

Immunologic changes during immunotherapy in asthmatic children: increased IL-13 and allergen-specific IgG4 antibody levels

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Background: The prevalence of allergic diseases such as asthma, allergic rhinitis, and atopic diseases has increased in recent years. Immunotherapy with allergens is a treatment documented to have an effect on regulating cytokine production and allergen-specific antibody production.

Objective: The aim of this study was to further investigate immunologic changes during immunotherapy and to explore the possible more efficient approach of immunotherapy.

Methods: Asthmatic children receiving house dust mite immunotherapy were followed to learn immunologic parameters such as allergen-specific antibody levels, proliferative response of peripheral blood mononuclear cells, and cytokine change during immunotherapy.

Results: The data suggested (1) IgG4 anti-mite antibody increased 8 months after immunotherapy while IgE antibody level remained the same; (2) allergen-induced, in vitro production of certain cytokines such as IL-4 and IL-10 decreased after immunotherapy; (3) IL-13 (which can induce IgG4 and IgE antibody production by B cells) increased after immunotherapy.

Conclusion: Although this needs more study, IL-13 might play an important role in the generation of IgG4-blocking antibody during immunotherapy.

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INTRODUCTION

Allergen-specific IgE and mast cells are the effector molecules and cells in triggering inflammatory responses in allergic diseases; but are under the overall control of allergen-specific T cells and their cytokines.¹ Both in vivo and in vitro evidence has suggested that type 2 T helper cell cytokines such as IL-4 are important for the immunoglobulin class switch from IgM to IgE, while IL-5 is critical of eosinophilia that causes more inflammatory response during the late stage of disease.²⁻⁴ Allergen-specific T cells derived from atopic patients are of type 2

T helper cell pattern predominantly. In contrast, antigen-specific T cells derived from nonatopic normals are predominantly of type 1 T helper cells.⁵⁻⁸

Allergen-specific immunotherapy was first introduced by Noon⁹ in 1911 and has become a widely used and effective treatment for patients with asthma and seasonal allergic rhinitis. The immunotherapeutic procedure consists of long-term regular immunization of the patients with gradually increasing doses of allergen, eventually resulting in the amelioration of allergic symptoms.¹⁰⁻¹³ Several studies have been reported concerning immunologic changes after immunotherapy. These changes include decreased production of platelet activating factor (PAF), prostaglandins and leukotriene C4, and histamine releasing factor (HRF).¹⁴⁻¹⁹ The precise mechanisms of hyposensitization are still not well defined although blocking antibodies, allergen-specific suppressor cells, and

change of cytokines pattern all have been suggested. Numerous studies have suggested that IgG4 allergen-specific antibodies, which are noted to increase after immunotherapy, were important for symptom relief.²⁰⁻²³ Class switch from IgM to IgG4 is regulated by TH-2 cytokines which is contradictory to the hypothesized mechanism of TH-2 to TH-1 switch in immunotherapy.

This study investigated further the changes of mite-specific antibodies and cytokines, including IL-2, γ -IFN, IL-4, IL-5, IL-10, and IL-13 during immunotherapy.

MATERIALS AND METHODS

Subjects

All patients with a diagnosis of asthma were followed up regularly at the Allergy Clinic, Department of Pediatrics, National Taiwan University Hospital. This study has been approved by the human research committee of this hospital, and informed consent was obtained from all study subjects. All the patients were sensitive only to mites (*Dermatophagoides pteronyssinus* and *D. farinae*) in terms of having a positive history of exposure (asthma attack during house cleaning), a skin test response greater than 2+ (wheal > 10 mm, by intracutaneous route with mite extract at a concentration of 10⁻⁶ g/mL), and a positive mite RAST result (CAP system, Pharmacia Diagnostics, Uppsala, Sweden). Routinely, a panel of 18 inhaled allergens including mites, cat, dog, pollens, cockroach, and molds was used in CAP testing and skin testing.

The protocol and effectiveness of immunotherapy was judged as described previously by comparing, from

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patient records, the sum of the frequency of asthma and the amount of medications consumed (total doses of β_2 agonists, theophylline, and steroids) in the year before immunotherapy with that 8 months after immunotherapy.²⁴ The result was considered to be excellent if the decrease of both parameters was greater than 90%, much improved with a 75% to 90% decrease, improved for a 50% to 75% decrease, slightly improved for a 25% to 50% decrease, and poor for a decrease of 25% or less. In this study good responders consisted of those patients showing an improvement greater than 50%, whereas poor responders were those showing an improvement less than 25%.

Isolation of Peripheral Blood Mononuclear Cells

Heparinized blood samples were collected before, and 8 months after immunotherapy. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque centrifugation. Briefly, cells at interface were collected and washed three times before further experiments. Most experiments were done in RPMI 1640 medium supplemented with 2% TCH (human serum replacement, Celox Corp., Hopkins, MN) and 2.5% human AB serum (Atlanta Biologicals, Norcross, GA), 4 mM L-glutamine, 25mM HEPES (pH 7.2), 5×10^{-5} M 2-mercaptoethanol, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 0.25 mg/mL amphotericin.

House Dust Mite (Der P)-Specific T Cell Proliferative Responses

To test mite-specific T cell proliferative responses, peripheral blood mononuclear cells were plated to a 96-well plate with the cell concentration of 2.5×10^5 cells/well. In addition, mite antigen was added to the well at a predetermined concentration of 5 μ g/mL, then incubated for seven days. Eighteen hours before harvest, 1 μ Ci H^3 -thymidine was added to each well. Cells were harvested onto filter paper and radioactivity was determined with a β -counter.

Sera Levels of Mite-Specific IgE, IgG1, IgG2 and IgG4 Antibodies

Mite antigen (100 μ g/mL) was coated to ELISA plates and incubated at 4 °C overnight. After three washes, the plates were blocked with 200 μ L of 1% bovine serum albumin and incubated at room temperature for two hours. Sera to be assayed were added to the plates and incubated at room temperature for two hours. Biotin-conjugated goat anti-human IgG1, IgG2, and IgG4 antibodies were added to the plate and incubated at room temperature for another two hours. 2,2'-Azino-bis-ethyl-benzthiazoline-6-sulfonic acid (ABTS) was used as the developing substrate and the reaction was stopped with 5% sodium dodecyl sulfate. IgE mite-specific antibody was determined with commercial CAP system and expressed as KU/mL. Throughout the study, sera with concentration determined by commercial CAP system was used as the positive control.

Cytokines Production by Mite-Stimulated T Cells

Peripheral blood mononuclear cells with the concentration of 1×10^6 cells/mL were set up in 24 well plates in the presence of mite antigen (5 μ g/mL, Pharmacia). After 48 hours incubation, the supernatant was collected and assayed for cytokines. Quantikine system (R&D system, MN) was used to determine the level of IL-2, IL-4, IL-5, IL-10, and IL-13 in the supernatant. An ELISA kit (Endogen Inc. Boston, MA, USA) was used for measuring γ -interferon (γ -IFN) production. The sensitivity of sandwich-ELISA used in our experiment is 10 pg/mL for IL-2, IL-4, IL-5, IL-13, and 20 pg/mL for IL-10 and γ -IFN, respectively.

Statistical Analysis

Student's *t* test and Chi square test were used to analyze the data throughout the study. A *P* value of $<.05$ was considered statistically significant.

RESULTS

Subjects

Among 19 asthmatic children (14 boys and 5 girls, mean age 11.1 ± 3.2 years)

followed, 14 of them (11 boys and 3 girls, mean age 10.9 ± 2.7 years) showed good response after immunotherapy. In contrast, five asthmatic children (three boys and two girls, mean age 11.5 ± 2.4 years) did not improve well after immunotherapy (poor response).

House Dust Mite (Der P)-Specific T Cell Proliferative Responses

These data suggested there was no significant difference in house dust mite-specific T cell proliferative response before and after immunotherapy (Fig 1). There was significant difference in mite allergen-specific proliferative response between the patients of good response and poor response groups ($P < .05$).

Sera Levels of Mite-Specific IgE, IgG1, IgG2, and IgG4 Antibodies

The results of mite-specific IgE, IgG1 IgG2, and IgG4 antibodies are summarized in Figure 2. The data suggested sera levels of mite-specific IgG4 increased in asthmatic children with good response to immunotherapy ($P < .001$). Although there was increasing mite-specific IgG4 in asthmatic children of poor response, it is not statistically significant ($.05 < P < .1$). In contrast, there is no difference in mite-specific IgG1, IgG2, and IgE level before and after immunotherapy in either poor or good response group.

Cytokine Production by Mite-Stimulated T Cells

The results of cytokine production are summarized in Table 1. For asthmatic children with good response to immunotherapy, both IL-4 and IL-10 decreased after immunotherapy ($P < .05$). In contrast, TH1-related IL-2 and γ -IFN remained similar before and after immunotherapy. Interleukin-2 and γ -IFN increased in asthmatic children with poor response ($P < .05$). Most strikingly, IL-5 produced by mite-stimulated peripheral blood mononuclear cells increased in asthmatic children of the poor response group ($P < .01$). In addition, the level of IL-5 after immunotherapy in asthmatic children of poor response group is higher com-

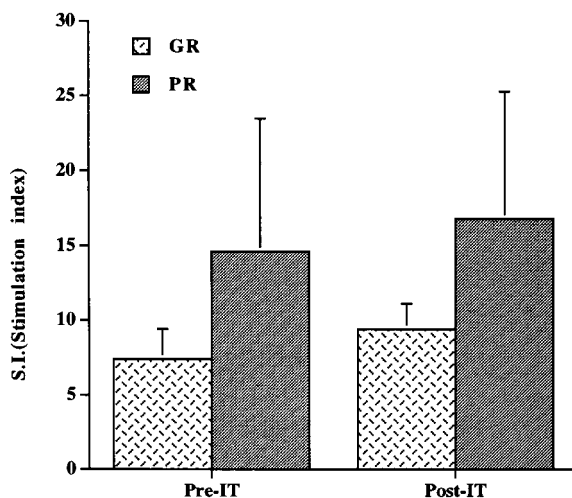


Figure 1. The result of house dust mite-specific proliferative response of peripheral blood mononuclear cells. There was no significant difference during immunotherapy in both good response (GR) and poor response (PR) groups. T cell proliferative response, however, is significantly higher in poor response group than that of good response group.

pared with that of the good response group. More studies are needed to investigate further if IL-5 accounts for lack of improvement in asthmatic symptoms.

Furthermore, IL-13 produced by mite-stimulated peripheral blood mononuclear cells increased after immunotherapy in asthmatic children of the good response group ($P < .01$). In contrast, no such finding was noted in asthmatic children of the poor response group. The level of IL-13 after immunotherapy was higher in asthmatic children of the good response group compared with that of the poor response group ($P < .05$).

DISCUSSION

One of the immunotherapeutic procedures is long-term regular desensitization of the patients with gradually increasing doses of allergen, eventually resulting in the amelioration of allergic symptoms.¹⁰⁻¹³ The precise mechanisms of hyposensitization are still not well defined; blocking antibodies, allergen-specific suppressor cells, and change of cytokines pattern all have been suggested. In addition, some studies showed allergen-specific suppressor cells developed during immunotherapy might be important for

downregulation of asthmatic attacks.^{25,26} The data demonstrated that there was no significant difference in allergen-specific T cell proliferative response during immunotherapy. IgG4 allergen-specific antibody increased significantly after immunotherapy, which is consistent with the results of many groups. Many studies have suggested that this immunotherapy-induced rise in IgG1 and IgG4 allergen-specific antibodies is important for symptom relief.²⁰⁻²³ In contrast, IgG1, IgG2, and IgE allergen-specific antibody levels were similar before and 8 months after immunotherapy.

The difference and interaction between type 1 and type 2 T helper cells has become a major subject of research in the past few years. It has become clear that addition of exogenous cyto-

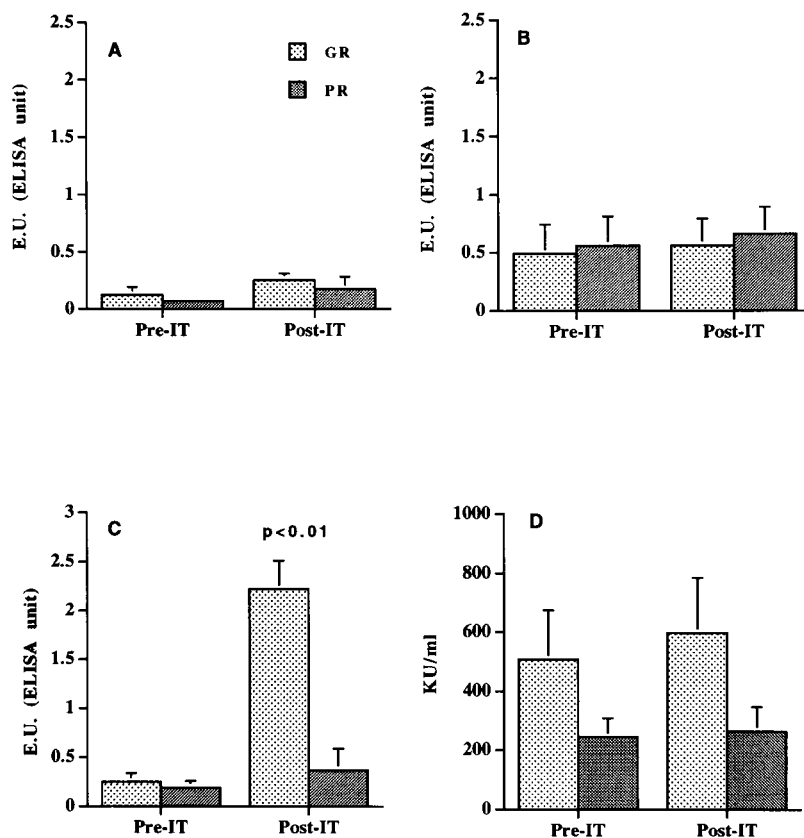


Figure 2. The concentrations of IgG1 (A), IgG2 (B), IgG4 (C), and IgE (D) mite-specific antibody in asthmatic children before and after immunotherapy. The level of IgG4 antibody was significantly increased ($P < .01$) after immunotherapy in asthmatic children with good response compared with that of before immunotherapy. GR: good response; PR: poor response (1 EU for IgG1: 818.2 $\mu\text{g/dL}$; 1 EU for IgG2: 508.2 $\mu\text{g/mL}$ and 1 EU for IgG4: 53.8 $\mu\text{g/mL}$, respectively).

Table 1. Cytokine Production by Mite-Stimulated Peripheral Blood Mononuclear Cells

Cytokine, pg/mL	Good Response		Poor Response	
	Pre-IT	Post-IT	Pre-IT	Post-IT
IL-2	173.9 ± 39.0*	165.5 ± 39.7	94.5 ± 23.5†	270.7 ± 90.6
γ-IFN	15.2 ± 4.3‡	11.4 ± 3.2	0.2 ± 0.1†	4.0 ± 1.6
IL-4	35.1 ± 19.0†	3.3 ± 2.2	10.5 ± 9.6	0
IL-5	1701.2 ± 397.6	1507.2 ± 415.2‡	1279.2 ± 284.4†	3983.3 ± 1592.5
IL-10	84.1 ± 18.8†	40.3 ± 8.9	76.6 ± 41.9	71.0 ± 31.2
IL-13	276.7 ± 41.5†	496.9 ± 93.9	230.7 ± 89.7	123.9 ± 93.7

* Mean ± SEM.

† The data was significantly different from that of post-immunotherapy ($P < .05$).‡ The data was significantly different from that of good response group ($P < .05$).

kines such as γ -IFN or IL-4 in vitro can affect the development of the T cells and their subsequent cytokine profile. These data suggested that the local concentration or types of cytokines might determine the fate of the T cells in vivo.^{27,28} Dysregulation between TH-1 and TH-2 has been noted and has contributed to many diseases such as acquired immunodeficiency syndrome (AIDS), systemic autoimmune diseases, chronic graft-versus-host disease, and certain protozoal infections. Immunotherapy targeted on cytokines has become an important research direction recently. This mechanism has also been strongly suggested in immunotherapy for allergic diseases. The data also demonstrated IL-4 and IL-10 decreased after immunotherapy in asthmatic children with good response. In contrast, neither IL-4, IL-5, or IL-10 showed the similar change after immunotherapy in those of poor response.

The cytokines IL-13 and IL-4 have been found to act as switch factors for IgG4 and IgE.^{29–31} Data have demonstrated increased IL-13 production in asthmatic children after immunotherapy. Most studies have demonstrated increased IgG4 allergen-specific antibody after immunotherapy. The level of IL-4 produced by allergen-stimulated peripheral mononuclear cells, however, decreased after immunotherapy.³² The data suggested that there is link between increasing IL-13 and mite-specific IgG4 antibody during immunotherapy. Although more studies are needed, it is possible that an

increased IL-13 level during immunotherapy might contribute to the increase of IgG4 anti-mite antibody, which is suggested to act as a blocking antibody. Furthermore, IL-13 may downregulate macrophage function and alleviate the inflammatory process during asthmatic attack.³³ Interleukin-13 has also been found to trigger the production of IgE, which declined gradually after immunotherapy but it is contradictory to the increased level of IL-13. It is speculated that IL-4 and IL-13 may exert the differential effect on class switch from IgM to IgE or IgG4 under the influence of other cytokines such as γ -IFN or IL-10.³⁴

This study details the changes of individual cytokines during immunotherapy. The changes of cytokines such as decreased IL-4 and IL-10, and increased IL-13 levels might contribute to the immunologic changes including increased allergen-specific IgG4 and symptom relief during immunotherapy.

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