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Stimulation of proliferation, differentiation, and function of human cells by primate interleukin 3

(colony-stimulating factor/eosinophil)

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ABSTRACT **Cloned gibbon interleukin 3 (gIL-3) was found to stimulate the proliferation and differentiation of human bone marrow cells to produce day-14 granulocyte, macrophage, granulocyte–macrophage, and eosinophil colonies in semisolid agar. In the presence of normal human plasma, gIL-3 stimulated megakaryocytes. In methylcellulose cultures, it stimulated erythroid colonies in the presence, but not in the absence, of erythropoietin. When mature human leukocytes were used, gIL-3 stimulated the function of purified mature eosinophils as measured by the capacity to kill antibody-coated target cells, to produce superoxide anions, and to phagocytize opsonized yeast particles in a manner similar to recombinant human granulocyte–macrophage colony-stimulating factor. In contrast, gIL-3 did not significantly stimulate any of the neutrophil functions tested, whereas human recombinant granulocyte–macrophage colony-stimulating factor was active in these assays. Among cytokines that are active on human hematopoietic cells, gIL-3 thus has a distinct set of functions and may predict the range of actions of the human molecule.**

The murine (m) cytokine interleukin-3 (IL-3) (1, 2), also known as multipotential colony-stimulating factor (3) and persistent cell-stimulating factor (4), is distinct among the cloned hematopoietic-stimulating factors in having the capacity to stimulate progenitor cell renewal. This, as well as its relative lack of lineage restriction, suggested that mIL-3 is active at a more primitive level than the colony-stimulating factors (CSF) granulocyte–macrophage (GM)-CSF, macrophage (M)-CSF, granulocyte (G)-CSF, or eosinophil differentiation factor (EDF) and that it may have a special role in leukemogenesis (5).

We have cloned the gene encoding a molecule active in hematopoiesis from a gibbon (g) cell line that is 29% homologous at the amino acid level and has a genomic organization similar to that of mIL-3 (6). These properties led to the designation of this molecule as gIL-3.

In this communication we describe some of the biological effects of gIL-3 and show that, to our knowlege, its spectrum of activities on human cells differs from all described hematopoietic factors.

METHODS

Cytokines. The gIL-3 used was in a COS cell-conditioned medium obtained by transfecting COS-1 cells with 5 μg of plasmid DNA containing the gIL-3 cDNA (pMLA-CSF) and harvesting the gIL-3-containing medium 72 hr after transfection (6).

Recombinant human (rh) GM-CSF, with endotoxin at <0.2 ng/ml, was purified from the conditioned medium of COS cells that had been transfected with cloned human GM-CSF cDNA in the p91203(B) vector as described (7). Silver-staining of the $NaDodSO_4$/polyacrylamide gels of the purified GM-CSF revealed a major band of 19 kDa.

rh tumor necrosis factor type α was a gift from Genentech, (South San Francisco, CA) and contained cytotoxic activity (3.6×10^7 units/mg) on actinomycin D-treated L929 mouse fibroblast cells and endotoxin at 0.8 ng/ml. rh tumor necrosis factor type α was produced in *Escherichia coli* (8) and purified to 99.8% purity.

Bone Marrow Cultures. *Erythroid colony-forming unit assay.* Light-density nonadherent bone marrow cells were obtained by separation on a Ficoll/Paque (Pharmacia, Sweden) density gradient followed by a 60-min incubation with carbonyl iron [200 mg of carbonyl iron per 15×10^6 cells in 10 ml of RPMI with 15% (vol/vol) fetal calf serum (FCS)] and removal of monocytes (containing attached or phagocytized iron particles) with a magnet. Cells were cultured in 0.9% methylcellulose (Fluka, Sweden) with Iscove's modified Dulbecco's medium (Commonwealth Serum Laboratories, Australia), 30% (vol/vol) FCS (GIBCO), 0.66% bovine serum albumin (fraction V, Sigma), and 20 μM 2-mercaptoethanol at a concentration of 5×10^4 cells per ml of culture medium. The cultures were stimulated with 1 unit of high-purity human urinary erythropoietin (EPO) per plate and phytohemagglutinin-stimulated leukocyte-conditioned medium (PHA-LCM) [5% (vol/vol)], rhGM-CSF (100 ng/ml), or gIL-3 (1:200 dilution). Control cultures with no added stimulus or with EPO alone were also prepared. The cultures were incubated in an atmosphere of 5% CO_2/95% air. Hemoglobin-containing colonies present after 14 days containing >100 cells were scored as large erythroid colonies, and those containing 40–100 cells were scored as small erythroid colonies.

Myeloid colony-forming unit assay. The same culture system was used except that 0.3% agar (Difco) replaced 0.9% methylcellulose and that EPO was omitted. Aggregates of >40 cells were scored as colonies after 14 days incubation. The agar discs were then fixed with 3% (vol/vol) glutaraldehyde and transferred onto individual 5 × 8 cm glass slides. The discs were dried at room temperature and stained with luxol fast blue and a combined specific and nonspecific esterase stain.

Megakaryocyte colony-forming unit assay. The same culture system was used except that EPO was omitted and that

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Abbreviations: CSF, colony-stimulating factor; EDF, eosinophil differentiation factor; EPO, erythropoietin; FCS, fetal calf serum; g, gibbon; G, granulocyte; h, human; HUVE, human umbilical vein endothelium; IL-3, interleukin 3; m, murine; M, macrophage; PHA-LCM, phytohemagglutinin-stimulated leukocyte conditioned medium; r, recombinant.

Table 1. Morphological types of colonies in human marrow cultures after 14 days of stimulation in agar

Marrow sample	Stimulus	Total number of colonies	Morphological type, % of colonies			
			G	GM	M	Eo
1	Medium	0	—	—	—	—
	PHA-LCM	303	44	9	32	16
	gIL-3	181	33	4	43	20
2	Medium	0	—	—	—	—
	PHA-LCM	96	55	10	18	17
	rhGM-CSF	103	59	7	28	5
	gIL-3	91	66	1	12	11

Eo, eosinophil.

Table 2. Enhancement of erythroid colonies by gIL-3

Stimulus	Mean number of erythroid colonies (large/small)		
	1	2	3
EPO	0/1	0/0	4/30
EPO/PHA-LCM	6/18	9/12	85/61
EPO/rhGM-CSF	2/14	0/5	4/53
EPO/gIL-3	0/4	1/7	20/65

The mean number of erythroid colonies from three experiments is reported. A small colony had 40–100 cells; a large colony had >100 cells.

15% (vol/vol) human plasma and 15% (vol/vol) FCS were substituted for 30% (vol/vol) FCS. After a 14-day incubation, each 1-ml culture was resuspended in Dulbecco's phosphate-buffered saline (PBS), and cytosmears were prepared on a cytocentrifuge. The cytosmears were examined for the presence of megakaryocytes using the alkaline phosphatase–monoclonal anti-alkaline phosphatase technique (9). The monoclonal antibody 25E11, which recognizes the platelet glycoprotein IIb/IIIa complex (10) served as the primary antibody.

Purification of Human Neutrophils and Eosinophils. Peripheral blood of healthy volunteers was centrifuged on a hypertonic gradient of metrizamide (Nyegaard, Oslo) as described (11) after dextran sedimentation. The purity was >95% for neutrophils and >92% for eosinophils. The cells were resuspended in Eagle's minimal essential medium supplemented with 10% (vol/vol) FCS, 20 mM Hepes buffer (pH 7.4), and antibiotics. In experiments involving adherence, blood was collected using 0.2% EDTA as anticoagulant. The neutrophils were isolated by density-gradient centrifugation in Ficoll/Paque, followed by sedimentation in dextran and hypotonic lysis of erythrocytes. The preparation contained >97% neutrophils and was suspended in 0.9% NaCl at 5×10^7 neutrophils per ml.

Antibody-Dependent Cell-Mediated Cytotoxicity Assay. ^{51}Cr-labeled, trinitrophenyl-coupled P815 cells (4×10^3 cells in 40 μl) were incubated with 24 μl of rabbit anti-trinitrophenyl (Miles-Yeda, Rehovot, Israel), 80 μl of purified human neutrophils or eosinophils (1.3×10^5 cells) as effector cells, and 16 μl of rhGM-CSF, gIL-3, or medium for 2.5 hr at 37°C in V-bottom microtiter plates. The percent cytotoxicity was calculated as described (12).

Phagocytosis Assay. Eosinophils were suspended at 2×10^7 cells per ml in PBS. Dried bakers' yeast (Tandaco, Melbourne, Australia) was suspended in PBS to an OD_{540} of 1.6. A 250-μl aliquot was mixed with 50 μl of stimulator (gIL-3, rhGM-CSF, or medium control), with 100 μl of fresh autologous serum, and with 100 μl of cells (13). Tubes were incubated for 1 hr at 37°C, placed on ice, and then centrifuged at 4°C. Cell pellets were resuspended in 50 μl of cold PBS. Smears were fixed in methanol and stained with Giemsa. The number of phagocytized yeast cells were counted in at least 100 eosinophils per preparation.

Neutrophil Adherence Assay. First- or second-passage human umbilical vein endothelial (HUVE) cells were isolated and maintained as described (14). HUVE cells were plated in RPMI 1640 medium with 20% (vol/vol) FCS into 641-mm diameter wells (Costar, Cambridge, MA) at 2×10^4 cells per well and grown to confluence. Prior to assay, the medium was removed, and the HUVE monolayer was washed once in RPMI 1640 containing 10% (vol/vol) FCS. To each well was added a total volume of 200 μl containing neutrophils and the activating substance. The monolayers were incubated at 37°C for 30 min in an atmosphere of 5% CO_2/95% air, and then the nonadherent cells were removed by aspiration, and each well was washed once. All medium was then removed, and 100 μl of a 0.25% solution of rose bengal in PBS, pH 7.3, was added

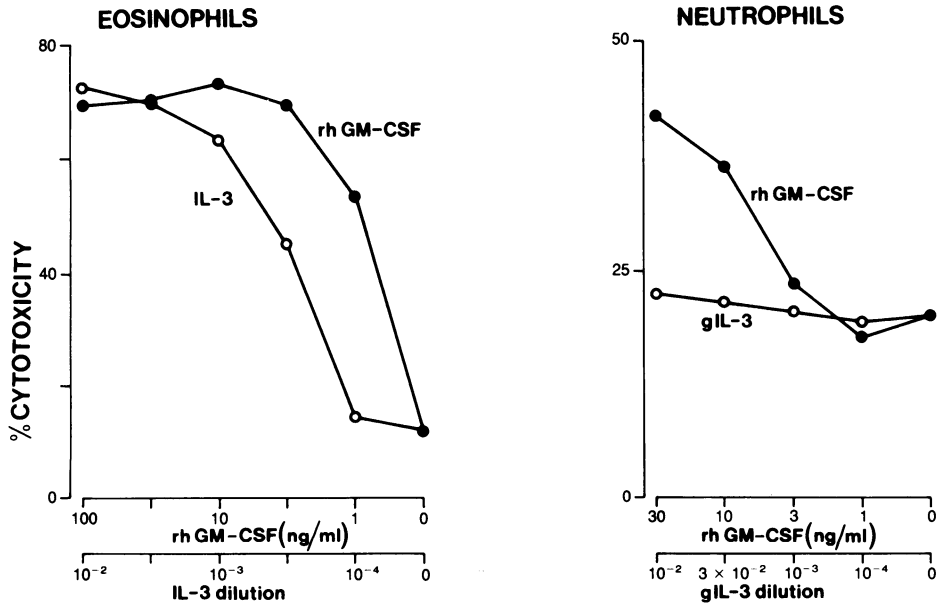

FIG. 1. Antibody-dependent cytotoxicity of trinitrophenyl-coupled P815 cells by human eosinophils and neutrophils in the presence of various concentrations of gIL-3 (○) and rhGM-CSF (●). The anti-trinitrophenyl IgG dilution was 1:300 for eosinophils and 1:3000 for neutrophils. No cytotoxicity was observed in the absence of antibody with or without gIL-3 or rhGM-CSF.

Table 3. gIL-3 stimulates human eosinophils to phagocytize serum-opsonized bakers' yeast

Fresh autologous human serum*	Stimulus	% of eosinophils containing phagocytized yeast cells 0	1	2
5% (vol/vol)	None	94.7 ± 0.9	4.3 ± 0.7	1 ± 0.6
	gIL-3†	84.5 ± 4.5‡	10.3 ± 1.2‡	5.3 ± 0.7‡
	rhGM-CSF	84 ± 1.1‡	12 ± 0‡	4 ± 1‡
2.5% (vol/vol)	None	97 ± 0.7	2 ± 0.6	0.7 ± 0.3
	gIL-3	84 ± 3.5‡	12.7 ± 2.3‡	3.3 ± 1.2‡
	rhGM-CSF	90 ± 1.5‡	7.7 ± 1.9‡	2.3 ± 0.3§

The percent of eosinophils containing zero, one, or two yeast cells is shown as the arithmetic mean ± SEM of triplicate determinations.
*In the absence of serum no phagocytosis was observed with any stimulus.
†gIL-3 was used at a final dilution of 1:300, while rhGM-CSF was used at 30 ng/ml.
‡Differs from no stimulus samples by $P \leq 0.05$.
§In this group alone there were several eosinophils with three particles.

to each well for 10 min at room temperature (15). After aspiration of the stain, each well was washed twice in assay medium and 200 μl of a 1:1 (vol/vol) solution of ethanol/PBS was added. When a uniform release of stain had occurred, 30 min later, the OD_{570} of each well was determined using an ELISA reader. Adherence is proportional to the difference between the OD of each well minus the OD of wells containing HUVE cells only.

Superoxide Production. Purified neutrophils or eosinophils were incubated with gIL-3, rhGM-CSF, or medium for various times at 37°C. Cells (150 μl) were then added to a mixture of 100 μl of freshly prepared cytochrome c (Sigma, type VI; 12.4 mg/ml), 100 μl of fMet-Leu-Phe (Sigma), and medium to 1 ml. The mixtures were incubated at 37°C for 5 min, after which the cells were rapidly cooled and pelleted at 4°C. The supernatants were transferred to plastic, disposable cuvettes. Superoxide production was measured in duplicate by the reduction of cytochrome c as described (16). In each experiment superoxide dismutase (Sigma) inhibited all superoxide generation.

Chemotaxis. The chemotactic response of neutrophils in a gradient of fMet-Leu-Phe was tested under agarose (17). Agarose (5 ml of 0.5% agarose; type II, Sigma) in RPMI 1640 with 2% (vol/vol) FCS and 20 mM Hepes was poured into a plastic Petri dish (50 mm, Kayline), and wells 2.4 mm in diameter were formed 2.4 mm apart in a horizontal line from the center to the edge of the plate. Neutrophils at 2.5×10^7 cells per ml in RPMI and 2% (vol/vol) FCS were preincubated for 45–60 min at 37°C in the presence or absence of rhGM-CSF at 100 ng/ml or gIL-3. Ten microliters of these cells was added to the center well; 10 μl of medium was added to the inner well; and 10 μl of fMet-Leu-Phe was added to the outer well. The Petri dish was then incubated for 2 hr at 37°C in 5% CO_2/95% air. Then, the cells were fixed in methanol at 4°C overnight, followed by 47% (vol/vol) formalin for 30 min at 25°C. Migration was measured under ×40 magnification with a calibrated graticule (1 division = 0.04 mm). Chemotaxis was the difference between directed and random movement.

RESULTS

Stimulation of Bone Marrow Cells. gIL-3 stimulated the formation of myeloid colonies in agar after 14 days of incubation (Table 1) and of very few clusters of cells after 7 days. Morphological examination of the stained agar cultures showed that gIL-3, like rhGM-CSF, stimulated granulocyte, macrophage, granulocyte–macrophage, and eosinophil colonies (Table 1). In two experiments megakaryocytes were identified in cytosmear preparations of gIL-3- but not of GM-CSF-stimulated colonies. gIL-3 also stimulated the formation of erythroid colonies in the presence but not in the absence of pure EPO (Table 2).

Stimulation of Human Granulocyte Function. *Antibody-dependent cell-mediated cytotoxicity assay.* gIL-3 stimulated eosinophils to kill antibody-coated tumor target cells in a dose-dependent manner (Fig. 1). The degree of stimulation was similar to that of rhGM-CSF, and comparable levels of killing were obtained with a 1:1000 dilution of gIL-3 and rhGM-CSF at 1 ng/ml. In contrast, neutrophil-mediated killing was not significantly enhanced by gIL-3 over the same dose range, whereas neutrophils did respond to stimulation by rhGM-CSF.

Phagocytosis. gIL-3 stimulated eosinophils to phagocytize serum-opsonized bakers' yeast (Table 3). The degree of

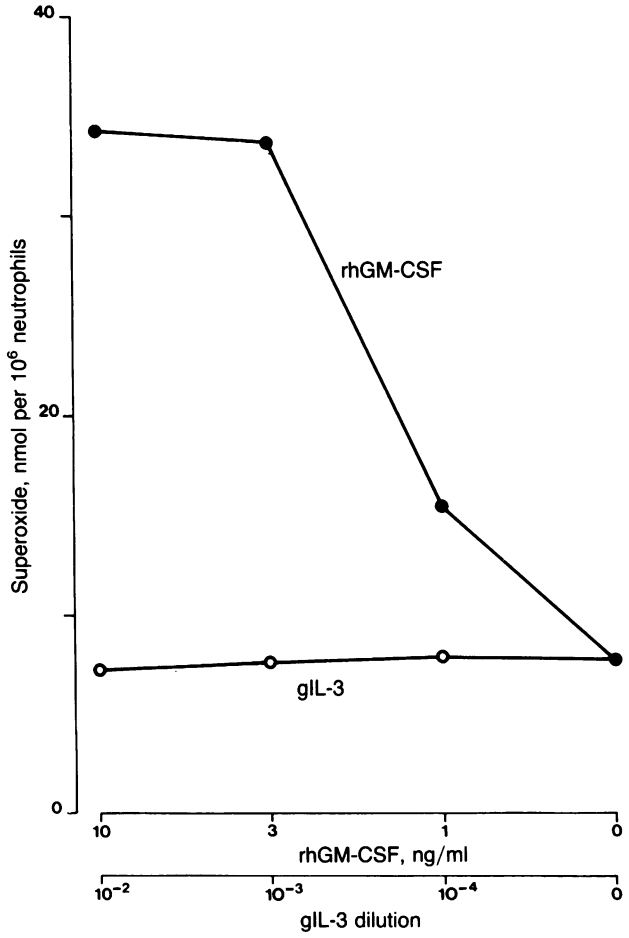

FIG. 2. rhGM-CSF but not gIL-3 enhanced fMet-Leu-Phe-stimulated superoxide production by human neutrophils. Points are arithmetic means of duplicate determinations, and SDs were always <15% of means.

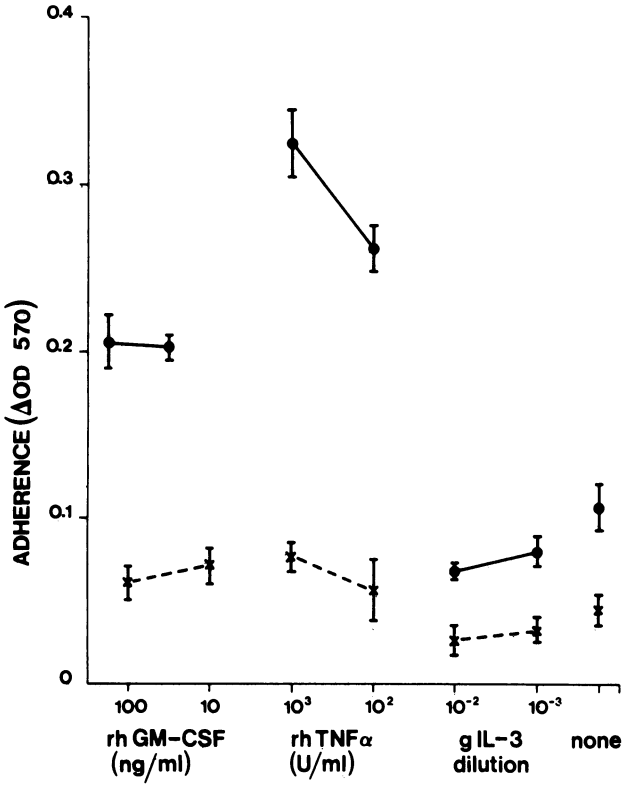

FIG. 3. Stimulation of neutrophil (●) and eosinophil (×) adherence to HUVE cells by rhGM-CSF, recombinant human tumor necrosis factor type α (rhTNF-α), and gIL-3. Each point is the arithmetic mean of three replicates ± SEM. Adherence is given as the change in OD_{570} of each well.

phagocytosis was similar to that obtained with rhGM-CSF, at the two concentrations of serum used.

Superoxide anion production. gIL-3 directly stimulated superoxide production by eosinophils (10.7 nmol per 10^6 cells per 15 min), which was comparable to the effect of rhGM-CSF (11 nmol per 10^6 cells per 15 min). By contrast neither cytokine directly stimulated superoxide production from neutrophils. Preincubation of neutrophils with rhGM-CSF, but not gIL-3, strongly enhanced their capacity to respond to fMet-Leu-Phe (Fig. 2).

Adherence. Because some cytokines have been shown to influence the adherence of neutrophils to endothelial cells, gIL-3 was tested in the HUVE cell adherence assay. gIL-3 did not stimulate either eosinophils or neutrophils to adhere to HUVE cells (Fig. 3). The control cytokines tumor necrosis factor type α and, to a lesser degree, rhGM-CSF stimulated

Table 4. Effect of rhGM-CSF and gIL-3 on neutrophil migration

Stimulus	Migration, mm			
	1 μM	0.1 μM	0.01 μM	None
		Total		
None	6.44 ± 0.06	5.4 ± 0.0	4.2 ± 0.1	4.00 ± 0
rhGM-CSF	5.86 ± 0.08*	4.54 ± 0.1*	4.14 ± 0.1	
gIL-3	6.5 ± 0.1	5.2 ± 0.1	4.1 ± 0.1	
		Chemotactic		
None	2.68 ± 0.06	1.38 ± 0.08	0.48 ± 0	0.32 ± 0
rhGM-CSF	2.18 ± 0.03*	0.84 ± 0.1*	0.18 ± 0.06	
gIL-3	2.5 ± 0.17	1.52 ± 0.06	0.24 ± 0.0	

Neutrophils were incubated with no stimulus, rhGM-CSF, or gIL-3, and fMet-Leu-Phe at 1 μM, 0.1 μM, or 0.01 μM was added as chemoattractant. As a control, medium without fMet-Leu-Phe was also used. Total migration and chemotactic migration [total migration − (random migration plus well diameter)] was measured.
*Differs from control sample by $P \leq 0.05$.

Table 5. Effect of various CSFs on human myeloid cells

CSF	Bone marrow colony type(s)	Neutrophil function	Eosinophil function
hGM-CSF	G, M, Eo	+	+
m- or hG-CSF	G	+	−
mEDF/Eo-CSF	Eo	−	+
gIL-3	G, M, Eo, megakaryocytic	−	+

Eo, eosinophil. +, Function present; −, function absent.

neutrophil adherence, while phorbol myristate acetate was the only stimulus tested that was effective on eosinophils.

Chemotaxis. The ability of gIL-3 to influence neutrophil movement was tested in a migration assay. gIL-3 did not stimulate or inhibit random migration of neutrophils or their unidirectional movement to a chemotactic gradient of fMet-Leu-Phe (Table 4). These functions, however, could be inhibited by rhGM-CSF.

DISCUSSION

Our results indicate that gIL-3 is a multipotential proliferative stimulus for human cells and strongly stimulates some functions of mature eosinophils but not of neutrophils.

gIL-3 stimulated normal human nonadherent bone marrow cells to produce predominantly day-14 colonies similar in morphology to those produced by GM-CSF (Table 1), thus suggesting that gIL-3 acts on a relatively primitive type of progenitor cell. In the presence of human plasma, gIL-3 also stimulated the formation of megakaryocytes, an effect comparable to that of mIL-3 on mouse bone marrow. On the other hand, its capacity to stimulate mast cell colonies remains to be elucidated. In the presence of EPO, gIL-3 stimulated erythroid colonies. The spectrum of activities of gIL-3, therefore, appears to be similar to GM-CSF except for the megakaryocyte-stimulating property. However, because only a single dose of each cytokine was used in these experiments and because gIL-3 was not purified, no conclusions can be drawn about their relative potency.

gIL-3, like rhGM-CSF, was a powerful stimulator of mature human eosinophil function as judged by enhancement of antibody-dependent cell-mediated cytotoxicity, superoxide production, and phagocytosis (Fig. 1 and Table 3). In contrast to the rhGM-CSF, however, gIL-3 had no detectable effect on any neutrophil function we studied. In this regard it resembled mEDF, although EDF is known to stimulate only eosinophil colonies from human bone marrow cells (18) whereas gIL-3 clearly stimulated neutrophil and macrophage maturation as well.

The failure of gIL-3 to stimulate mature neutrophil function was surprising in view of its ability to produce day-14 granulocyte colonies. However, since no day-7 granulocyte colonies were produced either, it is possible that human neutrophils lose their receptors or their responsiveness to IL-3 as they mature. In the mouse, autoradiographic studies with radiolabeled mIL-3 revealed a decreasing number of receptors on neutrophils and eosinophils during maturation; however, mature neutrophils and eosinophils were clearly labeled—eosinophils bound twice as many mIL-3 molecules as did neutrophils (19). An alternative, though unlikely, explanation for the lack of human neutrophil stimulation by gIL-3 is the fact that gIL-3 differs from hIL-3 by 11 amino acids (6), which may affect the interactions of the gibbon molecule with the human receptor.

It is clear from our experiments that IL-3, like other CSFs, has the property of stimulating granulocyte function. mIL-3 can stimulate murine macrophages *in vitro* (20) and *in vivo* (21), although no effect was observed on mature neutrophil

Table 6. Regions of homology between human IL-3 and mouse T-cell replacing factor/B-cell growth factor 2

	61																		78
hIL-3	G	E	D	–	–	–	Q	D	I	L	M	E	N	N	L	R	R	P	N	L	E
	*	*						*	*	*		·		·	*				·	*	
mTRF	G	E	I	F	G	G	L	D	I	L	K	N	Q	T	V	R	G	G	T	V	E
	63																				83
	125														138						
hIL-3	E	F	R	R	K	L	T	F	Y	L	K	T	L	–	E						
	*		*	*				*		*			*		*						
mTRF	E	R	R	R	T	R	Q	F	–	L	D	Y	L	Q	E						
	106														119						

Identical amino acids are indicated by asterisks and conserved changes with dots. mTRF, T-cell replacing factor.

and eosinophil function (21). Thus it remains to be seen whether there are fundamental differences between mouse and primate IL-3 in their capacity to bind and functionally activate mature neutrophils and eosinophils.

The biological activities of gIL-3 are compared to those of other CSFs known to stimulate human cells in Table 5. Clearly this molecule has a characteristic range of actions. It has broad specificity on relatively undifferentiated bone marrow cells, whereas it is active only on mature eosinophils and not mature neutrophils. It is also apparent that three cytokines have now been described with the capacity to stimulate mature human eosinophil function: rhGM-CSF (13, 16), mEDF (18), and gIL-3. We found some homologies between g- or hIL-3 and mouse T-cell replacing factor/B-cell growth factor 2, a molecule that is probably identical to mEDF (22). A computer comparison of hIL-3 (or gIL-3) and mouse T-cell replacing factor amino acid sequences revealed two regions that were 47% and 46% homologous (Table 6). The percentage homology was calculated over regions of >15 amino acids and includes conserved amino acid changes. It would be of interest to determine whether these regions of homology are related to eosinophil stimulation.

An eosinophil stimulatory molecule is produced by human mononuclear cells, probably monocytes, after culture (23, 24). It has been proposed that this molecule is similar but not identical to GM-CSF, or CSF-α (25). Our present results support the hypothesis that IL-3 is at least one of the factors produced under these culture conditions and is responsible for controlling some aspects of eosinophil stimulation. It is also possible that GM-CSF and IL-3 may regulate different aspects of eosinophil function; if this is the case, eosinophilia in allergy and in parasitism may be stimulated by different molecules and result in different clinical conditions reflecting the different roles of the eosinophil.

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1. Ihle, J. N., Keller, J., Oroszalan, S., Henderson, L. E., Copeland, T. D., Fitch, F., Prystowsky, M. B., Goldwasser, E., Schrader, J. W., Palaszynski, E., Dy, M. & Lebel, B. (1983) *J. Immunol.* **131,** 282–287.
2. Fung, M. C., Hapel, A. J., Ymer, S., Cohen, D. R., Johnson, R. M., Campbell, H. D. & Young, I. G. (1984) *Nature (London)* **307,** 233–237.
3. Cutler, R. L., Metcalf, D., Nicola, N. A. & Johnson, G. R. (1985) *J. Biol. Chem.* **260,** 6579–6587.
4. Clark-Lewis, I., Kent, S. B. H. & Schrader, J. W. (1984) *J. Biol. Chem.* **259,** 7488–7494.
5. Schrader, J. W. (1985) *Lancet* **ii,** 133–135.
6. Yang, Y.-C., Ciarletta, A. B., Temple, P. A., Chung, M. P., Kovacic, S., Witek-Giannotti, J. S., Leary, A. C., Kriz, R., Donahue, R. E., Wong, G. G. & Clark, S. C. (1986) *Cell* **47,** 3–10.
7. Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A. & Clark, S. C. (1985) *Science* **228,** 810.
8. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Eoddel, D. V. (1984) *Nature (London)* **312,** 724–729.
9. Cordell, J. L., Falini, B., Erber, W. N., Ghosh, A. K., Abdulaziz, Z., Macdonald, S., Pulford, K. A. F., Stein, H. & Mason, D. Y. (1984) *J. Histochem. Cytochem.* **32,** 219–229.
10. Burns, G. F., Cosgrove, L., Triglia, T., Beall, J. A., Lopez, A. F., Werkmeister, J. A., Begley, C. G., Haddad, A. P., d'Apice, A. J. F., Vadas, M. A. & Cawley, J. C. (1986) *Cell* **45,** 269–280.
11. Vadas, M. A., David, J. R., Butterworth, A., Pisani, N. T. & Siongok, T. A. (1979) *J. Immunol.* **122,** 1228–1236.
12. Vadas, M. A., Nicola, N. A. & Metcalf, D. (1983) *J. Immunol.* **130,** 795–799.
13. Metcalf, D., Begley, C. G., Johnson, G. R., Nicola, N. A., Vadas, M. A., Lopez, A. F., Williamson, D. J., Wong, G. G., Clark, S. C. & Wang, E. A. (1986) *Blood* **67,** 37–45.
14. Gamble, J. R., Harlan, J. M., Klebanoff, S. J. & Vadas, M. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8667–8671.
15. Chong, A. S. & Parish, C. R. (1985) *Cell. Immunol.* **92,** 277–289.
16. Lopez, A. F., Williamson, D. J., Gamble, J. R., Begley, C. G., Harlan, J. M., Klebanoff, S., Waltersdorph, A., Wong, G., Clark, S. & Vadas, M. A. (1986) *J. Clin. Invest.* **78,** 1220–1228.
17. Nelson, R. D., Quil, P. G. & Simmons, R. L. (1975) *J. Immunol.* **115,** 1650–1656.
18. Lopez, A. F., Begley, C. G., Williamson, D. J., Warren, D. J., Vadas, M. A. & Sanderson, C. J. (1986) *J. Exp. Med.* **163,** 1085–1099.
19. Nicola, N. & Metcalf, D. (1986) *J. Cell Physiol.* **128,** 180–188.
20. Crapper, R. M., Vairo, G., Hamilton, J. A., Clark-Lewis, I. & Schrader, J. W. (1985) *Blood* **66,** 859–865.
21. Metcalf, D., Begley, C. G., Johnson, G. R., Nicola, N. A., Lopez, A. F. & Williamson, D. J. (1986) *Blood* **68,** 46–57.
22. Sanderson, C. J., O'Garra, A., Warren, D. J. & Klauss, G. G. B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 437–440.
23. Veith, M. C. & Butterworth, A. E. (1983) *J. Exp. Med.* **157,** 1828–1843.
24. Vadas, M. A., Nicola, N., Lopez, A. F., Metcalf, D., Johnson, G. & Pereira, A. (1984) *J. Immunol.* **133,** 202–207.
25. Thorne, K. J. I., Richardson, B. A., Taverne, J., Williamson, D. J., Vadas, M. A. & Butterworth, A. W. (1986) *Eur. J. Immunol.* **9,** 1143–1150.