# MODULATION OF IMMUNE RESPONSE IN LONG-TERM USE OF INHALED CORTICOSTEROID IN CHILDHOOD ASTHMA RECEIVING SPECIFIC IMMUNOTHERAPY

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## ABSTRACT

***Background:*** *Inhaled corticosteroid is widely used in the management of mild to moderate persistent asthma including those receiving specific immunotherapy. Immunological mechanism underlie the improvement until now has not fully elucidated.* ***Objective:*** *To elucidate the modulation of immune response in long-term use of corticosteroid inhalation in asthmatic children receiving specific immunotherapy.* ***Materials and Methods:*** *Parents signed informed consent after receiving information of the study prior to enrollment. Randomization was done using "systematic random sampling". Patients were divided into three groups: group A receiving inhaled budesonide, group B receiving specific immunotherapy, and Group C receiving both specific immunotherapy and inhaled budesonide. Patients aged 6-16 years received either inhaled budesonide or specific immunotherapy for 3 months. The daily budesonide dose was 400 mg for children aged 6-11 years or 200 mg for children younger than 11 years. The primary outcome was IL-4, IL-5, IFN-$\gamma$ and IL-2 and FEV-1 reversibility. Ethical Committee of Dr. Soetomo Hospital Surabaya approved ethical clearance.* ***Results:*** *Significant differences were observed between pre and post treatment in all group ($p<0.05$). Patients receiving inhaled corticosteroid and immunotherapy showed attenuation of IL-4 and IL-5, elevation of IFN-$\gamma$ and IL-2, and improvement of FEV-1 reversibility. Analysis of discriminant yielded IL-2 as primary discriminator and correlated with the decrease of IL-5.* ***Conclusion:*** *Addition of inhaled corticosteroid to immunotherapy results in marked attenuation of IL-5 correlates with greater elevation of IL-2 and play an important role in the modulation of immune response resulting in the improvement of FEV-1 reversibility.*

***Keywords:*** *Childhood asthma, inhaled corticosteroid, immunotherapy, cytokine*

## INTRODUCTION

Asthma is a leading chronic illness in childhood. There is no universally accepted definition of asthma: it may be regarded as a diffuse, obstructive lung disease with hyper reactivity of the airways to a variety of stimuli and a high degree of reversibility of the obstructive process either spontaneously or as a result of treatment (Liu, 2004). House dust is the common inhalant allergen causing exacerbation of asthma. There is still no consensus in the management of asthma. In patients' sensitive to house dust, specific immunotherapy (SIT) with house dust extract is usually effective.

SIT seems likely cannot be a single treatment in the management of Asthma. Asthma is recognized as an inflammatory disease, and inhaled corticosteroids are the mainstay of treatment in patients with persistent disease. In children aged 12 moths-8 years, budesonide inhalation suspension is a glucocorticoid with high topical and low systemic activity, improves asthma symptoms and lung functions and decreases the need for breakthrough bronchodilator (Szefler, 2001). The newest double-blind randomized 3 years trial using budesonide turbuhaler showed that long-term once-daily treatment with low dose budesonide decreases the risk of severe exacerbations and improves asthma control in patients with mild persistent asthma of recent onset (Pauwels, 2003). This study was conducted in patients receiving conventional asthma therapy including SIT. The mechanism by which SIT and inhaled corticosteroid is effective in asthma remains incompletely defined. The objective of our study is to elucidate the alteration of cytokine profile in the use of budesonide inhalation in asthmatic children receiving immunotherapy.

## METHODS

### Patients

Between January, 2003 and Augustus, 2003, we recruited patients aged 6-14 years from Allergy Clinic Dr.Soetomo Hospital, Surabaya who had had symptoms of moderate Asthma with the onset of less than 2 years. Moderate Asthma was defined by wheeze, cough, dyspnea or chest tightening at least once per week, but not as often as daily, and FEV1 at the time of visit ranged between 60-80% of predicted normal value.

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Inclusion criteria included all patients who had reversible airway obstruction, defined as an increase in FEV1 of more than 12% after using a short-acting bronchodilator, a fall in FEV1 of more than 15% on exercise testing, or a variation of more than 15% between the two highest and two lowest peak expiratory flow rates during 14 days. Exclusion criteria were symptoms of asthma or asthma treatment for more than 2 years before entry to the study, more than 30 days of treatment with glucocorticoid, or more than one depot glucocorticoid injection per year, a decision by a treating physician that delay of inhaled glucocorticoid treatment was inappropriate, a prebronchodilator FEV1 less than 60% of that predicted, post bronchodilator FEV1 less than 80% of that predicted, or another clinically significant disease. Patients or their parents gave written informed consent, and local institutional review boards approved the study.

**Study Protocol**

Parents signed informed consent after receiving information of the study prior to enrollment. Patients were randomly allocated into group A receiving once daily budesonide, Group B receiving specific immunotherapy, and Group C receiving combination of specific immunotherapy and inhaled budesonide. Group A and B served as a control group. All group received conventional treatment for asthma. Randomization was stratified into two strata according to age, 6-11 years or more than 11 years. Within each stratum, patients were randomized in block of three in each group using systematic random sampling. Study site was Allergy Clinic Dr. Soetomo Hospital, Surabaya, Indonesia. The daily dose of inhaled budesonide was 400 mg or 200 mg for children younger than11 years at the time of randomization. Specific immunotherapy was house dust extract with the doses of 0.1 ml, 0.15 ml, 0.22 ml, 0.32 ml, 0.48 ml, 072 ml, 1 ml of 0.05 mg/ml solution, continued with 0.1 ml, 0.15 ml, 0.22 ml, 0.32 ml, 0.48 ml, 0.72 ml, 1 ml of 0.5 mg/ml solution with the interval of 1 week, continued with maintenance dose of 0.1 ml of 5mg/ml solution. At the end of three months lung function tests were performed again. The primary outcome were cytokines examination comprising IL-4, IL-5, IL-2, and IFN-γ. At all visits, we did spirometry using a Miniloop II spirometer (Micro Medical, Rochester, UK), measured post bronchodilator FEV1, 30 minutes after the inhalation of 0.5 mg terbutaline with turbuhaler, and FEV1 reversibility. At randomization and the end of study, we measured pre bronchodilator FEV1 and FEV1 reversibility. For the proportion of the predicted value, we measured height to the nearest cm at each visit. Ethical Committee of Dr. Soetomo Hospital, Surabaya approved the study protocol.

**Sample Size**

To meet normal distribution, sample size was determined more than 20 patients in each group. The sample size was calculated based on the formula for difference between proportions for independent groups with a power of 95%.

**Cytokine Measurements**

Cytokines were measured in serum by using ELISA. IL-4 was measured in samples of serum by using an ultra sensitive IL-4 ELISA with a mean limits of detection of less than 1pg/ml. IL-5 was measured in serum by using assays with a mean limit of detection of 2 pg/ml. IL-2 was measured in samples of serum by using an ELISA with a mean limit of detection of less than 1pg/ml. IFN-γ was measured in samples of serum by using an ELISA with a mean limit of detection of less than 0.8 pg/ml. Assay kits used were: Quantikine Human IL-4, IL-5, IFN-γ, IL-2 Immunoassay R&D Systems, Inc, Minneapolis, Minn. Each fluid was assayed at 1 to 3 dilutions in duplicate, and the result for each sample was calculated by averaging the results obtained from all dilutions followed by correction for concentration. When levels of cytokines were not detected, half of the lower limit of detection was used for statistical analysis.

**Statistical Analysis**

The MANOVA was applied to analyze the baseline data between groups A, B, and C, and comparing results of cytokine at baseline and at post treatment. Differences were considered to be at the $p<0.05$ levels.

**RESULTS**

Distribution of sex, age, history of other atopic disease, history of atopy in relative, eosinophil count, total IgE concentration, and FEV-1 %predicted normal were homogen in the three groups, p=0.870, 0.964, 0.593, 0.478 respectively.

Pre treatment examination showed normal distribution of variables in the three groups. Initial examination yielded IL-4 9.41 $\pm$ 5.27, IL-5 100.80 $\pm$ 11.45, IFN-γ 0.224 $\pm$ 1.27, IL-2 25.01 $\pm$ 14.41, FEV-1 reversibility 21.36 $\pm$ 7.54 in Group A; IL-4 9.30 $\pm$ 3.58, IL-5 98.41 $\pm$ 28.60, IFN-γ 0.224 $\pm$ 1.26, IL-2 20.86 $\pm$ 14.76, FEV-1 reversibility 21.25 $\pm$ 7.41 in Group B; IL-4 14.64 $\pm$ 12.29, IL-5 108.11 $\pm$ 17.06, IFN-γ 0.224 $\pm$ 1.27, IL-2 24.97 $\pm$ 14.41, FEV-1 Reversibility 21.08 $\pm$ 7.43 in Group C. (Figure 1).

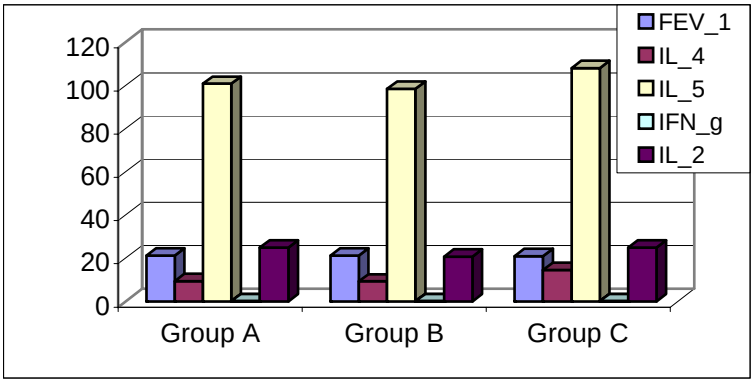


Figure 1. Results of pre treatment variables in each group.

Post treatment examination showed alteration of all variables: IL-4 1.20 ± 1.24, IL-5 63.56 ± 19.28, IFN-γ 0.224 ± 1.15, IL-2 10.65 ± 10.77, FEV-1 reversibility 7.36 ± 4.91 in Group A; IL-4 0.889 ± 0.994, IL-5 65.58 ± 15.42, IFN-γ 0.225 ± 4.02, IL-2 191.65 ± 81.31, FEV-1 reversibility 7.67 ± 4.83 in Group B; IL-4 1.78 ± 1.10, IL-5 66.76 ± 8.46, IFN-γ 0.225 ± 4.11, IL-2 206.33 ± 31.68, FEV-1 reversibility 5.79 ± 2.60 in Group C. (Figure 2).

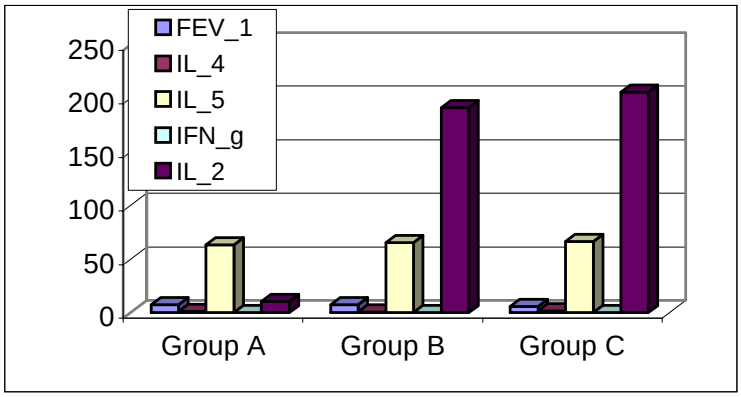


Figure 2. Results of post treatment variables in each group.

Significant differences were observed between pre and post treatment in all group ($p<0.05$). Patients receiving inhaled corticosteroid Group A) showed attenuation of IL-4 from 9.41 ± 5.27 to 1.20 ± 1.24, IL-5 from 100.80 ± 11.45 to 63.56 ± 19.28, IFN-γ from 0.224 ± 1.27 to 0.224 ± 1.15 , IL-2 from 25.01 ± 15.92 to 10.65 ± 10.77, and 29% failure of improvement FEV-1 reversibility. Patients receiving immunotherapy (Group B) showed attenuation of IL-4 from 9.30 ± 3.58 to 0.889 ± 1.24, IL-5 from 98.41 ± 28.60 to 65.58 ± 15.42, elevation of IFN-γ from 0.224 ± 1.26 to 0.225 ± 4.02, IL-2 from 80.26 ± 14.98 to 191.65 ± 81.31and 24% failure of FEV-1 reversibility. Patients receiving inhaled corticosteroid and immunotherapy (Group C) showed attenuation of IL-4 from 14.64 ± 15.92 to 1.78 ± 1.16 and IL-5 from 108.11 ± 17.06 to 66.76 ± 14.98, elevation of IFN-γ from 0.224 ± 1.27 to 0.225 ± 4.11 and IL-2 from 24.97 ± 14.41 to 206.33 ± 31.68, and 100% improvement of FEV-1 reversibility. (Figure 3).

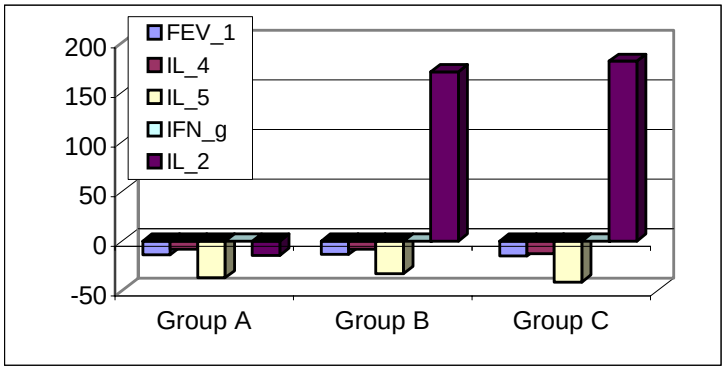


Figure 3. Difference of variables pre and post treatment.

Analysis of discriminant yielded IL-2 as primary discriminator and correlated with the decrease of IL-5. (R= 0.8, p= 0.00)

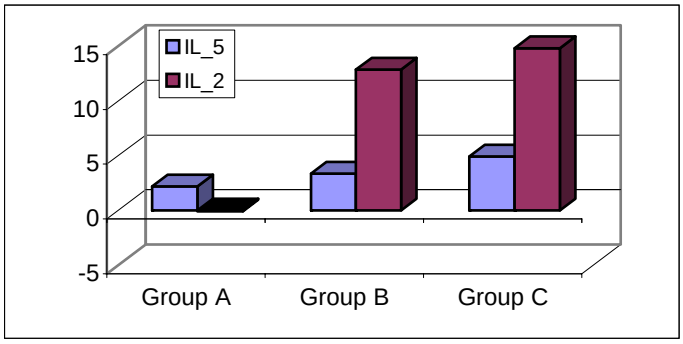


Figure 4. Analysis of discriminant showing the correlation between attenuation of IL-5 and elevation of IL-2.

**DISCUSSION**

SIT is a therapeutic intervention in which the patients are administered increasing doses of an extract consisting of the specific allergens to which the patient has been demonstrated to be allergic. In 1997, the World Health Organization and various allergy, asthma, and immunology society throughout the world met in Geneva to write guidelines for allergen immunotherapy. Panel members reach a consensus about the information to include in the WHO position paper "Allergen immunotherapy as a therapeutic vaccine for allergic disease" (Bousquet, 1998). Abramson first reported a Meta analysis of asthma SIT results in 1995. His report clearly showed that SIT was effective in relieving asthma symptoms and decreasing medication use for treatment of asthma. Otherwise, failure of this treatment still has been reported, in a single-blind placebo controlled, 2 years study of dust injection therapy reported that 78% of patients receiving SIT experiencing clinical improvement as compared with 34% of patients on placebo (Creticos, 2000).

The aim of allergen immunotherapy is to decrease sensitivity to allergens by the gradual administration of increasing doses of therapeutic allergen extract. A fundamental step of allergic process in asthma is the enhanced activity of lymphocyte of Th2 phenotype. The CD4+ T-helper lymphocyte population may be divided into at least two subpopulations: Th1 and Th-2. Each subpopulation promotes inflammatory responses with characteristics that contrast (Galli, 1997). The allergic response is associated with an up-regulated production of Th2 cytokines, such as IL-4, IL-5, IL-9, and IL-13.

Interleukins 13 and IL-4 regulate the differentiation Th-0 cells to develop a Th-2 phenotype. Interleukin-4 stimulates B-cell isotype switching to produce IgE. Interleukine-5 stimulates selective growth of eosinophils, responsible for the terminal differentiation and activation of this cell from its precursor in the bone marrow.

Studies in peripheral blood and within the target organ in allergic patients receiving specific immunotherapy have demonstrated a shift in the balance of T-cell

subsets away from Th-2-type producing particularly IL-4 and IL-5, in favor of a Th-1-type T-lymphocyte response with preferential production of IFN-γ and IL-2 (Durham, 1998) (Figure 5). Eosinophils are attracted to and retained at sites of allergic inflammation by the action of IL-5 and other chemo attractants. IL-5 also facilitates prolongation of eosinophil life span in allergic inflammatory cascade.

The IgE produced by B-cells binds with its Fc region to the high-affinity receptor (FceR1) on effectors mast cells or basophiles. IgE also binds to the low-affinity receptor on B cells and amplifies IgE production. Allergen immunotherapy provides the potential to down-regulate the inflammatory cascade, reduce IgE antibody production, and attenuates symptoms (Lane, 1996). This is accompanied by an increase in allergen-specific IgG blocking antibodies, although neither appears to correlate closely with clinical response to immunotherapy. One way in which allergen immunotherapy may act is by modifying the T-lymphocyte response to subsequent natural allergen exposure. Studies in peripheral blood and within the target organ have demonstrated a shift in the balance of T-cell subsets away from Th-2 type producing particularly IL-4 and IL-5, in favor of a Th-1 type T-lymphocyte response with preferential production of IFN-γ and IL-2 (Moss, 2000).

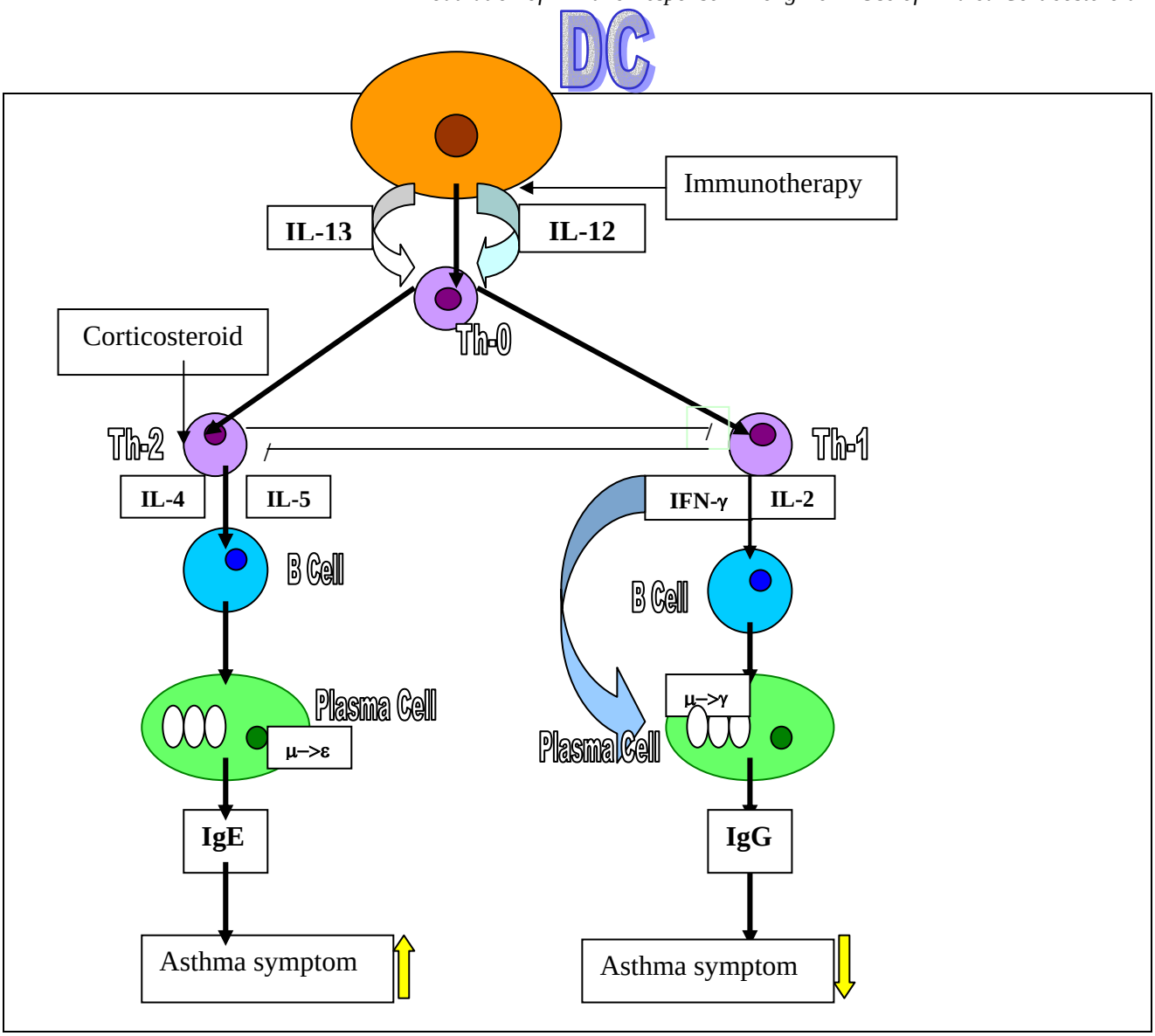


Figure 5. Visualization of concept. A fundamental step of allergic process in asthma is the enhanced activity of lymphocyte of Th-2 phenotype. The allergic response is associated with an up-regulated production of Th-2 cytokines, such as IL-4, IL-5, IL-9, and IL-13. Interleukins 13 and IL-4 regulate the differentiation Th-0 cells to develop a Th-2 phenotype. Interleukin-4 stimulates B-cell isotype switching to produce IgE. Interleukine-5 stimulates selective growth of eosinophils, responsible for the terminal differentiation and activation of this cell from its precursor in the bone marrow. Eosinophils are attracted to and retained at sites of allergic inflammation by the action of IL-5 and other chemo attractants. Allergen immunotherapy provides the potential to down-regulate the inflammatory cascade, reduce IgE antibody production, and attenuate symptoms.12 This is accompanied by an increase in allergen-specific IgG blocking antibodies. Addition of corticosteroid attenuates the production of IL-4 and IL-5 resulting in the potentiation of immunotherapy.

Baseline data from our study indicated that Group A, Group B, and Group C were comparable and in normal population as denoted by $p>0.05$ of all variables. Patients receiving corticosteroid inhalation showed attenuation of IL-4, IL-5, IFN-γ, IL-2 and 29% failure improvement of FEV-1 reversibility. Corticosteroid is an immunosuppressant of immune response of both Th-2 and Th-1 subset. If we look at the ratio of IFN-γ : IL-4 before treatment was 0.002 and after treatment was 0.16, indicating the shift of Th-1/Th-2 in favor of Th-1. Other study indicated, 3 days treatment with oral prednisone resulted in the inhibition both expression of mRNA protein of Th-2 especially IL-4, and IL-5; and mRNA protein of Th-1 especially IL-1, and TGF-a (Liu,

2001). In our study IFN-γ, IL-2 is to be examined instead of IL-1 and TGF-a because these latter cytokine do not responsible in the context of immunotherapy. The present study, using the relatively low dose of inhaled corticosteroids for 3 months, shows that inhaled corticosteroids reduces IL-4, IL-5, IFN-γ, and IL2. However, from other study revealed that total serum IgE levels remained high after the inhaled corticosteroid therapy compared with levels of IgE expected to be found in sera from patients with mild stable asthma. However, Settipane et al. reported that mean total serum IgE significantly decreased from 1007 IU/mL to 852 IU/mL after 2 weeks of treatment with a systemic administration of corticosteroids in mild stable asthma. Therefore, relatively high levels of IgE in those patients might exaggerate the reduction of total serum IgE levels induced by the corticosteroid therapy (Settipane, 1978). Laitinen et al. demonstrated that a treatment of inhaled corticosteroids for 3 months reversed a destructive process in the bronchial epithelium, a process that involves a change from a ciliated epithelium to goblet cell hyperplasic, metaplasia, and epithelial shedding in asthmatic patients (Laitinen, 1992). IgE synthesis is believed to require penetration of allergens through the airway epithelium, interleukin 4, and T cell-B cell interactions that involve the B cell antigen CD40 and its ligand expression on activated T-cells (Abba, 1991; Gagnon, 1993).

Therefore, inhaling budesonide for 3 months might improve the abnormalities of bronchial epithelium and reduce the opportunity for airborne allergen particles to contact cellular components of the immune system, resulting in a decrease in serum IgE in the present study. Alternatively, it is possible that the relatively high dose of inhaled budesonide might act through systemic effects and be equivalent to giving systemic corticosteroids. An improvement in asthmatic symptoms by treatment with inhaled budesonide correlated with a decrease in serum IL-4 and IL-5 levels in the present study, suggesting that one of the reasons for the clinical efficacy of inhaled corticosteroids may be their ability to reduce serum IgE levels in asthmatic patients. The damaged epithelium is reported to be associated with an increase in the number of bronchial epithelial eosinophils in asthma. Inhaled corticosteroids are reported to reduce the number of circulating eosinophils and that of eosinophils in the airway epithelium.

In present study, patients receiving immunotherapy showed attenuation of IL-4, IL-5, elevation of IFN-γ, IL-2 and 24% failure improvement of FEV-1 reversibility. Ratio of IFN-γ : IL-4 increased from 0.02 before treatment to 1.18 after treatment indicating that immunotherapy caused shift from Th-2 to Th-1 pattern more than corticosteroid done. This results accord with other study which reported a failure of 22% improvement of lung function test as a result of 2 year therapy in asthmatic children (Galli, 1997). The first study to describe a shift of allergen-induced T-cell responses in favor of Th-1 cytokine synthesis, after 1 year of a controlled trial of grass pollen immunotherapy, intradermal challenge with grass pollen extract was associated with a reduction in the cutaneous late-phase response (Till, 2004). When this site underwent biopsy at 24 hours, contrary to expectations, a significant reduction in numbers of IL-4 or IL-5 mRNA-expressing cells was not observed. On the other hand, modest but significant increases in IFN- and IL-2 mRNA-expressing cells were observed, consistent with local immune deviation in favor of a Th-1 response. Subsequently, skin biopsy specimens collected after 4 years of immunotherapy were examined for expression of mRNA encoding IL-12, a potent regulator of Th-1 responses (Varga, 2000). IL-12 mRNA expressions did indeed increase after immunotherapy and correlated positively with IFN- mRNA expression and inversely with IL-4 expression (Hamid, 1997). Other study confirms the present study findings, that specific immunotherapy induces a shift from the production of Th-2 type cytokines (IL-4 and IL-5) to the production of Th-1 type cytokines (IFN-γ and IL-2) (Maestrelli, 2004).

Patients receiving corticosteroid inhalation in addition to immunotherapy in present study showed greater attenuation IL-4, and especially IL-5, elevated IFN-γ, and especially IL-2 resulted in 100% improvement of FEV-1 reversibility. The IFN-γ/IL-4 ratio in this group increased from 0.02 before treatment to 1.26 after treatment indicating shift from Th-2 to Th-1 pattern the most if compared with group A receiving corticosteroid inhalation and Group B receiving immunotherapy alone. In a study involving the addition of specific immunotherapy to pharmacological treatment including budesonide inhalation and allergen avoidance was associated with a reduced use of rescue bronchodilators, a progressive increase in morning and evening PEF values, and a reduced skin reactivity to house dust mite extracts. These results suggest that specific immunotherapy added to appropriate medical care provides marginal but statistically significant additional clinical benefits, possibly by reducing the allergic response of asthmatic subjects sensitized to house dust mite. Controlled studies of allergic asthma in subjects with single allergy to house dust mite have shown that specific immunotherapy reduces symptoms and, in some cases, improves basal airway function (Maestrelli, 2004).

It seems likely that the improvement of FEV-1 reversibility is a cumulative result of 4 factors. Firstly,

in part by decreased IL-4, this enhances inhibition of IgE production. Secondly, decreased IL-5 enhances inhibition of eosinophil maturation in the stem cell, chemo taxis, and prolonged life span. Thirdly, increased IFN-γ enhances isotype switching of immunoglobulin production in favor of IgG subclass instead of IgE. Fourthly, increased IL-2 enhances the affinity maturation and differentiation of B cells giving rise to the generation of B memory cells and antibody secreting plasma cells producing IgG.

## CONCLUSION

Long-term use of inhaled corticosteroid in childhood asthma receiving immunotherapy results in elevation of IFN-γ and IL-2, diminish IL-4 and Il-5. Addition of inhaled corticosteroid to immunotherapy results in marked attenuation of IL-5 correlates with greater elevation of IL-2 and play an important role in the modulation of immune response resulting in the improvement of FEV-1 reversibility.

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