

Interleukin-10 regulation in normal subjects and patients with asthma

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Interleukin-10 or cytokine synthesis inhibitory factor has important antiinflammatory activities in immune diseases. We speculated that diminished IL-10 production in asthma would permit the unopposed synthesis of proinflammatory cytokines, contributing to the development and severity of asthma. Our data demonstrate constitutive secretion of IL-10 into bronchoalveolar lavage (BAL) fluid of normal, nonasthmatic subjects (130 ± 61 pg/ml; $n = 8$). Asthmatic patients' BAL fluid was characterized by diminished concentrations of IL-10 (9 ± 18 pg/ml; $n = 8$; $p < 0.01$ compared with that of normal subjects). By using the RNA-based polymerase chain reaction, we demonstrated that diminished IL-10 occurred as a result of inhibition of transcription. IL-10 transcription, but not protein, was observed at the time of the late asthmatic response. We speculate that the subsequent appearance of IL-10 protein could contribute to the resolution of the late asthmatic response. Similar to what was observed in the BAL fluid, peripheral blood mononuclear cells of patients with asthma demonstrated decreased spontaneous (0.01 ± 0.01 ng/ml—asthmatic and 0.09 ± 0.04 ng/ml—normal; $p < 0.05$) and stimulated (0.60 ± 0.22 ng/ml—asthmatic and 1.69 ± 0.49 ng/ml—normal; $p < 0.05$) IL-10 production compared with normal subjects. In support of the hypothesis that IL-10 mitigates the development of inflammation, we demonstrated that the addition of a neutralizing anti-IL-10 antibody to resting peripheral blood mononuclear cell cultures of normal subjects stimulated the spontaneous production of interferon- γ (10.4 ± 4.3 to 152.4 ± 23.6 ng/ml; $p < 0.01$). Finally, we reasoned that corticosteroids might exert at least part of their antiinflammatory activity through the induction of IL-10 secretion. However, methylprednisolone inhibited the lipopolysaccharide-stimulated production of IL-10 (2.34 ± 0.49 ng/ml IL-10 with lipopolysaccharide alone to 1.11 ± 0.38 ng/ml in the additional presence of 10^{-6} mol/L methylprednisolone; $p < 0.05$). (J Allergy Clin Immunol 1996;97:1288-96.)

Key words: Asthma, IL-10, bronchoalveolar lavage, inflammation, corticosteroids

Interleukin-10 is an intrinsic antiinflammatory peptide, originally identified and cloned as cytokine synthesis inhibitory factor, which has major downregulatory influences on inflammation. Human IL-10 is different from murine IL-10, which is primarily a T_{H2} product that contributes to T_{H1}/T_{H2} differentiation.^{1,2} Human IL-10 is produced by both T_{H1} and T_{H2} lymphocytes^{3,4}; however, blood

Abbreviations used

BAL:	Bronchoalveolar lavage
IFN:	Interferon
LAR:	Late asthmatic response
LPS:	Lipopolysaccharide
PBMCs:	Peripheral blood mononuclear cells
RT-PCR:	Reverse transcriptase-polymerase chain reaction
TNF:	Tumor necrosis factor

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monocytes and tissue macrophages may be its most important source. Human IL-10 is further differentiated from murine IL-10 by its broader spectrum of activity. In addition to its effects on inhibition of interferon (IFN)- γ and IL-2 production by T_{H1} lymphocytes, IL-10 acts to inhibit cytokine production by mononuclear phagocytes,⁵⁻¹⁰ natural killer cells,¹¹

and T_{H2} lymphocytes.³ Expression of IL-10 by antigen-presenting cells may have a role in lessening allergic inflammation through this ability to inhibit to synthesis of nonspecific proinflammatory cytokines such as IL-1, IL-6, tumor necrosis factor (TNF)- α , and IFN- γ , as well as cytokines associated with allergic inflammation including IL-4 and IL-5.³ This role for IL-10 in mitigating allergic inflammation is further supported by its ability to induce T-cell tolerance to allergens¹² and to inhibit eosinophil survival¹³ and IgE synthesis.¹⁴ We therefore attempted to determine whether decreased synthesis of IL-10 in the lungs of patients with asthma may contribute to the inflammation that is characteristic of this disease.

METHODS

Subjects

Assays were performed on cohorts of volunteers with asthma and normal control subjects (Tables I and II). Normal volunteers were individuals who lacked a medical history consistent with atopic disease including asthma, allergic rhinitis, or eczema. Skin prick test responses to a Colorado allergen panel were negative, and at the time of study volunteers' spirometry was normal. The asthmatic subjects met the criteria of the American Thoracic Society–American College of Chest Physicians for asthma¹⁵ and had reversible airway obstruction ($\geq 15\%$ improvement with bronchodilator and baseline FEV₁ $\geq 55\%$ of predicted value). All subjects reported a history of seasonal allergic rhinitis and demonstrated positive skin test responses (wheal >3 mm) to at least three of 13 common Colorado allergens. Asthma was controlled with β -adrenergics alone. Subjects were excluded from the study if they had other medical illnesses or recent upper respiratory tract infections, or if they had taken steroids (oral or inhaled) or cromolyn within 6 weeks of the study. All subjects gave informed consent, and the study was approved by the Institutional Review Board at the National Jewish Center. Four of the patients with asthma were screened and demonstrated to have a late asthmatic response (LAR) to an allergen challenge. Forty-eight hours after a baseline bronchoalveolar lavage (BAL) was performed, these four patients underwent an inhalation challenge with the relevant allergen. The dose given was determined from the preliminary testing as the total amount that previously caused a 25% decrease in FEV₁ from baseline. The dose was delivered in 3 aliquots, separated by 15 minutes. Serial pulmonary function testing was performed, and BAL was repeated 1 hour after a sustained (>2 consecutive $\frac{1}{2}$ hour time points) 15% or greater fall in FEV₁ was observed.

BAL

The lingula and right middle lobe were used for each BAL. Seven 40 ml aliquots were infused with gentle

TABLE I. Characteristics of normal subjects

Patient	Age (yr)	Sex	FEV ₁ (%)	Total cells*
BK	36	M	106	18.7
AG	30	F	104	15.8
CS	27	F	87	18.8
BE	22	M	93	9.4
CC	23	F	100	21.5

*More than 90% alveolar macrophages.

hand suction used to produce the effluent from each aliquot, as previously described.¹⁶ BAL fluid was centrifuged (600 g for 15 minutes), and both the supernatant and cell pellets were collected.

ELISA

IL-10 concentrations within the BAL fluid and from stimulated peripheral blood mononuclear cells (PBMCs) were assessed by ELISA.¹⁷ This ELISA uses a sandwich technique with two rat monoclonal antibodies, one of which is biotinylated. The sensitivity of this ELISA is 50 pg/ml. BAL fluid does not interfere with our capacity to measure IL-10 as demonstrated by studies in which we incubated authentic IL-10 protein (1 ng) with BAL fluid for 1 hour at 37°C (unpublished observations). Flat-bottomed polystyrene microtiter plates were coated with the anti-IL-10 capture antibody (4 μ g/ml; Pharmingen; San Diego, Calif.) in the presence of carbonate buffer (pH 9.6, 0.1 mol/L). Authentic IL-10 standards (100 pg to 2 mg; Genzyme, Boston, Mass.) and samples (PBMC supernatants neat and BAL fluid concentrated 10-fold by centrifuge filtration [Centriprep-30; Amicon, Inc., Beverly, Mass.]) were applied in duplicate and allowed to bind overnight at 4°C. After washing, the biotinylated anti-IL-10 detection antibody was applied (2 μ g/ml). After the additional application of avidin-peroxidase (1:400; Sigma Chemical Co., St. Louis, Mo.), substrate buffer (2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, Sigma) was introduced, and color change was determined on a Bio-Tek EL311 ELISA reader (Bio-Tek Instruments, Inc., Winooski, Vt.). Data are expressed as the concentration of cytokine per milliliter (for BAL fluid this was adjusted to concentration in the undiluted sample). Additional ELISAs were performed for IL-1 β (Cistron Biotechnology, Pine Brook, N.J.) and IFN- γ (Genzyme) by using commercial kits according to the manufacturers' instructions.

Reverse transcriptase–polymerase chain reaction

The presence of IL-10–specific transcripts was detected through the application of reverse transcription followed by the polymerase chain reaction (RT-PCR). Total RNA was extracted from the cell pellets in RNAzol (Tel-Test, Inc., Friendswood, Texas). The RNA (1 μ g) was washed, denatured (65°C for 5 minutes), and exposed to Moloney murine leukemia virus reverse

TABLE II. Characteristics of patients with asthma

Patient	Age (yr)	Sex	FEV ₁ (% pred)	LAR (%)*	Total cells × 10 ⁴		Eosinophils (%)		Lymphocytes (%)		Macrophages (%)	
					Pre	Post	Pre	Post	Pre	Post	Pre	Post
KG	29	M	56	13	18.8	13.1	16	15	2	4	78	79
SA	40	M	107	29	17.4	12.5	3	5	11	3	86	88
GS	41	M	71	5	22.5	19.4	0	0	1	9	97	88
SJ	27	M	67	27	8.8	8.4	5	2	3	0	90	97

*Percent decline in FEV₁ from baseline.

transcriptase (600 U; Life Technologies, Inc., Gaithersburg, Md.) in the additional presence of RNAsin (40 U; Promega, Madison, Wis.), oligo d(T) 12-18mer (Promega), deoxyribonucleoside triphosphate mix (10 mmol/L each; Promega) and the supplied RT buffer. The reaction was allowed to continue for 1 hour at 42°C, after which the RT product was denatured (95°C for 5 minutes). Aliquots of complementary DNA (1:40 dilution) were then subjected to the PCR in the presence of IL-10-specific primers (5': AAGCTGAGAACCAAGACCCAGACATC AAGGCG; 3': AGCTATCCCAGAGCCCCAGATCCG ATTTTGG; Clontech, Palo Alto, Calif.), Taq Polymerase (1 U, Promega), deoxyribonucleoside triphosphate mix (1.25 mmol/L each), and supplied reaction buffer. These primers expand a 328 nucleotide sequence from complementary DNA and cross at least one intron of genomic DNA. The DNA thermal cycler was programmed to perform 30 cycles with the following conditions: 94°C for 1 minute, 55°C for 2 minutes, 72°C for 3 minutes. At the completion of the PCR, 9 µl of PCR product was mixed with 1 µl of gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole, 30% glycerol) and electrophoresed in 1% agarose in the additional presence of 0.5 µg/ml ethidium bromide. The amplified DNA bands were visualized with a UV transilluminator. Data were normalized to the concentration of total RNA in the original RT mix and through the expansion of β-actin cDNA.

Modulation of IL-10 synthesis

We investigated spontaneous and stimulated IL-10 and IL-1β production by PBMCs from nonatopic nonasthmatic normal subjects and patients with asthma. Complete medium consisted of RPMI-1640 (Gibco, Grand Island, N.Y.) supplemented with penicillin (200 U/ml), streptomycin (200 µg/ml), L-glutamine (4 mmol/L), N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer (25 mmol/L, pH 7.20) and 10% Nuserum (Collaborative Research, Bedford, Mass.). Heparinized venous blood was obtained, and PBMCs were isolated by Ficoll-hypaque centrifugation (Pharmacia, Inc., Uppsala, Sweden). PBMCs (2 × 10⁶/ml) were either left in a resting state or stimulated with lipopolysaccharide (LPS) (10 µg/ml, Sigma). After 48 hours, supernatants were collected, and IL-10 and IL-1β concentrations were determined by ELISA. Further studies were performed

to evaluate the modulation of IL-10 production by corticosteroids. PBMCs were obtained from normal subjects, stimulated with LPS (10 µg/ml), and then exposed to methylprednisolone (10⁻⁶ to 10⁻⁴ mol/L, Sigma). Finally, we investigated the ability of neutralizing anti-IL-10 antibodies to stimulate spontaneous activation of T lymphocytes as manifested by IFN-γ secretion. The neutralizing antibody (R & D Systems, Minneapolis, Minn.) is a monoclonal murine antibody with a 50% neutralization dose of 0.01 to 0.03 µg/ml and was used at a concentration of 1 µg/ml. These studies were performed in nonatopic nonasthmatic subjects. Supernatants were collected at 48 hours, and IFN-γ concentrations were evaluated by ELISA.

Statistics

Results are presented as means ± SD for the replicate data points. Statistical analyses were performed by using Student's paired *t* test on a Macintosh IICx computer (Apple Computer Inc., Cupertino, Calif.) with JMP 3.0 software (SAS Institute, Cary, N.C.).

RESULTS

IL-10 and IL-1β protein in BAL fluid

IL-10 protein concentration in BAL fluid was measured by an ELISA. Patient characteristics are summarized in Tables I and II. IL-10 was readily measured in the BAL fluid of all of the normal subjects (130 ± 61 pg/ml; range, 41 to 211 pg/ml; *n* = 8). In contrast, only two of eight patients with asthma demonstrated IL-10 in the BAL fluid above the sensitivity of the ELISA, and even in these subjects, the concentration was lower than that observed in the normal subjects (9 ± 18 pg/ml; *p* < 0.01 compared with normal subjects; Fig. 1). None of the control subjects (*n* = 8) demonstrated IL-1β in their BAL fluid.

In four patients with asthma, BAL was also performed at the time of the LAR. No significant changes in the inflammatory cell infiltrate were observed (Table II). The LAR was associated with increased secretion of IL-1β by each subject (20.6 ± 11.2 to 54.4 ± 14.3 pg/ml concentrated BAL

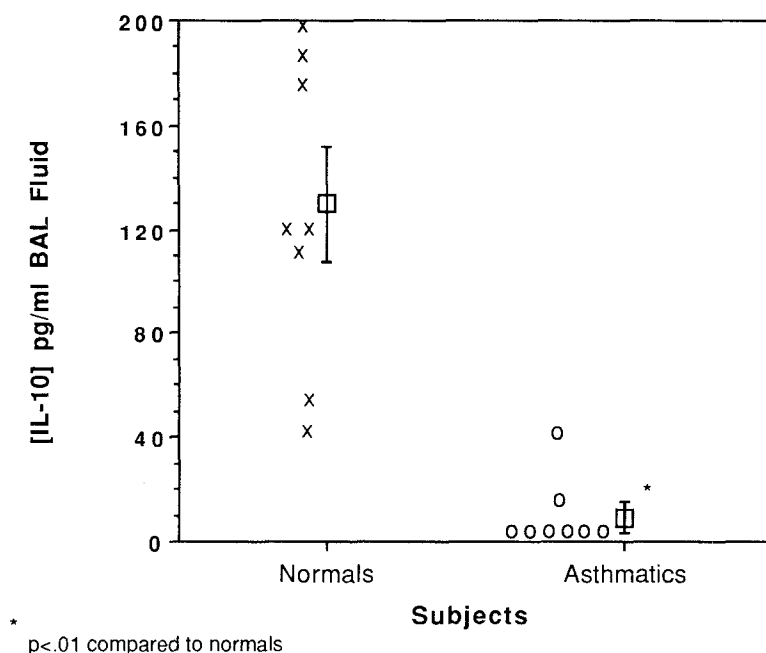


FIG. 1. IL-10 Protein in BAL fluid. BAL fluid was obtained from normal subjects and patients with asthma and concentrated 10-fold by filter centrifugation. IL-10 concentrations were determined by ELISA and are expressed as picograms per milliliter of the unconcentrated BAL fluid.

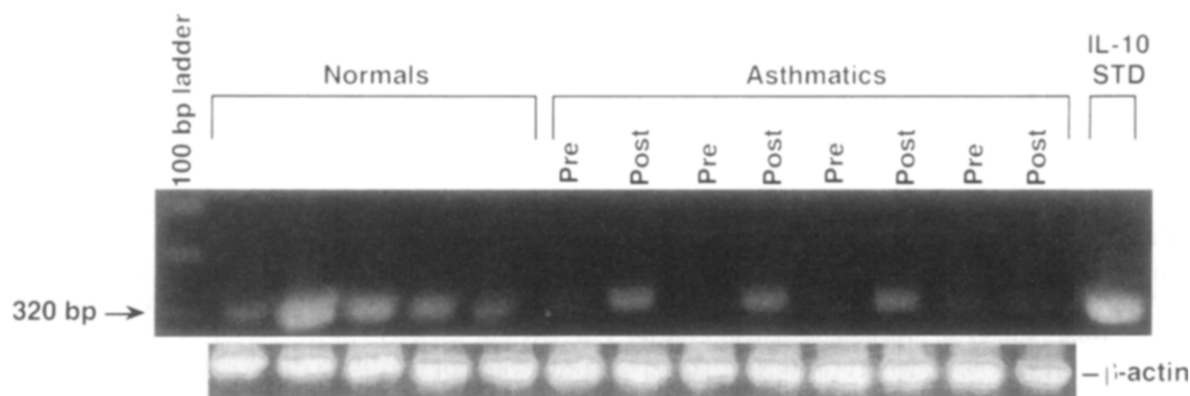


FIG. 2. IL-10 RT-PCR From BAL cell pellets. IL-10 transcripts were identified in BAL cell pellets from normal subjects and patients with asthma by RNA-based PCR. Additional studies were done on asthmatic BAL cell pellets obtained at the time of the late-phase response.

fluid; $p = 0.08$). None of the four patients had measurable IL-10 protein in their BAL fluid as determined by ELISA at the time of the LAR.

IL-10 RT-PCR on BAL cell pellets

We characterized expression of the human IL-10 gene by using the RNA-based PCR. Analyses were performed on a panel of five nonatopic nonasthmatic subjects and four patients with atopic asthma, and the data are displayed in Fig. 2. Patients with asthma were studied at baseline and at the time of their late-phase response to an allergen challenge. All five nonasthmatic subjects

demonstrated IL-10 transcripts. The four patients with asthma all demonstrated IL-10 transcripts at baseline as determined by RT-PCR; however, the concentration of PCR product was less in these four patients than in the six nonasthmatic subjects. Data are normalized to the expansion of cDNA for β -actin in the BAL cell pellets. After allergen challenge, there was an increase in IL-10 transcripts in three of the four patients with asthma.

IL-10 and IL-1 β synthesis by PBMCs

PBMCs obtained from 10 nonatopic, nonasthmatic subjects spontaneously secreted low concen-

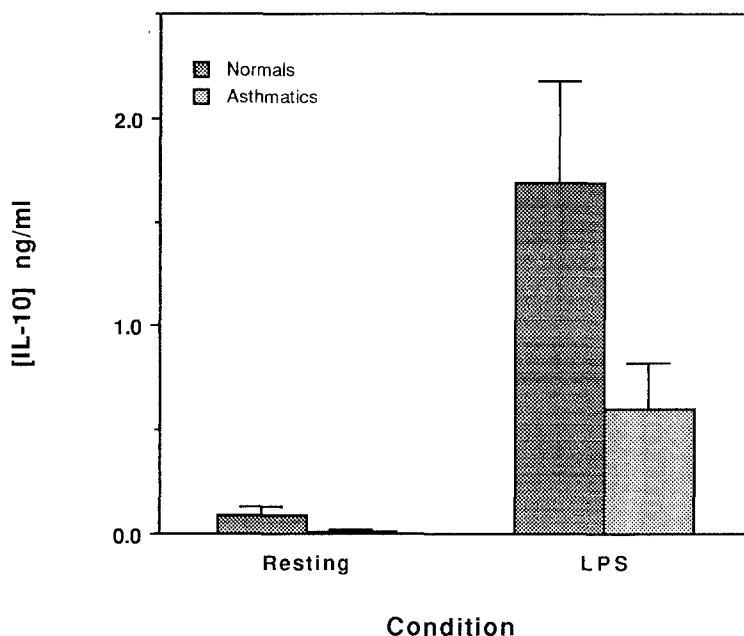


FIG. 3. IL-10 production by PBMCs. PBMCs were obtained from either nonatopic, nonasthmatic subjects or patients with asthma. Cells were either left in a resting state or stimulated with LPS (10 μ g/ml). Supernatants were collected after 48 hours and assayed for IL-10 concentrations by ELISA.

trations of IL-10 (0.09 ± 0.04 ng/ml; Fig. 3). This constitutive production of IL-10 was associated with the presence of IL-10 transcripts as shown by RT-PCR (data not shown). Less IL-10 secretion was observed from PBMCs obtained from patients with asthma (0.01 ± 0.01 ; $n = 8$; $p < 0.05$). LPS stimulated IL-10 production, however, in comparison with PBMCs from asthmatic subjects, PBMCs from normal subjects synthesized significantly more IL-10 (1.69 ± 0.49 ng/ml) (0.60 ± 0.22 ; $p < 0.05$; Fig. 3). In contrast to IL-10 no differences were observed in LPS-stimulated IL-1 β production by PBMCs derived from normal subjects (2.13 ± 0.42 ng/ml) and patients with asthma (1.65 ± 0.35 ng/ml).

Constitutive IL-10 production prevents the spontaneous synthesis of IFN- γ by PBMCs

To further evaluate a role for IL-10 in mitigating inflammation in the nonasthmatic population, we investigated the ability of a neutralizing anti-IL-10 antibody to permit spontaneous IFN- γ secretion (Fig. 4). Anti-IL-10 was a potent stimulus for IFN- γ secretion (10.4 ± 4.3 ng/ml in resting cells to 152.4 ± 23.6 ng/ml; $p < 0.01$), comparable to that observed with phytohemagglutinin (255.8 ± 101.1 ng/ml).

Corticosteroids inhibit IL-10 production

We hypothesized that corticosteroids might exert some of their antiinflammatory activity through the induction of IL-10 synthesis. Our data demonstrate that as observed for other cytokines, methylprednisolone inhibits (from 2.34 ± 0.49 ng/ml IL-10 with LPS alone to 1.11 ± 0.38 ng/ml in the additional presence of methylprednisolone; 10^{-6} mol/L; $p < 0.05$; $n = 7$) the LPS-stimulated production of IL-10. No further inhibition of IL-10 secretion was observed, even at concentrations of corticosteroids up to 10^{-4} mol/L (not shown).

DISCUSSION

Immune responses generated by cytokines are essential to the development of asthma. T lymphocyte-derived cytokines are required for the development of several phenotypic markers of asthma including IgE dysregulation (IL-4 and IL-13¹⁸⁻²⁰), eosinophilia (IL-5, granulocyte-macrophage colony-stimulating factor, and IL-3^{21, 22}), mast cell proliferation (IL-3, IL-9, stem cell factor²³⁻²⁵), and enhanced histamine release (IL-3, RANTES, and monocyte chemotactic and activating factor²⁶). Inflammation within the asthmatic lung has been associated with the expression of IL-1, tumor necrosis factor (TNF)- α , IL-6, and IL-8, cytokines

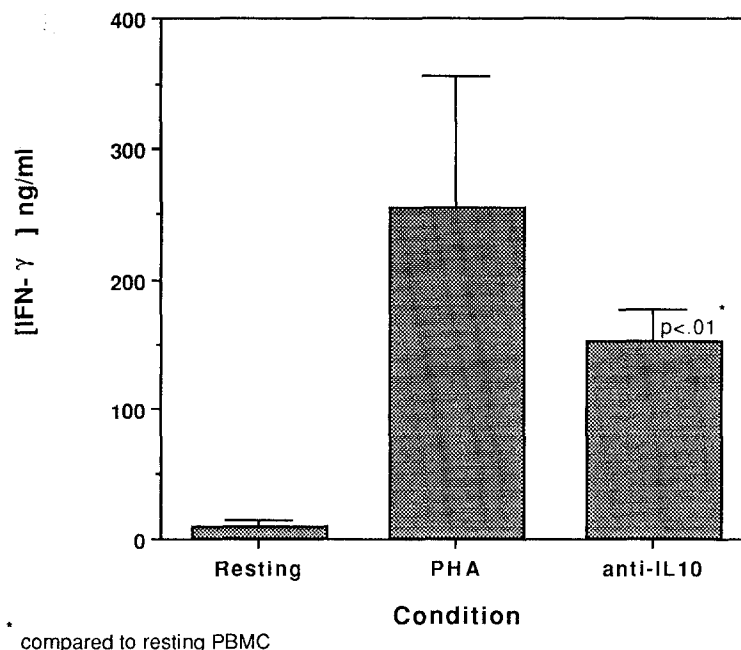


FIG. 4. Effect of anti-IL-10 on spontaneous IFN- γ production by PBMCs. PBMCs were either left in a resting state or stimulated with a neutralizing anti-IL-10 antibody (1:100) or phytohemagglutinin (0.3 μ g/ml). After 48 hours, supernatants were collected and assayed for IFN- γ by ELISA. PHA, Phytohemagglutinin.

that mediate inflammatory cell recruitment, mediator release, and adhesion molecule expression.^{27,28} Agents that modulate cytokine activity are active in controlling asthma. Corticosteroids owe much of their efficacy in the treatment of asthma to inhibition of cytokine release.^{27,29} Similarly, inhibition of cytokine secretion may contribute to the antiinflammatory mechanisms of cromolyn, nedocromil, and even methylxanthines.^{28,30}

An intrinsic physiologic mechanism for inhibiting cytokine synthesis is the cytokine IL-10. Human IL-10 is an antiinflammatory cytokine with major downregulatory influences in inflammation. Human IL-10, in contrast to murine IL-10, which is primarily a T_{H2} product, is produced by both T_{H1} and T_{H2} lymphocytes,^{3,4} as well as mononuclear phagocytic cells, which may be its most important source.^{7,8} Human IL-10 is also a product of other cells including cytotoxic T cells, B lymphocytes,³¹ and mast cells.³² Human IL-10 is further distinguished from murine IL-10 through its broader spectrum of activity. In addition to inhibition of IFN- γ and IL-2 production by T_{H1} lymphocytes, human IL-10 inhibits IL-4 and IL-5 production by T_{H2} lymphocytes³; IFN- γ and TNF- α production by natural killer cells¹¹; and IL-1 β , IL-6, IL-8, IL-12, and TNF- α production by mononuclear phago-

cytes.⁵⁻¹⁰ Mitigation of allergic inflammation by IL-10 is supported by its ability to inhibit IgE production, shorten eosinophil survival, and induce allergen-specific tolerance.¹²⁻¹⁴ The kinetics of IL-10 production differ from those of other cytokines. Although the message for IL-2 and IFN- γ can be identified 15 minutes to 4 hours after lymphocyte stimulation, the message for IL-10 does not appear until 8 hours after stimulation and is maximal at 12 to 24 hours. The appearance of IL-10 protein correlates with and may contribute to downregulation of IL-2, IL-4, and other proinflammatory cytokines.⁴ These inhibitory effects of IL-10 are in contrast to its ability to activate B lymphocytes³³⁻³⁵ and cytotoxic T cells.³⁶ Thus IL-10 inhibits cytokines associated with cellular immunity and allergic inflammation while stimulating humoral and cytotoxic immune responses.

We therefore reasoned that expression of IL-10 might be diminished in patients with asthma and that this dysregulation contributes to the inflammation of asthma. Our data demonstrate the constitutive production of IL-10 in the lungs of normal, nonatopic subjects (Fig. 2). This is similar to observations that IL-10 is constitutively produced by normal, unstimulated PBMCs (Fig. 3). This constitutive IL-10 pro-

duction in the lungs and peripheral blood of normal subjects may help prevent development of inflammation in the healthy state. This hypothesis is supported by the observation that anti-IL-10–neutralizing antibodies allow IFN- γ to be produced in freshly purified human mononuclear cell preparations (Fig. 4). In support of these observations, it was previously shown *in vivo* that anti-IL-10–treated mice had substantially elevated levels of TNF- α , IFN- γ , and IL-6.^{37,38} No differences were observed in IL-1 β production by PBMCs from normal subjects and patients with asthma. These data suggest that diminished production of IL-10 by PBMCs from patients with asthma is a specific effect and not a generalized downregulation of monokine production.

In contrast to normal subjects, there was a decrease in IL-10 expression in the BAL fluid of patients with asthma. Previous studies have demonstrated the presence of either protein or transcripts for numerous cytokines in BAL fluid, cell pellets, or bronchial epithelial biopsy specimens including those for IL-1 β , IL-4, IL-5, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor.^{27,28,39–42} The synthesis of these cytokines would be expected to be abrogated in the presence of IL-10. Consistent with this hypothesis is our observation that although IL-1 β was readily detected in the lungs of patients with asthma and was further upregulated after an allergen challenge, no IL-1 β was detected in the BAL fluid of normal subjects. This absence of IL-1 β in normal subjects confirms our earlier observations²⁷ and is consistent with the presence of antiinflammatory influences of IL-10. Together, these data support our hypothesis that the absence of IL-10 protein could lead to the unopposed actions of proinflammatory cytokines, contributing to the presence and severity of asthma.

These data are similar to a report of diminished IL-10 protein in cystic fibrosis.⁴³ In addition to the diminished IL-10 observed in the lungs, PBMCs obtained from patients with asthma demonstrated diminished constitutive and stimulated IL-10 production in comparison with normal subjects (Fig. 3). Therefore diminished IL-10 production may be a nonspecific feature associated with allergic inflammation.

Decreased IL-10 protein in the BAL fluid might reflect either decreased synthesis or increased clearance through proteolytic destruction or binding to receptors. That co-incubation of asthmatic BAL fluid with IL-10 for 1 hour at 37° C did not interfere with the ability to measure IL-10 in the ELISA suggests that proteases or secreted IL-10

receptors were probably not responsible for our inability to measure IL-10. However, binding to epithelial or other receptors can not be ruled out. Our data demonstrating diminished IL-10 transcripts in patients with asthma in comparison with normal subjects (Fig. 2) suggest that the decrease in IL-10 concentrations reflects, at least in part, inhibition of transcription. In contrast to the baseline state, IL-10 transcripts were induced after an allergen challenge. However, no IL-10 protein was observed at the 8-hour time point, and concentrations of IL-1 β were elevated relative to baseline. Previous studies have shown that IL-10 transcripts typically are only induced at 8 hours and peak at 12 to 24 hours, with the appearance of protein at these later time points.⁴ We speculate that the ultimate appearance of IL-10 protein may contribute to the resolution of the LAR.

RT-PCR does not permit determination of the cell source for the IL-10 in the lungs. In human beings, mononuclear phagocytic cells are the primary source, and we have recently shown that in the lungs, alveolar macrophages and elicited monocytes are the sources.^{7,8,44} We did not observe significant changes in the infiltrating cells present in the BAL cell pellets after the allergen challenge, with alveolar macrophages continuing to represent more than 80% of the cells present. As noted, T_{H1} and T_{H2} lymphocytes, cytotoxic T cells, B lymphocytes, and mast cells are known to produce IL-10, although the percentage of BAL cells in these subjects was always less than 10%. It is therefore unlikely that the appearance of IL-10 represented the influx of IL-10–producing cells.

Finally, we reasoned that corticosteroids may exert some of their antiinflammatory biologic activity through stimulation of IL-10 production. However, our data demonstrate that as with other cytokines,^{45–47} corticosteroids inhibit synthesis of IL-10. The effects of corticosteroids on IL-10 are complex, involving numerous positive and negative regulatory influences. It has been estimated that from 50% to 75% of the IL-10 secretion induced by LPS occurs as a result of induction of TNF- α , as shown by the ability of anti-TNF- α antibodies to partially abrogate LPS-induced IL-10 secretion.⁸ Inhibition of IL-10 secretion by corticosteroids may therefore reflect inhibition of TNF- α production by corticosteroids. However, there are likely to be additional direct effects of corticosteroids on IL-10 transcription as suggested by the presence of consensus AP-1 and NF κ B sequences in the IL-10 promoter, the activities of which would be inhib-

ited by corticosteroids,⁴⁸ in addition to effects mediated through a consensus glucocorticoid response element.^{49, 50} Inhibition of IL-10 production by methylprednisolone would be expected to exert antiinflammatory effects on humoral immune responses, given the ability of IL-10 to stimulate B-lymphocyte proliferation and differentiation^{31, 33-35, 37-38} and to activate cytotoxic T cells.³⁶

In summary, asthma is characterized by diminished concentrations of IL-10. The relative absence of IL-10 in asthma permits the continued secretion of allergenic cytokines such as IL-4 and IL-5, as well as other proinflammatory cytokines including IL-1, TNF- α , IL-6, and granulocyte-macrophage colony-stimulating factor. The later appearance of IL-10 after an allergen challenge may contribute to the resolution of the late-phase response.

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