

The Changes of Mite-Specific Immunoglobulin A and Transforming Growth Factor- β 1 During Immunotherapy of Children with Dust Mite Allergy

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ABSTRACT

Immunoglobulin A (IgA) is the most important immunoglobulin of the mucosa. It can protect the mucosal surface from invasion by foreign pathogens. However, only a few studies have mentioned the changes of allergen-specific IgA after immunotherapy in patients with bronchial asthma. In this study, we sought to investigate the levels of mite-specific IgA, IgE, and IgG4 as well as total IgE, IgG, IgA, and IgM, before and after immunotherapy. Serum levels of transforming growth factor β 1 (TGF- β 1), the major cytokine secreted by type 3 T helper (T_H3) cell and a contributing factor for IgA isotype switch, was also assayed. A total of 19 patients were enrolled in this study. The serum levels of total IgG, IgA, and IgM were measured by rate nephelometry. Total IgE, *Dermatophagoides pteronyssinus* (Der-p), and *Dermatophagoides farinae* (Der-f)-specific IgE, and mite-specific IgG4 were determined with commercial radioallergosorbent test (RAST) system. Mite-specific IgA and TGF- β 1 were measured by enzyme-linked immunosorbent assay (ELISA). The results showed a significant increase of mite-specific IgA and IgG4 mean of 7 months after immunotherapy ($p < 0.05$). TGF- β 1 decreases significantly ($p < 0.05$). There were no significant differences in total IgG, IgE, IgA, and IgM or mite-specific IgE after immunotherapy ($p > 0.05$). We hypothesized that the increased allergen-specific IgA and IgG4 might work as blocking antibodies at the mucosa and in serum, respectively, after immunotherapy. It might prevent the allergen from penetrating the mucosa or from competing with allergen specific IgE. However, further studies are needed to clarify the role of allergen-specific IgA and TGF- β in the working mechanisms of immunotherapy. (Pediatr Asthma Allergy Immunol 2003;16[1]: 15–22.)

INTRODUCTION

HYPOSENSITIZATION WITH ALLERGEN EXTRACT has been widely accepted for immunoglobulin (Ig) E-mediated inhalant allergy. The clinical efficacy has been confirmed in several studies.^{1–8} Various immunologic changes of allergen-specific immunotherapy have been documented. These include development of blocking antibodies, auto-anti-idiotypic antibodies and allergen-specific suppressor cells, decrease in al-

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lergen-specific IgE, and seasonal rise in IgE, and change of cytokine pattern.^{9,10} However, the precise mechanisms of hyposensitization are still not well defined.

Of these hypothesized mechanisms, more attention has been focused on the role of allergen-specific IgG and IgE during the past decades.^{11–14} The previous studies revealed that allergen-specific IgE increased initially, followed by gradual decrease after several years of immunotherapy.^{11–13} The levels of allergen-specific IgG₄ also increased considerably.^{14–15} It was thought to be the blocking antibody^{9,11} and considered as one of the mechanisms that makes immunotherapy work. Likewise, an increase of allergen-specific IgG and IgA in the nasal secretion after immunotherapy has been reported among patients with allergic rhinitis.¹⁶

Some investigators discovered the prevalence of allergic disease to be much higher in IgA-deficient children than in the total population.^{17–20} They proposed that more allergens could reach the lymphoid tissues in IgA-deficient patient, resulting in more synthesis of IgE. They speculated that reduced opsonization and phagocytosis of allergen might be the possible pathogenesis.²¹ The change of IgA antibody, a major immunoglobulin of mucosa, is seldom investigated among patients of bronchial asthma who received immunotherapy. Transforming growth factor β (TGF- β) is the major cytokine secreted by lymphocytes, macrophages, platelets, bones, kidney cells, and type 3 T helper (T_H3) cells. T_H3 cells are believed to play a critical role in mucosal immunity and contribute to IgA isotype switch.²² We wanted to clarify the relation between the serum level of allergen-specific IgA and TGF- β 1 after immunotherapy.

In this study, we investigated immunologic changes including mite-specific IgA, IgE, and IgG₄, and total IgG, IgA, IgM, IgE, and TGF- β 1 during immunotherapy. If the serum level of mite-specific IgA increased after immunotherapy, further studies are needed to correlate the mite-specific IgA level between serum and bronchoalveolar lavage. The role of allergen-specific IgA must be clarified further. It may be important in defense mechanisms of allergic diseases, especially at the mucosal level, and contribute to the therapeutic effect of allergen-specific immunotherapy.

PATIENTS AND METHODS

Patients

Nineteen patients were recruited from the Allergy Clinic, Department of Pediatrics of the National Taiwan University Hospital. All had a history of bronchial asthma with or without allergic rhinitis and received immunotherapy for house dust mite (HDM). All of the children with asthma included in the study were of moderate to severe persistent severity. All of the patients were sensitive to mites, *Dermatophagoides pteronyssinus* (Der-p), and *Dermatophagoides farinae* (Der-f), with a positive history of exposure, a skin test response greater than 2+ (wheal > 10 mm by intracutaneous route with mite extract at a concentration of 10^{-6} μ g/mL), and a positive mite radioallergosorbent test (RAST; Pharmacia Diagnostics, Uppsala, Sweden). Blood was collected before immunotherapy and 5–11 months (mean, 7.5 months) after therapy. All of the sera were separated and stored at -70°C until use.

Reagents

The allergen was prepared as described by Chiang et al.²³ Briefly, 1 g of lyophilized mite body was defatted with 100 mL of ether, then homogenized and stirred continuously in 100 mL of phosphate-buffered saline for 36 hours. After centrifugation ($10,000 \times g$), the crude extract was filtered through a $0.22\text{-}\mu\text{m}$ membrane, lyophilized, and stored at -70°C until use. Before use, the lyophilized extract was reconstituted in distilled water and the protein concentration of mite antigens was determined before use.

Serum levels of total IgG, IgA, IgM, and IgE antibodies

The serum levels of total IgG, IgA, and IgM were measured by Rate Nephelometry (Array Protein System, Beckman, Fullerton, CA) and expressed as micrograms per deciliter. Total IgE were determined with commercial RAST (CAP system, Pharmacia Diagnostics) and expressed as kilounit/liter (KU/l).

Serum levels of HDM-specific IgE, IgG4, and IgA antibodies

The Der-p and Der-f specific IgE were determined with commercial CAP system (Pharmacia Diagnostics) and expressed as KU/l. Mite-specific IgG4 was also determined with commercial CAP system (Pharmacia Diagnostics) and expressed as micrograms per liter. Serum HDM-specific IgA was measured by enzyme-linked immunosorbent assays (ELISA) and expressed as nanograms per milliliter. Briefly, microtiter ELISA plates (Costar-Corporation, Cambridge, MA) were coated with mouse anti-human IgA1/IgA2 monoclonal antibody (PharMingen San Diego, CA) 100 μ L/well at 5 μ g/mL in carbonated buffer (0.1 M NaHCO₃, pH 9.6) for standard curve evaluation, and with HDM 100 μ L/well at 25 μ g/mL for patients' sera determination overnight at 4°C. Each well was aspirated and washed with phosphate-buffered saline with 0.05% Tween 20 (PBST). Each well was blocked with 200 μ L blocking solution (PBS containing 5% Tween, 5% sucrose, and 0.05% NaN₃) for 2 hours at room temperature. Thereafter, each well was aspirated and washed with PBST; then, serial dilution of standard sample (purified human IgA, reagent grade, Sigma, Saint Louis, MO) on anti-human IgA-coated plates and serum sample (1:100 dilution) on HDM-coated plates were added overnight at 4°C. After aspiration and washing with PBST, each well was filled with biotin-conjugated mouse anti-human IgA1/IgA2 monoclonal antibody (PharMingen, San Diego, CA) 200 μ L/well at 2 μ g/mL and incubated at room temperature for 2 hours. After repeating the aspiration and wash as above, Extra-Avidin Peroxidase (Sigma, St. Louis, MO) was added and the plates were incubated at room temperature for 2 hours, then washed as in previous steps. Substrate buffer of 2,2'-azino-bis-3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) was added and absorbance was read at 420 nm on an ELISA reader (Anthos 2010, Anthos Labted Instrument, Ges.M.b.H, Salzburg, Austria). A range of standard samples from 40 ng/mL to 0.625 ng/mL was included on each assay plate. The standard curve calculated from this and the concentration of serum HDM-specific IgA was calculated by reference to this standard curve.

Serum level of TGF- β 1

Serum TGF- β 1 level was also checked by ELISA. Costar E.I.A/R.I.A microtiter ELISA plates were coated with mouse monoclonal anti-human TGF- β 1 (R&D Systems Inc., Minneapolis, MN) 100 μ L/well at 4 μ g/mL in carbonated buffer overnight at 4°C. Each well was aspirated and washed with PBST, then blocked with 300 μ L of blocking solution for 2 hours at room temperature. Thereafter, each well was aspirated and washed with PBST; then serum samples and serial dilution of standard samples (recombinant human TGF- β 1, R&D Systems Inc.) were added and incubated for 2 hours at room temperature. After aspiration and washing with PBST, each well was filled with chicken biotinylated anti-human TGF- β 1 (R&D Systems Inc. MN) 200 μ L at 100 ng/mL and incubated for 2 hours at room temperature. After repeating the aspiration and wash as above, ExtraAvidin Peroxidase (Sigma) was added and the plates were incubated at room temperature for 30 minutes, then washed as in previous steps. Substrate buffer (o-phenylenediamine dihydrochloride, Sigma) was added to each well and the plate was incubated at room temperature for 30 minutes. Absorbance was read at 420 nm on an ELISA reader. A range of standard samples from 2000 ng/mL to 16.125 ng/mL was included on each assay plate and the standard curve was calculated from this. The concentration of serum TGF- β 1 was calculated by reference to the standard curve. Because TGF- β is secreted in a biologically inactive form as TGF- β -latency associated peptide, *in vitro* activation can be achieved by transient acidification. The procedure of acidification was described in the previous study by Lin et al.²⁴

TABLE 1. IMMUNOLOGIC PROFILE OF PATIENTS WITH ASTHMA AND/OR ALLERGIC RHINITIS BEFORE AND AFTER IMMUNOLOGY

	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (KU/l)
Pre-IT	953.6 $\pm$ 234.3	181.3 $\pm$ 72.9	137.9 $\pm$ 76.0	1826.9 $\pm$ 1745.1
Post-IT	916.4 $\pm$ 250.0	181.0 $\pm$ 75.0	122.6 $\pm$ 53.1	2069.8 $\pm$ 2189.0
p value	0.14	0.20	0.23	0.14

Ig, immunoglobulin; KU/l, kilounit/liter; IT, immunotherapy.

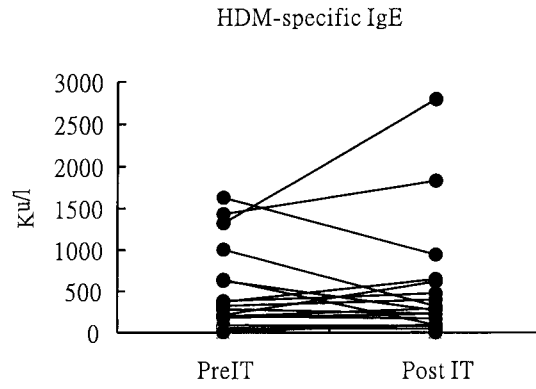
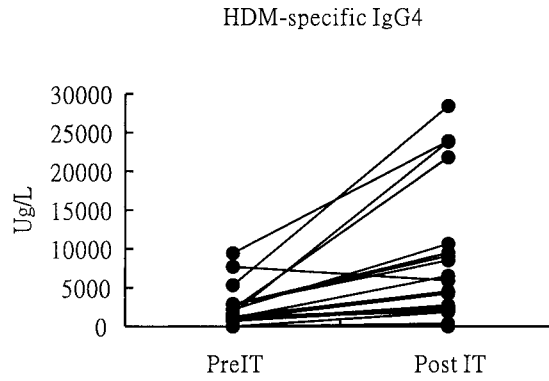
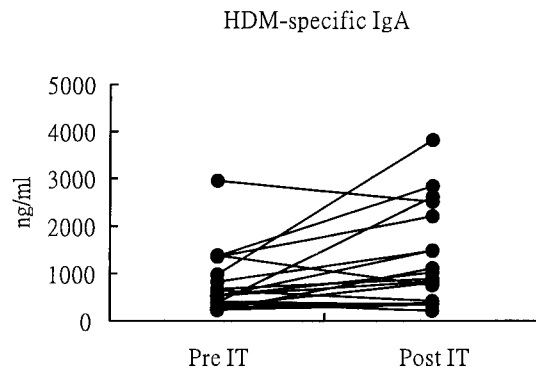
A**B****C**

FIG. 1. Serum levels of house dust mite (HDM)-specific immunoglobulin (Ig) E, IgG4, and IgA antibodies. **A.** There were no significant differences in sera level of HDM-specific IgE after immunotherapy ($p = 0.71$). **B.** The serum level of HDM-specific IgG4 increased significantly after immunotherapy ($p = 0.0005$). **C.** The serum level of HDM-specific IgA increased significantly after immunotherapy ($p = 0.02$).

Statistical analysis

The data are expressed by mean $\pm$ standard deviation (SD). The serum levels of all immunologic profiles before immunotherapy were compared to those after immunotherapy through the Wilcoxon matched pairs test. A p value of less than 0.05 is considered as statistically significant.

RESULTS*Patients*

Nineteen asthma patients with or without allergic rhinitis that is sensitive to HDM were enrolled. There were 5 girls and 14 boys. The mean age of the patients was 9.1 years, with a range of 5–22 years. All patients received immunotherapy of crude mites for an average of 7.5 months (range, 5–11 months).

Serum level of total IgG, IgA, IgM, and IgE antibodies

Data suggested there were no significant differences in sera level of total IgA, IgG, IgM, and IgE after immunotherapy (Table 1).

Serum level of HDM-specific IgE, IgG4, and IgA antibodies

Data showed that there were no significant differences in sera level of HDM-specific IgE after immunotherapy (Fig. 1A).

Before treatment, the level of HDM-specific IgG4 was $2322.1 \pm 2565.8 \mu\text{g/L}$ (mean $\pm$ SD). After immunotherapy, the level increased significantly to $8864.0 \pm 8894.5 \mu\text{g/L}$ ($p = 0.00046$) (Fig. 1B).

Before treatment, the level of HDM-specific IgA antibodies was $759.0 \pm 652.9 \text{ ng/mL}$ and it increased significantly after immunotherapy to $1283.5 \pm 1035.6 \text{ ng/mL}$ ($p = 0.02$) (Fig. 1C).

Serum level of TGF- β 1

Before treatment, the serum level of TGF- β 1 was $86.5 \pm 44.2 \text{ ng/mL}$. It decreased significantly after treatment to $59.4 \pm 41.2 \text{ ng/mL}$ ($p = 0.02$) (Fig. 2).

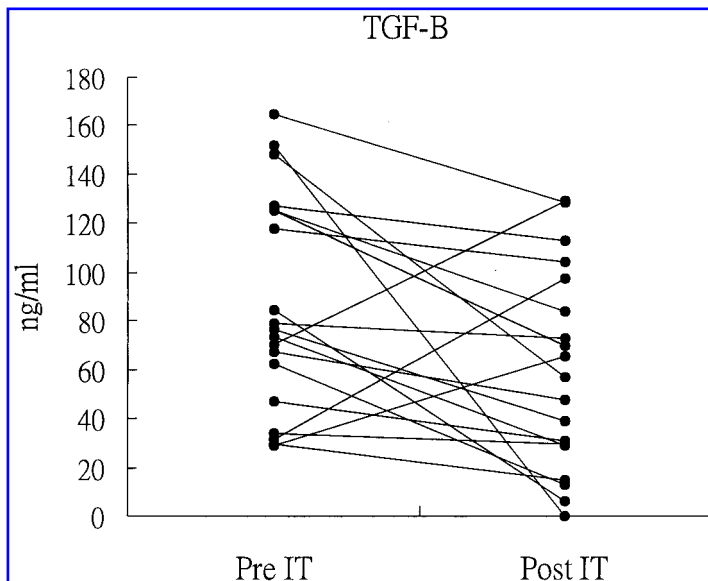


FIG. 2. The serum level of transforming growth factor (TGF)- β decreased significantly after immunotherapy ($p = 0.02$).

DISCUSSION

The results of our study revealed that the serum level of mite-specific IgA increased without significant change in total IgA after immunotherapy. The previous results also suggested that allergen-specific IgA increased in sera or in nasal secretions after immunotherapy of patients with allergic rhinitis.^{16,25} Sparholt et al.²⁶ demonstrated a significant rise in specific IgM- and IgA-secreting cells after immunotherapy of asthma patients. It has been suggested that the protective function of these partly differentiated plasma cells is mediated by allergen-specific secretory IgA. These results are consistent with ours. We speculated that allergen-specific IgA might work as blocking antibodies in respiratory mucosa. It could decrease the amount of allergen that reaches the regional lymphoid tissue via the airway. However, this hypothesis still has to be verified. Because serum levels cannot reflect the real amount of these lung antibodies, further studies are needed to demonstrate secretory IgA in bronchio-alveolar lavage of asthmatic patients after immunotherapy. Moreover, future studies should correlate the increase in allergen-specific IgA after immunotherapy to clinical symptoms.

TGF- β 1 is a cytokine secreted by lymphocytes, macrophages, platelets, bones, kidney cells, and T_H3 cells. It helps the IgA class to switch and suppress T_H1 and T_H2 cells.²⁷ Previous studies implicated TGF- β to be involved in airway remodeling of patients with asthma.^{28,29} They found that basal concentration of TGF- β 1 was significantly increased in patients with asthma than in control subjects.²⁸ A significant correlation was found between the number of epithelial or submucosal cells expressing TGF- β and the basement membrane thickness and fibroblast number in both asthma and chronic bronchitis.²⁹ Our results demonstrate a significant decrease in serum TGF- β 1 in contrast to the increase of allergen-specific IgA after immunotherapy. We hypothesized that a decrease in serum TGF- β 1 may lead to reduced airway remodeling. However, many confounding factors could affect the serum level of TGF- β 1. Thus, the serum level of TGF- β 1 could not stand for the real condition of airway. Further studies to evaluate the intracellular TGF- β level of mite specific T cell clones or the *in situ* hybridization for TGF- β in bronchial mucosal biopsies may be specified in order to define the change of cytokine in lungs.

The serum mite-specific IgG4 antibody level increased and the mite-specific IgE remained the same after immunotherapy for 5–11 months. This is consistent with the results of several other groups.^{30–32} Many studies have suggested that immunotherapy induces an increase in IgG4 allergen-specific antibodies because this was important for the relief of symptoms. In contrast, the levels of IgE allergen-specific antibody level were similar before and after immunotherapy.

According to the results of our study, there was an increase of mite-specific IgA and IgG₄ and a decrease of TGF- β 1 in serum after treatment. We hypothesize that the mite-specific IgA and IgG₄ work as blocking antibodies in the mucosa and serum, respectively. It might prevent the allergen from penetrating the mucosa or competing with allergen-specific IgE. However, further studies are needed to verify whether the decreased TGF- β 1 is related to the lesser degree of airway remodeling. Our results revealed a possible mechanism of mite-specific immunotherapy although the immune response may differ with other antigens.

In conclusion, the data here demonstrate that both allergen-specific IgA and IgG₄ increased significantly after 7 months of immunotherapy. The increase in allergen-specific IgA may be one of the possible mechanisms for the effectiveness of immunotherapy. However, the TGF- β 1 level was not correlated with the change in mite-specific IgA antibody. A further study including secretory IgA of bronchio-alveolar lavage in asthma patients, intracellular TGF- β level of mite-specific T cell clones, and the correlation between symptoms and the immunologic profile after immunotherapy are needed to clarify the possible mechanism of immunotherapy.

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