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Abstract

Using 6 fractions differing in molecular weight of Dermatophagoides pteronyssinus (Dp)-antigen, we measured by enzyme-linked immunosorbent assay (ELISA) the titers of specific IgE, IgG and IgG4 antibodies against Dp antigen in sera of allergic subjects who were sensitive to house dust mite. We intended to evaluate which Dp fraction acts as the major antigenicity for allergic subjects. Results were as follows: 1) In comparison with normal controls, the titer of IgE antibody specific to crude Dp antigen was evaluated, but no significant difference was found among the titers of IgE antibody against each Dp fraction. 2) The titer of IgG antibody against the fraction with a high molecular weight (190 KD, 95 KD) was significantly higher than the titer of the 15 KD fraction in the nasal allergy patients. 3) The 15 KD fraction induced significant elevation of the titer of IgG4 antibody. It suggests that the low molecular weight fraction may act as the major allergenicity of Dp-antigen for inducing both IgE and competitive IgG4 antibodies, although other fractions induce significant IgE responses in patients with nasal allergy.

KEYWORDS: IgG4, IgE, nasal allergy, Dermatophagoides pteronyssinus

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IgE, IgG, and IgG_4 Antiybody Titers to Fractionated House Dust Mite Antigens in Nasal Allergy Patients

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Using 6 fractions differing in molecular weight of *Dermatophagoides pteronyssinus* (Dp)-antigen, we measured by enzyme-linked immunosorbent assay (ELISA) the titers of specific IgE, IgG and IgG_4 antibodies against Dp antigen in sera of allergic subjects who were sensitive to house dust mite. We intended to evaluate which Dp fraction acts as the major antigenicity for allergic subjects. Results were as follows: 1) In comparison with normal controls, the titer of IgE antibody specific to crude Dp antigen was evaluated, but no significant difference was found among the titers of IgE antibody against each Dp fraction. 2) The titer of IgG antibody against the fraction with a high molecular weight (190KD, 95KD) was siginificantly higher than the titer of the 15KD fraction in the nasal allergy patients. 3) The 15KD fraction induced significant elevation of the titer of IgG_4 antibody. It suggests that the low molecular weight fraction may act as the major allergenicity of Dp-antigen for inducing both IgE and competitive IgG_4 antibodies, although other fractions induce significant IgE responses in patients with nasal allergy.

Key words : IgG_4, IgE, nasal allergy, *Dermatophagoides pteronyssinus*

Since 1960s antigens extracted from the genus *Dermatophagoides* have been shown to induce allergic diseases in humans (1). Since then many immunoserological studies have been conducted to uncover the characteristics of the mite allergens (2-11).

For the diagnosis and treatment of house dust mite allergy, it is necessary to isolate and analyze the allergens. For this purpose, we subjected *Dermatophagoides pteronyssinus* (Dp) to Sephacryl S200 column chromatography in accordance with the method reported by Ishii *et al.* (12). In a report which describes IgG_1, IgG_4, and IgE antybody responses of asthmatics of gel-fractionated and purified allergens of *Dermatophagoides farinae* (Df) (13), the authors reported that IgE antibodies reactive to low molecular weight fractions were prominent, while IgG_4 antibodies mostly responded to a high molecular weight fraction. Dp is a dominant species of the genus *Dermaphagoides* together with Df in Japan. Although Df and Dp might share common antigens, molecular analyses revealed considerable difference in amino acid sequences (14). It is, thus, somewhat controversial whether data obtained from Df may be directly applicable to

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Dp. Furthermore, asthma and nasal allergy are likely to have distinct pathogenesis, although both diseases are categoried as type I allergy.

In this study, we detected IgE, IgG and IgG_4 antibodies to 6 fractionated Dp antigens in patients with nasal allergy by using enzyme-linked immunosorbent assay (ELISA), and compared their Dp-specific antibodies with asthma patients and healthy controls. Based on those results, we investigated which fractions act as the target antigens of various isotype antibodies in nasal allergy.

Materials and Methods

Serum sample. Serum was separated from peripheral blood of 40 patients with nasal allergy examined at the Otorhinolaryngology Outpatient Clinic of Okayama University (aged 6–36 years, mean: 17.5 years), and from 60 asthmatics at the Pediatric Outpatient Clinic of Kochi Medical College (aged 2–15 years, mean: 8.0 years). Sera were stored at −20°C until use. The asthma patients had RAST score of 3–4 for Dp before desensitization. The nasal allergy patients showed a reaction to house dust mites only, and desensitization therapy had been performed for 3 months to 1 year with house dust (H.D. Torii Yakuhin Co., Tokyo). As controls, we used sera of 20 random healthy volunteers (21–30 years) of the Okayama University Medical School. From the preliminary ELISA experiments with sera from 86 healthy subjects, sera of 6 subjects with relatively high Dp-specific IgG titers and sera of 10 subjects with detectable Dp-specific IgE levels were pooled respectively, and were used as control pooled sera (data not shown). As second antibodies, horseradish peroxidase-conjugated (HRPO) rabbit anti-human IgG (MBL, Nagoya), HRPO goat anti-human IgE (TAGO, Burlingamme, USA), and HRPO mouse anti-human IgG_4 monoclonal antibody (Yamasa Shoyu, Tokyo) were used.

Extraction and fractionation of Dermatophagoides antigens. *Dermatophagoides pteronyssinus* (Dp) was cultured by the method of Miyamoto *et al.* (15). Dp antigen was prepared in accordance with the method of Kabasawa and Ishii (16). In brief, organisms were separated from the culture medium using saturated saline solution and homogenized by adding 6 ml of 0.1 M Tris-HCL buffer (pH 7.6) to 1 g of dried Dp. Following ultra-centrifugation at 60,000 *g* for 1 h, the supernatant was used as crude Dp antigen. The protein concentration was measured by the method used by Lowry *et al.* (17), and was 6.8 mg/ml. This crude Dp antigen solution (10 ml) was applied on a 2.5 × 100 cm Sephacryl S-200 column (Pharmacia, Uppsala, Sweden), and eluted with 0.1 M Tris-HCL buffer (pH 7.6). The eluate was collected in 5 ml fractions, and the absorbance at 280 nm was measured. Using protein molecular weight markers, eluates corresponding to 15, 25, 32, 53, 95 and 190 KD were obtained in 10 to 15 ml fractions to be examined for antigens.

Measurement of antibody titers. We measured antigen-specific antibody titers with ELISA (18–21). Crude Dp antigen or the fractionated antigens were diluted to a protein concentration of 10 μg/ml by adding 0.05 M carbonate buffer (pH 9.5). Then 100 μl aliquots of the mixed solution were added to the wells of a 96-well flat-bottomed polystyrene plate (Nunc, Denmark) and the plate was left overnight at 4°C. After washing 3 times with 0.01 M phosphate-buffered saline containing 0.05% Tween 20 (PBS/TW), PBS containing 1% BSA (BSA/PBS) was added to the wells and the wells were left overnight at 4°C. After washing 3 times, 100 μl (IgG) or 50 μl (IgE, IgG_4) of patient serum diluted × 40 (IgG), × 4 ((IgE) or × 2 (IgG_4) with BSA/PBS/TW was added, and the mixed solutions were incubated at 37°C for 1.5 h (IgG, IgE). For IgG_4 incubation was performed for 2 h at 37°C. After washing 3 times with PBS/TW, 100 μl (IgG) or 50 μl (IgE) of the second antibody diluted to 1:200 (IgG) or 1:20 (IgE) was added for incubation at 37°C for 1.5 h. For testing IgG_4 the second antibody was diluted to 1:1,000 and 50 μl was added for incubation at 4°C overnight. After washing 4 times 100 μl of a substrate solution prepared from 0.03% 2, 2′-Azinobis; 3-ethylene-thiazoline sulfonic acid (ABTS; Sigma, USA), 0.003% H_2O_2 and phosphate citrate buffer (pH 5.0) was added. After incubation for 30 min at 37°C, the reaction was stopped with 50 μl of 1.25% NaF and the OD was measured at 410 nm. For each experiment, control wells were prepared without antigen to give the background absorbance, and this was subtracted from the absorbance obtained for each well. Each specimen was measured in duplicate and the mean value was obtained. The IgG and IgE data were expressed as relative values in which OD values of control pool serum were taken as 100. IgG_4 data were expressed in absolute OD values because no positive control serum was available. In the IgG_4 ELISA, the background absorbance was able to be neglected.

Statistical analysis. The statistical significance was determined by the Student's t test (22).

Results

Dp fraction-specific IgG. In both the patients with nasal allergy and those with asthma, high values of antibody were shown in the 190 KD fraction, and low values in the lower molecular weight fractions; however, the healthy control subjects showed no such tendency (Fig. 1). Significant elevation of IgG titers to the 190 KD and 95 KD fractions was observed when compared with that to the 15 KD in the nasal allergy patients ($p < 0.01$), but the 32 KD, 25 KD, and

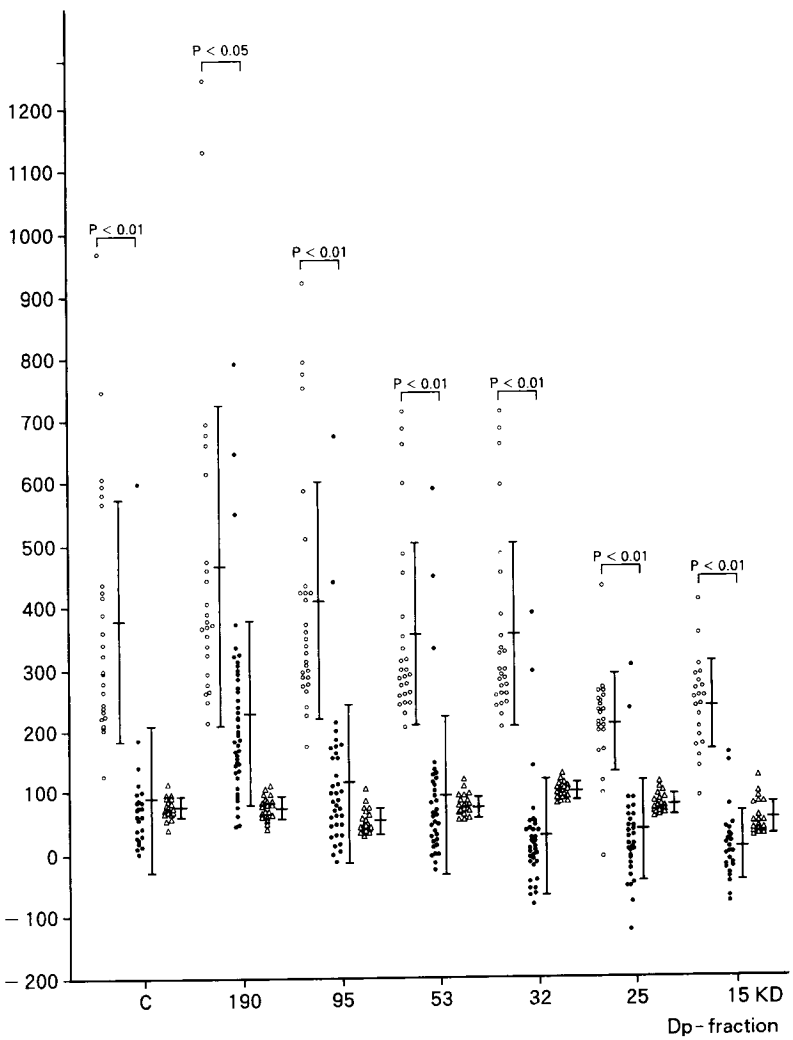


Fig. 1 Comparison of Dp fraction -specific IgG among three groups. Patients showed high values of antibody in the 190 KD fraction, and less antibodies were noted in the lower molecular weight fractions. The controle healthy subjects showed no such tendency. ○: patients with bronchial asthma, ●: patients with nasal allergy, △: Healthy controls. Data are expressed as ralative values in which OD values of the control pool serum were taken as 100.

15 KD fractions showed no significant difference. Specific IgG antibody titers were significantly higher in asthma patients than in those with nasal allergy for all Dp fractions tested. In nasal allergy patients, the mean antibody titer for the crude antigen was 60.57, and Dp-specific IgG antibody was not increased compared with the control.

Dp fraction-specific IgE. In both the patients with nasal allergy and asthma, the titers of Dp fraction specific IgE were variegated (Fig. 2), and no difference was present in the IgE titers among six fractions. The antibody titer against crude antigen was higher in both patients with nasal allergy and those with asthma than in the control pooled sera (176 for nasal allergy and 290 for asthma, $p < 0.01$).

Dp fraction-specific IgG_4. When IgG_4 ELISA was performed in the 40 patients with nasal allergy, an OD > 0.1 was found in 12 cases (Fig. 3). Both the 190 KD and 15 KD fractions showed significantly higher levels compared with the other fractions ($p < 0.05$).

Changes of fraction-specific IgG titers after desensitization therapy. In eight nasal allergy patients whose symptoms improved markedly with desensitization, antibody titers were compared between pre- and post-desensitization therapy for at least 3 months. It seemed likely that the antibody titers rose in the lower molecular weight fractions, but the difference was not statistically significant (Fig. 4).

Changes of the fraction-specific IgE titer after desensitization therapy. A comparison was made using the same patients tested for IgG (Fig. 5). The IgE antibody titer decreased by more than 50 % in all the fractions from 190 KD,

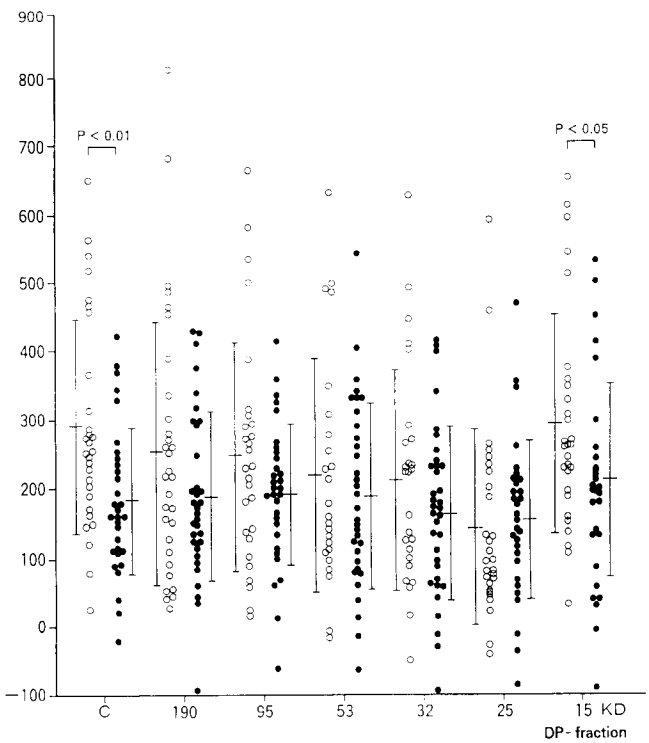


Fig. 2 Dp fraction-specific IgE levels in two allergic subjects. Both patients showed no peak response of IgE in any particular molecular weight fractions. The antibody titer against crude antigen was elevated in both the patients compared with the controle pooled sera. ●: patients with nasal allergy, ○: patients with bronchial asthma.

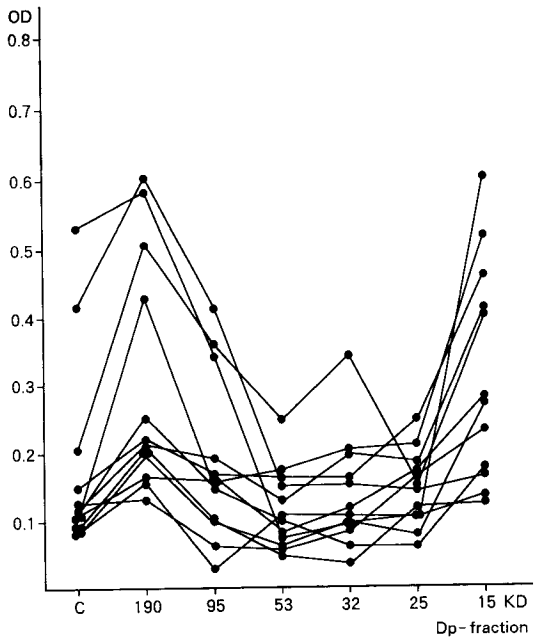
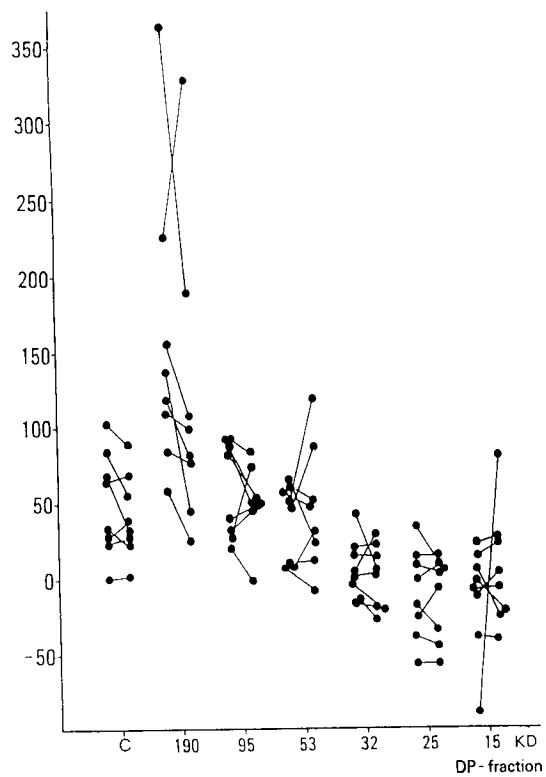
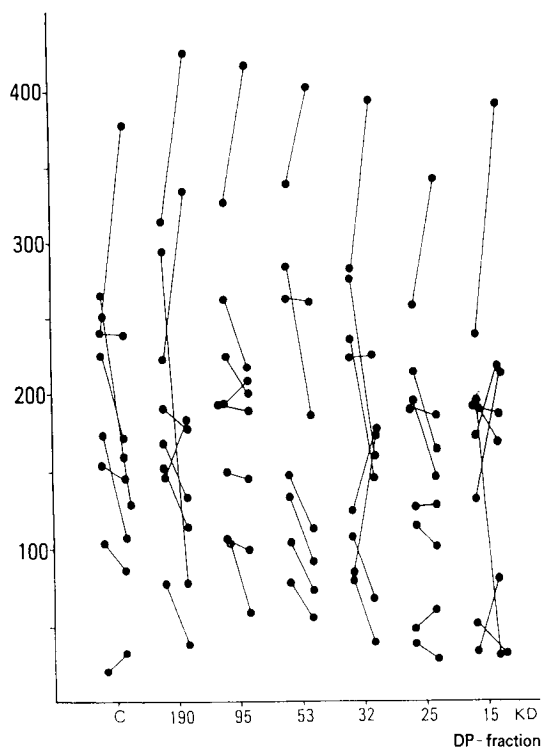


Fig. 3 (Upper left) Dp fraction-specific IgE_4 in patients with allergic rhinitis. Responses to both the 190 KD and 15 KD fractions showed significantly higher levels compared with the other fractions ($p < 0.05$).

Fig. 4 (Lower left) Changes of the fraction-specific IgG titer before and after desensitization therapy. Antibody titers rose in the lower molecular weight fractions, but the difference was not statistically significant.

Fig. 5 (Lower right) Changes of the fraction-specific IgE titer before and after desensitization therapy. The IgE antibody titer decreased by more than 50 % in all the fractions from 190 KD to 15 KD. The differences were, however, not statistically significant.





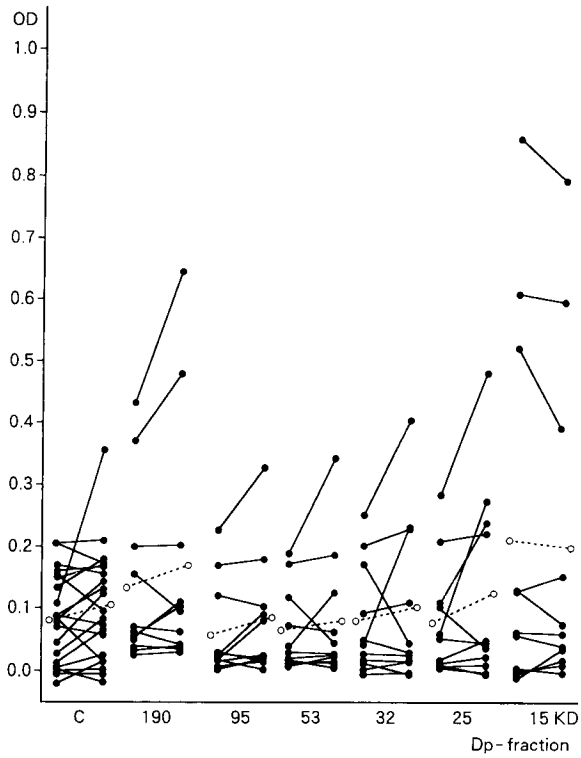


Fig. 6 Changes of the fraction-specific IgG_4 titer before and after desensitization therapy. Although fraction specific IgG_4 levels were compared, no significant change was observed. The mean OD value was show for each fraction (○-----○).

but there was no significant difference noted.

Changes of the fraction-specific IgG_4 titers after desensitization. When specific IgG_4 titers were compared before and after specific desensitization therapy (3M–6M) with house dust, no clear change was observed (Fig. 6).

Discussion

The most frequent air-borne allergen in Japan is house dust (H.D.) and its main component is *Dermatophagoides* (1, 23). Intradermal testing of patients with nasal allergy attending the Department of Otorhinolaryngology at Okayama University has shown a positive rate for H.D. of 74.5 % (24).

Desensitization therapy provides a crue for nasal allergy in some cases and is performed at present using house dust in Japan, while desensitization with the mite itself is not generally done. If house dust mite antigens could be identified as causative allergens for the disease, more effective desensitization therapy with fewer side effects may be developed.

Regarding the main allergenic determinations of the house dust mite, many reports showed their expression in the low molecular weight fractions (3–7,9). In 1987, Tovey (10) reported that 26 IgE-binding components were present in mite body extract among which 5 bands had an especially strong affinity.

Nakata *et al.* (13) performed ELISA with two fractions containing high and low molecular weight components. They suggested that the target antigens of IgE are in the low molecular

weight fractions, probably including "major mite allergens", whereas IgG_1 and IgG_4 are of a rather high molecular weight. The present study also showed that the total IgG level in patients with nasal allergy peaked in the high molecular weight fractions, whereas any IgG was hardly reactive to the low molecular weight fractions. The relationship between the level of IgG antibody production in response to high molecular weight fractions and the incidence of allergy is unknown at pesent. The IgE antibody response to each fraction was variegated, and the high titers were not always observed in the low molecular weight fractions. It may be due to differences in antigenicity between Dp tested in the present study and Df tested by Nakata *et al.* In our ELISA method, the protein content of each molecular weight fraction was set at 10 μg/ml, but the quantity of antigen in each fraction was not constant. Therefore, it must also be considered that intensity of the response to the antigen fractions may not reflect the actual response *in vivo*. However, gel filtration of crude Dp antigen showed no remarkable differences in protein content from 15 KD to 190 KD (25), so the differences observed between the fractions may not be spurious.

In the patients with nasal allergy, IgG and IgE titers were both lower compared with the asthma patients. In asthma patients, the mean age was 8.0 years, while is was 17.5 years for nasal allergy patients. It is generally considered that the antibody response of humoral immunity declines after 15 years of age. Moreover, asthma is accompanied by strong systemic symptoms and signs such as food allergy, dermatitis, rhintitis and peripheral eosinophilia, whereas nasal allergy rarely causes marked eosinophilia and the allergic inflammation tends to be localized to the nasal mucous membranes. This might reflect difference in antibody titer in the peripheral blood.

Human IgG_4 is a minute component of total IgG with a concentration of about 500 μg/ml in peripheral blood. Although it is still not conclusive whether IgG_4 inhibit or promote allergic manifestations, many reports have suggested that IgG_4 could bind allergens competitively with IgE (26–28). Nakata *et al.* (13) showed that IgG_4 mostly reacts with high molecular weight antigen, and that a low molecular weight antigen was detected only in one patient after desensitization therapy. However, our study showed that the IgG_4 response in patients with nasal allergy was often directed to the 15 KD as well as the 190 KD fractions. Considering that the 15 KD fraction of Dp has been defined as one of the major allergens (*Der P*II), one of the probable explanation for the findings is that the IgG_4 antibodies also recognize the major allergens, and subsequently act as blocking antibodies in nasal allergy.

There are many reports that, in the desensitization of asthma patients, the IgG titer increases and that of IgE decreases (28, 29). In our study, desensitization therapy reduced symptoms, however, there is no significant difference in antibody titers between pre- and post-desensitization therapy. Since the duration of sensitization was brief in the present study, we think that changes of IgE titers need to be observed over a longer term.

As mentioned above, serum antibody in patients with nasal allergy showed a high titer at 190 KD for IgG, whereas for IgE, the fractions showing high values differed among individuals. After desensitization, changes of the IgG and IgE titers were also not necessarily seen in asthma, and it was indicated that other factors are also concerned in the effect of desensitization. This is the first report on serum antybody titers in patients with nasal allergy determined in ELISA using fractionated Dp antigen. More detailed analysis is underway in the hope of devevoping new therapy for Dp-induced nasal allergy.

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