Allergology International (1996) **45**: 195–198

Original Article

Anti-*Dermatophagoides farinae* type I and II IgE antibodies in allergic rhinitis

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ABSTRACT

Sera from 27 patients with mite-sensitive allergic rhinitis, without atopic dermatitis and bronchial asthma, were examined for anti-*Der f* I and anti-*Der f* II IgE antibody contents by enzyme-linked immunosorbent assay (ELISA). Anti-*Der f* I and anti-*Der f* II IgE antibody levels were 14.78 ± 1.34 and 32.68 ± 0.88 ng/mL (mean ± SEM), respectively. The anti-*Der f* II IgE antibody was predominant over the anti-*Der f* I IgE antibody in these patients.

In comparison with the results of a previous study the present study indicates that the ratio between serum anti-*Der f* I and II IgE antibodies in patients with allergic rhinitis indicated the same pattern as in that of patients with bronchial asthma, while the inverse was the case in patients with atopic dermatitis.

These results indicate that immunological features and major allergen molecules could be different in different atopic diseases. At present it is not clear where this difference comes from, but the route of immunological sensitization (via respiratory tract *vs* via skin) might result in the difference.

Key words: *Der f* I, *Der f* II, IgE antibody, major allergen, mite.

INTRODUCTION

Allergy to house dust is an important cause for atopic diseases such as allergic rhinitis, bronchial asthma and atopic dermatitis in many countries. It has been well established that two different species of mite, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are the major sources of allergen in house dust.[1–4]

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Received 16 February 1996. Accepted for publication 17 September 1996.

The purification of mite allergen has been attempted by many investigators. At present, there is some general consent that the major allergens purified from both species may be grouped into two main groups of mite allergen, one group with a molecular weight of about 25 kDa and another with a molecular weight of about 15 kDa. According to the new allergen nomenclature system,[5] the major allergens for the house dust mites are to be designated as *Der p* I and *Der f* I for the large molecular-weight allergens, while the low molecular-weight allergens are called *Der p* II and *Der f* II, all isolated from either of the species.

Anti-mite IgE antibodies have been routinely measured in clinical practice for the management of mite-sensitive atopic patients, however the allergen preparations used for the assay were crude extracts of mite body or mite culture. This limitation prevented detailed analysis of anti-mite IgE antibodies in different atopic diseases. Recent progress in mite allergen research enabled us to analyze the anti-mite IgE antibodies directed to different allergen molecules in different atopic diseases. However, there have been few reports on anti-*Der f* I and anti-*Der f* II IgE antibodies in patients with allergic rhinitis. In this study, anti-*Der f* I and anti-*Der f* II IgE antibody contents were examined in patients with allergic rhinitis.

METHODS

Subjects

Twenty-seven patients with perennial mite-sensitive allergic rhinitis, without bronchial asthma and atopic dermatitis, were subjected to the study. The group was composed of 18 males and 9 females aged between 15 and 55 years (mean age 31.1).

Preparation of crude mite extract of *D. farinae*

Dermatophagoides farinae was defatted with ether, 20 mL of phosphate-buffered saline (2 mmol/L, pH 7.4) was added to 1 g of defatted mite and the mixture was rotated for 48 h at 4°C.

Preparation of *Der f* I and II

Isolation of *Der f* I was attempted by a method described previously[6] and isolation of *Der f* II by the method reported by Yasueda *et al.*,[7] with slight modifications. The columna such as CM-cellulose (CM-52, Whatman, Springfield Mill, Kent, UK) and Sephadex G-75 (Pharmacia, Uppsala, Sweden) were used when the isolation of *Der f* II was carried out. These were different from those of Yasueda *et al.*

Enzyme-linked immunosorbent assay for anti-*Der f* I and II IgE antibodies

For anti-*Der f* I and II IgE antibody measurements, micro-titer plates (Immulon 2; Dynatech Laboratories, Chantilly, VA, USA) were incubated with 100 μL/well of 10 μg/mL *Der f* I or 50 μg/mL of *Der f* II in 0.1 mol/L carbonate buffer (pH 9.6) overnight in the cold in a humidified box. After three washes with phosphate-buffered saline/Tween, protein-binding sites of the wells were blocked with 100 μL of Block Ace (Yukijirusi, Tokyo, Japan). Diluted 100 μL serum samples (1/2 dilution for IgE) were added to the wells. After incubation for 1 h at room temperature, the serum samples were removed and washed three times. Then 100 μL of 1/3000-diluted biotin-labeled affinity-purified goat antihuman IgE fragment (Tago, Burlingame, CA, USA) was added to each well and incubated at room temperature for 1 h. After three washes, 100 μL of 1/4000-diluted peroxidase-avidin D (Vector Laboratories, Burlingame, CA, USA) was added to each well and incubated further for 1 h. The wells were then washed three times with phosphate-buffered saline and 100 μL of o-phenylenediamine dihydrochlorine (Sigma Chemicals, St Louis, MO, USA) substrate solution was added, followed by the addition of 100 μL of 4 N sulfuric acid to terminate the reaction. Absorbance at 490 nm was read by an automatic ELISA reader (model MTP-32; Corona Electric, Ibaragi, Japan). A preparation of atopic serum which contained 526 ng/mL of anti-*Der f* I and 139 ng/mL of anti-*Der f* II IgE antibodies was employed as a standard. Absolute amount of IgE antibody was calculated on the intensity of coloring reaction by plotting on the standard curve prepared using a known amount of PS IgE myeloma protein.

Statistical analysis

Data were analyzed by applying paired sample *t*-test. *P* values <0.05 were considered statistically significant.

Results

Anti-*Der f* I and anti-*Der f* II IgE antibody levels in the sera of the patients with allergic rhinitis are shown in Fig. 1. Anti-*Der f* I and II IgE antibody levels were 14.78 ± 1.34 ng/mL, 32.68 ± 0.88 ng/mL (mean ± SEM), respectively. The ratio between serum anti-*Der f* I and II IgE antbodies (anti-*Der f* I:II ratio) was 0.45 ± 0.66 (Fig. 2). Anti-*Der f* II IgE antibody level was significantly higher than anti-*Der f* I IgE antibody level ($P < 0.01$).

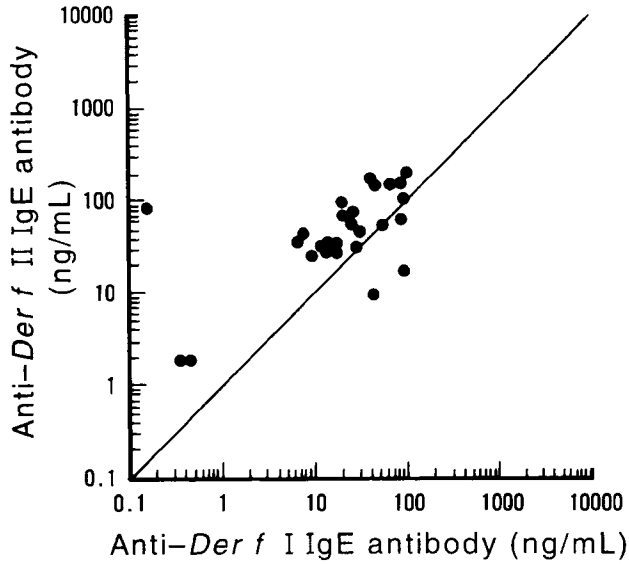

Fig. 1 Anti-*Der f* I and II IgE antibody levels in the sera of patients with allergic rhinitis.

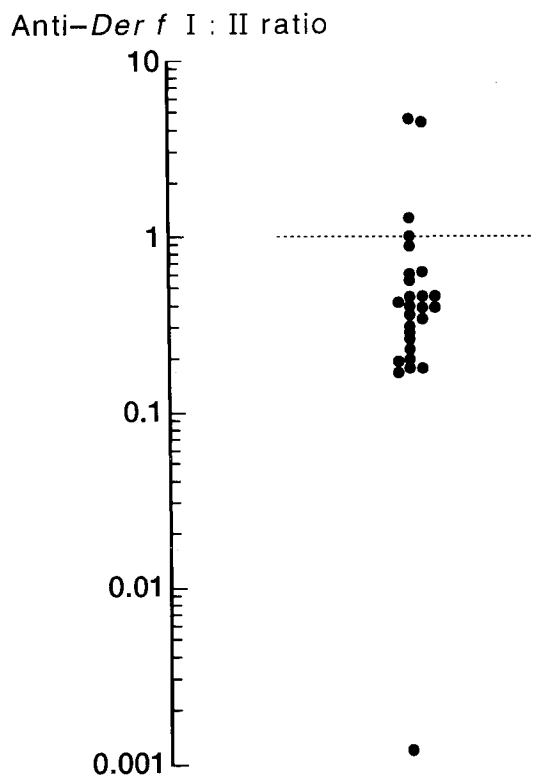

Fig. 2 The ratio between serum anti-*Der f* I and II IgE antibodies in the patients with allergic rhinitis.

Discussion

Since the discovery of the house dust allergen for atopic diseases around 1920, many investigators have sought its origin.

In 1967, Voorhorst *et al.* found that the house dust allergen content assessed by skin tests correlated well with the number of *Dermatophagoides* mites in house dust.[2] In 1968, Miyamoto *et al.* observed that IgE antibody titers against house dust and *Dermatophagoides* mite correlated very well.[3] Based on such findings, the importance of mite allergen in house dust allergy has been established gradually.

Subsequently, many investigators attempted the purification of mite allergen, but it took more than 12 years until Chapman and Platts-Mills reported purified antigen P_1 in 1980.[8] The difficulty of mite allergen research mainly derives from the multiplicity of mite allergens. Recently, several groups of studies have described the characterization and the method of isolation of the major allergen from *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Table 1).[8–18]

It was known that mite fecal particles as well as mite bodies were important sources of mite allergen, and it was speculated that a digestive enzyme secreted from the gastrointestinal tract may be one of the mite allergens. Chua *et al.* carried out sequence analyses of the *Der p* I cDNA clone and demonstrated the similarity of their amino acid sequences between *Der p* I and a group of cysteine proteases such as papain and cathepsin B.[19]

Ando *et al.* tried to detect, purify and characterize a cysteine protease in the crude extract of *D. farinae*.[6] According to their findings, comparison of the purified cysteine protease extract with purified *Der f* I led them to conclude that *Der f* I is actually a cysteine protease itself. On the other hand, recent investigations revealed that *Der f* II allergen is a protein derived from mite body composed of 129 amino acids containing six cysteine residues.[20] The predicted *Der f* II amino acid sequence has no potential N-glycosylation sites.

The detection of these major allergens enabled us to analyze the anti-mite IgE antibodies directed to different allergen molecules in different atopic diseases. In a previous study, sera from 23 patients with mite-sensitive bronchial asthma without atopic dermatitis and from 19 patients with atopic dermatitis without bronchial asthma, were examined for anti-*Der f* I and anti-*Der f* II IgE antibody contents.[21] The ratio between serum anti-*Der f* I and II IgE antibodies (anti-*Der f* I : II) was significantly higher ($P < 0.05$) in atopic dermatitis than in bronchial asthma patients. The mean ($\pm$ SEM) I : II ratio (85.17 ± 2.01) was significantly higher than 1.0 in atopic dermatitis patients, while it was 0.93 $\pm$ 0.16 in bronchial asthma patients. The results of this study of patients with allergic rhinitis without atopic dermatitis and bronchial asthma showed that the anti-*Der f* II IgE antibody was predominant over the anti-*Der f* I IgE antibody. That is to say, the data in this study on the ratio between serum anti-*Der f* I and II IgE antibodies in patients with allergic rhinitis indicated the same pattern as in that of patients with bronchial asthma, while the inverse was the case in patients with atopic dermatitis as reported in a previous study.[21]

In comparison with the results of the previous study, the present study indicates that the anti-*Der f* I : II ratio is lower in the case of patients with allergic rhinitis (0.45 ± 0.66) than in the case of patients with bronchial asthma (0.93 ± 0.16). These results indicate that immunological features and major allergen molecules could be different in different atopic diseases.

Thompson *et al.* also measured the IgE antibodies to two major allergens from *D. pteronyssinus*, *Der p* I and *Der p* II, among children with bronchial asthma, atopic dermatitis and allergic rhinitis, and reported that the anti-*Der p* I IgE antibody is predominant among the bronchial asthma group and the anti-*Der p* II IgE antibody is predominant in the atopic dermatitis and allergic rhinitis groups.[22,23]

The results of bronchial asthma and atopic dermatitis were contrary to the results of our previous study.[21] However, the result on allergic rhinitis was consistent with the result of the present study. The reason for the inconsistency in results of the former study is not clear, however, it could be related to the fact that the subjects Thompson *et al.*[22,23] studied were children;

Table 1. Major allergens of mite

WHO nomenclature	Original name	Molecular weight (Da)	Author	
Dermatophagoides pteronyssinus				
Der p I	P_1	25 000	Chapman *et al.*	1980
	Dpt 12	27 000	Stewart	1982
	Dp 42	25 000–30 000	Lind	1985
	DP 1	25 000	Yasueda *et al.*	1989
Der p II	Dp X	18 000–20 000	Lind	1985
	DP 2	15 000	Yasueda *et al.*	1989
Dermatophagoides farinae				
Der f I	Ag 11	28 000	Dandeu *et al.*	1982
	Df 6	28 000	Lind	1986
	DF 1	25 000	Yasueda *et al.*	1986
	Der f I	24 000	Heymann *et al.*	1986
	Me 2	27 000	Yamashita *et al.*	1989
Der f II	Me 1	17 000	Haida *et al.*	1985
	Ag 19/20 II a	14 500	Holck *et al.*	1986
	DF 2	15 000	Yasueda *et al.*	1986

the species of mite was *D. pteronyssinus* instead of *D. farinae*; the purification method of *Der* I and II was not the same as the method of our study; and the methods of measurement of IgE antibody were western blotting method and RAST method, which were different from our ELISA method. In the reports of Thompson *et al.*, only the pediatric cases were studied. Therefore, if we consider the matter of allergy march, the differences in IgE antibody levels between the group I and group II allergens may be attributed to aging. In order to clarify the inconsistency, comparative studies on IgE antibody levels between *Der p* and *Der f* may be necessary using the same subjects and method of measurement.

It is also not clear why the dominancy of IgE antibody to *Der f* I and II differs among the disease group. According to the report of Sakaguchi *et al.*, it could not be due to the difference in the amount of antigen of *Der f* I and II in the house dust.[24] Therefore, the differences in anti-*Der f* I and II IgE antibody levels may be related to the strength in immunogenicity and antigenicity of the *Der f* I and II allergens when the antigen is inhaled as in the case of the bronchial asthma, in the same manner as allergic rhinitis.

On the other hand, the defence wall of the skin is more rigid than the mucous membrane of the respiratory tract against the invasion of foreign substances. Although invasion of protein antigen is usually difficult, the invasion of mite antigen in the skin lesion was proved in the patients with atopic dermatitis. Therefore, the route through the skin could not be negated.[25] Further, another reason for the higher anti-*Der f* I IgE antibody levels compared to the levels of *Der f* II may be the ease with which invasion by *Der f* I of the skin occurs due to its protease activity, but detailed explanation is not yet clarified. It is unclear whether the local sites where antigen invades relate to the difference of interaction between the antigen and B cell.

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