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Allergens of the house dust mite, *Dermatophagoides pteronyssinus*, in patients with mite allergic rhinitis: a clinical investigation by intracutaneous skin tests and nasal provocation tests.

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Abstract

To determine the allergens of mite allergic rhinitis, we studied 31 patients with mite allergic rhinitis by skin tests and nasal provocation tests (15 for skin and 16 for nasal tests) using 6 fractions of *Dermatophagoides pteronyssinus* (Dp) extract differing in molecular weights (15, 25, 32, 53, 95 and 190 kDMW). In skin testing, patients showed intense positive reactions to the fractions of 15, 25, 32, 95 and 190 kDMW, among which the most patients showed positive reactions to the fractions of 15 and 25 kDMW. Significant differences were found in patients' positive reactivity among each fraction and between low (15 and 25 kD) and high (95 and 190 kD) molecular weight fractions as well. In nasal provocation tests, patients showed intense positive reactions to the fractions of 15, 32, 53 and 95 kDMW, especially to the fractions of 15 and 95 kDMW. Furthermore, the incidence of positive reactions to the 15 kDMW fraction was significantly higher than that to any other fraction in the skin tests ($P < 0.05$). From these results, the low molecular weight fraction, 15 kDMW, is considered to be the main allergen of this mite and the high molecular weight fractions, 95 and 190 kDMW, may also be considered to be allergens of this mite.

KEYWORDS: mite allergen, skin test, nasal provocation test, allergen rhinitis

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Allergens of the House Dust Mite, *Dermatophagoides pteronyssinus*, in Patients with Mite Allergic Rhinitis: A Clinical Investigation by Intracutaneous Skin Tests and Nasal Provocation Tests[†]

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To determine the allergens of mite allergic rhinitis, we studied 31 patients with mite allergic rhinitis by skin tests and nasal provocation tests (15 for skin and 16 for nasal tests) using 6 fractions of *Dermatophagoides pteronyssinus* (Dp) extract differing in molecular weights (15, 25, 32, 53, 95 and 190 kDMW). In skin testing, patients showed intense positive reactions to the fractions of 15, 25, 32, 95 and 190 kDMW, among which the most patients showed positive reactions to the fractions of 15 and 25 kDMW. Significant differences were found in patients' positive reactivity among each fraction and between low (15 and 25 kD) and high (95 and 190 kD) molecular weight fractions as well. In nasal provocation tests, patients showed intense positive reactions to the fractions of 15, 32, 53 and 95 kDMW, especially to the fractions of 15 and 95 kDMW. Furthermore, the incidence of positive reactions to the 15 kDMW fraction was significantly higher than that to any other fraction in the skin tests ($P < 0.05$). From these results, the low molecular weight fraction, 15 kDMW, is considered to be the main allergen of this mite and the high molecular weight fractions, 95 and 190 kDMW, may also be considered to be allergens of this mite.

Key words: mite allergen, skin test, nasal provocation test, allergen rhinitis

Since 1973, when Ishii (1) reported *Dermatophagoides pteronyssinus* (Dp) as an antigen causing

allergy, immunological and serological investigations of its antigens have been described (2-9). We previously reported specific IgE, IgG and IgG₄ antibodies to fractionated house dust mite antigens in mite nasal allergy patients (10): we found no significant differences between the titers of IgE antibody against each Dp fraction. Responses to both the 190 and 15 kDMW fractions were significantly higher than those observed with the other fractions in Dp fraction-specific IgG₄ in patients with allergic rhinitis.

The purpose of the present study was to investigate the allergenicity of the six mite fractions by the intracutaneous tests and nasal provocation tests.

Subjects and Methods

Patients

Patients given intracutaneous skin tests.

We studied 15 patients (8 males and 7 females aged 12-45 years; mean 32 years) with a confirmed diagnosis of mite allergic rhinitis. They had typical perennial symptoms of sneezing, nasal obstruction, and nasal hydrorrhea. Each showed a positive response to intracutaneous skin tests and nasal provocation tests with mite extract. Their specific IgE level was high (> 3.50 PRU/ml), and numerous eosinophils were observed in nasal smears. The increase in serum IgE from 87.1 to 4,644.0 U/ml (mean 905.1 U/ml) and elevated eosinophil counts in blood and nasal secretions strongly suggested allergic

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Table 1 Criteria for intracutaneous skin testing and nasal provocation testing

Tests	Grade of responses				
	(High) ##	(Middle) #	(Low) +	±	(Negative) -
Skin test					
Erythema	> 41 mm	40-21 mm	40~21 mm		< 20 mm
Wheal	> 15 mm	14-10 mm	< 9 mm		< 9 mm
Nasal provocation test	3 symptoms* (sneezing: ≥ 6 times/day)	3 symptoms (sneezing: < 6 times/day)	2 symptoms	1 symptom	No symptoms

* The 3 symptoms included: sneezing, pale swollen nasal mucosa and nasal hydropnea

rhinitis. Eleven of these patients had severe symptoms and family histories which included atopic diseases.

Patients given nasal provocation tests.

Sixteen patients with a confirmed diagnosis of mite allergic rhinitis (12 males and 4 females aged 19-22 years; mean 22 years) were studied. They had typical perennial symptoms and each showed a positive response to skin tests and nasal provocation tests with crude mite extract. In these patients, the specific IgE level was elevated (> 3.50 PRU/ml); and the serum IgE level ranged from 19.3 to 2,459 U/ml (mean 320.9 U/ml).

Informed consent was obtained from all patients.

Extraction and Fractionation of Dp Antigens

Dp extracts were prepared by the method of Miyamoto *et al.* (11). Dp antigen was prepared in accordance with the method of Kabasawa and Ishii (12). After the organism was separated from the culture medium with saturated saline, it was homogenized by adding 6 ml of 0.1 M Tris-HCl buffer (pH 7.6) to 1 g of dried Dp. After the homogenate was centrifuged (60,000 *g* for 1 h), the supernatant was used as crude Dp antigen. This crude Dp antigen solution (protein content 6.8 mg/ml) was applied to a Sephacryl S-200 column (Pharmacia, Uppsala, Sweden), and each was eluted with 0.1 M Tris-HCl buffer (pH 7.6). The eluate was collected in 5-ml fractions, and the absorbance at 280 nm was measured. Using protein molecular weight markers, eluates corresponding to 15, 25, 32, 53, 95 and 190 kDMW were obtained in 10- to 15-ml fractions for examination of antigens. Each of the six fractions was aseptically filtered, adjusted to a protein concentration of 1.7 μ g/ml, and

used for intracutaneous and nasal testings.

Intracutaneous Skin Tests

A volume of 0.02 ml of all six fractions and crude extracts of the mite were injected intracutaneously in the backs of 15 patients. Erythema and whealing were measured after 15 min. Intracutaneous reactions were evaluated according to the criteria shown in Table 1.

Nasal Provocation Tests

Round filter papers 2 mm in diameter immersed in saline were placed on the bilateral inferior turbinate. After confirming no allergic reaction, the nasal provocation test was performed. Filter papers immersed in each fraction of mite crude extract were placed on the bilateral inferior turbinate in 16 patients. After 15 min, the symptoms including sneezing, nasal hydropnea and nasal obstruction were observed and evaluated according to the criteria shown in Table 1.

Results

Response to intracutaneous skin tests. As shown in Fig. 1, fractions of 32, 25 and 15 kDMW showed intense positivity in many patients with mite allergic rhinitis.

Significant differences were found in patients' positive reactivities among each fraction and also between the low (15 and 25 kD) and the high (95 and 190 kD) molecular weight fractions. No relationship was observed between the molecular weight of the fraction that provoked a positive skin test reactions and patients' disease conditions, such as, the duration of illness, symptoms of nasal allergy, serum IgE level, or eosinophil counts in the

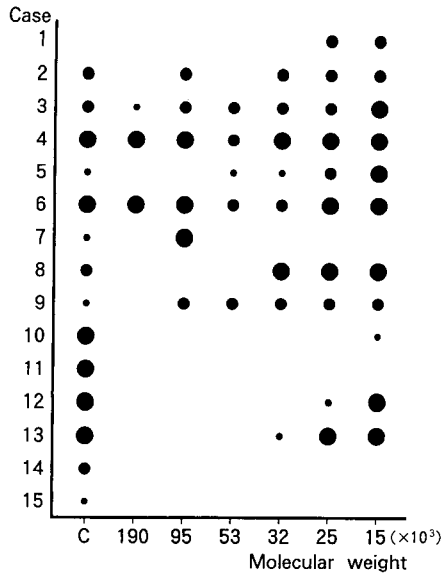


Fig. 1 Response to intracutaneous skin tests with six fractions of *Dermatophagoides pteronyssinus* (Dp) crude extract in 15 patients with mite nasal allergy (cases 1-15). Responses are shown as: large solid circle is high grade response, middle solid circle is middle grade response, and small solid circle is low grade skin test response, according to criteria in Table 1. C: Crude Dp antigen

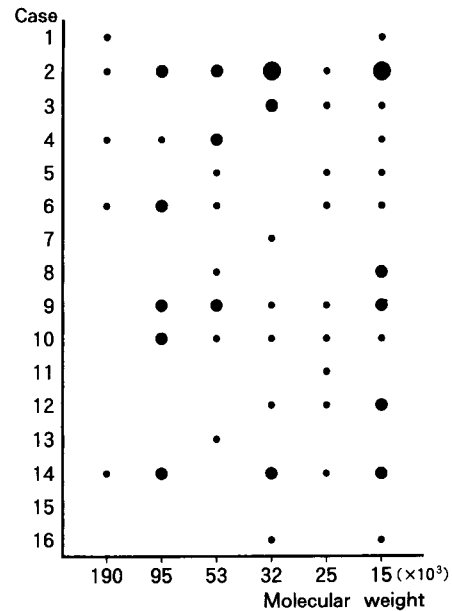


Fig. 2 Response to nasal provocation tests with six fractions of *Dermatophagoides pteronyssinus* crude extract in 16 patients with mite nasal allergy (cases 1-16). Response are shown as: large solid circle is high grade response, middle solid circle is middle grade response, and small solid circle is low grade provocation test response, according to criteria in Table 1.

blood or nasal smears.

The reactions to the 15-190 kDMW fractions in the 15 patients differed at 2.5 % level of significance by Friedman's two-way analysis of variance. The statistical value Q was 14.58, and since this was greater than the degrees of freedom in the χ^2 test or the limit of the 2.5 % probability (12.83), the reaction with each fraction was considered significantly different.

$$Q = 14.58 > \chi^2(5.0025) = 12.83 \\ P > 0.025$$

When the fractions were divided into those with molecular weight of 190 or 95 kD and those with molecular weight of 25 or 15 kD, the positive reactions to these two groups were differed at the 0.5 % level of significance by the Mann-Whitney U-test.

$$U_0 = 272 < U(0.005) = 275 \\ P > 0.005$$

Response to nasal provocation tests. Nasal provocation tests in 16 patients revealed an intense reac-

tion to the fractions of 15, 32, 53 and 95, and especially to 15 and 95 kDMW. Five patients showed a positive reaction to almost all fractions. Five patients (Cases 3, 7, 11, 12 and 16) reacted only to the fractions of low molecular weight, and no patient reacted to only the high molecular weight fractions (Fig. 2), while the incidence of the positive reactions to the fraction 15 kDMW was significantly higher than that to any other fraction (12/16 patients) ($P < 0.05$: Mann-Whitney U-test).

Discussion

In the previous studies, the main fractions of mite extract which were considered as allergens were those of low molecular weight: 20-30 kD by Nakagawa *et al.* (2), 24 kDMW by Chapmann *et al.* (3), 28 kD by Dandeu *et al.* (4), and 29 kD by Hoz *et al.* (5). In 1987, Tovey (6) reported that 26 IgE-binding components were present in mite body extract, and five bands showed especially strong binding. We evaluated mite allergens for their clinical relevance by intracutaneous tests and nasal

provocation tests with each fraction. From the results that there were differences in the reactions between the skin testing and provocation testing, it was considered that the fractions which induced an intracutaneous reaction did not always provoke allergic rhinitis. There were also individual differences in reactivity to each molecular weight fraction. In intracutaneous skin testing, there appeared to be three groups of patients in the degree of responses: (a) those reactive to the 15 and 25 kDMW fractions or to the 15, 25 and 32 kDMW fractions; (b) those reactive to only the 95 kD fraction or the 95 and 190 kDMW fractions; and (c) those reactive to the five fractions, 15, 25, 32, 95, and 190 kDMW fractions. Tovey (6) reported the 95, 32, 25 and 15 kDMW fractions as the main allergens and our clinical observations support their immunologic findings. However, we suggest that the allergens also exist in fractions of high molecular weight, namely, 95 and 190 kDMW. Some patients showed completely negative reactions while others except case 7 showed strongly positive reactions to these fractions as well as to other fractions. Most patients reacted positively to 15 and 25 kDMW; thus, these fractions are considered to be the main allergens of this mite.

Nasal provocation testing with each fraction is considered clinically reliable, but the results of both tests were not in agreement except for the reaction of 15 kDMW. Specific IgE production in response to each fraction may differ among patients. A combined study of immunological examinations of specific IgE production with clinical examinations in each patient could establish true allergens of this mite.

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