

RECOMBINANT HUMAN INTERLEUKIN 5 IS A SELECTIVE
ACTIVATOR OF HUMAN EOSINOPHIL FUNCTION

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Eosinophilia is an easily recognized clinical entity of diverse etiologies including allergic states such as atopy, asthma, and drug reactions, as well as helminth infestations. One of the striking features in many of these conditions is the selective nature of the eosinophilia, with other blood components found essentially in normal numbers. An increase in the state of activation of eosinophils can be observed concomitantly with an increase in eosinophil numbers in some of these syndromes. For example, eosinophils from patients with the hypereosinophilic syndrome show increased binding to antigen-antibody complexes (1), and eosinophils from patients with helminth infections and allergic conditions have a markedly increased capacity to adhere to and kill antibody-coated schistosomula of *Schistosoma mansoni* (2, 3) when tested in vitro.

The observation of selective eosinophilia has led many investigators to postulate the existence of an "eosinophilopoietin" molecule. We have recently shown that a murine cytokine, eosinophil differentiation factor (4), cross-reacted with human cells selectively stimulating the proliferation, differentiation, and function of eosinophils (5), predicting the existence of a human equivalent to this molecule. Although no native human factor has been characterized, a recombinant human (rh) molecule has now been identified and termed IL-5 (6, 7) which stimulates the production of eosinophils in cultures of human bone marrow (7). We show here that rhIL-5 is also a powerful and selective stimulator of human eosinophil function. IL-5 is thus the first hemopoietic factor whose elaboration in vivo can explain the selective eosinophilia and eosinophil activation seen in disease.

Materials and Methods

rhIL-5 and Granulocyte/Macrophage CSF (GM-CSF). The human gene encoding human IL-5 in the expression vector pcEXV-3 was transfected into COS cells by electroporation as previously described (7). The supernatant was collected after 4 d and concentrated by ultrafiltration. Mock-transfected control supernatants were prepared in the same way. rhGM-CSF, 99.4% pure, and with a specific activity of 10^6 U/ml, was a gift from Genetics Institute (Cambridge, MA).

Purification of Human Granulocytes. Peripheral blood was sedimented on dextran, and the leukocyte-rich supernatant was centrifuged on a gradient of hypertonic Metrizamide

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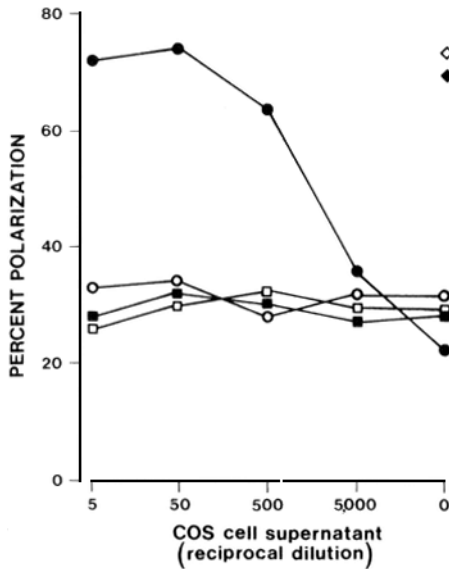


FIGURE 1. Titration of rhIL-5 (filled symbols) and mock-transfected COS cell supernatant (open symbols) on human eosinophil (circles) and neutrophil (squares) polarization. The percentage of polarized eosinophils (\diamond) and neutrophils (\blacklozenge) in the presence of 10 ng/ml rhGM-CSF is also shown.

(Nyegaard, Oslo, Norway) as previously described (8). The eosinophil and neutrophil preparations were always >90 and >95% pure, respectively.

Functional Assays. The antibody-dependent cell-mediated cytotoxicity, phagocytosis, superoxide anion production, and polarization assays used have been described in detail previously (9–11).

Results

rhIL-5 was found to induce morphological changes on eosinophils. Cells thus stimulated took on an irregular shape, the membrane ruffled, and the granules concentrated on one end (polarization); these are changes typically induced by other established activating agents (11, 12). Eosinophils were polarized by rhIL-5 in a dose-dependent manner and by rhGM-CSF but not by mock-transfected COS cell supernatant (Fig. 1). By contrast, neutrophils were polarized by rhGM-CSF but not by rhIL-5.

The morphological changes induced by rhIL-5 on eosinophils were accompanied by functional activation of these cells. rhIL-5 stimulated eosinophils but not neutrophils to kill antibody-coated tumor cells while rhGM-CSF stimulated both cell types (Fig. 2). rhIL-5 stimulated eosinophils in a dose-dependent manner and the levels of cytotoxicity were similar to those obtained with rhGM-CSF. Similar results were obtained when rhIL-5 was examined for its ability to stimulate phagocytosis of serum-opsonized baker's yeast. Eosinophils but not neutrophils were stimulated by rhIL-5 at 2.5 and 1.2% serum concentrations (Table I). Neutrophils, however, could be stimulated by rhGM-CSF.

The ability of rhIL-5 to stimulate the respiratory burst of eosinophils and neutrophils was examined by measuring the levels of O_2^- production. rhIL-5 stimulated directly the production of O_2^- by eosinophils and to the same extent as rhGM-CSF (Table II). In contrast, neither rhIL-5 nor rhGM-CSF directly stimulated O_2^- production by neutrophils. Because GM-CSF enhances the response of neutrophils to a subsequent stimulus, neutrophils were preincubated

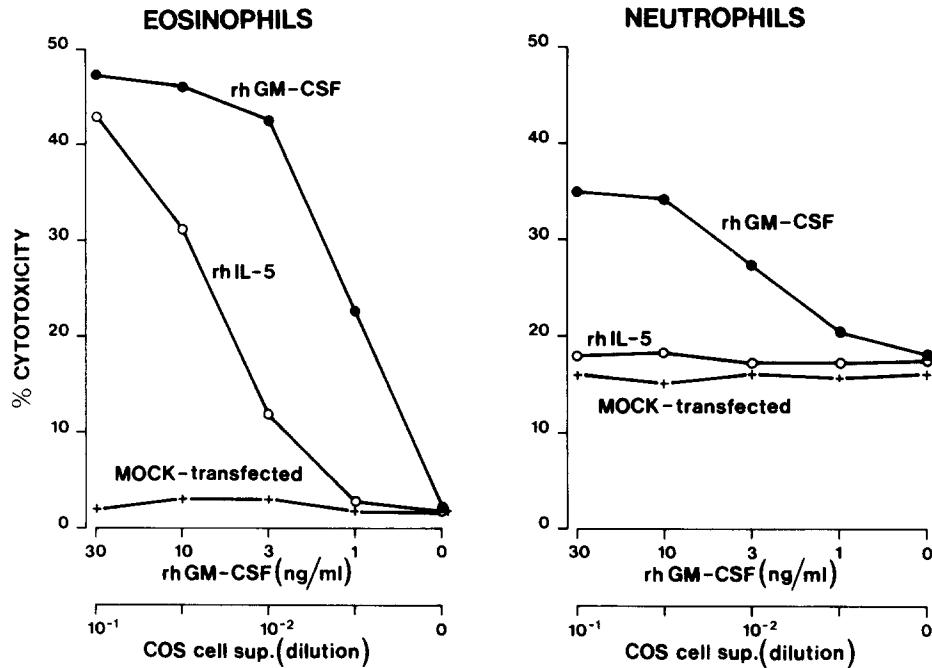


FIGURE 2. Titration of rhIL-5 (○) mock-transfected COS cell supernatant (+) and rhGM-CSF (●) on human eosinophil- and neutrophil-mediated antibody-dependent cell-mediated cytotoxicity of target cells.

TABLE I
rhIL-5 Stimulates Human Eosinophils but Not Neutrophils to Phagocytize Serum-Opsonized Baker's Yeast

Stimuli	Serum concentration	Number of phagocytized baker's yeast per cell					
		Eosinophils			Neutrophils		
		0	1, 2	3, 4	0	1, 2	3, 4
	%						
rhIL-5	2.5	37*	52.5	10.5	58	21	21
Mock-transfected COS cell supernatant	2.5	78.5	18.5	3	62	21.5	16.5
rhGM-CSF	2.5	55	32	13	13.5	31.5	55
rhIL-5	1.2	79.5	18	2.5	81.5	12	6.5
Mock-transfected COS cell supernatant	1.2	90.5	9	0.5	82	13.5	4.5
rhGM-CSF	1.2	80.5	16.5	3	48	27	25

rhIL-5 and mock-transfected COS cell supernatants were tested at 1:10, and rhGM-CSF was tested at 10 ng/ml final concentrations. Determinations were carried out in triplicate. In the case of eosinophils, values obtained with rhIL-5 and rhGM-CSF were significantly different from those obtained with mock-transfected COS cell supernatant ($p < 0.01$). In the case of neutrophils, rhGM-CSF, but not rhIL-5, values were significantly different from those obtained with mock-transfected COS cell supernatant ($p < 0.01$). Neither eosinophils nor neutrophils showed significant phagocytosis in the absence of serum.

* Percentage of cells containing different numbers of phagocytized yeast. A minimum of 200 cells were counted.

for 45 min at 37°C with rhIL-5, mock-transfected COS cell supernatant, or rhGM-CSF, and then stimulated with 10^{-7} M f-Met-Leu-Phe. The O_2^- release by neutrophils was enhanced by rhGM-CSF ($15.7 \text{ nmol}/10^6 \text{ cells}$) compared to

TABLE II
Effect of rhIL-5 on the Direct Production of Superoxide Anion by Human Eosinophils and Neutrophils

Exp.	Eosinophils				Neutrophils			
	rhIL-5	Mock-transfected COS cell supernatant	rhGM-CSF	PMA	rhIL-5	Mock-transfected COS cell supernatant	rhGM-CSF	PMA
1	4.5* ± 0.5 [‡]	1.0 ± 0.4	4.2 ± 0.3 [‡]	41.4 ± 0.9 [‡]	0.8 ± 0.1	1.4 ± 0.2	1.2 ± 0.3	46.5 [‡] ± 1.6
2	7.6 ± 0.5 [‡]	2.7 ± 0.1	6.3 ± 0.2 [‡]	50.2 ± 1.4 [‡]	1.9 ± 0.3	2.0 ± 0.2	2.2 ± 0.3	60.0 [‡] ± 0.9

rhIL-5 and mock-transfected COS cell supernatant were tested at 1:10 dilution. rhGM-CSF was used at 10 ng/ml. PMA was used at 30 ng/ml.

* Values in nmol O₂⁻/10⁶ cells followed by SEM.

[‡] Values significantly different from mock-transfected COS cells ($p < 0.05$).

medium control (8.5 nmol/10⁶ cells), but not by rhIL-5 (8.3 nmol/10⁶ cells) or mock-transfected COS cell supernatant (8.1 nmol/10⁶ cells).

Discussion

We show here that rhIL-5, in addition to stimulating eosinophil proliferation, is an activator of human eosinophil but not neutrophil function. Its selectivity for human eosinophils makes IL-5 the molecule most likely to be responsible for the increase in eosinophil numbers and for the state of activation of these cells observed in allergy, parasitic infections, and hypereosinophilic syndromes.

In the present experiments, rhIL-5 altered the morphological appearance of eosinophils to cells showing typical features of "polarization" including membrane ruffling, an elongated shape, and the granules concentrated towards one end. These morphological changes have been previously observed with chemotactic factors and rhGM-CSF on neutrophils and eosinophils (11, 12) and probably represent cytoskeletal changes occurring after cell activation (13). In addition, rhIL-5 selectively stimulated several effector functions on human eosinophils but not neutrophils.

Some of the functional effects of rhIL-5 reported in these experiments in vitro appear similar to those taking place in vivo as seen in eosinophils from patients with selective eosinophilia. For example, eosinophils from such patients have been shown to have increased oxidative metabolism (14, 15) and an altered morphology (1). Another feature of these eosinophils is their hypogranular appearance suggestive of degranulation in vivo (1); however, in these studies we have not examined whether IL-5 can directly induce degranulation of eosinophils in vitro.

The production of IL-5 in vivo may have important clinical implications. Firstly, a selective increase in eosinophils, a cell type effective against several parasites in vitro (8, 16), may facilitate the control of parasitic infestations. Secondly, the continuous presence of large numbers of circulating eosinophils may lead to tissue pathology as shown in cases of hypereosinophilic syndromes. In some of these cases peripheral blood eosinophils were degranulated and eosinophilic endomyocardial disease developed (17). Thirdly, IL-5 may be produced by certain tumors. Some cases of carcinoma of the lung are associated with eosinophilia (17, 18) and the extracted tumor can be shown to produce a substance that preferentially stimulates eosinophils in vitro (18). In cases of

lymphomas, eosinophilia has appeared before or at the time of diagnosis of the tumor with peripheral eosinophil counts decreasing during remission but rising again if a relapse occurs (19). Thus, IL-5 may be present in some paraneoplastic syndromes and its detection may serve diagnostic purposes.

Finally, IL-5 becomes the third type of human CSF after GM-CSF (11, 20) and IL-3 (21) capable of stimulating eosinophil proliferation and function in man. The property of activating eosinophils, also shared with TNF- α (22), is not obviously attributable to similarities in the primary structure of these molecules. However, rhIL-5 and rhIL-3 do show a significant homology in their primary sequence at the carboxy-terminal region—48% (including conservative substitutions) over 19 amino acids in a significantly hydrophilic region of the molecule—and it would be interesting to establish the relevance of this region for the eosinophil-activating function of these two molecules.

Summary

Human rIL-5 was found to selectively stimulate morphological changes and the function of human eosinophils. This molecule is thus a prime candidate for the selective eosinophilia and eosinophil activation seen in disease.

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