

Highly sensitive and specific ELISA with monoclonal antibody capture to measure *Dermatophagoides farinae* 1-specific IgE

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Background: *Dermatophagoides farinae* (Der f) 1 is a major allergen in Der f extract. Measurement of Der f 1-specific IgE has not been commonly used, because of the difficulty in obtaining large amounts of purified Der f 1.

Objectives: To improve the diagnosis of dust mite allergy, we wanted to develop an ELISA to measure Der f 1-specific IgE in which purified Der f 1 is not required.

Methods: Using a monoclonal antibody (mAb) against Der f 1, a mAb-capture ELISA was developed. Microplates coated with the mAb were sequentially incubated with crude Der f extract, serum samples, goat anti-human IgE, and conjugated rabbit anti-goat IgG. A conventional ELISA using purified Der f 1 as capture (Der f 1-ELISA) was developed to compare it with the mAb-capture ELISA.

Results: There was a significant correlation between the two ELISAs ($r = 0.89$, $P < .001$) with a sensitivity of 0.8 U/mL. Both the assays were inhibited by the crude Der f extract in a dose-dependent manner.

Using the two ELISAs, serum Der f 1-specific IgE was evaluated in 12 allergic patients with positive skin tests to Der f extract, 14 allergic patients with negative skin tests to Der f extract, and 15 healthy subjects. The mean Der f 1-IgE and the positivity rate of Der f 1-IgE were higher in the mite-allergic group and lower in the healthy group when the mAb-ELISA was used ($P < .01$) than when the Der f 1-ELISA was used ($P < .05$).

Conclusions: Der f 1-specific IgE can be measured by mAb-capture ELISA using crude Der f extract. The mAb-ELISA is more sensitive and specific than the conventional Der f 1-capture ELISA in diagnosing dust mite allergy.

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INTRODUCTION

House dust mite allergens are important in the pathogenesis of asthma, allergic rhinitis, and atopic dermatitis. Group 1 allergens of the mites *Dermatophagoides farinae* (Der f 1) and *Dermatophagoides pteronyssinus* (Der p 1) are the most significant

allergens; 80% to 95% of patients allergic to dust mites have an elevated IgE response to them.¹ Purification of Der f 1 and Der p 1 allergens has been achieved by a combination of 50% saturated ammonium sulphate precipitation and gel filtration² or by affinity chromatography with specific monoclonal antibodies.³ In most clinical studies, due to the difficulty of obtaining large amounts of purified group 1 allergens, dust mite-specific IgE has been measured by RAST (radioallergen sorbent test), ELISA (enzyme-linked immunosorbent assay), or Pharmacia CAP system using crude mite extracts. The conventional technique used in these assays is to immobilize mite allergens on a solid medium such as paper disks (RAST)

or polystyrene microtiter plates (ELISA), or hydrophilic polymers (CAP).

For 20 years, monoclonal antibodies (mAbs), especially anti-immoglobulins, have been used extensively in immunoassays to enhance specificity. Recently, a number of monoclonal antibodies to the major allergens of dust mite,⁴ cat,⁵ cockroach,⁶ *Alternaria*,⁷ grass pollen,^{8–10} *Penicillium*,¹¹ and latex,¹² etc, have been produced. These monoclonal antibodies are widely used to detect environmental allergens,^{3,5,6,13} standardize or quantitate allergen extracts,⁹ affinity-purify and characterize allergens,^{7,10} and analyze allergen cross-reactivity.⁸ Only in a few reports were these allergen-specific monoclonal antibodies used to measure specific antibodies.^{2,3,14–16} Although the mAb-capture assays are more sensitive than allergen-capture assays,^{14,15} the diagnostic value of the antibody-based assays has not been compared with the commonly used conventional allergen-capture assays.

In order to improve the sensitivity and specificity of immunoassays and to overcome the difficulty of preparing purified allergens, we developed a mAb-capture ELISA (mAb-ELISA) to measure Der p 1-specific IgE antibodies (Der p 1-IgE) using the mAb specific to Der f 1. We also developed a conventional Der f 1-capture ELISA (Der f 1-ELISA) and compared it with the mAb-capture ELISA. Using the two ELISAs, serum Der f 1-IgE levels were evaluated in allergic patients with or without skin reactions to the dust mite extract, and in non-allergic healthy subjects. We report the diagnostic value of the two ELISAs in dust mite allergy.

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MATERIALS AND METHODS

Materials

Monoclonal antibody (mAb) to Der f 1 (clone 6A8, a 50% saturated ammonium sulphate fraction of ascites, 10 mg/mL) and Der f 1 allergen (10 mg/mL, purified by affinity chromatography) were generous gifts from Dr. Martin D. Chapman, University of Virginia and the Indoor Biotechnologies, United Kingdom. Crude Der f whole body extract (1:100, wt/vol) was kindly provided by Dr. T. Wen, Shanghai Medical University. Goat anti-human IgE (P.S. myeloma-affinity purified) was a gift from Dr. N. Franklin Adkinson Jr, The Johns Hopkins Allergy and Asthma Center (Baltimore, USA). Alkaline phosphatase-conjugated rabbit anti-goat IgG was purchased from Jackson ImmunoResearch Laboratories, Inc. (PA, USA). Allergen extracts for skin prick tests were purchased from Bayer Allergy Products (Etobicoke, Ont). Polystyrene microplates were purchased from GIBCO Canada Inc (Burlington, Ontario). The THERMOmax microplate reader was obtained from Molecular Devices (California, USA).

Subjects

Twenty-six patients, 19 males and 7 females, aged 8 to 46 years, with allergic rhinitis and/or asthma in the Health Sciences Centre Allergy Clinics consented to skin tests and venipuncture. Diagnosis was based on medical history, examination, and one or more positive epicutaneous tests to eight common allergen extracts: cat, dog, weed, tree, mold, grass, house dust, and house dust mite (Der f, 50% glycerin, 30,000 AU/mL). Among the 26 patients, 12 had a positive skin test (mean of the largest and the smallest diameter of the wheal greater than 5 mm) to the dust mite extract, while 14 patients had a negative skin test to the mite extract. Fifteen healthy subjects with no history of allergic disorders also consented to skin tests and venipuncture. They had negative skin tests to the eight allergen extracts and were included as controls.

Enzyme-Linked Immunosorbent Assay (ELISA)

In the two ELISAs, optimal conditions for dilutions of mAb to Der f 1, purified Der f 1 and crude Der f extracts, serum, anti-human IgE, and conjugated antibody were chosen by checkerboard titration. Standardization of ELISA results among assays and the estimation of the relative amount of Der f 1-specific IgE in each sample was accomplished using a reference serum obtained from one subject with a high value of Der f 1-specific IgE. This serum was defined as 1,000 U/mL for Der f 1-specific IgE antibodies.

In the mAb-capture ELISA, polystyrene microtiter plates were coated with the mAb to Der f 1 (1 μ g/well) diluted in 0.05 M carbonate buffer (pH 9.6) and left overnight at 4 °C. All incubations were with volumes of 0.1 mL per well. After rinsing the plates 3 times with washing buffer (0.05% Tween 20 in 0.01 M PBS, pH 7.2) and blocking them with 2% bovine serum albumin (BSA) in washing buffer for one hour at room temperature, 0.1 mL of the crude Der f extract (10 μ g/well) diluted in ELISA buffer (0.2% BSA in washing buffer) was added to each well and the plates were incubated at 37 °C for one and one-half hours. The wells were washed; 0.1 mL of the test sample (1:20 and 1:40 diluted) or the reference serum (starting 1:20, 2-fold diluted, 11 dilutions) was added to each well, and the plates were incubated at 4 °C overnight. After washing, 0.1 mL of goat anti-human IgE (1:1,000) was added and incubated at 37 °C for one and one-half hours. After washing again, 0.1 mL of enzyme-conjugated rabbit anti-goat IgG (1:2,000) was added and incubated at 37 °C for one and one-half hours. After a final washing, the color was developed by adding 0.1 mL of the enzyme substrate (p-nitrophenylphosphate, 1 mg/mL in diethanolamine buffer, pH 9.8) and incubating the plates at 4 °C overnight. The reaction was then stopped by the addition of 0.1 mL of 1 N NaOH. Optical density

(OD) at 410 nm was read using the THERMOmax microplate reader.

In the conventional Der f 1-capture assay, microplates were coated with 0.1 mL of Der f 1 (0.1 μ g/well) followed by incubation with the samples or the reference serum. The remaining steps were the same as in the mAb-capture ELISA.

The value of Der f 1-specific IgE was calculated by interpolation from the dilution curve of the reference serum. Each IgE determination was performed on two dilutions of serum, with each dilution duplicated.

Inhibition Tests

In order to examine the specificity of the two assays, inhibition tests were conducted. The crude Der f extract was 10-fold sequentially diluted in ELISA buffer. Each dilution of the extract was mixed 1:1 with a diluted serum containing 20 U/mL of Der f 1-specific IgE and then incubated at 37 °C for two hours. Incubation of the serum dilution with ELISA buffer served as a positive control. The Der f 1-specific IgE in these samples was then measured by both the mAb- and Der f 1-capture ELISAs as described above. The percentage of inhibition was calculated as illustrated below: $\text{OD of the positive control} - \text{OD of the test sample} / \text{OD of the positive control} \times 100$.

Statistical Analysis

Analysis of the data was performed using the Number Crunch Statistical System software. Unpaired *t* tests were used between group comparisons of the IgE values. The Chi square test was used to compare the positive percentages between groups. Linear regressions were used for the analysis of the correlation of the specific IgE levels measured by mAb- and Der f 1-capture ELISAs.

RESULTS

ELISAs

The optimal concentrations obtained by checkerboard titration were 1 μ g/well for the mAb to Der f 1, 0.1 μ g/well for the purified Der f 1, 10 μ g/well for the crude Der f extract, 1:20 and 1:40 for

serum samples, 2-fold dilutions from 1:20 to 1:20,480 for the reference serum, 1:1,000 for goat anti-human IgE and 1:2,000 for conjugated rabbit anti-goat IgG. Under these conditions, the reference serum (as a positive control) had a high OD reaction, while the cord serum (as a negative control) and the background (ELISA buffer replaced serum sample) had a low OD reaction (<0.120 OD). The sensitivity of the two ELISAs was high: 0.8 U/mL, ie, even when the reference serum was diluted to 1:1,250, Der f 1-specific IgE was still measurable (Fig 1). Standard curves of the two ELISAs are shown in Fig 1, in which each dilution was assayed in triplicate. Low variability was obtained among wells, dilutions, and assays as previously reported for ELISAs.¹⁷

Inhibition Tests

As presented in Fig 2, the ELISA reactions in the measurement of Der f 1-specific IgE by the two ELISAs were inhibited by the crude Der f extract in a dose-dependent manner. The successful inhibition of the ELISA reactions indicates that the two ELISAs are specific for the dust-mite allergen.

Der f 1-Specific IgE Levels

The results obtained by the mAb-capture ELISA correlated significantly with those obtained by the Der f 1-capture ELISA ($P < .001$) (Fig 3). Using the mAb-capture ELISA, the geometric mean serum Der f 1-specific IgE level in the dust mite-allergic patients (23.9 U/mL) was significantly higher than that of either the mite non-allergic patients (7.4 U/mL) ($P < .04$) or the control group (4.0 U/mL) ($P < .001$). Using the Der f 1-capture ELISA resulted in the mean Der f 1-specific IgE level in the dust mite-allergic patients (37.1 U/mL) being significantly higher than that of the control group only (14.3 U/mL) ($P < .03$) and no significant difference between the mean Der f 1-specific IgE levels in the mite-allergic (37.1 U/mL) and mite non-allergic patients (19.9 U/mL). The mAb-capture ELISA resulted in a higher positive rate in the mite-allergic patients and a lower positive rate in either

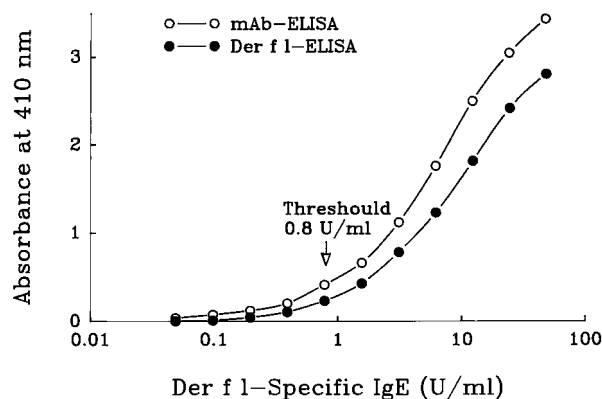


Figure 1. Standard curves of the 2 ELISAs for measurement of Der f 1-specific IgE. The sensitivity of both assays was 0.8 U/mL.

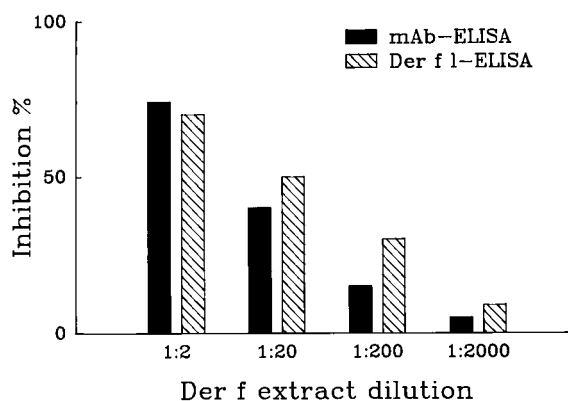


Figure 2. Inhibition tests. Before ELISA, the diluted serum was incubated with 10-fold serially diluted crude Der f extract for two hours at 37 °C. Incubation of the serum dilution with ELISA buffer served as a control. Der f 1-IgE was then measured using the two ELISAs. The percentage of inhibition was calculated as described in the "Methods."

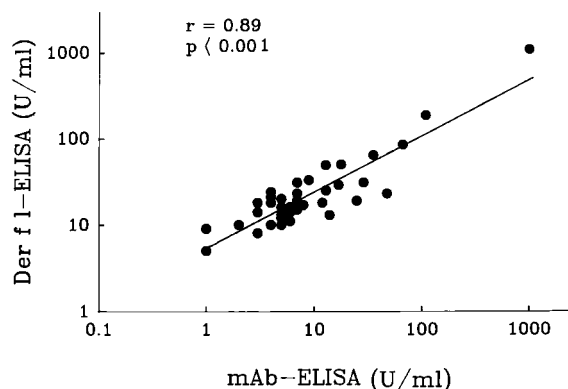


Figure 3. Correlation between mAb-capture and Der f 1-capture ELISAs in measurement of Der f 1-specific IgE in allergic patients and healthy subjects.

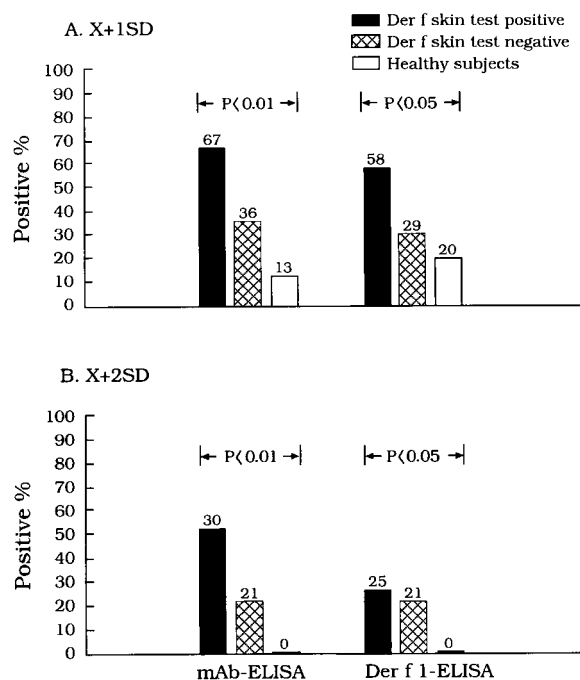


Figure 4. Positive percentage of serum Der f 1-specific IgE in allergic patients with or without a positive skin test to Der f extract and in healthy subjects.

the allergic controls or the healthy controls than the Der p 1-capture ELISA (Fig 4) when the results were expressed using positive percentages, whether a level greater than the mean plus 1SD or 2SD in the controls was considered as positive.

Diagnostic Values of the Two ELISAs

The results showed that mAb-capture ELISA was more sensitive and specific than conventional Der f 1-capture ELISA in the diagnosis of dust mite

allergy. The diagnostic values¹⁸ of the two methods are listed in Table 1.

DISCUSSION

The ELISA, first described by Engvall and Perlman,¹⁹ provides a safe, simple, and inexpensive method of measuring specific antibodies. To measure allergen-specific IgE, ELISA is marginally more sensitive than RAST.¹⁵ In our ELISAs, the sensitivity was 0.8 U/mL, ie, even when the reference serum was

diluted to 1:1,250, Der f 1-specific IgE was still measurable (Fig 1). The specificity of both ELISAs for Der f 1-specific IgE was demonstrated by successful inhibition tests (Fig 2). In the establishment of sensitive and specific immunoassays, it is important to ensure that there is standardization among the assays by using a reference serum. In our ELISAs, each microtiter plate contained a standard curve for calculation of test samples within the plate.

Utilizing the two ELISAs, serum Der f 1-IgE was evaluated from mite-allergic patients, mite non-allergic patients, and healthy control subjects to define the diagnostic values of the two assays. The results, whether expressed by positivity rate or geometric mean level, clearly indicate that the mAb-capture ELISA is more sensitive and specific than the conventional Der f 1-capture ELISA in the diagnosis of dust mite allergy. This suggests that a mAb-capture immunoassay could also be used to improve the diagnosis of other allergies as availability of monoclonal antibodies against major allergens increases. The use of allergen/antigen-specific monoclonal antibodies should not be limited to antigen measurements, but should be extended to measure specific antibodies. Although the Pharmacia CAP System assay has excellent analytic sensitivity and specificity,^{20,21} it would be optimized if monoclonal antibodies against major allergens, not the crude allergen extracts, were bound to the polymer carriers. In addition, the mAb-capture immunoassay avoids the time- and labor-consuming procedure of obtaining purified allergens. The mAb-capture ELISA method could be used with a wide range of different antigen-specific monoclonal antibodies in various immunoassays.

The reasons for the high sensitivity and specificity of the mAb-capture ELISA may include: (1) the monoclonal antibody binds more Der f 1 molecules than the microtiter plates; (2) in the mAb-ELISA the Der f 1 allergen that bound to the monoclonal antibody is more pure and less denatured than the chromatography-purified Der f 1 used in

Table 1. Diagnostic Values* of the Two ELISAs at Different Cutoff Levels (%)

Comparison Groups	Mean + 1SD		Mean + 2SD	
	mAb-ELISA	Der f 1-ELISA	mAb-ELISA	Der f 1-ELISA
Der f skin test positive patients and healthy subjects	154	138	150	125
Der f skin test positive and negative patients	131	129	129	104

* The reliability of a diagnostic test, that is, its diagnostic value, is the sum of its sensitivity and its specificity. It ranges from "failure" at 0% to "perfect" at 200%.¹⁸ The positive percentage in the mite-allergic group represents the sensitivity (the higher the percentage, the greater the sensitivity), while 1 minus the positive percentage of the negative group represents the specificity (the lower the percentage, the higher the specificity). The positive percentages are shown in Figure 4.

the Der f 1-ELISA; (3) the antigen may lose some of its biologic integrity when it binds to the plates²² and can also become detached from the plate.²³

The mean level and positivity rate of Der f 1-IgE in the allergic patients with negative skin tests to the mite extract were significantly lower than those in the mite-allergen group, but higher than those in the control group (Fig 4). Although these patients were not allergic to dust mite, they may have been sensitized to other allergens. Cross-reactivity among allergens has been reported in many studies. Shared allergens were found between the house dust mite (*Dermatophagoides pteronyssinus*) and the chironomid midge,²⁴ as well as among mites, shrimp, and insects.^{25,26} These allergens to which the patients are sensitized may have crossreactivity with Der f 1. Thus, the allergic patients with negative skin tests to dust mite may recognize some Der f 1-specific IgE.

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