

Specific Measurement of a Major Mite Allergen, *Der f* II, by an Enzyme-linked Immunosorbent Assay System Using Monoclonal Anti-*Der f* II Antibodies

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An enzyme linked immunosorbent assay system using a monoclonal antibody, 15E11, specific for a major allergen *Der f* II in house dust mite, was developed. This system detected only *Der f* II in the presence of *Der p* II and other allergens. The *Der f* II contents in several house dust samples significantly correlated with the numbers of the mites in the same house dust samples ($n=14$, $r=0.88$, $p<0.001$). These data showed that this system was useful for specific measurement of *Der f* II in house dusts.

Since the house dust mites (*Dermatophagoides* spp.) were found to be a major source of house dust allergens,¹⁾ their allergens have been physiochemically and immunologically studied.²⁻⁵⁾ These allergens cause allergic diseases such as bronchial asthma and allergic rhinitis.¹⁾ It has been recognized that *Dermatophagoides* mites have two groups of major allergens, the *Der* I group (25 kDa, mainly from mite fecal particles) and the *Der* II group (15 kDa, from mite bodies).^{4,6-7)} The major allergens from *D. farinae* were *Der f* I and *Der f* II corresponding to *Der p* I and *Der p* II from *D. pteronyssinus*, respectively.

The immunological measurements of the mite allergens can be useful for the assessment of their levels in house dusts and standardization of mite extracts for hyposensitization therapy.^{8,9)} Platts-Mills postulated that there may be advantages to measuring group II allergens because they are more stable than group I allergens.⁹⁾ Many monoclonal antibodies (Mabs) against each allergen have been produced for this purpose.¹⁰⁾ However, since none of them react specifically with *Der f* II or *Der p* II because of their structural and physiochemical homology,²⁻⁵⁾ no enzyme-linked immunosorbent assay (ELISA) systems that could species-specifically measure the group II allergen have been established.²⁾ We have prepared a *Der f* II-specific Mab, 15E11, which recognized *Der f* II but not *Der p* II, as previously described.¹⁴⁾ These studies were undertaken to establish an ELISA system specific for *Der f* II in house dust.

Materials and Methods

Alkaline phosphatase (EIA grade) was obtained from Boehringer Mannheim. *p*-Nitrophenyl phosphate was obtained from Sigma Chemicals Co. *Der f* II from *D. farinae* bodies and *Der f* I from *D. farinae* whole culture were purified as described previously.^{11,12)} *Der p* I and *Der p* II were a kind gift of Dr. H. Yasueda. Commercially available allergens such as ragweed pollen, cedar pollen, *Candida albicans*, *Aspergillus niger*, mosquito, housefly, and *D. pteronyssinus* were purchased from International Biologicals Inc. Other chemicals were of analytical grade.

Enzyme-labeled Mab. Preparation and characterization of anti-*Der f* II Mabs were described previously.¹⁴⁾ Purified Mabs 13A4 and 18G8

were labeled with alkaline phosphatase by conventional method using glutaraldehyde¹⁵⁾ (abbreviated as 13A4* and 18G8*).

The epitope specificity of Mabs with inhibition experiments. Microtiter plates (Immulon I, Dynateck Laboratories, Inc.) were coated with 1.5 µg/well of *Der f* II in phosphate-buffered saline, pH 7.4 (PBS), for 2 h. The wells were washed with PBS and blocked with PBS containing 1% bovine serum albumin (BSA) or 1% Tween 20 for 1 h. The wells were washed with PBS containing 0.05% Tween 20 (PBS-T), and then 25 µl of enzyme-labeled Mab (18 µg/ml) diluted with PBS-T was incubated with or without 25 µl of unlabeled Mab (0.002–10 µg/ml) diluted with PBS-T for 2 h. After the plates were washed, 100 µl of substrate (1 mg tablet/2.5 ml of diethanolamine buffer, pH 9.5) was added to the well. After 90 min, the reaction was stopped with 50 µl of 5N NaOH. Absorbance at 405 nm was measured by an autoreader (Colona MTP-12). All the incubations were at room temperature.

The extraction of antigens from the house dust samples. House dust samples were obtained from several houses using electric vacuum cleaners. Each house dust sample (0.1 g) was extracted with 5 ml of PBS-T for 10 min at room temperature. After centrifugation (7000 × *g*, 5 min), the supernatant was filtered with a 0.2-µm membrane filter and 50 µl of the filtrate was assayed by sandwich ELISA (see below).

Sandwich ELISA. The purified *Der f* II or extracts from house dust samples (50 µl) was added to each well of microtiter plates coated with 15E11 (0.025 µg/well) and incubated for 2 h. The wells were washed with PBS-T and alkaline phosphatase labeled-second antibody 13A4*, (9 µg/ml, 50 µl) was added to the wells. After incubation for 2 h, the wells were washed with PBS-T. The color development was done with *p*-nitrophenyl phosphate for 10 min. The absorbance at 405 nm was measured.

Counting the numbers of mite bodies in the house dust samples. A conventional method¹⁶⁾ was modified for counting the mites in the house dust samples. In brief, after extraction, the remained dusts in the centrifuge tube were washed with 50% ethanol and filtered with stainless mesh (355 µm). Methylene blue was added to the filtrate as a final concentration of 1%. After filtration with a 80 µm filter (Fuji Photo Film Co., Ltd.), *Dermatophagoides* mites were identified and counted under the microscope. The numbers of mites in 1 g of house dust were calculated.

Results

Epitope specificity of Mabs

The specificity of the six Mabs was summarized in Table

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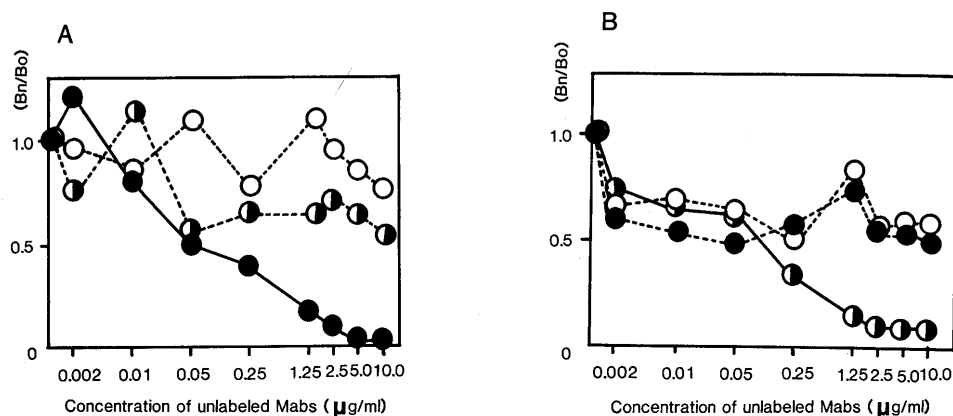


Fig. 1. Inhibition Binding Assay for the Monoclonal Antibodies.

A: Inhibition by unlabeled-15E11 (○), -18G8 (●) and -13A4 (●) of the epitopes on *Der f* II recognized by alkaline phosphatase-labeled 13A4. B: Inhibition by unlabeled-13A4 (●), -15E11 (○) and -18G8 (●) of those on *Der f* II recognized by enzyme-labeled 18G8. Bo, absorbance at 405 nm in the absence of unlabeled monoclonal antibodies; Bn, absorbance in the presence of unlabeled monoclonal antibodies.

Table I. Reactivities of six monoclonal antibodies for several mite allergens

Mabs	18G8	13A4	15E11	24B7	7C10	1B2
Host animal	Balb/c	Balb/c	Balb/c	Balb/c	A/J	A/J
Myeloma	NS-1	NS-1	NS-1	NS-1	SP-2	SP-2
Subclass	IgG1	IgG1	IgG1	IgM	IgG1	IgG1
<i>Der f</i> II	+++	++	+	+	++	++
<i>Der f</i> I	—	±	—	—	—	—
<i>Der p</i> I	—	±	—	—	—	—
<i>Der p</i> II	++	+	—	+	+	+

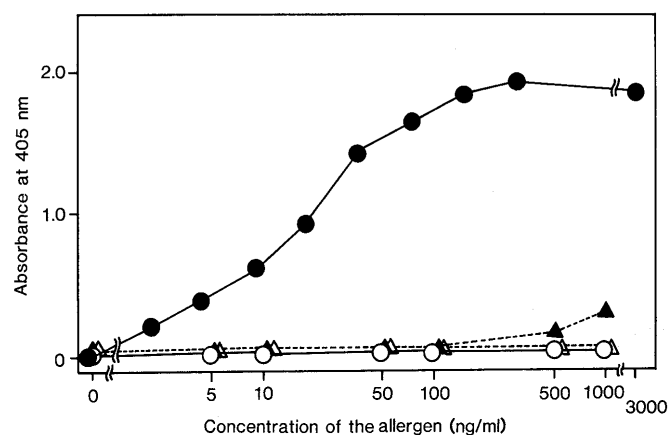


Fig. 2. Dose Response Curves for Several Mite Allergens in the Sandwich ELISA.

15E11 was used as solid phase antibody and alkaline phosphatase-labeled 13A4 was the second antibody. *Der f* I, ○; *Der f* II, ●; *Der p* I, △; *Der p* II, ▲.

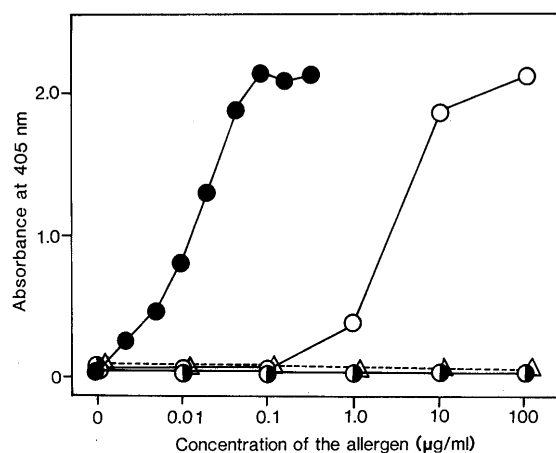


Fig. 3. Dose Response Curves for Other Allergens in the Sandwich ELISA.

D. farinae crude extracts were prepared from mite bodies. After each of allergens was extracted with PBS-T for 10 min, the filtrate (with 0.2-μm filter) was assayed by the Sandwich ELISA. *Der f* II, ●; *D. farinae* crude extract, ○; *D. pteronyssinus*, ○; other commercially available allergens (*Candida albicans*, *Aspergillus niger*, short rag weed pollen, cedar pollen mixture, house fly, and mosquito), △.

I. The inhibition binding assay was done to find whether the Mabs recognized the same or different epitopes on *Der f* II. Figure 1A showed that 15E11 does not inhibit the binding of 13A4* to *Der f* II. However, 18G8 did not completely compete with 13A4*. Similarly, 13A4 and 15E11 did not completely compete with 18G8 as shown in Fig. 1B. The reactivity of 1B2 and 7C10 was also the same as 18G8 (data not shown). These data suggested that 13A4 and 15E11 recognized different epitopes on the *Der f* II molecule. Actually, the sandwich ELISA could be done using 15E11 and 13A4* but other combinations failed to make an ELISA system.

Sandwich ELISA for measurement of mite allergens

Since 15E11 and 13A4 recognized different epitopes on *Der f* II, the sandwich ELISA system using these Mabs was examined. As shown in Fig. 2, when the reactivity of group I and group II of mite allergens were examined in the ELISA system, only *Der f* II was detected quantitatively at the detectable range of 0.5–100 ng/ml. Although *Der p* II slightly reacted at the concentration more than 500 ng/ml, its reactivity was negligible. The coefficient of variation of the ELISA system was 2.2% (at the concentration of *Der f* II of 9 ng/ml). Other mite allergens, *Der f* I and *Der p* I did not react at any concentration. As shown in Fig. 3, none of the commercially available allergens other than purified *Der f* II and a crude extract from *D. farinae* was detected at the concentration of 0–10 mg/ml in our ELISA system. Moreover, a dose response curve of crude extracts from *D. farinae* was parallel to that of purified *Der f* II.

Correlation between *Der f* II contents and numbers of mite bodies in the house dust samples

As the *Der f* II-specific assay system was established, a

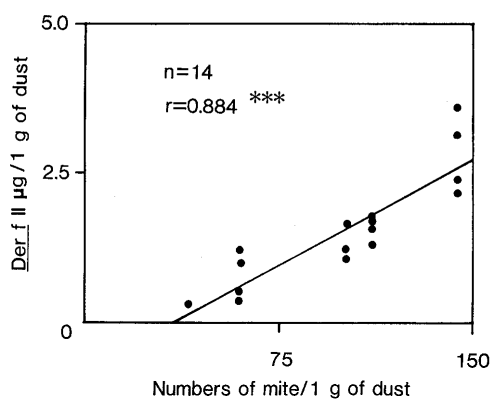


Fig. 4. Correlation between Mite-allergen Levels and Numbers of Mite in House Dust Samples.

House dust samples were collected from 14 different houses by the method mentioned in the text. The coefficient of correlation was statistically significant ($n=14$, $r=0.88$, $p<0.001$, shown as ***).

correlation between concentration of *Der f* II measured by the ELISA system and the number of mite bodies in the same house dust samples was examined. Figure 4 showed that a significant correlation between them was observed ($n=14$, $r=0.884$, $p<0.001$). These data suggest that the ELISA system not only measured *Der f* II contents but also estimated mite numbers in the house dusts.

Discussion

It has been reported that the measurement of individual mite allergens is important for monitoring of patients' allergen exposure and standardization of mite and house dust samples for hyposensitization therapy.^{17,18,21} For *Der f* I and *Der p* I, several immunological assay systems have been established using rabbit antisera^{17,18} or Mabs.¹⁹ On the other hand, no species-specific immunoassay systems for group II allergens (*Der f* II and *Der p* II) have been established, because species-specific antibodies have never been available.² The main reason for that is assumed to be because the amino acid sequences of *Der f* II and *Der p* II show as much as 88% homology (16 changes out of 129 amino acid residues)²⁰ and their sequences are highly conserved in these species.^{13,17,20}

In this study, we established a species-specific assay system of *Der f* II by the sandwich ELISA using Mabs, 15E11 and 13A4. As shown in a previous report,¹⁴ only 15E11 out of 6 Mabs reacted species-specifically with *Der f* II, others (containing 13A4) crossreacted with *Der p* II more or less by western blotting analysis. Therefore, the specificity of the ELISA system seems to be dependent upon that of 15E11. In the case of group II allergens, all the anti-group II Mabs have crossreacted and no species specific epitopes have been identified.²¹ The epitope of the *Der f* II molecule recognized by 15E11 should be further examined.

The limit of detection of our ELISA system was about

500 pg/ml. Luczynska *et al.*¹³ reported that the lowest detectable concentration of *Der f* I and *Der p* I by their ELISA system was 4 ng/ml and 10 ng/ml, respectively. Since the sensitivity for measurement of allergens in the house dusts required for the detection is assumed to be 1–10 µg/g dust,²¹ the sensitivity of our ELISA system is sufficient for that.

Furthermore, there was a significant correlation between *Der f* II contents and the numbers of *Dermatophagoides* mites in house dust samples ($n=14$, $r=0.88$, $p<0.001$). These data indicate that the ELISA system was able to estimate both *Der f* II contents and mite numbers in house dusts.

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