

Inhibition of effector T lymphocytes mediated through antagonism of IL-4

To the Editor:

IL-4 is important to the development of allergic inflammation. It induces the IgE isotype switch, upregulates low-affinity and high-affinity IgE receptors, and promotes eosinophilic inflammation through its induction of vascular cell adhesion molecule-1. Perhaps the most important biologic activities of IL-4 are derived from its influences on T lymphocytes. IL-4 differentiates naive T_H0 lymphocytes into the T_H2 phenotype. In the presence of IL-4, T lymphocytes produce IL-4, IL-5, IL-9, and IL-13 and become less likely to synthesize IFN- γ . Data derived primarily from murine studies suggest that IL-4 is also important in maintaining viability and activity of effector T helper cells. Activation of T lymphocytes typically leads to cell death. However, this is prevented by IL-4, in part secondary to its abilities to maintain levels of the survival-promoting proteins Bcl-2 and Bcl-x and through downregulation of the apoptosis-inducing receptor Fas.¹⁻³

Secreted forms of the IL-4 receptor (sIL-4R) occur naturally. Because they cannot transduce cellular activation but still bind IL-4, they are a naturally occurring homeostatic mechanism that can counter the effects of IL-4. Little is known regarding the importance of IL-4 to the functioning of established effector T_H2 -like lymphocytes in humans. We investigated inhibition of T-cell function and apoptosis in segmental allergen challenge–derived T helper lymphocytes during exposure to sIL-4R.

Segmental allergen challenges were performed on 6 subjects after obtaining informed consent under a protocol approved by the Human Investigations Committee at the University of Virginia. Subjects were selected with mild asthma managed on a β -agonist therapy as their only medication. Baseline bronchoscopy and segmental allergen challenges were performed as we have

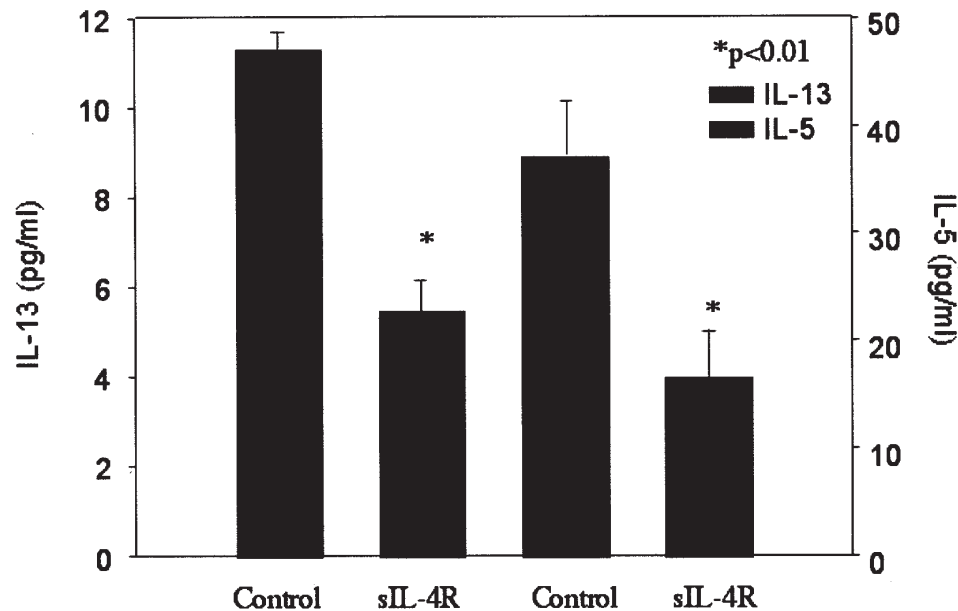


FIG 1. Modulation of T_H2 -like lymphocyte-derived cytokines by soluble IL-4R. Baseline bronchoscopy and segmental allergen challenges were performed as described. After 48 hours, bronchoalveolar lavage was performed. $CD3^+$ T lymphocytes were enriched by means of affinity purification and cultured for 4 days with or without the additional presence of sIL-4R. Culture supernatants were evaluated by ELISA for IL-5 and IL-13.

described.⁴ At screening visits, subjects underwent skin testing to a standardized allergen to which they demonstrated sensitivity with serial 10-fold dilutions (cat, dust mite [Der P12], or timothy; Greer Lenoir, NC). The segmental allergen challenges were performed with 5 mL of a 1:100 dilution of the lowest allergen concentration producing a positive prick skin test. On day 2, the bronchoscopy was repeated, and bronchoalveolar lavage was performed. $CD3^+$ T lymphocytes were obtained from bronchoalveolar lavage cell pellets by means of affinity column purification (Miltenyi Biotec, Auburn, Calif). The T lymphocytes were resuspended in complete medium supplemented with 10% autologous serum for 4 days with or without the additional presence of sIL-4R (10 μ g/mL). No additional allergen, cytokine growth factors, or mitogens were applied. For the proliferation assay, $CD3^+$ -enriched T lymphocytes (2×10^5) were aliquoted in quadruplicate in 96-well plates. Proliferation was quantified by means of the addition of CellTiter Aqueous One Solution (Promega, Madison, Wis). After 4 hours, proliferation responses were assayed with an EL808 microplate ELISA reader (Bio-Tek, Winooski, Vt). Cells committed to apoptosis were quantified by flow cytometry by their surface binding of annexin V, and data were analyzed according to cell viability as determined by their exclusion of propidium iodide. Cell expression of CD28, CTLA-4, Bcl-2, and Fas (Pharmingen, San Diego, Calif) were quantified by flow cytometry, with the flow cytometer gated to collect data on $CD4^+$ T helper lymphocytes. For the staining with Bcl-2, lymphocytes were permeabilized through exposure to Tween 20 (Sigma, St Louis, Mo). IL-5, IL-13, and IFN- γ were measured in the supernatants of the 4-day T lymphocyte cultures with

TABLE I. Modulation of expression of lymphocyte activation and apoptosis molecules by sIL-4R

	Control	sIL-4R	P value
Proliferation			
Mean fluorescence	0.57 $\pm$ 0.05	0.36 $\pm$ 0.04	<.01
Annexin V			
% Positive	52.9 $\pm$ 11.0	59.8 $\pm$ 9.0	n.s
CD28			
% Positive	59 $\pm$ 4	51 $\pm$ 4	n.s
Mean fluorescence	53.7 $\pm$ 26.9	72.8 $\pm$ 12.8	<.01
Fas (CD95)			
% Positive	71 $\pm$ 2	69 $\pm$ 2	n.s
Mean fluorescence	217.5 $\pm$ 35.4	269.7 $\pm$ 55.8	n.s
Bcl-2			
% Positive	58 $\pm$ 5	46 $\pm$ 5	<.05
Mean fluorescence	580.9 $\pm$ 23.9	390.8 $\pm$ 30.5	<.01
CTLA-4			
% Positive	56 $\pm$ 2	56 $\pm$ 2	n.s
Mean fluorescence	52.3 $\pm$ 8.0	77.2 $\pm$ 20.4	n.s

n.s., Not significant.

commercial ELISA kits (BioSource, Camarillo, Calif). Statistical analyses consisted of paired *t* tests comparison between the samples without and with the sIL-4R.

Soluble IL-4R was associated with significant inhibition of T-cell proliferation ($35.0\% \pm 0.5\%$; $P < .01$; Table I). However, the presence of sIL-4R did not influence either cell viability (propidium iodide exclusion) or apoptosis (annexin V expression). The additional presence of sIL-4R was associated with a significant downregulation of expression of the activating B7 (CD80/CD86) ligand CD28 on segmental allergen challenge-derived T lymphocytes. Although associated with a trend toward

increased expression, no significant difference in the inhibitory B7 ligand, CTLA-4, was observed. Similar to the apoptosis studies, sIL-4R was not associated with a significant upregulation of the apoptosis-inducing ligand Fas (CD95) on these T cells. However, the presence of sIL-4R was associated with a significant downregulation of the apoptosis inhibitory protein Bcl-2. We next evaluated influences of sIL-4R on cytokines associated with T_H1 -(INF- γ) and T_H2 -like lymphocytes (IL-5, IL-13). Soluble IL-4R induced significant inhibition of both IL-5 and IL-13 production (Fig 1). Inhibition of these cytokines by soluble IL-4R did not represent T_H2 to T_H1 immune deviation insofar as no increase in INF- γ production was observed (2.7 ± 0.2 in control samples to 2.2 ± 0.2 pg/mL in sIL-4R-exposed samples).

Inhibition of T_H2 lymphocyte differentiation is not likely to be relevant to the beneficial effects of IL-4 antagonism observed when these agents are administered after the establishment of asthma. However, in addition to the influences of IL-4 on the differentiation of T_H2 lymphocytes, IL-4 has interactions with established T_H2 cells. The current studies establish influences of IL-4 antagonism in activated effector human T helper lymphocytes, including significant inhibition of proliferation. As effector T lymphocytes mature, they evolve from a state in which they express CD28 on their surface membrane and are activated by B7 (CD80/CD86) on antigen-presenting cells to a state in which they express CTLA-4, and engagement of B7 leads to inhibition. Our studies suggested a trend toward increased expression of this inhibitory B7 counter-ligand CTLA-4. A shift toward a CTLA-4-positive phenotype is supported by the significant downregulation of the activating B7 counter-ligand, CD28.⁵ Inhibition of metabolic activity of established T_H2 -like lymphocytes is supported by the dramatic inhibition of production of cytokines associated with these lymphocytes, IL-5 and IL-13. Although IL-4 antagonism is known to inhibit differentiation of T_H2 -like lymphocytes and thereby, secondarily, the secretion of these T_H2 -associated cytokines, this mechanism was clearly not relevant to these results. These studies used established, highly activated, and spontaneously proliferating T effector lymphocytes and demonstrated a biologic effect of IL-4 antagonism in these cells. Together these data establish in humans that IL-4 is important in maintaining function of T_H2 -like effector lymphocytes. In contrast to the IL-5 and IL-13 data, no changes were observed in INF- γ production, inveighing against immune deviation of established T_H2 -like lymphocytes into T_H1 cells. It is intriguing to speculate that the effects of IL-4 antagonism on proliferation that we observed were specific for T_H2 -like cells and did not reflect any modulation of proliferation of functioning of INF- γ -producing T_H1 -like cells.

An alternative mechanism by which IL-4 antagonism might modulate function of established T lymphocytes is through promotion of apoptosis. Surprisingly, our studies were unable to demonstrate an influence of IL-4 antagonism on T lymphocyte viability or annexin V expression by T cells. We did confirm in humans an influence of IL-

4 on Bcl-2 through the capacity of the IL-4 antagonist to significantly diminish expression of this anti-apoptotic factor. It remains plausible that although our studies did not demonstrate increased apoptosis, in the presence of diminished Bcl-2 and an appropriate pro-apoptotic signal (eg, FasL), increased elimination of T_H2 lymphocytes might have been observed.

In summary, antagonism of IL-4-inhibited proliferation of established effector T lymphocytes. Soluble IL-4R inhibited release of cytokines associated with T_H2 -like lymphocytes (IL-5, IL-13) without influencing the T_H1 cytokine INF- γ . This was associated with a significant downregulation of CD28 and a trend toward upregulation of CTLA-4, phenotypic markers of inactivated cells. We could not demonstrate an influence of IL-4 antagonism on T-cell apoptosis; however, inhibition of the anti-apoptotic factor Bcl-2 was observed. These data establish important roles for IL-4 in maintaining function and activity of effector T_H2 -like lymphocytes.

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