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Influence of Age on IgE Responsiveness to *Dermatophagoides farinae*: An Immunoblot Study

Key Words

Dermatophagoides farinae
Der f I
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IgE Immunoblotting
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Abstract

IgE responsiveness to *Dermatophagoides farinae* body extract was compared by immunoblotting among four groups of mite-sensitive asthmatic children: group I aged 0–3, group II aged 4–7, group III aged 8–11 and group IV aged 12–15. In the group I subjects, the 15-kD component was bound by 88% of sera and strongly by 56% of sera. The 25-kD component was bound by 40% of sera, but most of the binding reactions were faint. IgE binding to other components was generally low. In the group II subjects, 15- and 25-kD components were bound by 96% and 56% of sera, 30-kD and 110-kD components by 76% and 60% of sera, respectively. In addition, 18-, 40-, 59- and 80-kD components were bound by more than 30% of sera. In group III and IV subjects, the frequencies of IgE binding to these components were not significantly changed as compared with those of group II subjects. The rabbit antisera against *Der f* I and *Der f* II specifically reacted with 25- and 15-kD components, respectively. These results suggest that the 15-kD *Der f* II allergen is the most important antigenic constituent associated with the early IgE response to house dust-mites in mite-reactive asthmatic children.

Introduction

Allergic reactions to house-dust mites are often believed to play an important role in the pathogenesis of asthma in children, because high levels of IgE antibody against the mite antigen show significant correlations not only with a clinical history of asthma, but also with provocation test results with this antigen [1–4].

The nature of house-dust mite antigen has been extensively studied, and now more than 30 IgE-binding proteins

have been identified in body and fecal extracts from the genus *Dermatophagoides* [5–14]. Although asthmatic children are often sensitized to house dust mites during early childhood [15–17], it is not clear what antigenic constituents of mites are associated with the initial sensitization of these children. In this study, we examine the influence of age on IgE responsiveness to *Dermatophagoides farinae*, a dominant species of house dust mite in this country, by immunoblot analysis.

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Materials and Methods

Antigen

The lyophilized mite body extract prepared from isolated mite bodies of *D. farinae* [9] was kindly supplied by Torii Co., Ltd. (Tokyo), and used as *D. farinae* antigen. The *Der f* I and *Der f* II contents of this antigen were 0.53% and 0.48% on a weight basis, respectively.

Sera for Immunoblotting

The sera were obtained from mite-sensitive asthmatic children visiting the Allergy Clinic of the University Hospital of Tsukuba. The diagnosis of asthma was based on the definition of the American Thoracic Association [18], and patients with two or more episodes of wheezing with shortness of breath during the past year were considered as having bronchial asthma. Specific IgE antibody levels against *D. farinae* were determined by the Sepharose RAST methods as described previously [19], using twofold serial dilutions of sera. The maximum dilution of serum producing RAST positivity was expressed as a RAST titer. High RAST sera for *D. farinae* were first screened from a total of 526 allergic sera saved in our laboratory by the Sepharose RAST method using 128-fold diluted sera. The 173 sera showing positive RAST reactivity to *D. farinae* (RAST titers $\geq 1:128$) were selected as high RAST sera for *D. farinae*. Finally, 25 high RAST sera for *D. farinae* were chosen from each of the following age groups: group I aged 0–3, group II aged 4–7, group III aged 8–11, and group IV aged 12–15. The average ages of groups I, II, III and IV were 3.2 ± 0.6 , 6.2 ± 0.9 , 10 ± 1.2 , and 14 ± 0.8 , respectively.

A total of 100 sera, 25 samples each group, were serially diluted from 1:128 to 1:4,096 with PBS containing 2% HSA, and RAST titers were determined. All sera were adjusted to have the same RAST titer of 1:128, and then 0.1 ml of the sample sera were mixed with 0.9 ml of PBS containing 2% HSA (the final RAST titer was 1:12.8), and subjected to immunoblot analysis.

IgE Immunoblot Analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously [20], with 12% homogeneous gels. The 90 × 40 mm mini-gel was used, and the sample gel was divided into two lanes by the use of a special comb: one 3.5 mm wide for molecular weight marker standards (Calibration kit proteins, Pharmacia, Sweden) and the other 80 mm wide for sample application.

The gels were loaded with 200 µg of protein, and electrophoresed at 30 mA per gel in the unreduced condition. After electrophoresis, gels were electroblotted onto a polyvinylidene difluoride membrane (Immobilon®, Millipore, Bedford, Mass.) in a Tris-glycine-SDS-methanol buffer at 0.8 mA/cm. After the transfer, the lane containing the molecular weight marker standards was removed and sequentially stained with amido black. The blotted samples were blocked for 3 h in PBS buffer containing 0.01% Tween 20 and 3% bovine serum albumin. The blots were then washed five times in PBS-Tween, and cut into 26 strips, each being 0.3 mm wide. The strips were put into special strip-incubation tubes, incubated overnight with 1 ml of the test sera, washed again, and incubated for 4 h with 0.2 µCi of ^{125} I-labeled anti-human IgE obtained from Phadebas RAST kits (Pharmacia). The membrane was washed again, dried, mounted and autoradiographed with X-OMAT AR film (Eastman Co., Rochester, N.Y., USA).

The molecular weights of the IgE binding components were measured according to the calibration curve constructed by plotting the relative migration distance of the calibration kit proteins.

The intensity of radiostaining of IgE binding bands was graded as 0 to 11, referring to a sensitivity control gray scale for X-ray film (Wex-jex Inc., Iuyland, Penn., USA). When the intensity was grade 2 or more, the reaction was defined as positive. The positive reactions were further divided into two groups; weakly positive (grade 2–4) and strongly positive (grade ≥ 5).

Identification of *Der f* I and *Der f* II Allergens

The electroblotted membrane strips were incubated with 1:600 diluted rabbit antisera against *Der f* I and *Der f* II, which were kindly supplied by Dr. Tohru Ando, Torii Research Laboratory, Japan, at 37 °C for 3 h. The strips were then washed three times in PBS-Tween, and further incubated with peroxidase, conjugated goat anti-rabbit IgG (Cappel, USA) solution (1:6,000) in PBS for 1 h at 37 °C. Then, the strips were washed again, and transferred into freshly prepared substrate buffer (10 mg of 3,3-diaminobenzidine 4 HCl, 40 ml of PBS, 15 µl of 30% H₂O₂) and developed at room temperature.

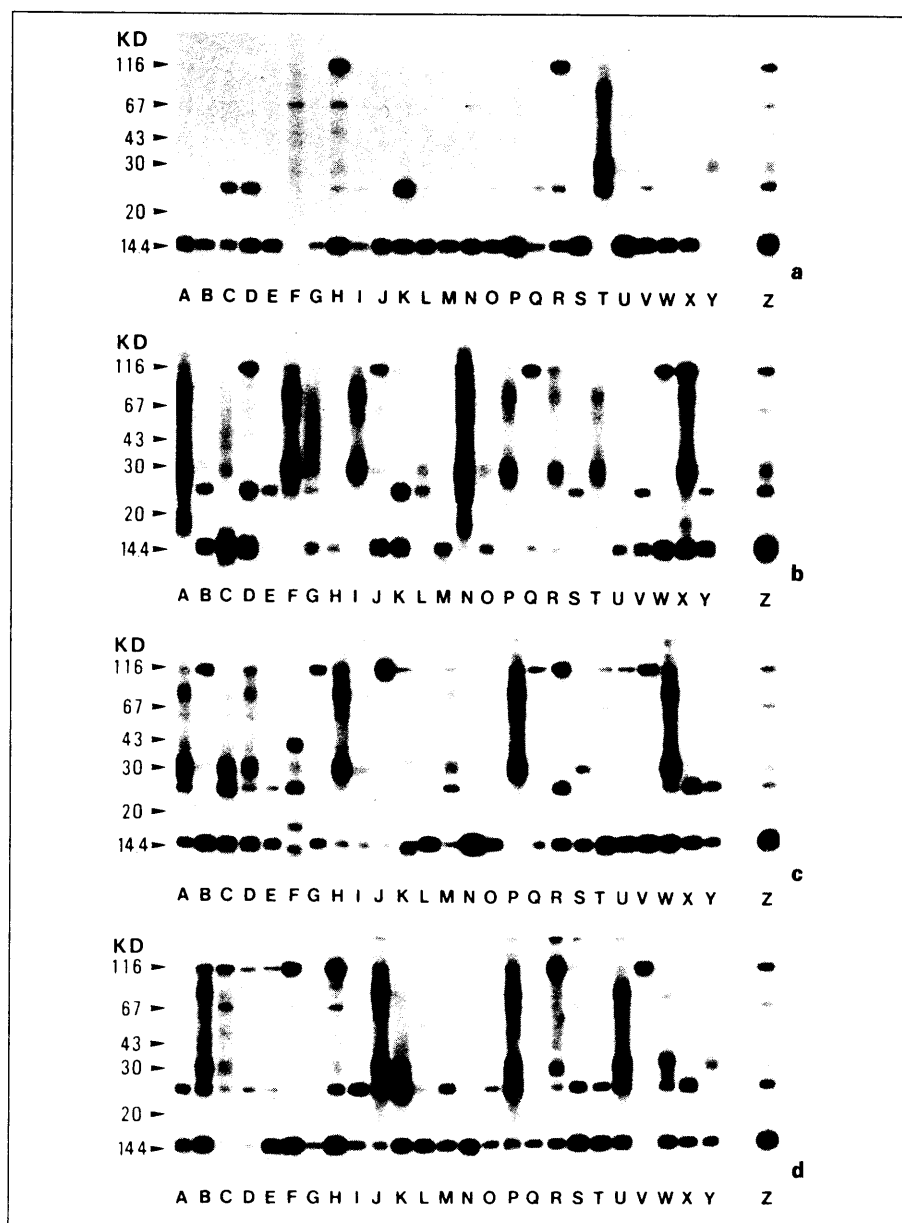
Results

IgE binding reactions to *D. farinae* body extract were compared by immunoblotting among four groups of mite-sensitive asthmatic children of different ages. The immunoblot profiles of groups I, II, III and IV sera are illustrated in figure 1a–d, respectively. As determined visually, the sera from group I subjects aged 0–3 preferentially bound to low molecular weight components of less than 30-kD, and especially to 15-kD components (fig. 1a). The number and strength of IgE binding bands to *D. farinae* increased remarkably in group II patients aged 4–7 (fig. 1b), but the IgE reactivities did not differ considerably in group III and IV subjects as compared with those of group II.

The *D. farinae* antigen demonstrated at least 13 distinct IgE binding bands with most of the staining between 12 and 120 kD. The molecular weights of the reactive bands were 12, 15, 16, 18, 25, 30, 40, 48, 59, 67, 80, 95 and 110 kD. Of these IgE binding components, those of 15, 25, 30 and 110 kD were bound by more than 50% of sera in the older children (groups II, III), and therefore were considered to be major allergenic components. The broad bands were seen in many cases in the molecular weight region between 28 and 33 kD. Here, the bands located in this lesion were expressed as a 30-kD component.

The frequencies of positive IgE binding reaction to the 13 *D. farinae* components in the four groups are summarized in figure 2. In the group I subjects aged 0–3, a 15-kD component was bound by 22/25 sera (88%) and strongly by 14/25 sera (56%). A 25-kD component was bound by 10/25 sera (40%), but most of the binding reactions were weak. In contrast, IgE binding to higher molecular weight components of more than 30 kD were generally low. In the

Fig. 1. Comparison of IgE binding of individual sera to *D. farinae* body extracts among four groups of mite-sensitive asthmatic children: group I aged 0–3 (**a**), group II aged 4–7 (**b**), group III aged 8–11 (**c**) and group IV aged 12–15 (**d**). Lanes A–Y: 25 individual sera from *D. farinae*-sensitive asthmatic children; lane Z: positive control serum pooled from 5 *D. farinae*-sensitive asthmatic patients.



group II subjects aged 4–7, 15- and 25-kD components were bound by 24/25 (96%) and 14/25 (56%), 30- and 110-kD components by 19/25 (76%) and 15/25 (60%), respectively. In addition, more than 30% of the group II sera bound to other components with molecular weights of 18, 40, 59 and 80 kD. In the group III and IV subjects, the frequencies of IgE binding to most components did not significantly change as compared with those of group II.

To confirm that the 25-kD allergen is *Der f* I, and that 15-kD allergen is *Der f* II, the electroblotted strips were stained with either rabbit antisera or human IgE antibodies. As shown in figure 3, the antisera against *Der f* I and

Der f II specifically reacted with 25-kD and 15-kD components of *D. farinae*, respectively, which were also major allergenic components recognized by human IgE antibodies.

Discussion

The *D. farinae* extract demonstrated at least thirteen distinct IgE binding bands on immunoblotting, with apparent molecular weights of 12, 15, 16, 18, 25, 30, 40, 48, 59, 67, 80, 95 and 110 kD. Of these bands, the 15-, 25-, 30- and

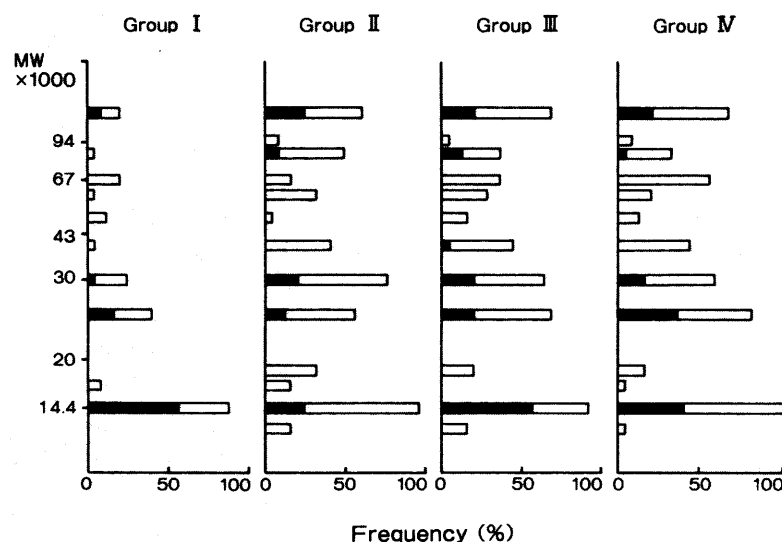


Fig. 2. Frequencies of IgE binding to *D. farinae* body extracts in four groups of asthmatic children. □ = Weakly bound; ■ = strongly bound.

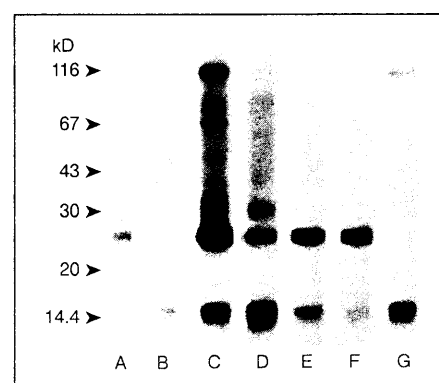


Fig. 3. Immunoblot analyses of *D. farinae* body extract with either rabbit anti-*Der f* I and anti-*Der f* II sera or human IgE antibodies. Lane A: a staining with rabbit anti-*Der f* I serum and peroxidase-conjugated anti-rabbit IgG; lane B: a staining with rabbit anti-*Der f* II serum and peroxidase-conjugated anti-rabbit IgG; lanes C–G: stainings with individual sera from *D. farinae*-sensitive asthmatic children and radiolabeled anti-IgE.

110-kD bands were found in more than 50% of sera from mite-allergic asthmatic patients. Tovey et al. [5] reported that a total of 26 IgE binding bands were demonstrated by immunoblotting of the *Dermatophagoides pteronyssinus* body extract. Nakanishi et al. [6] also identified 29 IgE binding components in the *D. farinae* body extract. The lower incidence of IgE binding bands in this study may be due to the fact that the sera for IgE immunoblot analysis were obtained from children, and a mini-gel was used for SDS-PAGE. However, the pattern of the IgE reactivity of *D. farinae* antigen based on the frequency histogram was similar to that described in the previous studies [5, 6].

Major groups of allergens have been defined from the genus *Dermatophagoides* [7–14]. The group I (*Der* I), group II (*Der* II), group III (*Der* III) and group IV (*Der* IV) allergens are shown to have molecular weights of 24–25, 15, 30 and 56 kD, respectively [5–11]. Our immunoblot study using specific rabbit antisera has demonstrated that the

25-kD and 15-kD IgE binding bands in this study correspond to the *Der f* I and *Der f* II allergens, respectively.

Our results have shown that younger children aged less than 4 years old produce IgE antibodies to a smaller range of *D. farinae* component proteins than those of older age groups, and the protein to which they react most is a 15-kD protein.

The *D. farinae* body extracts used in this study contained almost equal amounts of *Der f* I and *Der f* II allergens. It is therefore unlikely that the difference in the IgE reactivity to *D. farinae* components with 15 kD dominance is due to a difference in the loading doses of the two allergens on the electrophoresis.

It has been shown that *Der* I allergens are more easily denatured than *Der* II allergens by heating, acid-treatment, reduction and alkylation, and lose their allergenic activity to a variable extent [21, 22]. In this study, however, the *D. farinae* allergens were freshly prepared, lyophilized

and stored at -70°C until used, they were never heated or acidified, and they were separated by SDS-PAGE under nonreducing conditions. The possibility may remain that SDS-PAGE or electric transfer may degrade *Der f I* allergen. We found, however, on performing immunoblot analyses with specific rabbit antisera, that the final dilutions of anti-*Der f I* and anti-*Der f II* sera producing positive staining bands with 25- and 15-kD components were both 1:1,200 (data not shown). Since both antisera are shown to have the same precipitating antibody titers of 1:8 dilution on immunodiffusion in Ouchterlony [pers. commun. from Dr. H. Ando], it seems unlikely that 25-kD *Der f I* allergen is denatured by the SDS treatment on electroblotting.

Several explanations may be possible for the age-dependent difference in IgE response to mite allergens with the apparent dominance of 15-kD protein (*Der f II*) in young asthmatic children. First, the *Der f II* allergen predominates in house dust in this country. Yasueda et al. [23], however, have shown that the ratio of *Der I*:*Der II* is not significantly different in the house dust samples from Japanese asthmatic patients. Second, because *Der f II* is a low molecular weight allergen, it can more easily penetrate the airway membrane than other higher molecular weight allergens, resulting in the earlier IgE response in young children. Third, *Der f II* has an increased number of immunogenic carrier determinants for T cells rather than haptenic determinants for B cells as compared with *Der f I*. It has been generally accepted that most allergens are thymus dependent, and require activated T cells in order for B cells to produce antibody [24, 25]. It is therefore likely that the age-dependent difference in the IgE response to mite components may reflect the number or functional capacity of the T cell carrier determinants of the allergen molecules.

The number of *D. farinae* components to which the subjects responded increased rapidly from 4 to 7 years of

age. This rapid progress of IgE responses to a variety of *D. farinae* components in the preschool-aged children may be associated with the functional maturation of immunologic systems, especially the T cell systems [26].

The immunoblot analyses were carried out with only high RAST sera for *D. farinae* ($\geq 1:128$) in all age groups, because the analytical sensitivity of the IgE immunoblot methods was limited. Our preliminary study has shown that most mite-sensitive asthmatic children aged 3 or less had lower RAST titers for *D. farinae* ($\leq 1:64$). Therefore, to obtain high RAST sera for *D. farinae* from young children, we had to screen a large number of serum samples.

More than 80% of mite-allergic adult patients have demonstrable IgE antibodies against both *Der I* and *Der II* on RAST or skin testing [10–12, 27]. On the other hand, Calkhoven et al. [15] have shown in a RAST study using longitudinal serum samples in young children that more than half have no detectable IgE antibody against *Der p I* and *Der p II*, suggesting that the early IgE antibody response to house-dust mite is induced by components distinct from these two major allergens. To investigate the early IgE responses to mites in very young children with low RAST values on immunoblotting, more sensitive methods are required.

In conclusion, the data presented here show that the 15-kD component of *D. farinae* is an important antigen associated with the initial sensitization to house dust mite in children. Further epitope analyses of the 15-kD antigen using sera from young asthmatic children should provide a better understanding of the molecular determinants of IgE responsiveness, and may thus contribute to future immunotherapy.

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