

Comparisons of IgE, IgG, and IgG4 responsiveness to *Dermatophagoides farinae* in children by immunoblotting

Noguchi E, Shibasaki M, Isoyama S, Takita H. Comparisons of IgE, IgG, and IgG4 responsiveness to *Dermatophagoides farinae* in children by immunoblotting.

Allergy 1996; 51: 907–913. © Munksgaard 1996.

Although asthmatic children are often sensitized to the house-dust mite (HDM) during early childhood, it is not clear what antigenic components are associated with the early immune response of these children. To investigate this problem, we evaluated IgE, IgG, and IgG4 antibody responses to *Dermatophagoides farinae* (Df) by immunoblotting among three groups of children: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years. In the group I subjects, positive IgE-binding reactions to 15- and 25-kDa components were found in 76% and 44% of sera, respectively. Those to other components were generally low in frequency. In contrast, positive IgG-binding reactions to 15- and 25-kDa components were found in 44% and 24% of sera, and those to 30- and 110-kDa components in 48% and 88% of sera, respectively. Positive IgG4 reactions to 15- and 25-kDa components were found in 48% and 24%, respectively, and those to other components were very low. Positive IgE-binding reactions to these components gradually became more frequent with increasing age in groups II and III, while IgG and IgG4 reactivities were not markedly different in these age groups. These results suggest that the 15- and 25-kDa proteins of Df are important antigens associated with the initial IgE response to HDM in early childhood, while the 30- and 110-kDa proteins are associated with IgG and 15-kDa components with IgG4.

**E. Noguchi, M. Shibasaki,
S. Isoyama, H. Takita**

Department of Pediatrics, Institute of Clinical
Medicine, University of Tsukuba, Tsukuba, Japan

Key words: asthma; *Dermatophagoides farinae*;
house-dust mite; IgE; IgG; IgG4; immunoblotting.

Masanao Shibasaki, MD
Department of Pediatrics
Institute of Clinical Medicine
University of Tsukuba
Tsukuba, Ibaraki-ken 305
Japan

Accepted for publication 25 June 1996

Allergic sensitization to house-dust mite (HDM) is believed to play an important role in the development of asthma in children, because high levels of IgE antibodies against the HDM antigen show significant correlations not only with a clinical history of asthma, but also with a provocation test result with this antigen (1–4). The HDM antigen has been shown to be a complex material containing more than 30 IgE-binding components, and several major antigenic proteins have been isolated from body and fecal extracts of the genus *Dermatophagoides* (3, 5–13). On the other hand, it has been shown that asthmatic patients have increased levels of IgG antibody against HDM as well as IgE antibody (14), and that there is a quantitative correlation between IgG and IgE antibodies (15, 16), suggesting that IgE antibodies are produced as a part of the immune response that

also includes IgG antibodies. Furthermore, IgG4 antibody against HDM has been shown to be increased in atopic asthma (17, 18). The clinical significance of the allergen-specific IgG antibodies remains unclear. However, since the IgG antibody is a dominant class of immunoglobulin, it is likely that the IgG-binding proteins of HDM may express important antigenic determinants involved in the induction of the immune response to HDM allergens.

Although asthmatic children are often sensitized to HDM during early childhood (16, 19), it is not clear what antigenic components are associated with this early sensitization. To investigate this problem from a developmental aspect, we evaluated IgE, IgG, and IgG4 antibody responses to *Dermatophagoides farinae* (Df) in asthmatic children of different ages by immunoblot analysis.

Material and methods

Antigen

The lyophilized mite-body extract prepared from isolated bodies of Df was kindly supplied by Torii Co., Ltd (Tokyo, Japan), and used as Df antigen (6). The Der f 1 and Der f 2 contents of this antigen were 0.53% and 0.48%, respectively, by weight.

Sera for immunoblotting

Test sera having a high RAST titer and high ELISA levels for Df were obtained from HDM-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 (20), and patients with two or more episodes of wheezing with shortness of breath during the past year were regarded as having bronchial asthma. No patients had received desensitization therapy. High-RAST sera for Df were first screened from 1136 sera of Df-sensitive asthmatic children by the Sepharose RAST method, as described previously (21): the 1 : 128 diluted sera were subjected to the Sepharose RAST for Df, and only sera showing positive RAST reactivity were selected as high-RAST sera. A total of 270 sera were included.

High-ELISA sera for Df, having an IgG antibody level of more than 250 ELISA units (EU)/ml, were further selected from the 270 high-RAST sera for Df by the ELISA method, as described below (22). A total of 192 sera were selected.

Finally, 25 high-RAST and high-ELISA sera for Df were randomly chosen from each of the following age groups: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years. The average ages of groups I, II, and III were 2.5 ± 0.9 , 7.9 ± 1.3 , and 11.7 ± 1.4 years, respectively. The sera were stored at -20°C , and adjusted to have either the same RAST titer of 1 : 128 or the same ELISA levels of 250 EU/ml just before use.

Quantification of Df-specific IgG

In this assay, 100 μl of Df antigen dissolved in carbonate coating buffer (sodium carbonate/bicarbonate 0.1 M, pH 9.6) at 10 $\mu\text{g}/\text{ml}$ protein concentration were placed in the wells of flat-bottom, microtiter plates (Sumitomo, Japan), and incubated for 90 min at 37°C . After three washes with washing buffer (phosphate-buffered saline [PBS] containing 0.05% Tween 20), the plates were blocked for 1 h at 37°C with 250 $\mu\text{l}/\text{well}$ of PBS containing 1% bovine serum albumin (BSA), and washed with washing buffer.

Serum samples were diluted to 1 : 100 with diluting buffer (PBS containing 0.1% Tween 20 and 1% BSA), and 100 μl of each was added to the well. The plates were incubated for 1 h at 37°C . After washing with washing buffer, 100 μl of horseradish peroxidase (HRP)-conjugated goat polyclonal anti-human IgG (Cappel, Durham, NC, USA) diluted to 1 : 2000 with diluting buffer, the plates were added to the wells, and incubated for 1 h at 37°C . After three washes, they were further incubated with 100 μl of 0.2 M citrate-phosphate buffer (pH 5.0) containing 0.4 mg/ml *o*-phenylenediamine dihydrochloride (OPD; Sigma Chemical Company, USA) and 4 μl of 30% H_2O_2 per 20 ml for 30 min at room temperature. After the reaction was stopped with 2 M H_2SO_4 , absorbance was measured with an Immunoreader (Japan Intermed Co., Ltd, Japan) using a test wavelength of 490 nm and a reference wavelength of 630 nm.

Serial twofold dilutions of a standard serum were made from 1 : 100 to 1 : 12 800 for IgG assay of Df. The absorbancy and the reciprocal of the dilution titer were plotted on logarithmic paper. The absorbancy at the serum dilution of 1 : 400 was arbitrarily defined as 250 EU/ml, and IgG concentrations of the samples were quantified by reference to this standard.

IgE, IgG, and IgG4 immunoblotting

SDS-PAGE was carried out as previously described (23). The 90×40 mm minigel 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 for IgE blotting and 400 μg for IgG and IgG4 blotting, and electrophoresed at 30 mA per gel in the unreduced condition. After electrophoresis, gels were electroblotted onto a polyvinylidene difluoride membrane (Immobilon[®], Millipore, Bedford, MA, USA) 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% BSA. The blots were then washed five times in PBS-Tween, and cut into 26 strips, each being 3 mm wide. For detection of specific IgE antibodies, the test sera, previously adjusted to a RAST titer of 1 : 128, were further diluted 10-fold with PBS buffer (final RAST titer 1 : 12.8), and 1 ml of each diluted serum was incubated with strips overnight in the special strip incubation tubes with 1 ml of

the test sera, washed again, and incubated for 4 h with 0.2 μ Ci of ^{125} I-labeled antihuman IgE obtained from Phadebas RAST kits (Pharmacia). The strips were washed, dried, and autoradiographed with a Fujix BAS2000 Imaging Analyzer (Fuji Photo Film, Tokyo, Japan).

For IgG immunoblot analysis, the test sera previously adjusted to have the same ELISA level as 250 EU/ml were diluted 30 times with dilution buffer. The strips were put into tubes containing 1 ml of the diluted serum, and incubated overnight at room temperature. After washing five times with washing buffer, the strips were further incubated with HRP-conjugated goat polyclonal antihuman IgG (Cappel) diluted to 1:1000 with dilution buffer for 3 h at room temperature, washed again, substrated with diaminobenzidine tetrahydrochloride (DAB) (Wako, Japan), and developed for 20 min. The reaction was stopped by washing in distilled water.

For IgG4 immunoblot analysis, the same sera as used in the IgG immunoblot analysis were diluted to 1:5 with dilution buffer, and incubated with the strips overnight at room temperature. After washing, the strips were incubated with HRP-conjugated mouse monoclonal antihuman IgG4 (Binding Site, Birmingham, UK) diluted to 1:500 with dilution buffer for 3 h at room temperature. Next, the strips were substrated with DAB and developed.

Quantification of band intensity

The intensity of IgE-, IgG-, and IgG4-binding bands was graded as 0 to 11, referring to the sensitivity control gray scale for x-ray film (Wejtec Inc., Iuyland, PA, USA). When the intensity was grade 2 or more, the reaction was defined as positive. Two independent laboratory investigators interpreted the immunoblot analysis in a blind fashion for each patient with this intensity-rating scale as a reference.

Results

IgE reactivity to Df

IgE-binding reactions to Df body extract were compared by immunoblotting among three groups of mite-sensitive asthmatic children of different ages. The immunoblot profiles of groups I, II, and III sera are illustrated in Fig. 1. As determined visually, the sera from group I subjects aged 0–4 years preferentially bound to low-molecular-weight components of less than 30 kDa, especially to the 15-kDa component. The number and strength of IgE-binding bands to Df, especially to the 15-, 25-,

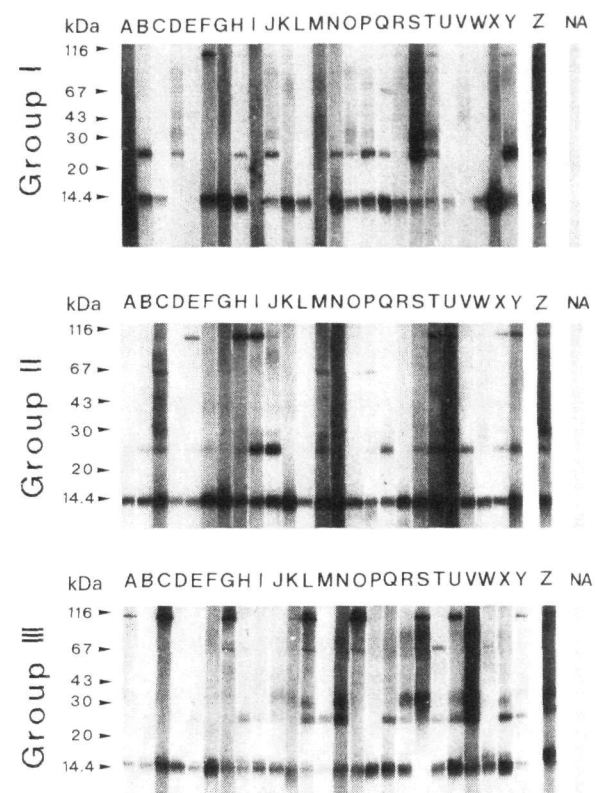


Fig. 1. Comparison of IgE binding of individual sera to Df body extracts among three groups of mite-sensitive asthmatic children: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years. Lanes A–Y) 25 individual sera from Df-sensitive asthmatic children; lane Z) positive control serum pooled from three Df-sensitive asthmatic patients. NA: negative control serum from nonallergic subject.

and 110-kDa components, increased gradually in group II subjects aged 5–9 years. The IgE reactivities to high-molecular-weight components of more than 30 kDa increased remarkably in group III subjects aged 10–14.

The Df antigen presented at least 10 distinct IgE-binding bands with molecular weights of 12, 15, 25, 30, 40, 48, 59, 67, 80, and 110 kDa. Of these IgE-binding components, those of 15 and 25 kDa were bound by more than 50% of sera in both young and older children, and therefore were considered to be the major allergenic components.

IgG reactivity to Df

IgG-binding reactions to Df were examined with the same panel of sera. The immunoblot profiles of groups I, II, and III are shown in Fig. 2. In group I subjects aged 0–4 years, IgG antibody bound strongly to the 110-kDa component, and less strongly to the 30-kDa component. Although IgG bound to high-molecular components of 67 and 95 kDa and low-molecular components of 15 kDa, most binding reactivities were faint. The pattern of IgG reactivity to Df components did not differ considerably among groups I, II, and III, except that the number and intensity of the IgG-binding reactions to 30- and 110-kDa components increased remarkably in group III subjects.

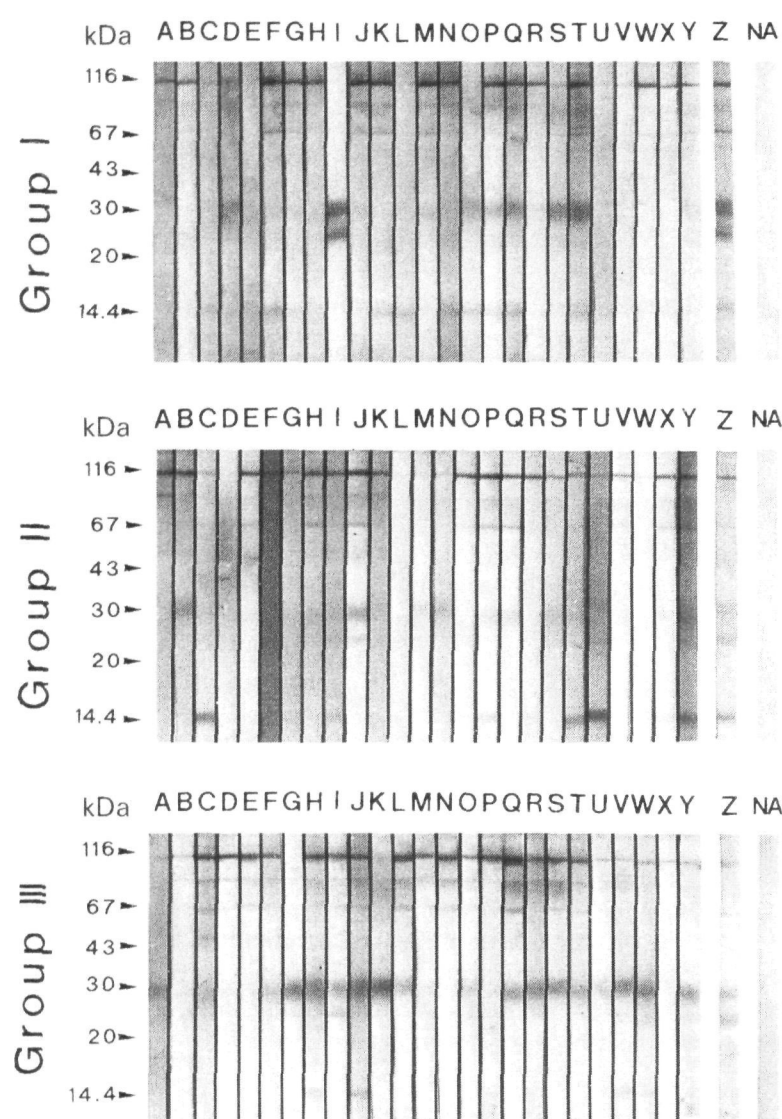


Fig. 2. Comparison of IgG binding of individual sera to Df body extracts among three groups of mite-sensitive asthmatic children. Same panel of sera was used in both IgE and IgG immunoblot analysis: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years. Lanes A–Y) 25 individual sera from Df-sensitive asthmatic children; lane Z) positive control serum pooled from three Df-sensitive asthmatic patients. NA: negative control serum from nonallergic subject.

The Df antigen demonstrated at least 11 IgG-binding bands, with molecular weights of 12, 15, 25, 30, 40, 48, 59, 67, 80, 95, and 110 kDa. Of these components, the 30- and 110-kDa bands were found to be the major IgG-binding components.

IgG4 reactivity to Df

IgG4-binding reactions to Df were also examined in the same sera as used in the IgG immunoblot analysis. The immunoblot profiles of groups I, II, and III are shown in Fig. 3. In group I, IgG4 bound mainly to low-molecular components, especially a 15-kDa protein. The number of IgG4-binding bands to Df increased in groups II and III, and IgG4 showed considerable reactivity to the 110-kDa protein.

The Df antigen demonstrated four IgG4-binding bands, with molecular weights of 15, 25, 30, and 110

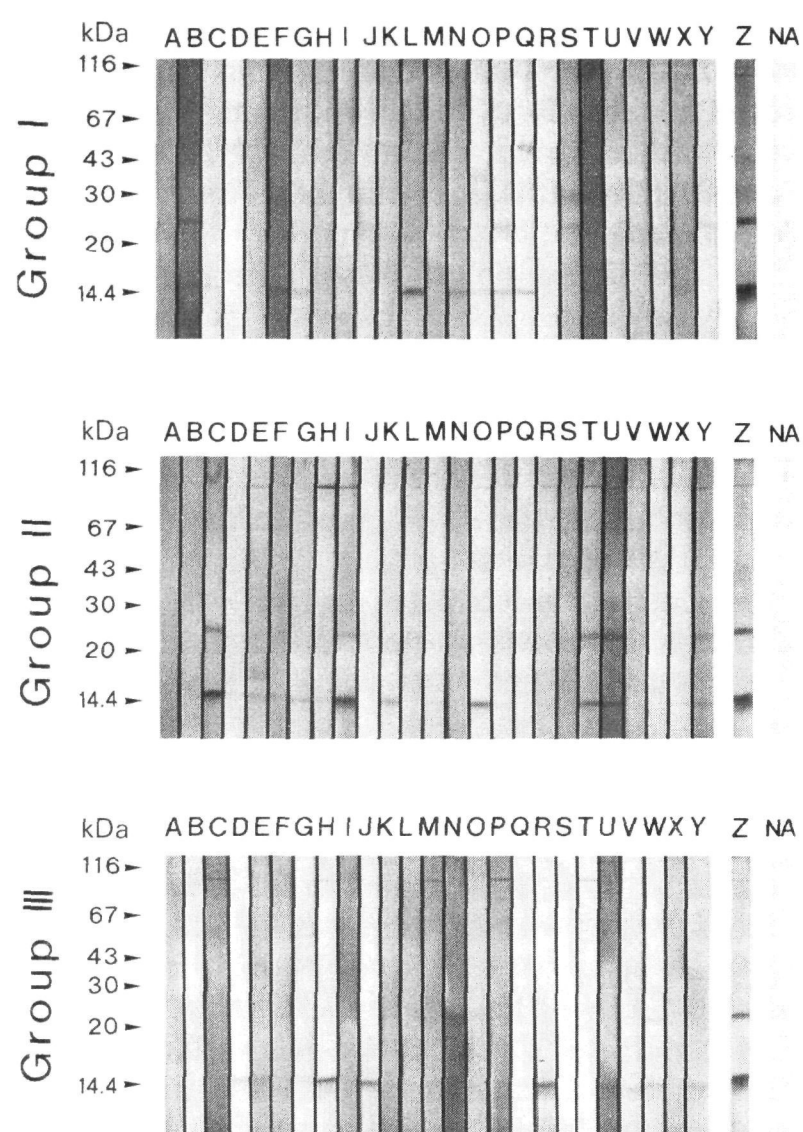


Fig. 3. Comparison of IgG4 binding of individual sera to Df body extracts among three groups of mite-sensitive asthmatic children. Same panel of sera was used in both IgE and IgG immunoblot analysis: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years. Lanes A–Y) 25 individual sera from Df-sensitive asthmatic children; Z) positive control serum pooled from three Df-sensitive asthmatic patients. NA: negative control serum from nonallergic subject.

kDa. Of these components, the 15-kDa bands were found to be a dominant IgG4-binding component.

Comparison of IgE-, IgG- and IgG4-binding reactions to Df

The frequencies of positive IgE-, IgG-, and IgG4-binding reactions in children of groups I, II, and III are summarized in Fig. 4. In the group I subjects aged 0–4, the positive IgE-binding reactions to 15- and 25-kDa components were found in 76% and 44% of sera, respectively. Those to other components were generally low in frequency. The positive IgG-binding reactions to 15- and 25-kDa components were found in 44% and 24%, and those to 30- and 110-kDa components in 48% and 88%, respectively. The positive IgG4-binding reactions to 15- and 25-kDa components were found in 48% and 24%, respectively, and those to the 30-kDa component were found in 8% of sera.

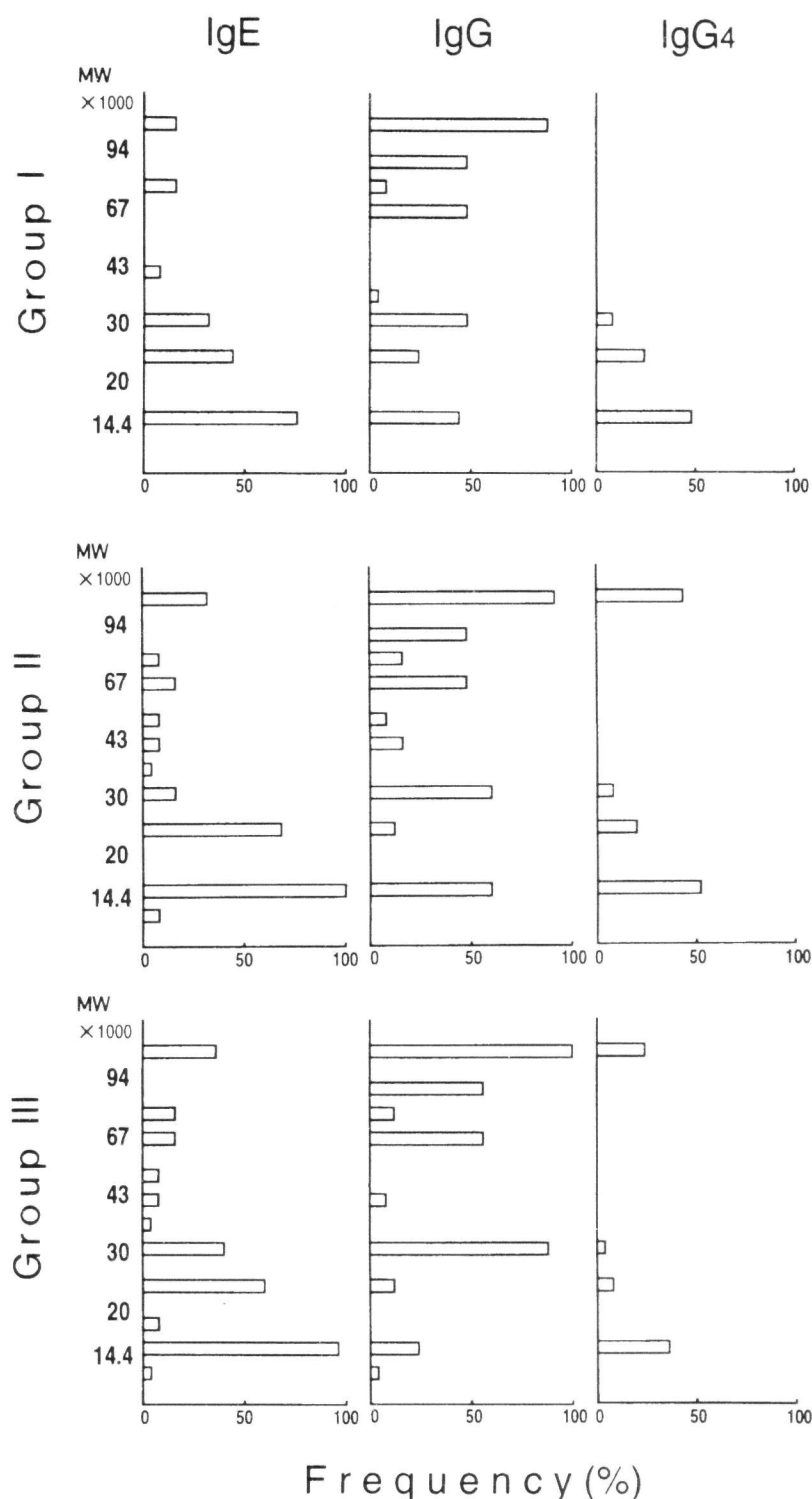


Fig. 4. Frequencies of IgE, IgG, and IgG4 binding to Df body extracts in three groups of asthmatic children: group I aged 0–4 years, group II aged 5–9 years, and group III aged 10–14 years.

In the group II subjects aged 5–9 years, positive IgE-binding reactions to 15- and 25-kDa components were found in 100% and 68%, and those to 30- and 110-kDa in 16% and 32%, respectively. Positive IgG-binding reactions to 15-, 25-, 30-, and 110-kDa components were found in 60%, 12%, 60%, and 92%, and the positive IgG4 reactions to these components in 52%, 20%, 8%, and 44%, respectively.

In the group III subjects aged 10–14 years, positive IgE-binding reactions to 15- and 25-kDa components were found in 96% and 60%, and to 30- and 110-kDa components in 40% and 36%, respectively. Positive IgG-binding reactions to 15-,

25-, 30- and 110-kDa components were found in 24%, 12%, 88%, and 100%, respectively. The positive IgG4 reactions to these components were found in 36%, 8%, 4%, and 24%, respectively.

Discussion

The IgE immunoblot study with Df body extract demonstrated at least 10 distinct IgE-binding bands, with apparent molecular weights of 12, 15, 25, 30, 40, 48, 59, 67, 80, and 110 kDa. Of these bands, the 15- and 25-kDa bands were found to be major IgE-binding components, to which more than 50% of HDM-sensitive sera reacted. Our previous study has shown that the 25- and 15-kDa proteins correspond to the group 1 and group 2 allergens of Df, respectively (24). On the other hand, the Df extract showed 11 IgG-binding bands, with molecular weights of 12, 15, 25, 30, 40, 48, 59, 67, 80, 95, and 110 kDa. Of these, the 30- and 110-kDa bands were found to be major IgG-binding components.

Tovey & Baldo (12) have reported that a total of 26 IgE-binding bands can be demonstrated by immunoblotting of the *D. pteronyssinus* body extract, including the five most strongly binding components with molecular weights of 15, 25, 28–32, 53, and 95 kDa. Nakanishi & Shimokata (10) have shown that 29 IgE- and 26 IgG-binding components can be identified in the Df body extract by immunoblotting, and that both IgE and IgG antibodies bind preferentially to 13–16-, 25–30- and 43–60-kDa components. In this study, the pattern of IgE and IgG reactivities to these allergens were similar to these previous observations, except that 95% of IgG antibody of sera tested bound to the 110-kDa component of Df body extracts. Recently, however, Lin et al. (25) have shown that the 110-kDa component is a potent IgE-binding protein, like the 15- and 25-kDa components, and is capable of reacting with 50% of mite-sensitive sera. Our results indicate that the 110-kDa component is also a unique antigen capable of inducing IgG response in man.

It remains possible that the low incidence of IgE-binding reaction to the 110-kDa component is due to the interference of IgG antibody with the 110-kDa protein. However, our preliminary study has shown that the preincubation of the blotted strips with 250 EU/ml of IgG antibody preparation, purified by protein A-Sepharose, fails to inhibit IgE binding to this antigen. Therefore, it seems unlikely that Df-specific IgG exerts any influence on the IgE immunoblot (data not shown).

It has been shown that more than 80% of HDM-sensitive asthmatics demonstrate IgE antibodies

against both Group 1 and Group 2 allergens of Df on RAST (7, 9, 13). In this study, the prevalence of IgE antibody against Der f 1 was less than 70% in school-aged children. Since the Df extracts used contained almost equal amounts of Der f 1 and Der f 2 allergens, it is unlikely that the difference in the IgE reactivity to Df components with 15-kDa dominance is attributable to a difference in the loading doses of the two allergens on electrophoresis. On the other hand, it has been shown that Group 1 allergens of Df are more easily denatured than Group 2 allergens by heating, acid treatment, reduction, and alkylation (26, 27). In this study, although Df allergens were neither heated nor acidified, and were electrophoresed under nonreducing conditions, it is still possible that SDS-PAGE or electric transfer degraded Group 1 allergen of Df.

It has been reported that continuous low-dose exposure to an antigen such as HDM can elicit the IgE response in susceptible patients (28), whereas high-dose exposure, as in immunotherapy of atopic patients, may induce the elevation of specific IgG (29, 30), suggesting that both the dose of antigen and the route of immunization are crucial for the development of IgE responses. However, our asthmatic patients had never had immunotherapy with a high-molecular-weight fraction of Df such as the 110-kDa protein. Therefore, our results, showing that IgE predominates in response to 15- and 25-kDa proteins and IgG to 30- and 110-kDa proteins, seem to suggest that the physicochemical nature of the antigenic proteins is an important factor which distinguishes IgE responses from IgG responses to allergen.

An increased level of IgG4 antibody is often associated with atopic disease (17, 18). Production of IgE and of IgG4 has been shown to be regulated by the same cytokine, interleukin-4 (31). Recent studies have also demonstrated that IgE and IgG4 antibodies directed toward the same antigens occur in parallel in patients allergic to pollen and mosquito saliva (32, 33). Our results are not consistent with these studies, since the 15-kDa protein frequently reacted with both IgE and IgG4 antibodies, but the 25-kDa protein, which is another major IgE-binding component, reacted less frequently with IgG4 antibodies.

In this study, younger children aged under 4 years had IgE antibodies to a smaller range of Df components than older age groups, while they had IgG antibodies to a wide range of Df components, and thereafter the IgG reactivities were not markedly different. This indicates that IgE antibody responses are more likely to be influenced by age than are IgG responses. It has been demonstrated that IgE antibody production does not normally

occur in atopic children until after 2 years of age, and then increases gradually from 2 to 8 years (16, 34). On the other hand, natural exposure to a common inhalant antigen has been shown to be able to evoke an HDM-specific IgG response in an unselected group of babies during the first year of life (35). Therefore, it is likely that the IgG synthetic system matures more rapidly than the IgE system.

References

1. Aas K. Bronchial provocation tests in asthma. *Arch Dis Child* 1970;45:221-8.
2. Berg T, Bennich H, Johansson SG. *In vitro* diagnosis of atopic allergy. I. A comparison between provocation tests and the radioallergosorbent test. *Int Arch Allergy Appl Immunol* 1971;40:770-8.
3. McNichol KN, Williams HE. Spectrum of asthma in children. II. Allergic components. *BMJ* 1973;4:12-16.
4. Stenius B, Wide L. Reaginic antibody (IgE), skin, and provocation tests to *Dermatophagoides culinae* and house dust in respiratory allergy. *Lancet* 1969;2:455-8.
5. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980;125:587-92.
6. Haida M, Okudaira H, Ogita T, et al. Allergens of the house dust mite *Dermatophagoides farinae*: immunochemical studies of four allergenic fractions. *J Allergy Clin Immunol* 1985;75:686-92.
7. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83:1055-67.
8. Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House dust mite-derived amylase: allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991;87:1035-42.
9. Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985;76:753-61.
10. Nakanishi K, Shimokata K. Immunoblot analysis of *Dermatophagoides farinae* antigen. *Ann Allergy* 1990;64:219-23.
11. Stewart GA. Isolation and characterization of the allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 1982;69:224-30.
12. Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;79:93-102.
13. Yasueda H, Mita H, Yui Y, Shida T. Isolation and characterization of two allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1986;81:214-23.
14. Rizzo MC, Arruda LK, Chapman MD, et al. IgG and IgE antibody responses to dust mite allergens among children with asthma in Brazil. *Ann Allergy* 1993;71:152-8.
15. Chapman MD, Rowntree S, Mitchell EB, Di Prisco FM, Platts-Mills TAE. Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. *J Allergy Clin Immunol* 1983;72:27-33.
16. Ogura Y, Ogura H, Morita H, Kurashige T. Specific IgG, IgM, IgA and IgE antibodies to *Dermatophagoides farinae* in asthmatic and healthy children – their changes with age. *Jpn J Allergol* 1988;37:1057-64.

17. Gwynn CM, Smith JM, Leon GL, Stanworth DR. Role of IgG4 subclass in childhood allergy. *Lancet* 1978;1:910-11.
18. Shakib F, McLaughlan P, Stanworth DR, Smith E, Fairburn E. Elevated serum IgE and IgG4 in patients with atopic dermatitis. *Br J Dermatol* 1977;97:59-63.
19. Calkhoven PG, Aalbers M, Schilte PP, et al. Newly generated IgE antibodies to *Dermatophagoides pteronyssinus* in children are directed against components distinct from *Der p* I and *Der p* II. *J Allergy Clin Immunol* 1991;88:265-71.
20. American Thoracic Society. Definition and classification of chronic bronchitis, asthma and pulmonary emphysema. *Am Rev Respir Dis* 1962;85:419-26.
21. Shibasaki M, Tajima K, Morikawa A, Mitsuhashi M, Sumazaki R, Tokuyama K. Relation between frequency of asthma and IgE antibody levels against *Dermatophagoides farinae* and total serum IgE levels in schoolchildren. *J Allergy Clin Immunol* 1988;82:86-94.
22. Kemeny DM, Urbanek R, Richards D, Greenall C. Development of a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for detection of human IgG subclass antibodies. *J Immunol Methods* 1987;96:47-56.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
24. Shibasaki M, Isoyama S, Takita H. Influence of age on IgE responsiveness to *Dermatophagoides farinae*: an immunoblot study. *Int Arch Allergy Immunol* 1994;103:53-8.
25. Lin KL, Wang SY, Hsieh KH. Analysis of house dust mite-specific IgE, IgG4, and IgG antibodies during immunotherapy in asthmatic children. *Ann Allergy* 1991;67:63-9.
26. Ford SA, Tovey ER, Baldo BA. Effect of reduction and heat on the detection of house dust mite (*Dermatophagoides pteronyssinus*) allergen *Der p* I by protein blotting. *Int Arch Allergy Appl Immunol* 1989;89:318-20.
27. Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD. Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. *J Immunol* 1990;144:1353-60.
28. Arruda LK, Rizzo MC, Chapman MD, et al. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433-9.
29. McHugh SM, Lavelle B, Kemeny DM, Patel S, Ewan PW. A placebo-controlled trial of immunotherapy with two extracts of *Dermatophagoides pteronyssinus* in allergic rhinitis, comparing clinical outcome with changes in antigen-specific IgE, IgG, and IgG subclasses. *J Allergy Clin Immunol* 1990;86:521-31.
30. Piazza I, Bizzaro N. Humoral response to subcutaneous, oral, and nasal immunotherapy for allergic rhinitis due to *Dermatophagoides pteronyssinus*. *Ann Allergy* 1993;71:461-9.
31. Nusslein HG, Spiegelberg HL. Interleukin-4 induces both IgG4 and IgE secretion by peripheral blood B cells. *J Clin Lab Anal* 1990;4:414-19.
32. Brummer KH, Lappalainen P, Reunala T, Palosuo T. Detection of mosquito saliva-specific IgE and IgG4 antibodies by immunoblotting. *J Allergy Clin Immunol* 1994;93:551-5.
33. Jeep S, Meysel U, Kunkel G. IgE, IgG, IgG1 and IgG4 patterns in yellow jacket allergic patients during immunotherapy with a venom depot extract. *Clin Exp Allergy* 1992;22:297-302.
34. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727-35.
35. Mariani F, Price JF, Kemeny DM. The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of the immune system following natural exposure. *Clin Exp Allergy* 1992;22:29-33.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.