

Proinflammatory effects of IL-4 antagonism

To the Editor:

The related cytokines IL-4 and IL-13 are important to the development of allergic inflammation (see review¹). Arguably, the most important biological activity of IL-4 in this process, which is distinct from IL-13, is its ability to drive the differentiation of naive T_H0 lymphocytes into the T_H2 phenotype. In contrast with the proinflammatory effects, IL-4 has prominent anti-inflammatory influences on mononuclear phagocytic cells. IL-4 inhibits antigen processing and downregulates IL-1 α , IL-1 β , and TNF- α production.^{2,3}

Our previous studies investigated influences of IL-4 antagonism on established antigen-specific T_H2-like cell lines and segmental allergen challenge (SAC)-derived T_H lymphocytes.⁴ IL-4 antagonism inhibited T-cell proliferation. More importantly, inhibition of lymphocyte function was specific for T_H2-like cells, because inhibition of IL-5 and IL-13 production was observed without changes in IFN- γ . The current studies addressed modulation of anti-inflammatory cytokines including IL-10 and TGF- β .

Segmental allergen challenges were performed on 6 subjects with informed consent under a protocol approved by the Human Investigations Committee at the University of Virginia. Subjects were selected with mild asthma treated with β -agonist therapy as their only medication. Baseline bronchoscopy and SAC were performed as previously described.^{4,5} SAC was performed using 5 mL 1:100 dilution of the lowest allergen concentration

producing a positive skin prick test to either standardized timothy or cat. On day 2, bronchoscopy was repeated, and bronchoalveolar lavage (BAL) was obtained. BAL cell pellets underwent Ficoll-Hypaque centrifugation (Amersham Biosciences, Uppsala, Sweden), and mononuclear cells were resuspended in complete medium supplemented with 10% autologous serum for 4 days. For these studies, IL-4 activity was antagonized with the soluble IL-4 receptor (sIL-4R, 10 μ g/mL; Amgen, Seattle, Wash) as described.⁴ IL-10 and TGF- β were measured in the supernatants using commercial ELISAs (BioSource, Camarillo, Calif). IL-4 antagonism significantly increased secretion of IL-10 and TGF- β compared with spontaneous secretion in the absence of sIL-4R (Fig 1). Alveolar macrophages are the dominant source of IL-10 in BAL pellets⁵ and made up ~20% to 30% of cells. Subsequent experiments used peripheral blood-derived monocytes to delineate further the role of mononuclear phagocytic cells in IL-10 production.

PBMCs were obtained from 8 healthy control subjects and isolated through Ficoll-Hypaque density gradient centrifugation from heparinized venous blood. CD14⁺ monocytes were enriched (>90%) by negative selection using magnetic bead affinity chromatography (Miltenyi Biotec, Auburn, Calif). Monocytes were resuspended in complete medium with 10% autologous serum and cultured with IL-4 (10 ng/mL) or IL-13 (10 ng/mL; BD Pharmingen, San Diego, Calif) with or without the additional presence of LPS (1 μ g/mL; Sigma, St Louis, Mo). Further studies were performed with or without the

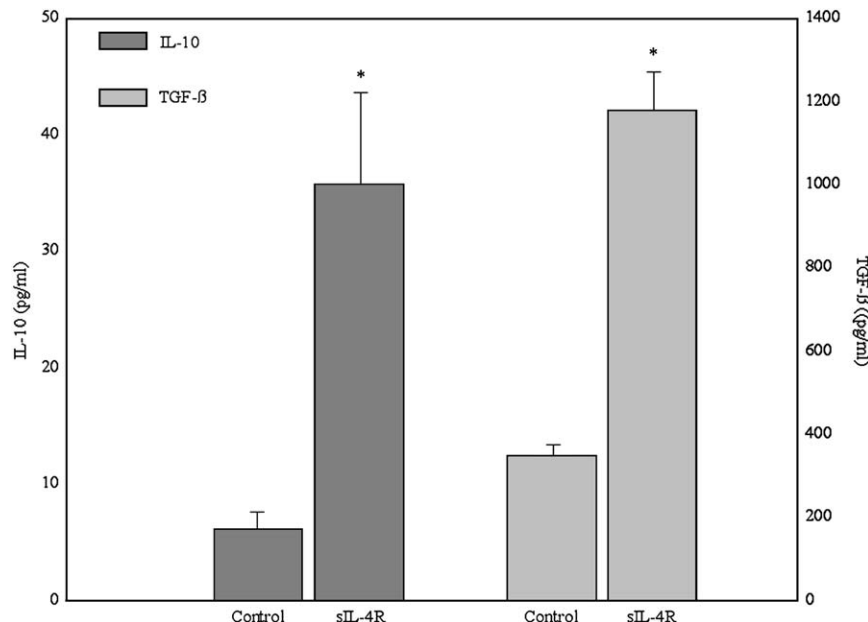


FIG 1. Bronchoalveolar lavage cell pellets were collected 48 hours after segmental allergen challenge and mononuclear cells obtained by Ficoll-Hypaque centrifugation. Cells were subsequently cultured for 48 hours with or without the soluble IL-4 receptor and supernatants assayed for IL-10 and TGF- β production. Data represent means $\pm$ SEMs of 6 replicate experiments. * P < .01.

TABLE I. Cytokine production by monocytes (pg/mL)

Cytokine	Resting	+IL-4 (10 ng/mL)	+IL-13 (10 ng/mL)	+Anti-IL4R α antibodies
IL-1 β	22.7 $\pm$ 2.1	15.3 $\pm$ 2.9*	17.7 $\pm$ 3.9	24.3 $\pm$ 2.6
IL-6	26.8 $\pm$ 0.3	22.5 $\pm$ 1.3*	27.2 $\pm$ 0.3	26.4 $\pm$ 0.3
IL-8	28.6 $\pm$ 0.2	28.3 $\pm$ 0.3	28.8 $\pm$ 0.4	28.6 $\pm$ 0.5
IL-10	6.3 $\pm$ 2.3	2.0 $\pm$ 0.7*	7.2 $\pm$ 4.1	7.0 $\pm$ 2.4
TNF- α	11.6 $\pm$ 2.8	4.8 $\pm$ 1.1*	10.7 $\pm$ 2.7	12.1 $\pm$ 2.7
LPS-stimulated monocytes (pg/mL):				
IL-10	19.3 $\pm$ 2.7	16.9 $\pm$ 2.9*	Not done	26.9 $\pm$ 6.9*

* $P < .01$.

presence of the IL-4/IL-13 dual antagonist, anti-IL-4 receptor α (IL-4R α) antibody (100 ng/mL; R&D Systems, Minneapolis, Minn). After 24-hour incubation, supernatants were collected and evaluated for cytokine production (IL-1 β , TNF- α , IL-6, IL-8, and IL-10) using a cytometric bead assay (Bioplex; BioRad, Hercules, Calif).

Monocytes exposed to IL-4, but not IL-13, demonstrated inhibition of constitutive and LPS-stimulated IL-10 production (Table I). IL-4 antagonism had minimal influences on resting monocytes but demonstrated a statistically significant increase in IL-10 production from LPS-stimulated monocytes. Although not identifiable in supernatants of these monocyte-enriched populations, IL-4 activity in LPS-stimulated samples may be predicted from the presence of IL-4 mRNA transcripts (data not shown). To discern whether this was a unique property of IL-4 on IL-10 or whether this reflected a general biological effect of IL-4 on mononuclear phagocytic cells, supernatants were screened for expression of other cytokines. Inhibition of IL-10 by IL-4 reflected the anti-inflammatory effects reported for this cytokine^{2,3} demonstrated by decreases in IL-1 β , IL-6, and TNF- α (Table I).

Segmental allergen challenges are effective in generating IL-4-producing T_H2-like lymphocytes.⁶ Therefore, we used this technique to investigate influences of IL-4 antagonism. Although the importance of IL-4 for differentiation of T_H2-like lymphocytes is well established, our previous studies showed that IL-4 is important to the ongoing function of these cells.⁴ We were surprised by our observation that SAC-derived cell pellets dramatically induced secretion of the anti-inflammatory cytokines IL-10 and TGF- β 1 when exposed to an IL-4 antagonist (Fig 1). We studied monocytes to delineate the source of this IL-10. Although derived from many cells, the most important human source of IL-10 is mononuclear phagocytic cells. We speculated that enhanced IL-10 secretion was derived from alveolar macrophages, which made up ~20% to 30% of the BAL cell population after SAC. With IL-4 antagonism of LPS-activated peripheral blood monocytes, we were able to recapitulate the increase in IL-10 observed with the BAL cells (Table I). This effect of IL-4 antagonism is consistent with published observations and our current studies showing that IL-4 inhibits cytokine production by monocytes.^{2,3}

The source of IL-4 in monocyte-enriched cultures was not immediately obvious, although its presence was supported by the presence of mRNA transcripts. IL-4 is

difficult to measure in cell cultures, reflecting its primary tendency to function in a paracrine fashion within the immune synapse. The presence of IL-4 transcripts in our resting and LPS-stimulated mononuclear cell population could reflect contaminating naive T_H0 or T_H2 effector cells or natural killer T cells, which are important sources for IL-4, but it is also plausible that monocytes secrete IL-4.

We inhibited the biological activity of IL-4 in the SAC studies with soluble receptors and, when that reagent became unavailable, antireceptor antibodies. Soluble IL-4 receptors can bind to and sequester IL-4 but cannot transduce cellular activation. In contrast with sIL-4R, the antireceptor antibody also blocks binding of IL-13 to its receptor. It is unlikely that the effects of the antireceptor antibody on monocytes were mediated through IL-13 antagonism because we did not observe anti-inflammatory effects of IL-13 in the monocyte cultures (Table I). This may reflect the existence of a second class of IL-13 receptor on monocytes functioning as a decoy receptor, blocking its activity.

Proinflammatory effects of IL-4 antagonism might have contributed to the failure of IL-4 inhibitors in asthma. These clinical trials were associated with the infrequent development of flulike symptoms including low-grade fever, chills, myalgias, and arthralgias (Unpublished data, Immunex Corp, Seattle, Wash). Although not the focus of these studies, it is interesting that IL-4 antagonism also enhanced TGF- β 1 secretion. Although it has anti-inflammatory properties, enhanced production of TGF- β 1 is problematic in regard to asthma therapeutics, because this cytokine may promote airway remodeling.

In summary, we demonstrate that IL-4 antagonism enhances IL-10 production and suggest that the source for this IL-10 is mononuclear phagocytic cells. Inhibition of IL-10 production by IL-4 reflects a generalized anti-inflammatory action of this cytokine, because it occurred in parallel with decreased production of IL-1 β , IL-6, and TNF- α , implying that a parallel increase in these cytokines could occur with IL-4 antagonism. These proinflammatory effects of IL-4 antagonism may confound attempts to use IL-4 antagonists as therapeutic agents in T_H2-mediated diseases such as asthma.

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Is the Allergic Rhinitis and its Impact on Asthma classification useful in daily primary care practice?

To the Editor:

In 2001, the Allergic Rhinitis and its Impact on Asthma (ARIA) initiative proposed a new classification for allergic rhinitis (AR) on the basis of the duration of symptoms and their effect on the quality of life and formulated evidence-based guidelines for the treatment of this disease.¹ AR is a major primary health care problem, and the ARIA guidelines were developed primarily to assist general practitioners (GPs) in their management of this condition. The classification scheme is the basis of the stepwise treatment recommendations and should be tailored to the AR patient population in daily general practice.

During the pollen season of 2003, we conducted a cross-sectional survey in general practice in Belgium. Ninety-five Belgian GPs enrolled 804 patients who presented with symptoms of AR. For each patient, a questionnaire covering the duration, severity, symptomatology, and management of the disease was completed. In a previous article, we described the characteristics of the patients in the different ARIA classification groups, and we compared the management of AR in daily primary care practice with the ARIA recommendations.² The results demonstrated that the classification of AR as persistent and intermittent, on the basis of the duration of disease,

TABLE I. Symptom scores in the new mild, moderate, and severe AR groups*

	Mild AR (165)	Moderate AR (369)	Severe AR (270)	P
Rhinorrhea†	52.1	60.7	61.5	NS
Nasal congestion†	42.4	61.5	72.2	<.0001
Nasal itch†	35.1	51.8	50.4	.007
Sneeze†	55.8	61.0	67.4	.01
Eye symptoms†	33.3	37.9	45.9	<.0001
Headache†	4.2	12.5	19.2	<.0001
Somnolence†	1.8	9.8	13.3	.0001
Troublesome symptoms (%)	47.9	81.4	90.4	<.0001

NS, Not significant.

*Statistical analyses with χ^2 test for trend (Medcalc, version 8.1.0.0, Medcalc Software, Mariakerke, Belgium).

P < .05 = statistically significant.

†Each symptom score is expressed as the percentage of patients with a score of 3 or 4 (symptoms were scored on a 4-point scale evaluating whether AR manifests by these symptoms: 1 = never/rarely, 2 = occasionally, 3 = frequently, or 4 = always).

is not equivalent to the previous, unsatisfactory classification of AR as perennial or seasonal. These findings are similar to those from large epidemiological studies in the general population,^{3,4} in primary care practice,⁵ and in specialty practice.⁶

On the other hand, the usefulness and validity of the classification of AR severity as mild or moderate-severe, on the basis of the impact of AR on quality of life, are not verified with data. The results of our survey indicate that moderate-severe AR indeed represents a higher burden of disease than mild AR, as reflected by the higher scores for nasal and nonnasal symptoms accompanying AR and the more complicated diagnostic and therapeutic management in moderate-severe compared with mild rhinitis.² An important finding in our data is the disproportionate size of the diagnostic groups, with the preponderance of subjects classified as moderate-severe rhinitis, 89.3%, and only 10.7% classified as mild. These data are similar to results of a year-round assessment in general practice in France, in which 93% of the 3052 patients with AR enrolled were classified with moderate-severe rhinitis.⁷

We address the imbalance between mild and moderate-severe AR and propose a refinement of the current ARIA classification for AR severity.

Allergic Rhinitis and its Impact on Asthma defines the severity of AR on the basis of 4 quality of life items: (1) impairment of sleep; (2) impairment of daily activities, sports, or leisure; (3) impairment of school or work; and (4) troublesome symptoms. If 1 or more of these items are present, rhinitis is classified as moderate-severe.¹ In our study, abnormal sleep was reported by 37.1% of the patients; impairment of daily activities, sports, or leisure by 71.3%; impairment of work or school by 53.2%; and troublesome symptoms by 77.6%.² Because 86.9% of the patients with moderate-severe AR considered their symptoms troublesome and only 9.8% of the patients reported troublesome symptoms in the absence of impairment of daily activities/sports/leisure, school/work, or