 min and the reaction was stopped by adding 0.2 M H₂SO₄.

Mite allergen content was assayed by the indirect method using β -D-galactosidase-labelled anti-human IgE antibody (Kainos, Japan). The dust extracts were adsorbed on CNBr-activated paper discs and pooled human serum with high anti-mite IgE titer was used at 50 times dilution to react with mite allergen on the paper discs. The

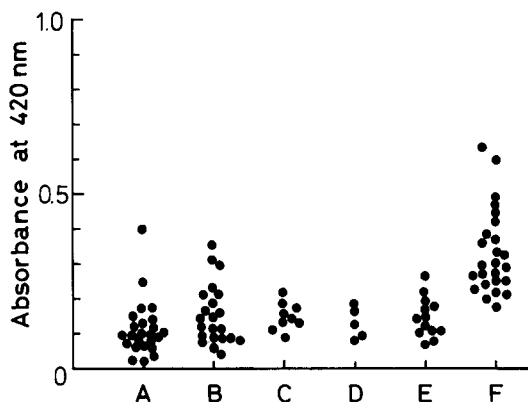


Fig. 1 Mite allergen contents in house dust. Mite (*Dermatophagoides pteronyssinus*) allergen contents in house dust extracts were assayed by ELISA using β -galactosidase-labelled anti-human IgE antibody as described in the text. House dust samples were obtained from homes of normal subjects (A), of patients with allergy to mites and house dust (B), to mites (C), to house dust (D) and to other allergens (E), and of patients with Kawasaki disease (F).

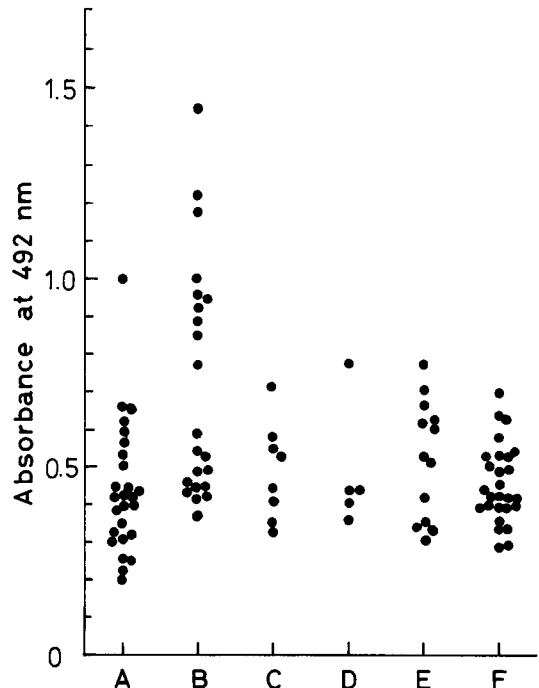


Fig. 2 Mite antigen contents in the house dust. Mite antigen contents in house dust extracts were assayed by ELISA using peroxidase-labelled anti-mite (*Dermatophagoides pteronyssinus*) rabbit antibody as described in the text. House dust samples were obtained from homes of patients and of normal subjects as described in Fig. 1.

pooled serum was examined to contain only negligible amount of IgE to allergens other than dust mites, since cross reacting allergen may interfere in the assay. The substrate used was *o*-nitrophenyl- β -D-galactopyranoside solution. The allergen content in mite culture medium was used as a standard and the allergen content was found to be proportional to the number of mites in the medium.

Dust samples in electric sweepers were collected as described previously (4) from homes of 48 patients allergic to mites and other allergens, 26 normal controls and 26 patients with Kawasaki disease (mucocutaneous lymphnode syndrome, MCLS), mostly from the Kyushu district.

The results of allergen content measure-

ments are shown in Fig. 1. Fig. 2 shows the results of antigen content measurements. The allergen values of dusts of allergic patients were not different among allergic patients. The value of MCLS dusts was statistically higher (analysis of variance and Scheffe method) than that of control samples. On the other hand, the antigen content of dust samples of patients allergic to both mites and dust was statistically high, but that of MCLS was not.

As a whole, however, viewing the overlapping of value points in the figures, we consider that these results suggest that there is not much difference in antigen and allergen contents of house dust as environmental factors. Our previous ecological studies showed little difference in mite fauna and mite numbers in dust samples (3). The only difference so far detected was that the number of mites was statistically low in dust samples from skin test negative patients (4, 5). Still, the present method for measuring mite derived allergen in dust samples provides useful information about

the daily environmental condition of atopic patients.

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