

Interleukin-4 Production in Asthmatic Patients During Allergen Immunotherapy: A Preliminary Study

SHAHID SHEIKH, M.D.,¹ NANJUNDAIAH KUMAR, M.D.,² MYRON ZITT, M.D.,²
DENNIS BOUBOULIS, M.D.,² SOO FANG WANG, B.S.,² SAVITA PAHWA, M.D.,³
and MARIANNE FRIERI, Ph.D., M.D.²

ABSTRACT

Background: Chronic inflammation and cytokine amplification play a major role in the pathogenesis of asthma. Allergen immunotherapy in atopic asthma has been shown to be effective, but the precise immunologic mechanisms for clinical improvement are poorly understood. CD4+T-cells from atopic asthmatics produce increased levels of the cytokines such as interleukin-4 (IL-4), compared with CD4+T-cells from nonatopic nonasthmatic individuals and these cytokines are believed to be directly involved in allergic disease process. It is not clear if IL-4 secretion patterns change during immunotherapy.

Objectives: Monitoring IL-4 levels may be very useful in evaluating the effectiveness and response to allergen immunotherapy.

Methods: Peripheral blood mononuclear cells were obtained from healthy nonasthmatic controls (n = 5) and from atopic asthmatic patients (n = 5) prior to immunotherapy and at 3 and 6 months after initiation of immunotherapy to monitor IL-4 secretion in unstimulated and grass allergen-specific stimulated mononuclear cells. Changes in IL-4 secretions were related to clinical response to immunotherapy.

Results: Mean IL-4 secretion was lower in controls than in asthmatics but the difference was not statistically significant. In asthmatics 3 months after starting immunotherapy mean IL-4 secretion significantly decreased ($p < .002$) in both grass antigen-specific stimulated cultures and un-stimulated cells. This pattern persisted at 6 months.

Conclusion: In atopic asthmatics mean IL-4 secretion was significantly decreased during immunotherapy however this change was not allergen-stimulation dependent at the dose response chosen. (Pediatr Asthma Allergy Immunol 1997;11(1)17-22)

INTRODUCTION

CHRONIC INFLAMMATION AND BRONCHIAL HYPERREACTIVITY in pathogenesis of asthma involves a complex interaction of genetic and environmental factors and many cell types especially CD4+ T-cells.^(1,2) Airway inflammation also includes bronchial epithelial cell-cytokine interaction and cytokine regulation of

¹Pediatric Pulmonary Division, University of Louisville, Kentucky, ²Department of Medicine and Pathology, Nassau County Medical Center, East Meadow, New York, and The State University of New York at Stony Brook, and ³North Shore University Hospital, Manhasset, New York.

airway epithelial cell function.⁽³⁾ It is known that the increased production by CD4+ T cells, of cytokines like interleukin-4 (IL-4), which is critical in the induction of immunoglobulin E (IgE) synthesis,⁽⁴⁻⁹⁾ and interleukin-5 (IL-5), which is a significant eosinophil differentiation factor,⁽¹⁰⁻¹²⁾ determines whether allergic disease develops in a given individual.⁽¹³⁾ Allergen-specific CD4+ T cells from patients with allergic diseases such as asthma and allergic rhinitis produce higher levels of IL-4 and IL-5 compared with allergen-specific CD4+ T-cells from nonallergic individuals.⁽⁴⁻²²⁾ It has been demonstrated that allergen immunotherapy in patients with allergic rhinitis reduces the production of IL-4 in allergen specific CD4+ T cells to the levels observed with T cells from nonallergic individuals, or to levels induced by nonallergenic antigens, such as tetanus toxoid.⁽²³⁾ It has also been shown that IL-4 synthesis in CD4+ T cells is downregulated by high concentrations of allergen and/or when antigen is presented by monocytes rather than by B cells.⁽²⁴⁾ IL-4 also inhibits expression of Fc γ receptors and downregulates nitric oxide, interleukin-1 (IL-1), interleukin-6 (IL-6), and other cytokines.⁽²⁵⁾

Immune responses that produce large amounts of IL-4 by allergen-specific CD4+ T cells are associated with increased IgE production and excessive IgE production is associated with allergic diseases.^(14-18,26) Allergen immunotherapy is considered effective in the treatment of allergic diseases such as severe allergic rhinitis.⁽²⁷⁻³²⁾ although specific immunologic mechanisms are not fully understood. Studies have demonstrated that the immunologic effects of immunotherapy include changes in both the secretion of cytokines and changes in lymphocyte proliferative responses.⁽³³⁾

We serially monitored circulating and specific allergen-induced IL-4 production by peripheral blood mononuclear cell (PBMC) in asthmatic subjects before immunotherapy and at 3 months and 6 months while receiving immunotherapy and correlated cytokine levels during immunotherapy with clinical scores, including frequency of exacerbation and need for medications. In a previous study we also observed modulation of interferon- γ (IFN- γ) and interleukin-10 (IL-10) during allergen immunotherapy.⁽³⁴⁾ Our hypothesis was that monitoring these cytokine levels may be useful in evaluating the effectiveness and response to immunotherapy.

MATERIALS AND METHODS

We initially evaluated five adult patients with moderate asthma as defined by the Global Initiative for Asthma Guidelines,⁽¹⁾ not receiving systemic steroids because these agents inhibit cytokine production. A comparable number of nonatopic, nonasthmatic healthy individuals with negative skin testing were used as controls. Prick tests were performed with standard extracts of 1:20 weight-to-volume concentration in 50% glycerine, a diluent and histamine control (Nelco Laboratories, Deer Park, NY). The skin test was considered positive at 3+ to 4+ if a wheal developed, sized 3 to 8 mm with erythema or greater than 8 mm with pseudopods, measured within 10 to 30 minutes of administration. In skin prick-negative subjects, intradermal skin testing was performed by standard methods using standard aqueous solutions of 1:100 and 1:1000 protein nitrogen units (PNU) per milliliter concentrations. Patients were evaluated by a careful history, physical examination, skin testing to a panel of appropriate allergens, and symptom scores. Patients on systemic steroids 1 month prior to entry were excluded. All five patients were on daily inhaled steroids at the time of initial evaluation and underwent immunotherapy for atopic asthma. Two patients also had allergic rhinitis and were on daily cromolyn sodium nasal spray twice a day. Allergen extracts for grass used for immunotherapy were standard extracts (Nelco Laboratories, Deer Park, NY) and were not mixed with mold allergens because proteases in mold extracts can denature pollen antigens.⁽³⁵⁾ All five patients had positive skin testing to grasses and were given immunotherapy. After each dose of immunotherapy patients were observed for 30 minutes for any reaction to immunotherapy and there were no systemic reactions noted during immunotherapy. Two out of five patients developed only local reactions and doses were increased very slowly. The protocol was approved by the Nassau County Medical Center Internal Review Board.

Venous blood was taken after informed consent from healthy nonasthmatic controls and from atopic asthmatic patients at baseline (prior to immunotherapy) and from asthmatic patients at serial time periods at 3 months and 6 months after initiation of immunotherapy to monitor IL-4 secretion by PBMC with and without allergen-specific stimulation. PBMCs were prepared by the standard Ficoll Hypaque technique. Two

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milliliters of 2×10^6 PBMC per milliliter were incubated in complete Rosewell Park Memorial Institute (RPMI) media for 24 hours with and without 20 $\mu\text{L/mL}$ of 1:10 weight-to-volume preservative-free grass specific allergen extract. The PBMCs were then centrifuged and supernatants were harvested for cytokine determination by standard enzyme-linked immunosorbent assays (ELISA) techniques for IL-4 (Genzyme, Cambridge, MA). Cytokine assays were performed in duplicates and results were reproducible. Total serum IgE was performed in asthmatic patients and healthy controls at time of enrollment. Usage of all medications was closely monitored throughout 6 months on immunotherapy in all patients. Episodes of wheezing, difficulty in breathing, and night-time cough reported by patients were used as symptom scores, and improvement in these parameters was considered as clinical improvement. The dose of inhaled corticosteroids (triamcinolone) was titrated at 100 μg activation based on improvement either in clinical status or peak flow rate. Immunotherapy was done using standard alum-precipitated extracts for grasses standardized in protein nitrogen units per milliliter (PNU/mL) (Nelco Laboratories, Deer Park, NY). Immunotherapy was started at 5 PNU, with dose progression individualized for each patient, and a standard schedule was followed.⁽³⁶⁾

STATISTICS

Mean and standard deviations (SD) were calculated using the EPISTAT network. Student's *t*-test assuming unequal variance were performed, including *p* value determination, for IL-4 in controls versus asthmatics before immunotherapy, and for unstimulated and grass-specific allergen stimulated PBMCs before immunotherapy and at 3 and 6 months after initiation of immunotherapy.

RESULTS

Cytokine evaluations for IL-4 from PBMCs cells after 24-hour incubation with either RPMI media alone (spontaneous) or with grass-specific allergens were performed in five asthmatic patients at the time of enrollment into the study and were compared with cytokine secretion from PBMCs after 24-hours incubation with media (spontaneous) or with grass allergen-specific stimulation from five healthy nonatopic, nonasthmatics labeled as controls. There was no statistically significant difference in IL-4 secretion from spontaneous and antigen-specific stimulated PBMCs in controls. Median total IgE in controls in milligrams per deciliter (mg/dL) was $52 \text{ mg/dL} \pm 137 \text{ mg/dL}$ and median total IgE in asthmatics was $129 \text{ mg/dL} \pm 93 \text{ mg/dL}$. This difference was not statistically significant. All five patients were started on immunotherapy and cytokine secretion was evaluated at 3 and 6 months after initiation of immunotherapy. At 3 months of immunotherapy the mean dose for grass antigen was $220 \pm 107 \text{ PNU/mL}$ and at 6 months of immunotherapy the mean dose for grass antigen was $1800 \pm 979 \text{ PNU/mL}$. The pattern of cytokine secretion was also compared with clinical response to immunotherapy.

Mean IL-4 secretion (controls versus asthmatics before starting immunotherapy) were compared (Table

TABLE 1. MEAN IL-4 SECRETION (CONTROLS VS. ASTHMATICS) BEFORE IMMUNOTHERAPY

	<i>Controls</i>	<i>Asthmatics before Immunotherapy</i>	
		<i>Grasses</i>	<i>PBMC</i>
IL-4	13.6 ± 3.4	15.1 ± 1.2	13.2 ± 1.5
<i>p</i> -value		NS	NS

Values are expressed as the mean $\pm$ SD and measured in pg/mL, NS = nonsignificant.

TABLE 2. CHANGES IN MEAN IL-4 SECRETION IN ASTHMATICS WITH IMMUNOTHERAPY

<i>IL-4 secretion</i>	<i>Grasses</i>	<i>PBMCs</i>
Before IT	15 $\pm$ 1.2	13.2 $\pm$ 1.5
At 3 months	1.8 $\pm$ 1.7	1.2 $\pm$ 1.2
<i>p</i> -value	<0.002	<0.002
At 6 months	<1	<1
<i>p</i> -value	<0.002	<0.002

Values are expressed as the mean $\pm$ SD and measured in pg/mL.

1). All values are measured in picograms per milliliter and are expressed as the mean $\pm$ SD. Mean IL-4 secretion was lower in controls (13.6 $\pm$ 3.4 pg/mL) than in asthmatics after grass antigen-specific stimulation of PBMCs (15.1 $\pm$ 1.2 pg/mL), but the difference was not significant.

Mean IL-4 secretion from asthmatics was compared, before starting immunotherapy and after 3 and 6 months of immunotherapy (Table 2). At 3 months on immunotherapy mean IL-4 secretion was significantly decreased both for grass allergen-specific stimulated PBMCs 1.8 $\pm$ 1.7 pg/mL (p < .002) and for unstimulated PBMCs 1.2 $\pm$ 1.2 pg/mL (p < .002). As there was significant change in IL-4 secretion from peripheral blood mononuclear cells without antigenic stimulation, this decrease in IL-4 was not an antigen dependent response. At 6 months of immunotherapy mean IL-4 secretion was undetectable both for grass-specific stimulated PBMCs and for unstimulated PBMCs (p < .002). As there was significant change in IL-4 secretion from PBMCs without antigenic stimulation, this decrease in IL-4 was either an antigen independent response or a higher dose response may have shown a difference.

When decrease in IL-4 secretion with immunotherapy was compared with the clinical response to immunotherapy, it was noted that all patients had improvement in wheezing episodes and night-time cough. None of the patients had any exacerbation of acute asthma during immunotherapy. Before initiating immunotherapy all patients were on daily inhaled steroids (triamcinolone), doses ranging from 600 μ g/day divided twice a day to 1000 μ g/day divided twice a day, median daily dose in was 760 $\pm$ 149 μ g/day. At 6 months of immunotherapy, two patients (receiving 600 μ g/d of triamcinolone) were able to discontinue the use of inhaled steroids without any increase in use of β_2 agonists and median dose of inhaled corticosteroids in micrograms per day was 240 $\pm$ 293 μ g/day. This improvement was statistically significant (p < .02). There was no statistically significant change noted in pulmonary function tests (FVC, FEV₁, FEF₂₅₋₇₅) in patients before, after 3 and 6 months of immunotherapy. Two patients with allergic rhinitis were taking cromolyn sodium nasal spray on a daily basis, felt clinical improvement in nasal irritation and discharge, and were able to discontinue this nasal spray at 6 months on immunotherapy.

DISCUSSION

Evidence suggests that T-cell responses and cell-cytokine interactions are very important in allergic disorders such as asthma. We evaluated the role of immunotherapy in modulating cytokine release from PBMCs, without stimulation and in response to grass allergen-specific stimulation, in five moderate to severe asthmatics. Although the number of our patients was limited, and a placebo effect cannot be ruled out, we observed that the treatment of atopic asthmatics with allergen immunotherapy was associated with significant reduction in IL-4 synthesis *in vitro*. The reduction in IL-4 synthesis *in vitro* from PBMCs of patients receiving immunotherapy was not allergen specific at the dose evaluated. Decrease in IL-4 levels in patients receiving immunotherapy was noted both in PBMCs harvested with media alone and PBMCs harvested with grass allergen. This reduction in IL-4 levels with immunotherapy was associated with improved clinical symptoms and decrease in asthma medications. Immunotherapy may accomplish its clinical effects by cytokine modulation including reduction in IL-4 synthesis in PBMC. As there was no true control group in

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our study such as matched atopic asthmatics not receiving immunotherapy, it is not possible to determine the role of other factors that might be contributing to the clinical improvement, and changes in other cytokine profiles. Other factors such as the pollen season and environmental control must also be considered. More studies in larger numbers of patients are needed to confirm these findings.

Although there have been recent improvements in the symptomatic treatment of patients with atopic asthma due to inhaled steroids, treatment with allergen immunotherapy, which appears to alter the underlying immunologic basis of the disease may be a more beneficial long-term modality. Immunotherapy is important in moderate and severe asthmatics with well-documented allergic components and it is useful both immunologically and clinically in controlling asthma. Downregulation of IL-4 correlates with clinical improvement in atopic asthmatics and can be used as a marker for successful immunotherapy.

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Address reprint requests to:
Marianne Frieri Ph.D., M.D.
Associate Professor of Medicine and Pathology
State University of New York at Stony Brook
Nassau County Medical Center
2201 Hempstead Turnpike
East Meadow, NY 11554