

## Measurement of IgE and IgG Subclass Antibodies to Whole Body Antigen and two Major Allergens (*Der fI* & *Der fII*) of *Dermatophagoides Farinae* in Normal Subjects and Asthmatics

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House dust mites have been known as the most important allergen in respiratory allergic diseases. Among several mite allergens, group I and group II antigens were recognized as major allergens. We measured specific IgE and IgG subclass antibodies against whole body antigen (WBA) and two major allergens of *Dermatophagoides farinae* (*Der fI* and *Der fII*) in sera from 66 adults with asthma (asthma group) and 34 normal subjects (healthy group) by ELISA. The mean O.D. values of WBA-specific IgE and IgG subclass antibodies in 100 studied sera were significantly higher than those of the two major allergens ( $p < 0.001$ ) and the level of *Der fII*- IgG1, IgG4 and IgE were higher than those of *Der fI* but IgG2 of *Der fI* was higher than that of *Der fII* ( $p < 0.001$ ). The level of IgG4 of WBA were significantly higher in the atopic group than in the nonatopic group ( $1280 \pm 0.634$  vs.  $0.8290 \pm 0.388$ ,  $p < 0.001$ ), but the WBA- IgG1, IgG2, IgG3 were not different between the two groups. Among IgG subclass antibodies of *Der fI*, IgG2 was significantly higher in the nonatopic group than in the atopic group ( $17770 \pm 0.255$  vs.  $1.636 \pm 0.390$ ,  $p < 0.05$ ) but there were no differences in IgG1, IgG3, and IgG4. Among IgG subclass antibodies of *Der fII*, IgG2 ( $1534 \pm 0.380$  vs.  $1.3010 \pm .431$ ,  $p < 0.05$ ) and IgG4 ( $1.09650 \pm .567$  vs.  $0.708 \pm 0.146$ ,  $p < 0.001$ ) were significantly higher in the atopic group than in the nonatopic group. IgE antibodies to WBA, *Der fI* and *Der fII* were significantly higher in the atopic group ( $p < 0.001$ ). There were significant correlations between the levels of IgE and IgG4 of WBA ( $r = 0.60$ ), *Der fI* ( $r = 0.33$ ) and *Der fII* ( $r = 0.72$ ). Even though there were no differences in the levels of allergen specific IgE and IgG subclass antibodies between nonatopic healthy and nonatopic asthmatic groups, the number of sera with prominent level of IgG2 of WBA were more common in the nonatopic asthmatic group (69% in nonatopic asthma group vs. 28% in nonatopic healthy group,  $X^2$ -test,  $p < 0.01$ ). When compared with the atopic healthy group, the levels of IgG4 of WBA, IgE of *Der fI* and IgG1 of *Der fII* were 5.23 times, 9.26 times and 271 times higher in the atopic asthma group respectively and the sera with prominent levels of IgG4 of WBA (74%) and IgE of *Der fI* (78%) were more common in the atopic asthma group (38% and 19% in atopic healthy group respectively,  $X^2$ -test,  $p < 0.01$ ). With these results, the authors conclude that IgE and IgG subclass antibodies responses to house dust mite antigens in the atopic asthma group may be different from those of the atopic healthy group and that nonatopic asthma group may also have different immune responses of IgG subclasses to house dust mite antigens than the nonatopic healthy group.

**Key Words:** *Dermatophagoides farinae*, whole body antigen, *Der fI*, *Der fII*, IgG subclasses, IgE

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Since 1960s house dust mites have been known as the most common allergen causing asthma and other allergic diseases (Voorhost *et al.* 1969; Miyamoto *et al.* 1968). Among the family Pyroglyphidae, *Dermatophagoides farinae* is the dominant species in Korea (Cho and Houh 1977; Cho 1991; Lee and Cho 1984). More than 50 protein bands could be identified in crude mite extracts by SDS-PAGE (Krilis *et al.* 1984; Alian *et al.* 1987), and about thirty IgE-binding components in *Dermatophagoides* species have been identified by cross radioimmuno-electrophoresis and immunoblottings (Tovey and Baldo 1987, 1985; Lind and Lowenstein 1983). Two groups of mite allergens were recognized as the major allergens because 80~90% of mite allergic patients had measurable IgE antibody in their sera (Platts-Mills and Chapman 1987, Heyman *et al.* 1989) and have been well characterized. Group I allergen (*Der fI*) with molecular weights of 24 kDa was excreted by mites into fecal material. Group II allergens (*Der fII*) with molecular weights of 15kDa was a body component of mites. Both major allergens have been purified and their amino acid sequences were identified (Baldo *et al.* 1989; Heymen *et al.* 1989).

The immune responses to mite allergens have been extensively studied in allergic diseases and IgE is regarded as the most important immunoglobulin, but other immunoglobulins such as IgG and IgG4 have recently been reported to play some roles in allergic diseases. In this study, we measured the level of specific IgE and IgG subclass antibodies to purified two major allergens and whole body antigen of *D. farinae* in the sera of asthmatics and healthy subjects and tried to analyze differential points of IgG subclass and IgE immune responses to whole body antigen and two major allergens of *Dermatophagoides farinae* between asthmatic and healthy subjects, and between atopic and nonatopic groups.

## MATERIAL AND METHODS

### Patient sera

Sera from 66 asthmatics who visited the De-

partment of Internal Medicine in Severance Hospital of Yonsei University College of Medicine and who did not have immunotherapy were selected for the asthma group. As the control group, we used the sera of 34 healthy volunteers among medical students of the Yonsei University College of Medicine. Healthy volunteers were carried out skin prick tests with *Dermatophagoides farinae* and cat hair, and asthmatics were tested with 50 inhalant allergens (Bencard Co., England). According to the skin reactivity, we made the asthmatics and the volunteers who showed  $\geq 2+$  skin reactivity to *D. farinae* as the atopic group and the cases who showed  $< 2+$  reaction to *D. farinae* and other allergens as the nonatopic group. Therefore, we made both asthmatics and healthy volunteers into the subgroup as follows: Group 1-nonatopic healthy group (n=18), Group 2-atopic healthy group (n=16), Group 3-nonatopic asthma group (n=26), Group 4-weak atopic asthma group (n=13); asthma patients with mild skin reactivity (2+) to *D. farinae* and other allergens, Group 5-atopic asthma group (n=27); asthmatic patients with strong skin reactivity to *D. farinae* ( $\geq 3+$ ). The sera were stored at  $-20^{\circ}\text{C}$  until used.

### Allergens

**Whole body antigen (WBA) of *D. farinae*:** The purely cultured house dust mites (*D. farinae*) were defatted with ethylether and dried. 100 ml of phosphate buffered saline (PBS) was added to 1 gm of defatted house dust mite and stirred continuously for 48 hours at  $4^{\circ}\text{C}$ . Then the extract was centrifuged at 10,000 g for one hour at  $4^{\circ}\text{C}$  and the supernatant was dialyzed in a large amount of distilled water for 48 hours. The supernatant were lyophilized and stored in a freezer until its use.

**Two major allergens (*Der fI* & *Der fII*) of *D. farinae*:** *Der fI* and *Der fII* were generously donated by Dr. Okudaira, Department of Medicine and Physical therapy, University of Tokyo School of Medicine.

**The determination of IgE and IgG subclass antibodies**

Specific IgE and IgG subclasses in serum

were measured with ELISA. Wells of microtiter plate were coated overnight with allergen solutions (WBA: 2 µg/well, *Der fI* and *Der fII*: 10 ng/well) in 50 µl of 0.05M carbonate buffer (pH 9.6) at 4°C. The plates were washed once with PBS-T, incubated for 1 hour with 1% BSA/PBS-T, and then washed twice with PBS-T. Fifty microliter of patient's serum (1:10 diluted serum for IgG1-3 measurement and undiluted serum for IgG4 and IgE measurement) were put to wells of the plates and incubated for 1 hour at room temperature and the plates were washed three times with PBS-T. 50 µl of biotinylated monoclonal anti-human IgG subclass antibodies (SIGMA Co, 1:500 v/v diluted) or biotinylated polyclonal anti-human IgE antibodies (epsilon chain specific, Vector Laboratories, Inc., 30 Ingold Road, Burlingame, CA 94010) were incubated for 1 hour and washed with PBS-T 3 times. The plates were incubated with 50 µl of streptavidin-peroxidase (SIGMA Co, 1:500 v/v) for 30 min and were washed five times with PBS-T. 100 µl of ABTS [2,2-azino-di-(3ethyl-benzthiazoline sulfonic acid)] solution was put as substrate for color development [55 mg of ABTS was dissolved in 100 ml of 70mM citrate phosphate buffer (pH 4.2)]. After 5 min at room temperature the reaction was stopped by the addition of 100 µl of 2mM sodium azide. The color reaction was measured the optical density at 410 nm by Auto-ELISA reader (Dynatec Lab, Alexandria, Virginia, USA).

### Statistical analysis

The statistical significance was determined by independent t-test and oneway analysis.

## RESULTS

### Determination of allergen specific IgE and IgG subclass antibodies to *Dermatophagoides farinae*

We compared the logarithmic mean values of O.D.(×1,000) of whole body antigen (WBA), *Der fI* and *Der fII* specific immunoglobulines of all studied sera (Table 1). The O.D. values of specific IgG subclass and IgE antibodies of

**Table 1. Comparison of logarithmic mean values of O.D.(×1000,) value of WBA, *Der fI* and *Der fII* specific IgE and IgG subclasses(n=100)**

Specific IgG & IgE	WBA	<i>Der fI</i>	<i>Der fII</i>
IgG1	1.527±0.934*	0.624±0.604	1.091±0.417*
IgG2	2.489±0.437*	1.699±0.342 <sup>†</sup>	1.430±0.418
IgG3	1.844±0.498*	1.145±0.510	1.192±0.498
IgG4	1.092±0.575*	0.370±0.331	0.925±0.475*
IgE	1.610±0.745*	0.838±0.640	1.345±0.988*

\*: significantly higher than those of the two major allergen specific immunoglobulines (p value < 0.001)

<sup>†</sup>: significantly higher than that of *Der fI* specific immunoglobulines (p value<0.001)

<sup>‡</sup>: significantly higher than that of *Der fII* specific immunoglobulines (p value<0.001)

**Table 2. The mean O.D. value of IgE and IgG subclass antibodies to WBA of *D.farinae* in atopic and nonatopic groups**

IgG & IgE of WBA	Nonatopy (n=44)	Atopy (n=56)	p value
IgG1	1.459±0.937*	1.582±0.936	>0.05
IgG2	2.453±0.454	2.518±0.426	>0.05
IgG3	1.827±0.522	1.857±0.483	>0.05
IgG4	0.829±0.388	1.280±0.634	<0.001
IgE	1.075±0.195	2.030±0.750	<0.001

\*: Log<sub>10</sub> (O.D×1,000)±S.D.

WBA were significantly higher than those of *Der fI* and *Der fII* (p<0.001). The level of *Der fII* specific IgG1, IgG4 and IgE was significantly higher than those of *Der fI*. The level of *Der fII* specific IgG4 was 0.925±0.475 and about 3.6 times higher compared with 0.370±0.331 in *Der fI* specific IgG4 (p<0.001). But level of *Der fI* specific IgG2 was 1.699±0.342 and 1.86 times higher when compared with *Der fII* and there was no significant difference between *Der fI* and *Der fII* specific IgG3.

### Comparison of IgE and IgG subclass antibodies to whole body antigen between atopic and nonatopic groups

The logarithmic mean values of O.D.(×1000)

of IgE and IgG subclass antibodies to WBA are summarized in table 2. In the atopic group, the mean value of IgG4 was  $1.280 \pm 0.634$  and significantly 2.80 times higher than that of nonatopic group ( $p < 0.001$ ). But we could not find significant differences in the levels of IgG1, IgG2 and IgG3 to WBA between the atopic and nonatopic group. The level of IgE to WBA was about 9.02 times higher in the atopic group than the nonatopic group ( $2.030 \pm 0.750$  v.s.  $1.075 \pm 0.195$ ,  $p$  value  $< 0.001$ ).

#### Comparison of IgE and IgG subclass antibodies of *Der fI* between atopic and nonatopic groups

Table 3 shows the level of *Der fI* specific immunoglobulins between the atopic and

nonatopic groups. When compared the levels of IgG subclasses to *Der fI* between the two groups, there were no differences in the level of IgG1, IgG3 and IgG4 but the level of IgG2 was  $1.777 \pm 0.255$  in the nonatopic group and was significantly higher than that of the atopic group ( $1.636 \pm 0.390$ ,  $p < 0.05$ ). In the atopic group, the levels of IgE to *Der fI* were also significantly higher than the nonatopic group ( $0.541 \pm 0.252$  v.s.  $1.071 \pm 0.749$ ,  $p < 0.001$ ).

#### Comparison of IgE and IgG subclass antibodies of *Der fII* between atopic and nonatopic subjects

The levels of *Der fII* specific immunoglobulins between the atopic and nonatopic groups are presented in Table 4. No significant difference in the logarithmic mean val-

Table 3. The mean O.D. value of IgE and IgG subclass antibodies of *Der fI* of *D. farinae* in atopic and nonatopic group

IgG & IgE of <i>Der fI</i>	Nonatopy (n=44)	Atopy (n=56)	p value
IgG1	$0.617 \pm 0.589^*$	$0.628 \pm 0.621$	$> 0.05$
IgG2	$1.777 \pm 0.255$	$1.636 \pm 0.390$	$> 0.05$
IgG3	$1.179 \pm 0.481$	$1.117 \pm 0.534$	$> 0.05$
IgG4	$0.348 \pm 0.270$	$0.388 \pm 0.375$	$< 0.05$
IgE	$0.541 \pm 0.252$	$1.071 \pm 0.749$	$< 0.001$

\*:  $\text{Log}_{10} (\text{O.D} \times 1,000) \pm \text{S.D.}$

Table 4. The mean O.D. value of IgE and IgG subclass antibodies of *Der fII* of *D. farinae* in atopic and nonatopic group

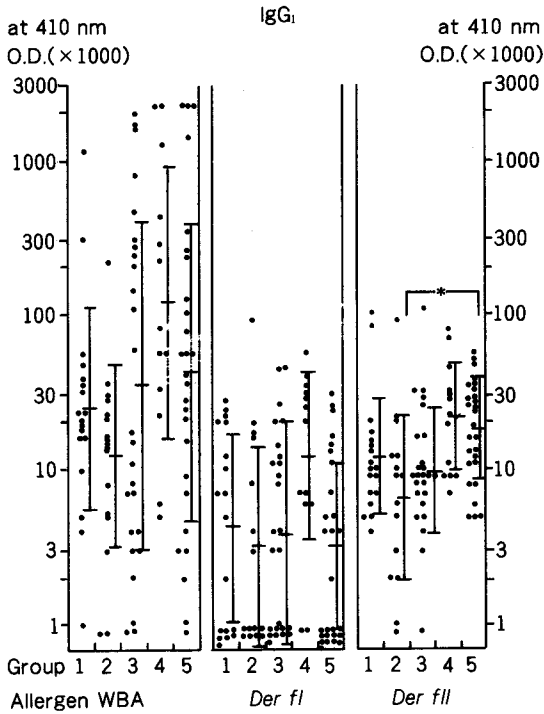
IgG & IgE of <i>Der fII</i>	Nonatopy (n=44)	Atopy (n=56)	p value
IgG1	$1.025 \pm 0.380^*$	$1.144 \pm 0.440$	$> 0.05$
IgG2	$1.301 \pm 0.431$	$1.534 \pm 0.380$	$> 0.05$
IgG3	$1.238 \pm 0.428$	$1.156 \pm 0.548$	$> 0.05$
IgG4	$0.708 \pm 0.146$	$1.096 \pm 0.567$	$< 0.001$
IgE	$0.734 \pm 0.227$	$1.804 \pm 1.088$	$< 0.001$

\*:  $\text{Log}_{10} (\text{O.D} \times 1,000) \pm \text{S.D.}$

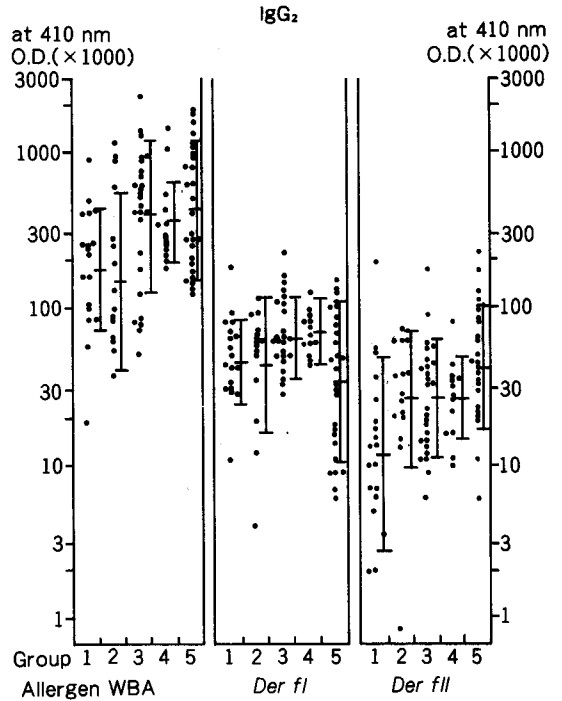
Table 5. Correlation coefficient between specific IgG4 and IgE to WBA, *Der fI* and *Der fII* of *D. farinae*

	Specific IgG4 to			Specific IgE to		
	WBA	<i>Der fI</i>	<i>Der fII</i>	WBA	<i>Der fI</i>	<i>Der fII</i>
Specific IgG4 to						
WBA	1.00					
<i>Der fI</i>	0.56*	1.00				
<i>Der fII</i>	0.72*	0.43*	1.00			
Specific IgE to						
WBA	0.60*	0.21	0.65*	1.00		
<i>Der fI</i>	0.59*	0.33*	0.53*	0.81*	1.00	
<i>Der fII</i>	0.57*	0.25*	0.72*	0.90*	0.79*	1.00

\*:  $p < 0.005$



**Fig. 1.** Distribution of O.D. value of IgG1 to whole body antigen (WBA) and two major allergens (*Der fI* and *Der fII*) of *D. farinae* in each groups. Group 1 is the nonatopic healthy group, groups 2 is the atopic healthy group, group 3 is the nonatopic asthma group, group 4 is the weak atopic group and group 5 is the atopic asthma group. (\*: *P* value between two groups <0.05)



**Fig. 2.** Distribution of O.D. value of IgG2 to whole body antigen (WBA) and two major allergens (*Der fI* and *Der fII*) of *D. farinae* in each groups. Group 1 is the nonatopic healthy group, groups 2 is the atopic healthy group, group 3 is the nonatopic asthma group, group 4 is the weak atopic group and group 5 is the atopic asthma group.

ues of O.D. of *Der fII* specific IgG1 and IgG3 was found. But unlikely with *Der fI* specific IgG2, the level IgG2 of *Der fII* was  $1.534 \pm 0.380$  in the atopic subjects and was significantly higher compared with  $1.301 \pm 0.431$  in the nonatopic group (*p* value <0.05) and both IgG4 and IgE to *Der fII* allergen were significantly higher in the atopic group than the nonatopic group (*p*<0.001).

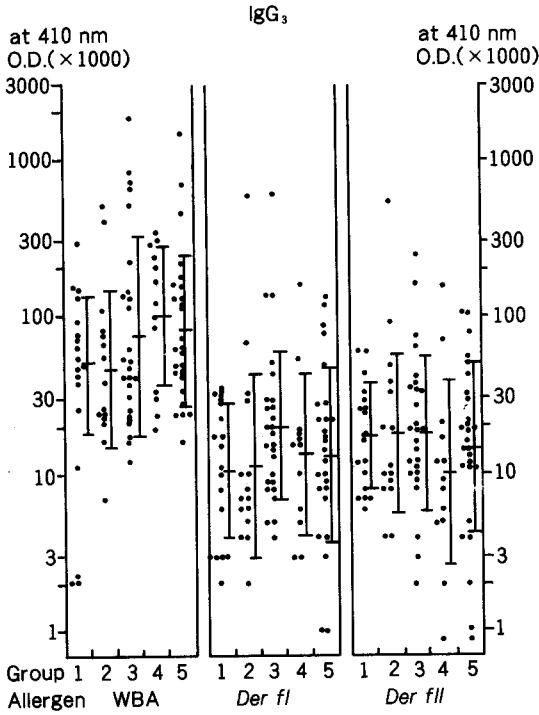
#### Correlation between specific IgE and IgG4 against whole body antigen and *Der fI* and *Der fII* of *D. farinae*

When the O.D. value of IgE and IgG4 against WBA and two major allergens were

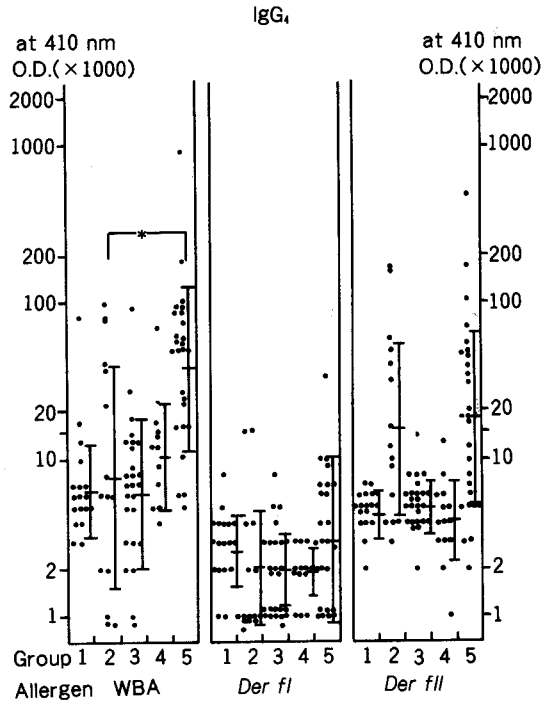
compared, modest correlations between specific IgE and IgG4 were observed in WBA ( $r=0.60$ ), *Der fI* ( $r=0.33$ ) and *Der fII* ( $r=0.72$ ). Furthermore, we could find excellent association among the specific IgE of WBA, *Der fI* and *Der fII* and modest correlation among the specific IgG4 of these three allergens (Table 5).

#### Comparison of IgE and IgG subclass antibodies of whole body antigen and *Der fI* and *Der fII* between healthy subjects and asthmatics

The logarithmic mean values of O.D. of IgG subclass and IgE antibodies of WBA, *Der fI*



**Fig. 3.** Distribution of O.D. value of IgG3 to whole body antigen (WBA) and two major allergens (Der fl and Der fII) of *Dfarinae* in each groups. Group 1 is the nonatopic healthy group, groups 2 is the atopic healthy group, group 3 is the nonatopic asthma group, group 4 is the weak atopic group and group 5 is the atopic asthma group.

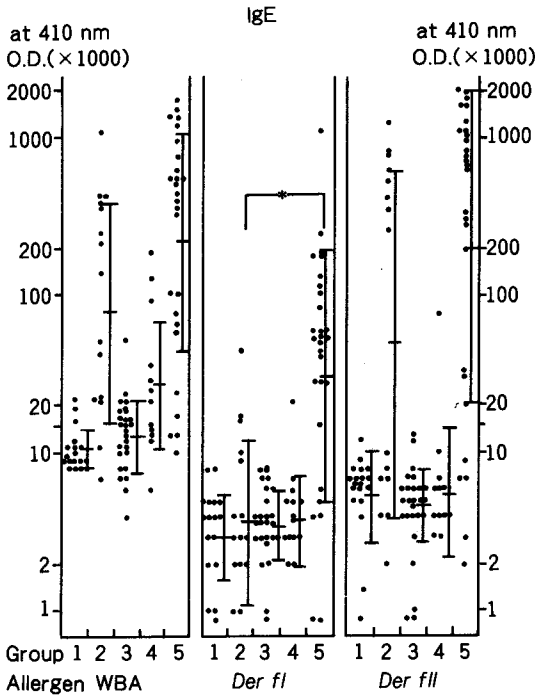


**Fig. 4.** Distribution of O.D. value of IgG4 to whole body antigen (WBA) and two major allergens (Der fl and Der fII) of *Dfarinae* in each groups. Group 1 is the nonatopic healthy group, groups 2 is the atopic healthy group, group 3 is the nonatopic asthma group, group 4 is the weak atopic group and group 5 is the atopic asthma group. (\*: P value between two groups <0.05)

and *Der fII* of *D. farinae* in asthma groups and healthy groups are illustrated at Figure 1, 2, 3, 4 and 5. We compared the mean values of allergen specific immunoglobulins between nonatopic asthma group (group 3) and nonatopic healthy group (group 1) and couldn't find any significant differences in the levels of specific IgG subclass and IgE antibodies of three kinds of allergens (Fig. 1~5). But the number of sera with prominent level of IgG2 of WBA (O.D.  $\geq 0.300$ ) were more common in the nonatopic asthma group than the nonatopic healthy group; 69% of the sera in group 3 noted prominent level of IgG2 compared with 28% of the sera of the group 1 ( $X^2$ -test,  $p$

<0.01) (Fig. 2).

We compared the mean values of allergen specific immunoglobulins between the atopic asthma (group 5) and atopic healthy (group 2) groups. IgG1 level of *Der fII* in the atopic asthma group (group 5) was  $1.245 \pm 0.323$  and about 2.71 times higher compared with that of the atopic healthy group (group 2,  $0.813 \pm 0.534$ ,  $p < 0.05$ , Fig. 1). But there were no significant differences of the levels of IgG2 and IgG3 of WBA, *Der fl* and *Der fII* between atopic asthma and atopic healthy groups. The IgG4 level of WBA and IgE level of *Der fl* were significantly higher in the atopic asthma group than in the atopic healthy group ( $p <$



**Fig. 5.** Distribution of O.D. value of IgE to whole body antigen (WBA) and two major allergens (*Der fl* and *Der fII*) of *D.farinae* in each groups. Group 1 is the nonatopic healthy group, groups 2 is the atopic healthy group, group 3 is the nonatopic asthma group, group 4 is the weak atopic group and group 5 is the atopic asthma group. (\*: P value between two groups <0.05)

0.05). Logarithmic mean values of O.D. of IgG4 to WBA and IgE to *Der fl* in the atopic asthma group were  $1.622 \pm 0.502$  and  $1.565 \pm 0.727$  respectively, compared with  $0.904 \pm 0.726$  and  $0.598 \pm 0.485$  in the atopic healthy group and the level of IgG4 of WBA and IgE of *Der fl* in the atopic asthma group were higher in 5.23 times and in 9.26 times respectively. But the levels of *Der fII* specific IgG4 and IgE were not different between the two atopic groups.

As we compared frequencies of the sera with prominent specific IgE (O.D.  $\geq 0.020$ ) and IgG4 (O.D.  $\geq 0.020$ ) to *D. farinae* allergens, between two atopic asthmatic and atopic

healthy groups, the numbers of sera with prominent high level of IgG4 to WBA and IgE to *Der fl* were more common in the atopic asthma than the atopic healthy groups : 74% and 78% of the sera of atopic asthma group had a prominent level of IgG4 to WBA and IgE to *Der fl* respectively, compared with 38% and 19% of the sera in the atopic healthy group ( $X^2$ -test,  $p < 0.05$  in both, Fig. 4, 5). But we couldn't find any significant differences in frequencies of the sera with prominent high level of IgE and IgG4 to *Der fII* between two atopic groups.

## DISCUSSIONS

House dust mites (HDM) are well known as an allergen responsible for respiratory allergic diseases. IgG antibodies to HDM have been demonstrated in sera from HDM allergic patients by radioimmuno-electrophoresis (Platts-Mills and Chapman. 1987; Soliman and Rosenstreich 1986; D'Souza et al. 1973; Gabriel et al. 1977). And IgG binding activities to pollen allergen also have noted in hay fever patients (Platts-Mills et al. 1978). There are several detection methods of allergen specific antibodies such as radioimmunoassay and enzyme immunoassay. The most sensitive method for measuring IgG antibody to allergens is the double antibody or antigen binding techniques. Antigen binding technique requires purified or at least partially purified allergens (Chapman and Platts-Mills 1978). Therefore, radio-allergosorbent test or ELISA with solid phase allergens are popular to detect allergen specific immunoglobulins.

In this study, for the evaluation of the roles of IgG subclass antibodies in the pathophysiology of HDM allergic diseases, we measured specific IgE and IgG subclass antibodies to WBA, *Der fl* and *Der fII* of *D. farinae* in the sera of adult asthmatics and healthy subjects by ELISA method and evaluated their relationship between the levels of IgE antibody and the levels of IgG subclass antibodies against *D. farinae* allergens.

Chapman and Platts-Mills (1978) measured

specific IgG against house dust mite allergens using partially purified mite antigens containing *Der pI* and *Der pII*. IgG antibodies to the mite antigens were detected in sera from 94% of mite allergic patients and from 30% of nonallergic subjects. In the measurement of IgG subclass antibodies of HDM (Etievant *et al.* 1979), the levels of IgG1 antibody were slightly greater in atopic patients and IgG2 levels were similar in various observed population and IgG3 level was greater in the group with clinical improvement after immunotherapy. And they noted enhanced titer of specific IgG4 in atopic patients.

The several considerations about the roles of allergen specific IgG and/or IgG4 on allergic responses have been reported. IgG or IgG4 antibodies of allergens were increased after immunotherapy and was thought as blocking antibodies to protect allergic reactions after allergen exposure in the patients with immunotherapy (Alberse *et al.* 1983a; Urbanek *et al.* 1986). High level of allergen specific IgG4 antibodies have been noted in some nonimmunized atopic asthmatics and healthy subjects such as those in this paper. Early high IgG4 levels, measured within 3 months after initiation of immunotherapy, was strongly associated with treatment failure after 1-2 years of immunotherapy (Djurup and Malling, 1987). In special occupational conditions, IgG antibodies to occupational environmental agents were thought as an indicator for the degree of exposure of sensitized agents (Platts-Mills *et al.* 1987; Botham *et al.* 1989). There are also some evidences to suggest that IgG4 antibodies are synthesized in response to the chronic exposure to antigen (Homburger *et al.* 1986, Aarberse *et al.* 1983b). Kemenny *et al.* (1989) reported that all subjects were capable of recognizing and mounting an IgG1 antibody response to natural exposure to both HDM and pollens. It was the level of IgG4 antibody which seems to distinguish the nonatopics from atopics. But IgG2 and IgG3 was detected in only two children in 20 HDM allergic children, 80 adults and 32 nonatopic controls. Djurup (1984) noted ELISA can detect IgG antibody in higher rate (94% atopic and 97% non-atopic to HDM, 81% and 100% to

grass pollen, respectively) due to the ability of the ELISA to detect antibodies to lower affinity than those detected by radioimmunoassay, in which IgG antibodies to inhalant allergens in only 20~30% of non-atopic individuals.

About the clinical significances of HDM specific IgG or subclass antibodies, Gwynn *et al.* (1982) showed that patients with HDM specific IgG4 developed a delayed response and patients with both classes of antibody against the same allergen exhibited a dual response. Ito *et al.* (1986) reported that there was a close correlation of the presence of high IgG1 antibodies with propensity to develop late asthmatic responses. And Ito *et al.* (1989) also noted that IgG1 and IgG4 antibodies to HDM were higher in mite sensitive steroid-independent patients than HDM sensitive steroiddependent patients and Oshika *et al.* (1992) reported that all IgG subclass antibodies in patients with bronchial asthma were higher than those in control group and the level of *Der pII* specific IgG1 and IgG4 in asthmatic patients were 1.97 and 2.20 times higher respectively than those in control group. And there were reports about the importances of natural immunity to dust mites in adult chronic asthma (Soliman and Rosenstreich, 1986) and chronic rhinosinusitis (Freudenberger *et al.* 1988). However clinical study of desensitization therapy by specific antigen displayed an increase of specific IgG4 antibodies and concomitant improvement of clinical symptoms. IgG4 antibodies have been considered to be blocking antibodies in immediate allergic reactions (Moss *et al.* 1987; Aalberse *et al.* 1983a; van der Giessen *et al.* 1976; Devey *et al.* 1976; Nakagawa *et al.* 1983).

In this paper we measured specific IgE and IgG subclass antibodies of WBA and major allergens (*Der fI* and *Der fII*) of HDM in various clinical groups. There were no specific differences of the levels of IgG1, IgG2 and IgG3 to WBA between atopic and nonatopic groups. IgG1 and IgG3 levels of two major allergens were not significantly different between two groups. IgG2 of *Der fI* was higher in nonatopic group than in the atopic group, but IgG2 of *Der fII* was higher in the atopic



group than in the nonatopic group. IgG4 of *Der fII* and IgE of both two major allergens were prominent in the atopic group as similar as those of WBA of HDM.

We evaluated if there would be any differences on immune responses to antigens or allergens of HDM between asthma and healthy groups according to skin reactivity. The levels of IgG subclasses to WBA, *Der fI* and *Der fII* were not significantly different between nonatopic asthma and nonatopic healthy groups (group 1 and group 3). But the number of sera with prominent level of IgG2 of WBA was significantly higher in nonatopic asthma group (69%) than in nonatopic healthy group (28%). In atopic asthma group (group 5) IgG1 of *Der fII*, IgG4 of WBA, and IgE of *Der fI* were significantly higher than in atopic healthy group (group 2). And the sera numbers with prominent levels of IgG4 of WBA (74%) and the sera numbers with prominent level of IgE of *Der fI* (78%) were more higher in atopic asthma group (group 5) than atopic healthy group (group 2, 38% and 19% respectively). These results suggested that the skin reactivities to HDM were mainly associated with IgE and IgG4 antibodies to whole body antigen and *Der fII* of HDM. And in nonatopic asthma groups some role of IgG2 ( $p=0.00634$ ) and IgG1 ( $p=0.0582$ ) to WBA of HDM were suggestive also. And the atopic asthma group would have some specific immune responses of IgE and IgG4 to *Der fI* and WBA rather than *Der fII*.

Furthermore, between specific IgE and IgG subclass antibodies, we could find cross-relationship between the levels of specific IgG4 and IgE of all HDM antigens but there were no association between HDM specific IgG1-3 and IgE. This finding suggests that HDM specific IgE and IgG4 will be under common immuno-regulatory control.

In studies of ragweed pollen allergy all the sera with specific IgE had detectable IgG to major and minor allergens (Platts-Mills *et al.* 1981). There were the heterogeneity of antigen recognized by IgG, IgA and IgM classes from a normal individual, as compared to a pollen sensitive patient (Peltre *et al.* 1982). Desvaux *et al.* (1989) noted on immunoprint that there

was obvious relationship between IgE and IgG4. On separate immunoprints with isotype-specific antibodies, binding patterns of IgG4 and the majority of IgG1 and IgA2 antibodies in the IgG4+ plasma group very closely paralleled the binding patterns produced by the IgE antibodies from the same plasma and were described as the 'allergen repertoire' (Batard *et al.* 1993). They proposed that allergenic agents selectively would trigger the expression of the locus controlling region (LCR) element of the second duplication unit which subsequently would control immune responses involving IgG4, IgE and IgA2 antibodies.

The different immune responses to HDM antigens have been suggested on the development of asthma in atopic and nonatopic subjects according to this work. Even though natural immune responses to HDM were also reported in chronic nasal allergy (Freudenberger *et al.* 1988), our findings of the distinctive IgE and IgG subclass antibody responses to HDM antigens according to respiratory symptoms and atopic status would be an indirect evidences that atopic asthmatics will have different antibody responses to common environmental allergens from atopic healthy subjects and that nonatopic asthmatics may also have different IgG antibody responses from nonatopic healthy control.

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