

Changes in the levels of house dust mite specific IgG₄ during immunotherapy in asthmatic children

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Summary

Serum levels of IgG subclass and house dust mite (*Dermatophagoides pteronyssinus*, *Dpt*) specific IgG₄ were evaluated during immunotherapy in asthmatic children. Asthmatic children undergoing long-term immunotherapy (more than 2 years) posed a mean value of total serum IgG₄ or *Dpt*-specific IgG₄ antibodies significantly higher than that of patients prior to receiving immunotherapy, asthmatic (placebo) controls, or patients undergoing short-term immunotherapy (less than 1 year) ($P < 0.05$). The mean levels of serum *Dpt*-specific IgG₄ in all asthmatic groups were also significantly higher than in the non-allergic controls ($P < 0.01$). Moreover, the mean level of *Dpt*-specific IgG₄ tended to increase during immunotherapy. A significant correlation between total serum IgG₄ and *Dpt*-specific IgG₄ antibodies was noted ($r = 0.6243$; $P < 0.001$). Serial follow-up reveals that *Dpt*-specific IgG₄ levels usually rose significantly with clinical improvement in asthmatic children during immunotherapy. These results suggest that the anti-mite-specific IgG₄ antibody may serve as an indicator for clinical outcome of mite allergy during immunotherapy.

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Introduction

House dust mite, *Dermatophagoides pteronyssinus* (*Dpt*) or *D. farinae*, has been identified as one of the main sources of house dust allergen and has been shown to play an important allergenic role in asthmatic children in many countries [1–3] as well as in Taiwan [4–6]. There are reports of clinical improvement with routine immunotherapy with house dust extracts [7,8]. Although the beneficial effects of immunotherapy on allergic asthma have remained unproven, clinical improvement has been reported by several studies [7–11].

The immunological changes of allergic disease during immunotherapy indicate that there is a decrease in specific serum IgE antibodies to pollen [12–15] or house dust [16,17] and a rise in specific IgG antibodies [10,15,18,19] that are called 'blocking antibodies'. Immunologically, IgG₄ antibodies may compete for sites on the surface of tissue mast cells and reduce the efficiency of IgE allergen binding. IgG₄ may also compete physically with IgE for circulating allergen, thus reducing the amount of

allergen actually coming in contact with mast cell surface bound IgE antibody. Although IgG₄ does not precipitate antigen well due to lack of interaction with the complement system, the specific IgG₄ in allergic disease may be important in acting as an allergen-neutralizing blocking antibody which compete with the anaphylactic antibodies to antigen [20], thereby having a favourable effect on disease [21,22]. Therefore, the measurement of total IgG₄ and allergen-specific IgG₄ antibody may be useful in monitoring or evaluating therapeutic effects of hypo-sensitization.

The purpose of this study was to determine the change of IgG subclasses and specific IgG₄ antibody against *Dpt* during immunotherapy in house-dust-sensitive asthmatic children.

Subjects and methods

Subjects

This study included 98 asthmatic children (aged 5–13 years, mean 9.3) sensitive to house dust or house dust mites, who had attended the paediatric outpatient Allergy Clinic of the Veterans General Hospital in Taipei.

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Table 1. Serum IgG subclass concentration in the asthmatic children during immunotherapy

IgG subclass	Non-allergic control (n = 15)	Patients prior to receiving immunotherapy (n = 17)	Receiving immunotherapy			Placebo control (n = 15)
			1 year (n = 20)	2 years (n = 21)	3 years (n = 25)	
IgG ₁	8.36 ± 2.59	7.10 ± 1.52	7.91 ± 1.05	8.53 ± 1.96	6.84 ± 1.20	7.83 ± 2.67
IgG ₂	3.16 ± 2.55	2.96 ± 2.11	1.92 ± 1.67	1.89 ± 1.64	2.64 ± 2.33	2.45 ± 1.79
IgG ₃	1.63 ± 1.80	1.29 ± 1.21	0.81 ± 0.66	1.27 ± 1.38	1.58 ± 1.55	1.34 ± 1.16
IgG ₄	0.91 ± 0.87	0.65 ± 0.36	0.52 ± 0.24	1.49 ± 1.08*	1.36 ± 1.20*	0.68 ± 0.31

Mean ± s.d. values (mg/ml) of serum IgG subclass.

*Significant difference ($P < 0.05$) was noted as compared with the asthmatics prior to immunotherapy, patients after 1 year of immunotherapy, or asthmatic controls.

Table 2. *Dpt*-specific IgG₄ antibody titre in the asthmatic children during immunotherapy

Non-allergic controls (n = 15)	Patients prior to receiving immunotherapy (n = 17)	Receiving immunotherapy			Placebo control (n = 15)
		1 year (n = 20)	2 years (n = 21)	3 years (n = 25)	
0.138 ± 0.04	0.309 ± 0.192	0.29 ± 0.156	0.47 ± 0.194*	0.496 ± 0.243*	0.316 ± 0.174

Mean ± s.d. values (mg/ml) of serum *Dpt*-specific IgG₄ antibody titre was reading from the value of the optical density at 414 nm in ELISA assay.

*Significant difference ($P < 0.05$) was noted, as compared with the patients after 1 year of immunotherapy, or with pretreated or placebo controls.

Patients were diagnosed on the basis of history, skin test and specific IgE antibody determination. They all had a well-documented history of asthma assessed by symptoms and medication, and the treated patients were randomly chosen to receive therapy of varying duration and to collect venous blood at the end of the course of immunotherapy. There were 17 asthmatic children whose blood was collected only before beginning a course of immunotherapy as untreated controls. Blood samples were drawn from each treated group 1 year, 2, or 3 years after the course of immunotherapy. In the placebo control group, 15 asthmatic patients did not receive immunotherapy but had also attended the paediatric outpatient Allergy Clinic for 1–3 years of medication. Another group of 15 normal children of both sexes in the same age group were chosen as normal (non-allergic) controls. House dusts were collected by vacuum cleaner. The average number of mites/g of house dust in the family couch and bedroom floor from patients' houses was 390 ± 361 (mean ± s.d.) and 585 ± 462 in the Taipei area, respectively [24]. There were approximately 10 mites in 1

ml of house dust extract. Immunotherapy consisted of weekly injections of 0.1 ml of house dust extract (Tawian Allergy Center, Taipei). The dose was given continuously for 6 weeks for each of four concentrations: 0.1 µg, 1 µg, 10 µg and 100 µg of protein content. After 24 weeks of immunotherapy, injections of 100 µg were given once a month until the clinical condition improved. For at least 2 weeks before blood was drawn, none of the patients were given inhaled steroids even for moderate-to-severe asthma. The parameter of the clinical improvement was judged by the frequency of attacks and medication used. Sera were separated and stored at -70°C . All serum samples were tested simultaneously.

Allergen extracts

Extraction of the *Dpt* allergens (Allergon, Engelholm, Sweden) was performed as previously described [24]. Briefly, 1 g of mites was mixed with 100 ml of cold phosphate-buffered saline (PBS, 0.01 M, pH 7.2) and stirred for 48 hr at 4°C . The mixture was then centrifuged for 1 hr

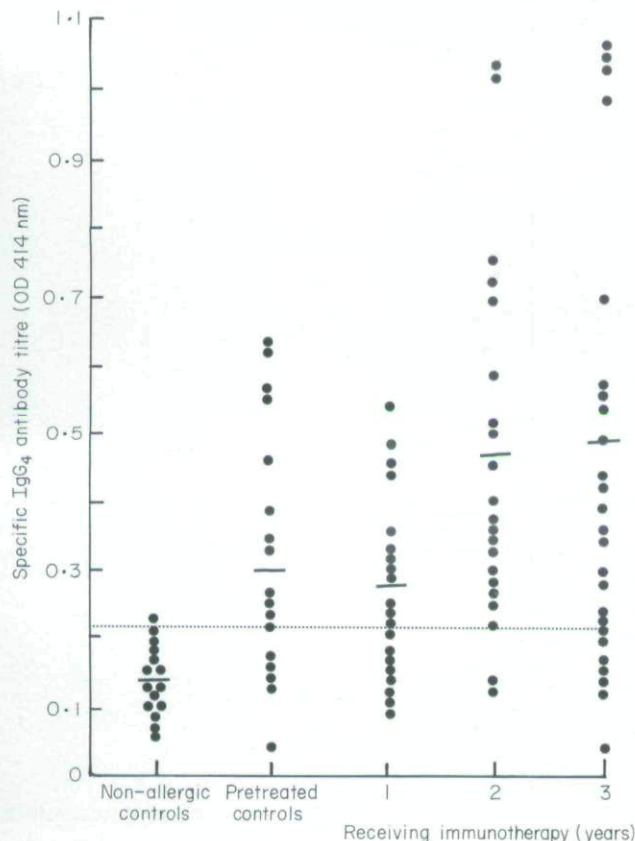


Fig. 1. Changes of specific IgG₄ antibody levels to *D. pteronyssinus* in house-dust-sensitive asthmatic children during hypo-sensitisation.

at 12 000 r.p.m. and the supernatant was dialysed against distilled water for 48 hr at 4°C. The crude extract was filtered through a 0.45 µm-millipore filter and then lyophilized and stored at 4°C. The stocked extracts were assayed by a protein assay kit (BioRad, Richmond, CA), and a final concentration of 2 mg/ml of protein was used in ELISA. The immunochemical characteristics of mite extract were analysed by polyacrylamide gel electrophoresis and autoradiography [5]. The molecular weight of the most apparent protein of *Dpt* was 10–35 kD, and the major allergens were 15.8 and 27–35 kD.

IgG subclasses

A monoclonal ELISA kit (Biomakor, Rehovot, Israel) was used quantitatively to determine the human IgG subclasses in the serum of asthmatic children. The procedure was performed according to the manufacturer's instructions. Briefly, antibody to human IgG in coating solution (100 µl) was coated onto each microtitre plate well and incubated overnight at 37°C. After washing, 100 µl of diluted serum samples (1/20 000, 1/40 000

and 1/80 000) and reference sera were incubated at room temperature for 2 hr in the microtitre plate. Alkaline-phosphatase-conjugated monoclonal antibodies to human IgG₁, IgG₂, IgG₃ or IgG₄ were then added to the washed plate and incubated again. Fifty microlitres of the enzyme substrate *P*-nitrophenyl phosphate were then added to react with the alkaline phosphatase for 30 min. Another 50 µl of 3 M NaOH solution was added to stop the reaction and optical density (OD) was read at 405 nm in an ELISA reader (Flow Multiskan Spectrophotometer, Flow Lab., Irvine, U.K.). Each sample was assayed in duplicate.

IgG₄ ELISA

In a previous study we established a calibration for the measurement of IgE antibody levels to mite allergen [24]. A modified technique was employed to determine the mite-specific IgG₄ antibodies in this study. Microtitre plates were coated with 50 µl (0.1 µg of protein content) of *Dpt* extract per well in 0.1 M sodium carbonate buffer (pH 9.6) overnight at 4°C. After washing, a 250-µl aliquots of 1% bovine serum albumin in PBS (BSA-PBS) was used for blocking at 37°C for 1 hr and the plates were then washed with PBS-Tween solution (0.05% Tween in PBS, pH 7.0). Fifty microlitres of undiluted serum were added to the wells and incubated at 37°C for 3 hr. After washing, 50 µl of diluted peroxidase-conjugated murine monoclonal anti-human IgG₄ (Janssen Biochemica, Olen, Belgium) in 0.1% BSA-PBS was added and incubated for a further 2 hr. Then 50 µl (0.68 mg/ml) of ABTS (2,2'-Azinobis(3-ethylbenz-thiazoline sulphonic acid)) substrate in citrated buffer (pH 4.2) containing 0.018% H₂O₂ were added to each well and incubated for 30 min at room temperature in darkness. Optical density was measure at 414 nm by an ELISA reader. To obtain the background reading, BSA-PBS was added instead of the serum sample. Each sample was also assayed in triplicate and the background reading subtracted.

Statistical analysis

Two-way chi square analysis was used to analyse differences in distribution. The significant levels of difference between mean values were determined by Student's *t*-test.

Results

The IgG subclass concentration in asthmatic children who had undergone a course of immunotherapy, in non-allergic children and in asthmatic controls are listed in Table 1. After 1 year, no significant difference in the mean value of the total serum IgG₁, IgG₂, IgG₃ and IgG₄ levels

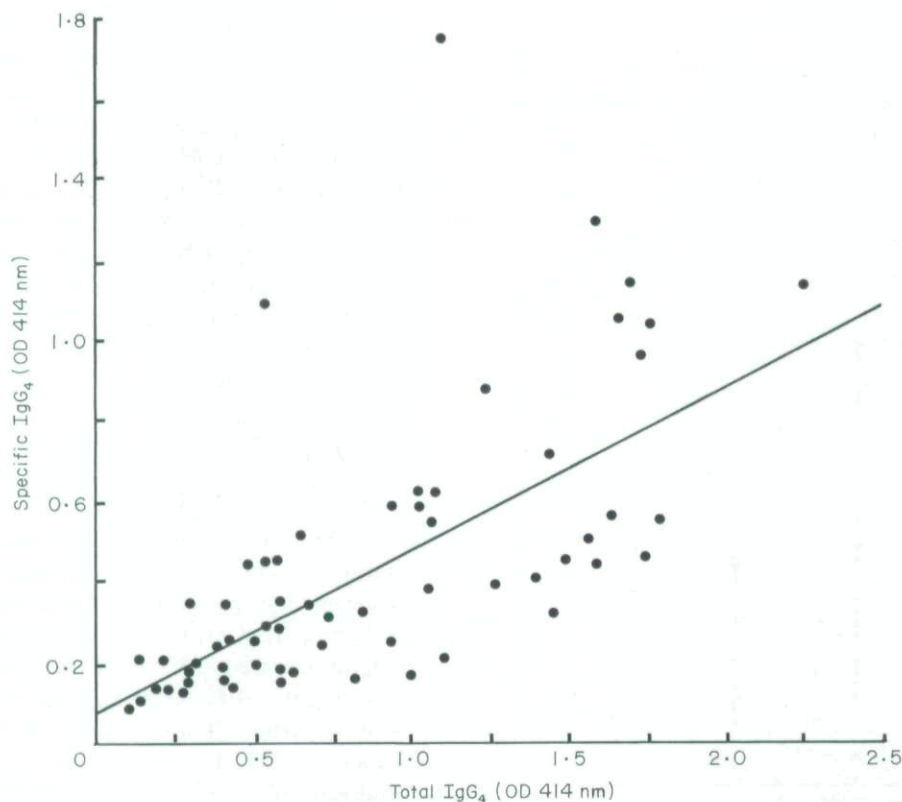


Fig. 2. Relationship between total serum IgG₄ level and the IgG₄ antibody titre specific to *D. pteronyssinus* in house-dust-sensitive asthmatic children.

was found between non-allergic controls and pretreated or placebo controls, or in patients who had had 1 year of immunotherapy ($P > 0.1$). However, there was a significantly higher value of total IgG₄ after 2 years (1.49 ± 0.18 , mean $\pm$ s.d. OD) and 3 years (1.36 ± 1.20) of immunotherapy than after 1 year of immunotherapy (0.52 ± 0.24) ($P < 0.02$), or in previously treated (0.65 ± 0.36) or placebo controls (0.68 ± 0.31) ($P < 0.05$). All samples were run simultaneously. The coefficient of variation of intra-assay for IgG subclass levels in patient serum was lower than 9.6%.

Table 2 and Fig. 1 shows the mean values and the distribution of *Dpt*-specific IgG₄ antibody titre in the asthmatic children. These results showed that the serum levels of specific IgG₄ in all groups of asthmatic children was significantly higher than in non-allergic controls ($P < 0.01$). However, the mean value of *Dpt*-specific IgG₄ tended to increase during immunotherapy. There were significantly higher mean levels in asthmatics after 2 or 3 years of immunotherapy than in patients after 1 year of immunotherapy, or in pretreated or placebo controls ($P < 0.05$). The significant correlation between the total IgG₄ and the IgG₄ antibody specific to *Dpt* in 62 asthmatics is shown in Fig. 2 ($P < 0.001$). The correlation coefficient was 0.6243.

Ten house dust sensitive asthmatic children receiving immunotherapy had serial determinations of their *Dpt*-specific IgG₄ antibody (Fig. 3). Two of these patients showed no significant change on *Dpt*-specific IgG₄ levels during immunotherapy. However, eight out of ten patients who received 2 or 3 years of immunotherapy had significantly elevated levels of the specific IgG₄ antibody. In addition, judging by the frequency of asthmatic attacks and medication used, clinical improvement and reduction of allergic symptoms were noted in 43 out of 46 (95.6%) patients after 2 or 3 years of immunotherapy.

The coefficient of variation of intra- and inter-assay for specific IgG₄ level in patients' serum was 4.0% (0.258 ± 0.011) and 6.2% (0.262 ± 0.016), respectively.

Discussion

Immunotherapy with a specific allergen has been shown to be effective in the treatment of respiratory allergy [7–11,25]. Mechanisms for the beneficial effect of immunotherapy, such as reduction in serum specific IgE antibody [12,16], decreased reactivity of target cells to the antigen [26], decreased sensitivity of basophils to antigen [17] and formation of blocking antibody, have been suggested [11,21,22,27].

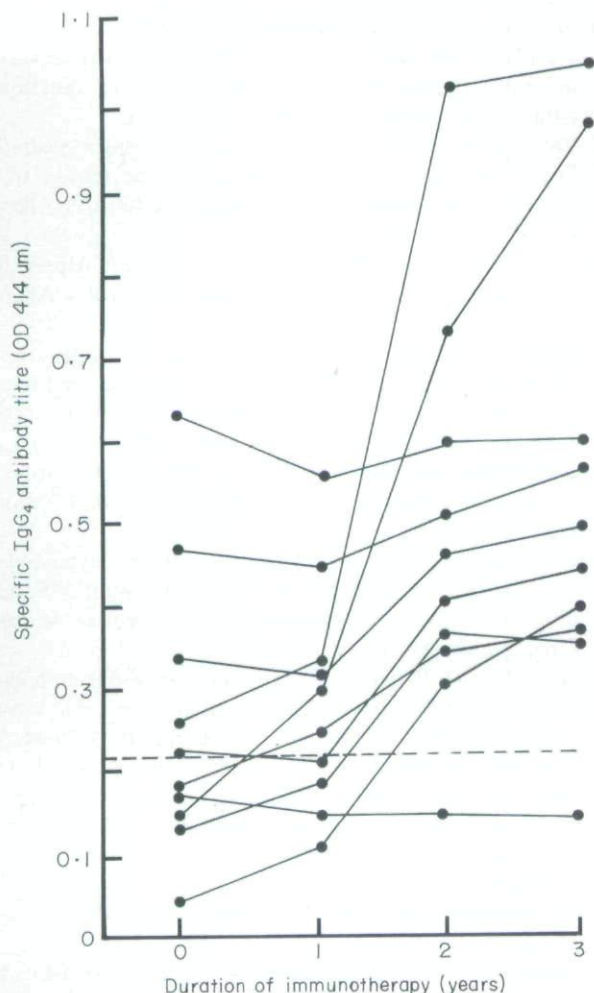


Fig. 3. Follow-up study of the IgG₄ antibody specific to *D. pteronyssinus* in house-dust-sensitive asthmatic children during hypsensitization.

D. pteronyssinus is one of the main components of house dust and is a major allergen associated with asthma, allergic rhinitis and atopic eczema in Europe, Japan and Australia [3] as well as in Taiwan [4,5]. Immunotherapy using extracts of the house dust in various institutions in Taiwan has resulted in immunological changes with varying degrees of effectiveness. It has been suggested that allergen-specific IgG₄ antibody levels provide a rough indication of protection against symptoms [11,14]. In another report, studies of the subclasses of the IgG antibody response during 2 years of immunotherapy with timothy grass pollen suggested that only IgG₁ and IgG₄ responses were induced [19]. IgG₄ has been suggested as a marker of allergy because of vast high IgG₄ antibody response to the relevant allergens in the vast majority of patients who were successfully treated by immunotherapy, without a significant increase on IgG₁,

IgG₂ and IgG₃ levels [22,28,29]. It has also been suggested that the specific IgG₄ antibodies may act as blocking antibodies in IgE-mediated immediate allergic reactions [21,30]. The data presented here indicate that in asthmatic children not only the total IgG₄ level but also the *Dpt*-specific IgG₄ antibody titre increased following immunotherapy. Although some reports have shown that high IgG₄ levels in patients who have undergone immunotherapy are not associated with clinical efficacy [31,32], others have indicated the importance of IgG₄ antibodies associated with a decrease in synthesis of IgE antibodies to insect venom [33]. However, the reduction of IgE antibodies to mite allergens in our previous study [16] and the elevation of IgG₄ blocking antibodies during hypsensitization have been mentioned [8,22]. The elevation of specific IgG₄ in our present data showed that the specific IgG₄ antibodies may act as blocking antibodies that produce favourable effects following immunotherapy. Theoretically, IgG₄ antibodies may compete with IgE antibodies at sites on the surface of tissue mast cells which may reduce the amount of histamine released by a mast cell and therefore reduce allergic symptoms.

A previous study undertaken by us also shows that there is a positive correlation between total serum IgE and IgE antibody specific to mite allergens in asthmatic children [24]. In the present study we have observed that total serum IgG₄ levels and *Dpt*-specific IgG₄ antibody titres are related ($r=0.6243$; $P<0.001$). Asthmatic symptoms were reduced in most patients (95.6%) after 2 years of immunotherapy. The increasing levels of *Dpt*-specific IgG₄ also correlate with clinical improvement in eight out of 10 asthmatic children after receiving immunotherapy in a follow-up study (Fig. 3). Therefore, specific IgG₄ antibody may be a valuable marker of allergy [22,28] and clinical improvement of allergic asthma during immunotherapy.

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