

Modulation of Cytokine Production During Immunotherapy in Atopic Asthmatics: A Potential Role for Cytokine Antagonists. A Preliminary Study

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ABSTRACT

Background: The pathogenesis of asthma involves hyperreactivity and chronic inflammation of airways in which CD4+ T cells play a major role. In atopic asthmatics, Th1 responses due to interferon-gamma (IFN- γ) are depressed, and Th2 responses due to interleukins (IL-4, IL-10) are predominant. It is not clear if cytokine secretion patterns change with clinical improvement during immunotherapy.

Objectives: Monitoring IFN- γ and IL-10 may be very useful in evaluating the effectiveness and response to allergen immunotherapy and in developing new therapeutic interventions with specific cytokine antagonists or peptides.

Materials and 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 cytokine secretion (IFN- γ , IL-10) in unstimulated and grass and ragweed allergen-specific stimulated mononuclear cells. Changes in cytokine secretions were related to clinical response to immunotherapy.

Results: Controls had significantly higher mean IFN- γ secretion compared to asthmatics ($P < 0.01$). Mean IL-10 secretion was lower in controls than in asthmatics, but significant levels were noted only with grass allergen ($P < 0.03$). In asthmatics, 3 months after starting immunotherapy mean IFN- γ secretion significantly increased ($P < 0.01$) in both stimulated and unstimulated cells, which persisted at 6 months. Although there was no significant change in IL-10 secretion at 3 months, mean IL-10 secretion at 6 months was significantly decreased in allergen-stimulated cells ($P < 0.02$).

Conclusion: In atopic asthmatics, mean IFN- γ secretion significantly increased and allergen-specific IL-10 secretion was significantly decreased during immunotherapy.

INTRODUCTION AND BACKGROUND

THE PATHOGENESIS OF ASTHMA involves a complex interaction of genetic and environmental factors and many cell types leading to chronic inflammation and bronchial hyperreactivity.^(1,2) T cells, especially

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CD4⁺ T cells, play a major role in the pathogenesis of atopy and asthma. CD4⁺ T-helper cells can be phenotypically divided into two subgroups (Th1 and Th2) depending on their specific pattern of cytokine secretion.⁽³⁾ When CD4⁺ T cells, in response to an antigenic stimulation, secrete predominantly interferon-gamma (IFN- γ) and interleukin-2 (IL-2), a Th1 response will result, but if the same CD4⁺ T cells, in response to the same antigenic stimulation, predominantly secrete IL-4, IL-5, IL-6, and IL-10, a Th2 response will result. Both Th1 and Th2 responses cross-regulate each other, and in healthy individuals, these responses are balanced. However, certain disease processes lead to impairment of this delicate balance, resulting in the predominance of one response over the other such as asthma and atopic dermatitis in which T cells exhibit Th2 responses and more IL-4 and IL-10 are secreted.⁽⁴⁾ Our understanding of the immunologic basis of allergic disease suggests that the production by CD4⁺ T cells of increased quantities of cytokines such as IL-4, which is critical in the induction of IgE synthesis,⁽⁵⁻⁷⁾ and IL-5, which is a significant eosinophilic differentiation factor,⁽⁸⁻¹⁰⁾ determines whether allergic disease develops in a given individual.⁽¹¹⁾ It has been demonstrated that 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 to allergen-specific CD4⁺ T cells from nonallergic individuals.⁽¹²⁻¹⁶⁾ Allergen immunotherapy in patients with allergic rhinitis has been shown to reduce the production of IL-4 in allergen-specific CD4⁺ T cells to the levels observed in T cells from nonallergic individuals, or to levels induced by nonallergenic antigens such as tetanus toxoid.⁽¹⁷⁾

Allergen immunotherapy is considered to be effective in the treatment of allergic diseases such as severe allergic rhinitis,⁽¹⁸⁻²¹⁾ although specific immunologic mechanisms are not fully understood. Studies have revealed that immunotherapy induces a rise in IgG blocking antibodies, especially of IgG4.^(20,22) Other studies have demonstrated that CD8⁺ suppressor T cells are increased during immunotherapy, which modulates allergen-specific IgE production,⁽²³⁾ although both of these findings are still controversial. Studies have demonstrated that the immunologic effects of immunotherapy include changes both in the secretion of cytokines and in lymphocyte proliferative responses.⁽²⁴⁾ The presence of activated T cells in blood, bronchial biopsies, and broncho alveolar lavage (BAL) from asthmatics supports the central role of T cells in the pathogenesis of asthma.⁽²⁵⁻²⁶⁾

We have previously reported increased specific antigen-stimulated IL-4 levels with undetectable IFN- γ levels from several patients with asthma.⁽²⁷⁾ Lower levels of IFN- γ are detected from peripheral blood mononuclear cells (PBMCs), lymphocytes, and serum of patients with atopy, asthma, and atopic dermatitis as compared to nonatopic, nonasthmatic controls.⁽²⁸⁻³¹⁾ PBMCs and lymphocyte cultures from patients with atopy and asthma revealed a decline in dust mite-specific IFN- γ levels following immunotherapy,⁽³²⁾ and it has been suggested that following IFN- γ levels in patients on immunotherapy may be useful.⁽³³⁾

In the murine system, IL-10 is produced selectively by Th2 clones and is shown to be a potent inhibitor of the synthesis of IFN- γ by Th1 clones but not of IL-3 or IL-4 by Th2 clones both in vitro and in vivo.⁽³⁴⁻³⁶⁾ This inhibition is primarily because of the action of IL-10 on antigen-presenting cell functions rather than on the T-helper cells themselves.⁽³⁵⁾ Similarly, human IL-10 has been shown to inhibit functions of Th1 cells in humans. Although in humans IL-10 can be produced by both Th1 and Th2 clones,⁽³⁷⁾ it may be preferentially associated in vivo with predominantly Th2 responses.^(38,39) Because of its relatively selective production by Th2 cells as well as its differential effects on Th1 cytokine synthesis, IL-10 has been thought to play a major role in cross-regulation of Th1 and Th2 development and activation, perhaps helping to control the balance between immune responses induced by these helper T-cell subsets.^(40,41) These earlier observations prompted this study.

We serially monitored circulating and specific allergen-induced IL-10 and IFN- γ production in asthmatic subjects before and during immunotherapy at 3 months and 6 months and correlated both circulating and allergen-induced PBMC cytokine levels during immunotherapy with clinical scores, including frequency of exacerbation and need for medications. Our hypothesis was that monitoring these cytokine levels may be useful in evaluating the effectiveness and response to immunotherapy or therapeutic intervention with specific antagonists.

MATERIALS AND METHODS

We initially evaluated five adult patients with moderate asthma as defined by the Global Initiative for Asthma (GINA) guidelines.⁽⁴²⁾ These patients were not on systemic steroids, because these agents inhibit

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cytokine production. A comparable number of nonatopic, nonasthmatic healthy individuals with negative skin tests 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 with a diluent and histamine control, 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 and ragweed used for immunotherapy were standard extracts (Nelco Laboratories) and were not mixed with mold allergens, because proteases in mold extracts can denature pollen antigens. All five patients had positive skin tests to grass and ragweed extracts and were given immunotherapy for both allergens. After each dose of immunotherapy, patients were observed for half an hour for any reaction to immunotherapy, and there were no systemic reactions noted during immunotherapy. Two 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 obtaining informed consent from healthy nonasthmatic controls and from asthmatic patients at baseline (prior to immunotherapy) and at serial time periods 3 and 6 months after initiation of immunotherapy to monitor cytokine secretion by specific allergen-stimulated mononuclear cells. PBMCs were prepared by the standard Ficoll Hypaque technique. Two mL of 2×10^6 PBMCs per milliliter were incubated in complete RPMI media for 24 hours with and without 20 $\mu\text{L/mL}$ of 1:10 weight-to-volume specific preservative-free grass and ragweed allergen extract. The PMBCs were then centrifuged and supernatants were harvested for cytokine determination by standard enzyme-linked immunosorbent assay (ELISA) techniques for IL-10 and IFN- γ (Cytoscreen; Biosource, Camarillo, CA; and Genzyme, Cambridge, MA). Cytokine assays were performed in duplicate and results were reproducible. Total serum IgE by ELISA was performed in asthmatic patients and healthy controls at the time of enrollment. Use of all medications was closely monitored before starting immunotherapy and throughout 6 months of immunotherapy in all patients. Episodes of wheezing, difficulty in breathing, and nighttime 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. Pulmonary function testing (spirometry) was performed before starting immunotherapy and after 3 and 6 months on immunotherapy with instrumentation by Sensomedics (Yorba Linda, CA), approved by the American Thoracic Society. Immunotherapy was done using standard alum-precipitated extracts for grasses and ragweed standardized in 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.⁽⁴³⁾ Patients were observed for 20 minutes after immunotherapy on each visit. Other than occasional mild local reactions, no significant local or systemic (immediate or protracted) reaction was noted.

Statistics

Mean and standard deviations (SDs) were calculated using the EPISTAT network. Student's *t* tests assuming unequal variance were performed, including *P* value determination, for IFN- γ and IL-10 in controls versus asthmatics before immunotherapy and for each antigen at 3 months and at 6 months after initiation of immunotherapy.

RESULTS

Cytokine evaluations for IFN- γ and IL-10 from PBMCs after a 24-hour incubation with either RPMI media alone (spontaneous) or with specific allergens (grasses and ragweed) were performed in five asthmatic patients at the time of enrollment into the study. Results were compared with results of cytokine secretion from

TABLE 1. MEAN IFN- γ AND IL-10 SECRETION (CONTROLS VERSUS ASTHMATICS)^a

Cytokines	Controls	Asthmatics Before Immunotherapy		
		Grasses	Ragweed	PBMCs
IFN- γ	600.8 $\pm$ 130	49.4 $\pm$ 2.4	54.6 $\pm$ 8.5	46.6 $\pm$ 1.8
<i>P</i> value		<0.01	<0.01	<0.01
IL-10	28.4 $\pm$ 18.5	305.4 $\pm$ 165	135.6 $\pm$ 146	12.6 $\pm$ 25.3
<i>P</i> value		<0.03	0.16	0.3

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

PBMCs after a 24-hour incubation in RPMI media (spontaneous) or with specific allergen stimulation (grasses and ragweed) from five healthy nonatopic, nonasthmatic individuals labeled as controls. There was no statistically significant difference in IFN- γ and IL-10 secretion from spontaneous and allergen-specific stimulated PBMCs in controls. Median total IgE in controls was 52 mg/dL $\pm$ an SD of 137 mg/dL and median total IgE in asthmatics was 129 mg/dL $\pm$ an SD of 93 mg/dL. This difference was not statistically significant. All patients were started on immunotherapy, and cytokine secretion was evaluated at 3 and 6 months after initiation of immunotherapy. At 3 months on immunotherapy, the mean dose for grass allergen was 220 $\pm$ 107 PNU/mL and at 6 months on immunotherapy, the mean dose for grass allergen was 1800 $\pm$ 979 PNU/mL. At 3 months on immunotherapy, the mean dose for ragweed allergen was 190 $\pm$ 150 PNU/mL and at 6 months on immunotherapy, the mean dose for ragweed allergen was 1760 $\pm$ 1015 PNU/mL. The pattern of cytokine secretion was also compared with the clinical response to immunotherapy.

Mean IFN- γ and mean IL-10 secretion values (controls versus asthmatics before starting immunotherapy) were compared (Table 1). All values are measured in picograms per milliliter and are expressed as the mean plus or minus the SD. Significantly more IFN- γ secretion was noted from unstimulated PBMCs from nonasthmatic controls (600.8 $\pm$ 130 pg/mL) as compared to PBMCs from asthmatics with or without allergen-specific stimulation; e.g., grass allergen stimulation (49.4 $\pm$ 2.4 pg/mL, P < 0.01), ragweed-specific allergen stimulation (54.6 $\pm$ 8.5 pg/mL, P < 0.01), and from unstimulated cells (46.6 $\pm$ 1.8 pg/mL, P < 0.01). Significantly less IL-10 secretion was noted from unstimulated PBMCs from nonasthmatic controls (28.4 $\pm$ 18.5 pg/mL) as compared to PBMCs from asthmatics after grass-specific allergen stimulation (305.4 $\pm$ 165 pg/mL, P < 0.03) but was not significant after ragweed-specific stimulation (135.6 $\pm$ 146 pg/mL, P = 0.16) or from unstimulated cells (12.6 $\pm$ 25 pg/mL, P = 0.3).

Mean IFN- γ and IL-10 secretions from asthmatics were compared before starting immunotherapy and after 3 months and 6 months on immunotherapy. Mean IFN- γ secretion from PBMCs (with or without allergen-specific stimulation) was significantly increased at 3 months (p < 0.001), and this significance persisted after 6 months on immunotherapy (P < 0.001), independent of allergen-specific stimulation (Table 2).

Mean IL-10 secretion from PBMCs with or without allergen stimulation was not significantly changed at 3 months. At 6 months on immunotherapy, mean allergen-specific IL-10 secretion was significantly decreased both for grass (20.3 $\pm$ 2.1 pg/mL, P < 0.02) and for ragweed (36.6 $\pm$ 14.7 pg/mL, P < 0.02). Interestingly, there was no significant change in IL-10 secretion from PBMCs without allergen stimulation (Table 3), which means that this decrease in IL-10 was an allergen-specific response.

TABLE 2. CHANGES IN MEAN IFN- γ SECRETION IN ASTHMATICS WITH IMMUNOTHERAPY^a

IFN- γ Secretion	Grasses	Ragweed	PBMCs
Before	49.4 $\pm$ 2.4	54.6 $\pm$ 8.5	46.6 $\pm$ 1.8
3 mo.	100.5 $\pm$ 6.1	102.4 $\pm$ 5.6	99.2 $\pm$ 3.2
<i>P</i> value	<0.001	<0.001	<0.01
6 mo.	98.6 $\pm$ 1.1	97.6 $\pm$ 1.2	97.6 $\pm$ 1.2
<i>P</i> value	<0.001	<0.001	<0.001

^aValues are expressed as mean $\pm$ SD and measured in pg/mL.

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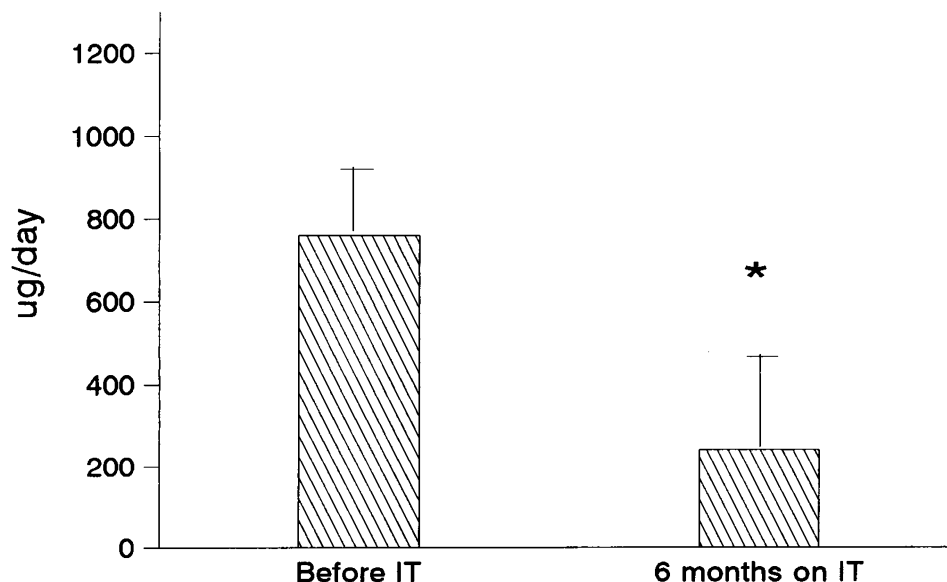


FIG. 1. Median daily dose of inhaled steroids before and at 6 months on immunotherapy. Values are expressed as the median $\pm$ SD, an asterisk denotes significant ($p < 0.02$).

When an increase in IFN- γ and a decrease in IL-10 secretion with immunotherapy were compared to the clinical response to immunotherapy, it was noted that all patients had improvement in wheezing episodes and nighttime cough. None of the patients had any exacerbation of acute asthma during immunotherapy. Before initiating immunotherapy, all patients were started on daily inhaled steroids (Triamcinolone), with doses ranging from 600 $\mu\text{g/d}$ divided into two doses per day to 1000 $\mu\text{g/day}$ divided into two doses per day. The median dose was $760 \pm 149 \mu\text{g/d}$. At 6 months on immunotherapy, two patients (receiving 600 $\mu\text{g/d}$ of Triamcinolone) were able to discontinue the use of inhaled steroids without any increase in use of β_2 agonists, the median dose of inhaled corticosteroids was $240 \pm 293 \mu\text{g/d}$ (Fig. 1), and improvement was statistically significant ($P < 0.02$). There was no statistically significant change noted in pulmonary function tests [Forced Vital Capacity (FVC); Forced Expiratory Volume in one second (FEV_1); Forced Expiratory Flow rate between 25 and 75 percent of vital capacity (FEF_{25-75})] in patients before, after 3 months, and after 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 after 6 months on immunotherapy.

DISCUSSION

Evidence suggests that T-cell responses are very important in allergic disorders such as asthma. T cells (T-helper cells) can be phenotypically divided into two groups (Th1 and Th2) based on their patterns of cy-

TABLE 3. CHANGES IN MEAN IL-10 SECRETION IN ASTHMATICS WITH IMMUNOTHERAPY^a

<i>IL-10 Secretion</i>	<i>Grasses</i>	<i>Ragweed</i>	<i>PBMCs</i>
Before immunotherapy	305.4 $\pm$ 165	135.6 $\pm$ 146	12.6 $\pm$ 25
3 mo.	214.5 $\pm$ 134	172 $\pm$ 77.5	56 $\pm$ 89
<i>P</i> value	0.4	0.6	0.4
5 mo.	20.3 $\pm$ 2	36.6 $\pm$ 14.7	17 $\pm$ 11.7
<i>P</i> value	<0.02	<0.02	0.7

^aValues are expressed as the means $\pm$ SD and measured in pg/mL.

tokine release. If CD4⁺ T cells predominantly secrete IL-4, IL-5, IL-6, and IL-10, they are called Th2 cells, but if they secrete more IFN- γ and IL-2, they are called Th1 cells. IL-4 stimulates B cells to secrete more IgE, thus increasing the risk of allergic diseases such as asthma. In healthy individuals, both responses cross-regulate each other. It is suggested that in allergic diseases, e.g., asthma and atopic dermatitis, this delicate balance is altered and Th2 responses predominate; therefore, more IgE is secreted. It is hypothesized that if we reverse or modify these alterations in cytokine secretion, we may be able to block or even reverse worsening of allergic disease such as asthma.

We evaluated the role of immunotherapy in modulating cytokine release from PBMCs in response to specific grass and ragweed allergens in five moderate-to-severe asthmatics. Although the number of our patients was limited, we observed a significant increase in mean IFN- γ (Th1 response) secretion after 3 months on immunotherapy ($P < 0.01$ in each case), and this increase was persisted at 6 months on immunotherapy ($P < 0.01$), whereas allergen-specific IL-10 secretion (Th2 response) decreased ($P < 0.02$). This modulation of cytokine secretion was closely correlated with marked clinical improvement in all patients. The number of our patients was small, and a placebo effect cannot be ruled out. There was a significant reduction in the amount of inhaled steroids in that two patients discontinued their use, and thereby, the median dose was statistically significantly lower. Because there is no true control group in our study (with 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, decrease in use of medications, and changes in T-cell cytokine profile (such as being out of the allergy season). Studies in larger numbers of patients are needed to confirm these preliminary findings.

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REFERENCES

1. Creticos PS. Immunology in asthma. *J Allergy Clin Immunol* 1989;83:554–562.
2. National Asthma Education Program, Expert Panel Report. Guidelines for the diagnosis and management of asthma. National Heart, Lung, Blood Institute (NHLBI) Publication No. 91-3042, p. 10, August, 1991.
3. Frieri M. The role of cytokines in asthma. *Asthma Management* 1993;2:25–31.
4. Knapik M, Frieri M. Altered cytokine production in atopic dermatitis. A preliminary study. *Pediatr Asthma, Allergy Immunol* 1993;7:127–133.
5. Tepper RI, Levinson DA, Stanger BZ, Campos-Torres J, Abbas AK, Leder P. IL-4 induced allergic-like inflammatory disease and altered T-cell development in transgenic mice. *Cell* 1990;62:457–467.
6. Finkelman FD, Urban JF, Jr, Beckmann MP, Schooley KA, Holmes JM, Katons IM. Regulation of murine in vivo IgG and IgE responses by a monoclonal anti-IL-4 receptor antibody. *Int Immunol* 1991;3:599–607.
7. DeKruyff RH, Turner T, Abrams JS, Palladino MA, Jr, Umetsu DT. Induction of human IgE synthesis by CD4⁺ T-cell clones: Requirement for interleukin 4 and low molecular weight B-cell growth factor. *J Exp Med* 1989;170:1477–1493.
8. Walker C, Virchow JC, Jr, Bruijnzeel PLB, Blaser K. T-cell subsets and their soluble products regulate eosinophilia in allergic and non-allergic asthma. *J Immunol* 1991;146:1829–1835.
9. Stern M, Meagher L, Savill J, Haslett C. Apoptosis in human eosinophils: Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. *J Immunol* 1992;148:3543–3549.
10. Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D. Antibody to IL-5 inhibits helminth-induced eosinophilia in mice. *Science* 1989;245:308–310.
11. Umetsu DT, Bocian RC, DeKruyff RH. Restricted lymphokine synthesis in CD4⁺ helper T cells: New insights

IMMUNOTHERAPY IN ASTHMA

- into the regulation of IgE synthesis and into the pathogenesis of allergic disease. *Pediatr Asthma, Allergy Immunol* 1992;6:1.
12. Gaga M, Frew AJ, Varney VA, Kay AB. Eosinophil activation and T lymphocyte infiltration in allergen-induced late phase skin reactions and classical delayed-type hypersensitivity. *J Immunol* 1991;147:816–822.
 13. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
 14. Kapsengerg ML, Wierenga EA, Bos JD, Jansen HM. Functional subsets of allergen-reactive human CD4+ T cells. *Immunol Today* 1991;12:392–395.
 15. Marshall J, Wen Y, Abrams JS, Umetsu DT. In vitro synthesis of IL-4 by human CD4+ T cells requires repeated antigenic stimulation. *Cell Immunol* 1993;152:18–34.
 16. Durham SR, Ying S, Varney VA, Jacobsen MR, Sudderick RM, Mackay IS, Kay AB, Hamid QA. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: Relationship to tissue eosinophilia. *J Immunol* 1992;148:2390–2394.
 17. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin-4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993;178:2123–2130.
 18. Varney VA, Gaga M, Frew AJ, Kay VR, Durham SR. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. *BMJ* 1991;302:530–531.
 19. Norman PS. Immunotherapy for nasal allergy. *J Allergy Clin Immunol* 1988;81:992–996.
 20. Bousquet J, Becker WM, Hejjaoui A, Cour P, Chanal I, Lebel B, Dhivert H, Michel FB. Clinical and immunological reactivity of patients allergic to grass pollens and to multiple pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. *J Allergy Clin Immunol* 1991;88:43–53.
 21. Van Metre TE, Marsh DG, Adkinson NF, Jr. Immunotherapy for cat asthma. *J Allergy Clin Immunol* 1988;82:1055.
 22. Reid MJ, Moss RB, Hsu YP, Kwasnicki JM, Commerford TM, Nelson BL. Seasonal asthma in northern California: Allergic causes and efficacy of immunotherapy. *J Allergy Clin Immunol* 1986;78:590–600.
 23. Rocklin RE, Sheffer A, Greineder DK, Melmon KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980;302:1213–1219.
 24. Rocklin RE. Clinical and immunologic aspects of allergen-specific immunotherapy in patients with seasonal allergic rhinitis and/or allergic asthma. *J Allergy Clin Immunol* 1983;72:323–334.
 25. Gerblich AA, Salik H, Schuyler MR. Dynamic T-cell changes in peripheral blood and bronchoalveolar lavage after antigen bronchoprovocation in asthmatics. *Am Rev Respir Dis* 1991;143:533–537.
 26. Azzawi M, Bradley B, Jeffrey PK, Frew AJ, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407–1413.
 27. Castillo L, Knapik M, Frieri M. Gamma interferon and interleukin-4 levels in stimulated PBMC from atopic patients evaluated for food hypersensitivity [abstract]. *J Allergy Clin Immunol* 1992;89:203a.
 28. Hashimoto S, Amemiya E, Horie T, et al. Elevation of soluble IL-2 receptor and IL-4, and non-elevation of IFN-gamma in sera from patients with allergic asthma. *Ann Allergy* 1993;71:455–458.
 29. Tang ML, Coleman J, Kemp AS. Interleukin-4 and interferon-gamma production in atopic children with asthma. *Clin Exp Allergy*. 1995;25:515–521.
 30. Noma T, Yoshizawa I, Yata J, et al. Pattern of cytokine production by T cells from adolescents with asthma in remission, after stimulation with *Dermatophagoides farinae* antigen. *Pediatr Res* 1995;38:187–193.
 31. Tang ML, Kemp A, Varigos G. Interleukin-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993;92:120–124.
 32. Ellaurie M, Yost SL, Rosenstreich DL. A simplified human whole blood assay for measurement of dust mite-specific gamma interferon production in vitro. *Ann Allergy* 1991;66:143–147.
 33. Walker C. T lymphocytes and cytokines in intrinsic asthma. Presented at the AAAI/EAACI Cytokine Symposium, Orlando, FL, March 11, 1992.
 34. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T-helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989;170:2081–2095.

35. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Hoard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cells to inhibit cytokine production by Th1 cells. *J Immunol* 1991;146:3444-3451.
36. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mossman TR. Interleukin-10. *Annu Rev Immunol* 1993;11:165-190.
37. Del Prete G, De Carli M, Almerigonga F, Giudici MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both Th1 helper T clones and Th2 helper T clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;150:353-360.
38. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, Bloom BR. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T-cell clones. *Science* 1991;254:279-282.
39. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, Modlin RL. Defining protective responses to pathogens: Cytokine profiles in leprosy lesions. *Science* 1991;254:277-279.
40. Paul WE, Metcalfe DD, Busse WW, Reece ER. Asthma (NIH Conference). *Ann Intern Med* 1994;121:698-708.
41. Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin-4 deficient mice. *Science* 1991;254:707-710.
42. Global Initiative for Asthma. NIH/NHLBI Publication No. 95-3659, 1995.
43. Fischer TJ, Entis GN, Winant JG, Bernstein IL. Basic principles of therapy for allergic disease. In Lawlor GL, Fischer TJ, eds. *Manual of Allergy and Immunology*, 2nd edition. Boston: Little, Brown and Company, 1988, pp 92-95.

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