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Specific IgE, IgG and IgG4 antibodies against house dust mite in patients with bronchial asthma.

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Abstract

Serum levels of total IgE, specific IgE, IgG and IgG4 against house dust mite were measured in mite-sensitive asthma patients receiving immunotherapy with house dust. Serum levels of total IgE, mite specific IgE and IgG did not significantly change during the course of hyposensitization. Increased levels of mite specific IgG4 were observed in patients during immunotherapy. The increase in specific IgG4 was dependent on the total dose of house dust administered in both children (r = 0.636, p less than 0.001) and adults (r = 0.629, p less than 0.01). However, the increase of specific IgG4 in adults was not as apparent as in children. These results might suggest that mite specific IgG4 is a useful immunological marker in the immunotherapy for allergic asthma, and that IgG4 antibody acts as a blocking antibody in atopic bronchial asthma.

KEYWORDS: house dust mite, bronchial asthma, hyposensitization, specific IgG4

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Specific IgE, IgG and IgG4 Antibodies against House Dust Mite in Patients with Bronchial Asthma

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Serum levels of total IgE, specific IgE, IgG and IgG4 against house dust mite were measured in mite-sensitive asthma patients receiving immunotherapy with house dust. Serum levels of total IgE, mite specific IgE and IgG did not significaltly change during the course of hyposensitization. Increased levels of mite specific IgG4 were observed in patients during immunotherapy. The increase in specific IgG4 was dependent on the total dose of house dust administered in both children ($r = 0.636$, $p < 0.001$) and adults ($r = 0.629$, $p < 0.01$). However, the increase of specific IgG4 in adults was not as apparent as in children. These results might suggest that mite specific IgG4 is a useful immunological marker in the immunotherapy for allergic asthma, and that IgG4 antibody acts as a blocking antibody in atopic bronchial asthma.

Key words : **house dust mite, bronchial asthma, hyposensitization, specific IgG4**

Mites, especially *Dermatophagoides farinae* and *Dermatophagoides ptoronyssinus* are universal allergens in atopic bronchial asthma (1). Recently molecular nature of these allergens have been investigated and their sequences have been reported (2-7). In spite of these advances in the research of causative agents, there is little progress in the specific therapy of bronchial asthma. Immunotherapy using allergens was introduced 70 years ago (8) and is still the sole specific therapy in bronchial asthma. Several inventigators reported 70-80 % efficacy of immunotherapy with house dust in mite-sensitive asthma (9, 10). Efforts were made to determine the mechanisms of its efficacy. Cook *et al.* (11) reported the presence of a serum factor capable of inhibiting the Prausnitz-Küstner response in hyposensitized patients and termed it a blocking antibody. This antibody was later found to be specific IgG for administered allergens (12). Recently specific IgG4 for allergens has been investigated. Although some researchers postulated it as an allergic antibody (13, 14), others reported that the increased level of specific IgG4 was observed in patients receiving immunotherapy (15). In order to clarify immunological responses to immunotherapy, we measured serum levels of total IgE, IgG and IgG4 antibodies against house dust mite in patients hyposensitized with house dust. Difference in IgG4 production

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during hyposensitization was also compared between child and adult patients.

Subjects and Methods

Subjects. Subjects were 32 children (11 females and 21 males) and 13 adults (9 females and 4 males) with bronchial asthma. Mean age of these groups of patients were 11.2 ± 3.1 years old (range; 4–17) and 48.7 ± 14.0 years old (range; 28–68), respectively. They were diagnosed as having house dust mite-sensitive asthma at Misasa Medical Branch on the basis of clinical history, physical examination, skin test, specific IgE and pulmonary function test. They have been treated with weekly subcutaneous injections of house dust (Torii Pharmaceutical Co., Tokyo), the 10-times diluted solution of which contains 100 mg dry house dust extracts in 1 ml physiological saline. Sera were collected from patients and stored at − 70°C until use.

IgE and specific IgE. Total IgE was determined by using Pharmacia IgE RIA kit and specific IgE to house dust was measured with Pharmacia RAST RIA kit.

Enzme-linked immunosorbent assay (ELISA). Specific IgG to *Dermatophagoides* farinae (DF) was tested by ELISA as described previously (16). Briefly, wells of 96-well microtiter plates were coated with DF extracts overnight. The wells were blocked with PBS containing 5% BSA and incubated with 1000 times diluted sera at 4°C for three hours. After washing twice with PBS containing tween-20 the wells were further incubated with alkaline phosphatase conjugated anti-human IgG (Miles Co., USA) at 4°C for three hours. Then the washed wells were developed with paranitrophenol phosphate. Optical density of the wells were determined at 405 nm. For measuring mite specific IgG4, murine monoclonal anti-human IgG4 (Yamana Shoyu Co., Chiba) were used instead of anti-human IgG. The wells were further incubated with peroxidase-conjugated anti-mouse IgG and developed with O-phenylenediamine. Optical density of the wells were determined at 500 nm as described by Nakagawa *et. al* (15). These assays were carried out in duplicate. The results of specific IgG4 were expressed as arbitrary units per milliliter against a positive serum pool.

Results

Characteristics of patients. The mean age of the patients at the dianosis were 11.2 ± 3.1 in child cases and 48.7 ± 14.0 in adult cases, respectively. The mean serum IgE were 1110 ± 840 IU/ml in children and 514 ± 339 IU/ml in adults. The mean specific IgE to house dust were 10.1 ± 4.9 PRU/ml in children and 4.4 ± 4.7 PRU/ml in adults. All children were able to be controlled without corticosteroid therapy, whereas two adult patients were receiving systemic corticosteroid (Table 1). All subjects were hyposensitized with house dust.

Total IgE, specific IgE, IgG and IgG4 against house dust mite in children. Serum levels of total IgE, specific IgE, IgG and IgG4 against house dust mite during hyposensitization were investigated in children. Total IgE did not show a significant correlation with total dose of house dust administered (Fig 1).

Mean specific IgE against house dust was 10.1 ± 4.9 PRU/ml before treatment and 10.9 ± 5.0 PRU/ml at the dose 100 mg or more of house dust administered (Fig. 2). Increased levels of specific IgE was observed at 1–9 mg, but not significantly higher than the pretreatment levels. No significant decrease of specific IgE was observed during the course of hyposensitization.

Serum levels of specific IgG against DF

Table 1 Patients with mite-sensitive bronchial asthma

Patients	Number of cases	Age	Total IgE (IU/ml)	Specific IgE for house dust (PRU/ml)	Number of cases with steroid therapy
Children	32	11.2 ± 3.1	1110 ± 841	10.1 ± 4.9	0
Adults	13	48.7 ± 14.0	514 ± 339	4.4 ± 4.7	2

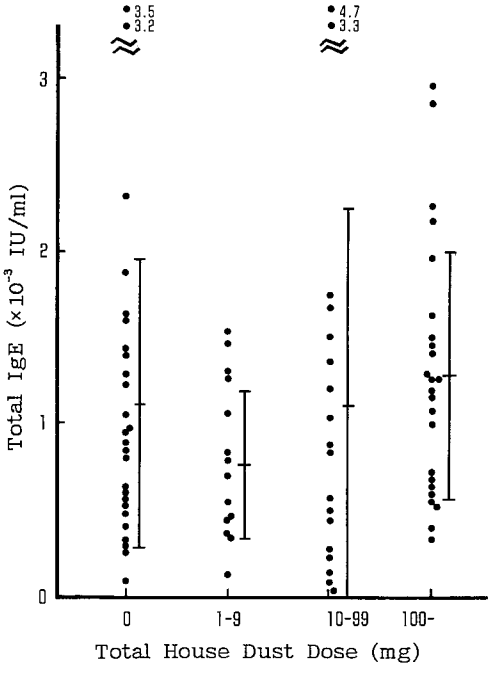

Fig. 1 Correlation betweed total dose of house dust administered and total IgE in the patient serum. Mean IgE ± standard deviation are as follows. 1110 ± 840 IU/ml at 10–99mg before treatment, 760 ± 430 IU/ml at 1 − 9mg, 1110 ± 1140 IU/ml at 10 − 99mg and 1280 ± 710 IU/ml at 100mg or more.

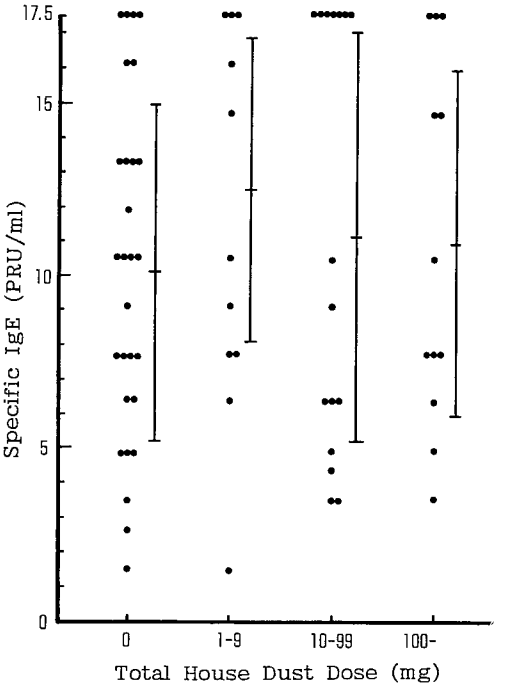

Fig. 2 Correlation between total dose of house dust and specific IgE. Mean specific IgE ± standard deviation are as follows. 10.1 ± 4.9 PRU/ml before treatment, 12.5 ± 4. 4 PRU/ml at 1–9mg, 11.1 ± 5.9 PRU/ml at 10–99 mg and 10.9 ± 5.0 PRU/ml at 100 mg or more.

extracts at various doses of house dust administered during hyposensitization were shown in Fig. 3. Decreased levels of specific IgG was observed at higher dose of house dust. However, no signigicant difference was detected among three groups with different doses of house dust.

Furthermore, no significant correlations were found between these three parameters and duration of hyposensitization (data not shown). These results suggest that total IgE, specific IgE and IgG are not good immunological markers in the immunotherapy of house dust mite-sensitive asthma.

Next, specific IgG4 against mites in the sera of hyposensitized patients was tested by ELISA. This assay was carried out by using monoclonal anti-human IgG4, which was highly specific to human IgG4 and did not crossreact with other subclasses of human IgG (17). As shown in Fig. 4, levels of specific IgG4 had a positive correlation with the total dose of house dust administered ($r = 0.636$, $p < 0.001$). Changes of specific IgG4 levels in several patients are illustrated in Fig. 5. Levels of specific IgG4 increased as the total house dust dose increased.

Specific IgG4 response in adults. Correlation between specific IgG4 and total dose of house dust was also investigated in adult patients with mite-sensitive asthma. As shown in Fig. 6, positive correlation was also observed in adults between specific IgG4 and total house dust dose ($r = 0.629$, $p < 0.01$). However, the increase of IgG4 in adults was less than that in children, suggesting that more total dose of house dust is

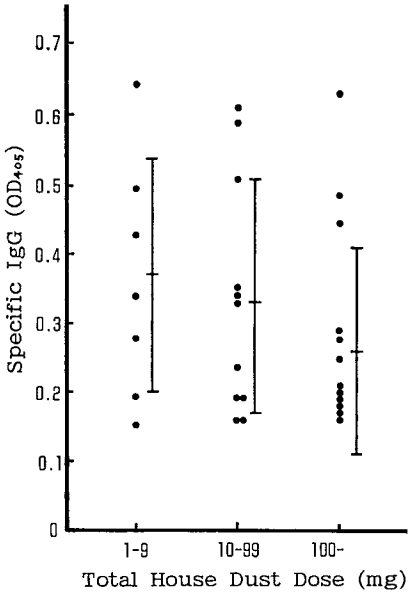

Fig. 3 Correlation between total dose of house dust and specific IgG. Mean specific IgG ± standard deviation are as follows. 0.37 ± 0.17 at 1-9mg, 0.33 ± 0.16 at 10-99mg and 0.26 ± 0.15 at 100 mg or more.

needed for adult cases to induce increased production of specific IgG4 than for child cases.

Discussion

In order to clarify immunological markers reflecting responses to hyposensitization in asthmatic patients, we measured serum levels of total IgE, specific IgE, IgG and IgG4 against house dust mite. Specific IgG4 showed a house dust dose dependent increase in both children and adults during hynosensitization. However, the increase of IgG4 in adults was not higher than that in children. Levels of total IgE, specific IgE and IgG did not change significantly during hyposensitization. It has been reported that specific IgE titers decreased by long term hyposensitization (18). This phenomenon was not observed in our study.

Immunotherapy using allergens was introduced 70 years ago. 70-80 % efficacy was reported in house dust mite-sensitive asthma (9,

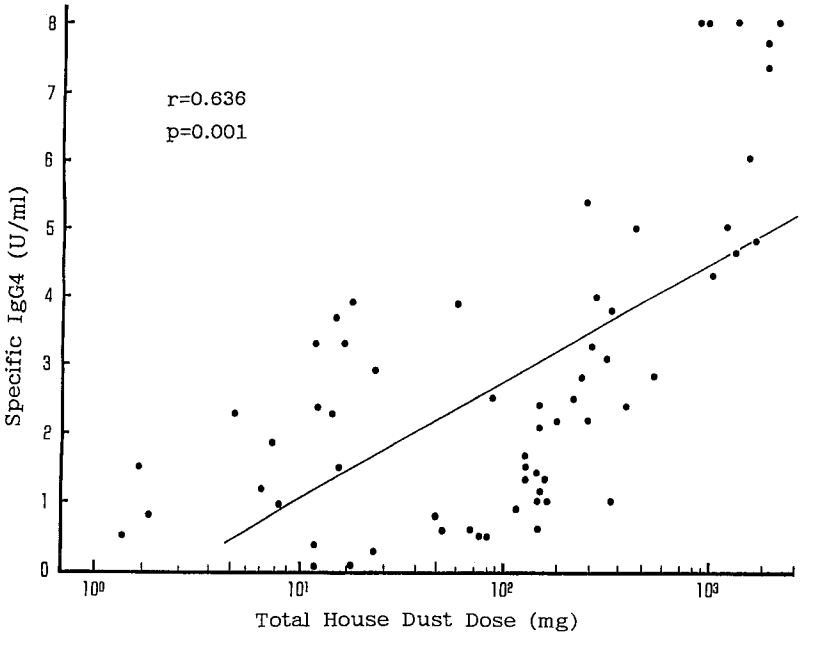

Fig. 4 Correlation between total dose of house dust and specific IgG 4 in children mite-sensitive asthma. A positive correlation ($r = 0.636$, $p < 0.001$) was observed.

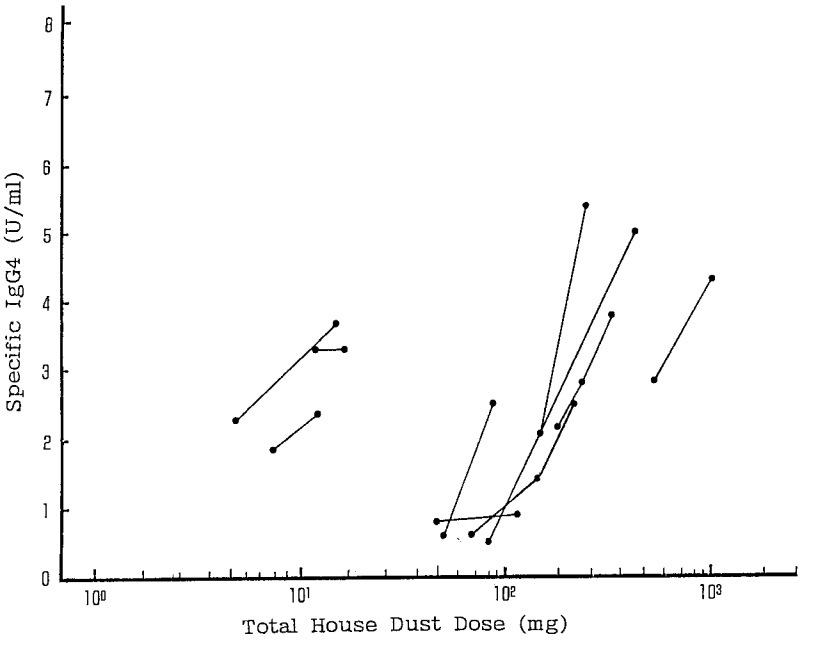

Fig. 5 Changes in specific IgG4 levels in several child patients.

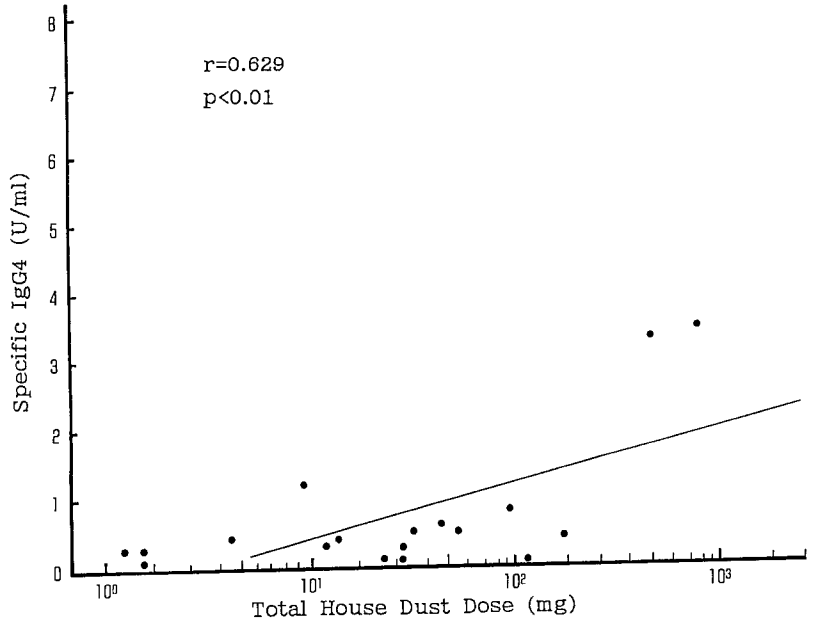

Fig. 6 Correlation between total dose of house dust and specific IgG4 in adult patients with mite-sensitive asthma. A positive correlation ($r = 0.629$, $p < 0.01$) was observed.

10). Cooke *et al.* demonstrated the presence of a serum factor capable of inhibiting the Prausnitz-Küstner response in hyposensitized patients and termed it a blocking antibody (11). This antibody was later found to be specific IgG for administered allergens (12). On the other hand, IgG, which participates in immediate allergic reactions, was first described by Parish (19), showing that IgG could act as short-term sensitizing antibodies. Since then, IgG4 antibodies have been noticed as an allergic antibody (13, 14). However, no evidence of the participation of IgG4 in allergen-triggered histamine release and anti-IgG 4 induced release of histamine have been reported (20, 21). There are several reports showing that IgG4 could function as a blocking antibody in natural exposure and hyposensitization treatment (15, 22, 23). The question as to whether IgG4 is an allergic or a blocking antibody, or both still remains to be clarified.

The present study showed that mite specific IgG4 was a useful immunological markers in the immunotherapy of mite-sensitive bronchial asthma patients. However, total mite specific IgG did not show a significant increase in the course of hyposensitization. Therefore, we postulate that IgG4 subclass antibody might play a major role as a blocking antibody in the total specific IgG.

Recent studies using an immunoblotting method revealed major allergens of *Dermatophagoides* species. Specific IgE was frequently found to be directed against *Der* I with a molecular weight of 24,000 and *Der* II with a molecular weight of 15,000 (2, 3, 24). In order to determine IgG4 specific molecules in the mite extracts we are coducting further investigations via immunoblotting.

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