MURINE EOSINOPHIL DIFFERENTIATION FACTOR

An Eosinophil-specific Colony-stimulating Factor with Activity for Human Cells

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Eosinophilia is a characteristic feature of certain parasite infections and allergic conditions in which eosinophils may be involved in the elimination of the parasite, and in the production of tissue damage. In most of these cases, the occurrence of eosinophils is selective, suggesting that mechanisms must exist to regulate the production of these cells independently of other members of the granulocyte-macrophage series.

The mechanism of induction of selective eosinophilia is not known. Colony-stimulating factors (CSFs)[1] have been shown (1, 2) to regulate the proliferation, differentiation, and functional activation of hematopoietic cells, including eosinophils. One of the most important properties of CSFs is their lineage specificity. Thus, in the mouse, macrophage-CSF (M-CSF) (3) and granulocyte-CSF (G-CSF) (4, 5) act predominantly on the monocyte-macrophage and neutrophil lineages, respectively. Granulocyte-macrophage CSF (GM-CSF) (5–7) acts on both of these lineages, and at high concentrations also stimulates the growth of eosinophil colonies. In man, analogs of the murine GM-CSF have been described, either as semipurified CSF-α (8), or as purified recombinant GM-CSF (9), and have been shown to stimulate neutrophil, eosinophil, and macrophage progenitors (8–10), as well as mature neutrophils and eosinophils (11, 10). The human analog of murine G-CSF is probably CSF-β, which has been semipurified and biologically characterized (12).

Hitherto there have been no descriptions of a purified eosinophil-CSF (Eo-CSF) from human or murine sources. Conditioned media from PWM-stimulated murine spleen cells (spleenocyte-conditioned medium; SCM) (13) and from

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[1] *Abbreviations used in this paper:* β_2m, β_2 microglobulin; BCM, bladder carcinoma–conditioned medium; CSF, colony-stimulating factor; EDF, eosinophil differentiating factor; Eo, eosinophil; GFA, granulocyte functional antigen; GM, granulocyte/macrophage; HPCM, human placenta–conditioned medium; IMDM, Iscove's modified Dulbecco's medium; MLCM, mouse lung–conditioned medium; SCM, splenocyte-conditioned medium.

murine alloreactive T cell clones (14) have been shown to cause proliferation of human eosinophil progenitors. SCM also enhanced the metabolic activity of mature eosinophils (15). However, these conditioned media did not support the growth of murine eosinophils in the colony-forming assay in agar (13).

The recent isolation of a mouse T cell hybrid line (NIMP-TH1) selected for the production of a lymphokine with eosinophil differentiation activity without concomitant production of IL-2, IL-3, IFN-γ, G-CSF, or GM-CSF (16, 17), and the subsequent purification of the molecule, termed eosinophil differentiation factor (EDF), have enabled us to examine whether this murine lymphokine has the properties expected of an Eo-CSF. In this paper, we show that murine EDF selectively stimulated the clonal proliferation and differentiation of murine eosinophil progenitors, establishing this molecule as an Eo-CSF. In addition, murine EDF stimulated the growth of human eosinophil progenitors and selectively activated mature human eosinophils but not neutrophils in functional assays. Thus the existence of a murine eosinophil-specific growth regulator is established, and the data strongly suggest that there is a human analog of murine EDF (Eo-CSF).

Materials and Methods

Preparation of EDF and Other Hematopoietic Growth Factors. Purified EDF was prepared from conditioned media from the T cell hybrid line (NIMP-TH1) (16, 17) by the following steps: ammonium sulfate precipitation, elution from a lentil lectin column, chromatography on phenyl-Sepharose CL-4 B, gel filtration on AcA54-Sepharose, ion-exchange chromatography on DEAE-Sepharose, followed by reverse-phase chromatography on a C_{18}-bondapak column. The full details of the purification procedure will be published elsewhere. The degree of purification was estimated to be 10,000-fold, and the activity titrated to a dilution of 1:10,000 in the mouse liquid bone marrow culture system (17).

In every series of experiments, purified EDF was used. In few individual experiments, crude NIMP-TH1-conditioned medium, which we have shown previously (17) contains only EDF, was used; no differences were ever found whether the purified material or NIMP-TH1-conditioned medium was employed.

The other hematopoietic growth factors used were human placental–conditioned medium (HPCM) (8), conditioned medium from the human bladder carcinoma cell line (U5637) (BCM), and semipurified CSF-α and -β obtained after fractionation of the latter by hydrophobic chromatography (12). These were generously provided by Dr. N. A. Nicola, Walter and Eliza Hall Institute of Medical Research. Mouse lung–conditioned medium (MLCM) was obtained as serum-free medium conditioned for 48 h by whole-lung tissue from mice injected with endotoxin (18). Mouse SCM was obtained by stimulating spleen cells from BALB/c mice with PWM as previously described (13). All preparations of CSF were pretitrated and diluted to deliver a supramaximal stimulus. No preparation exhibited high-dose inhibition.

Animals. Inbred C57BL and CBA mice were maintained in the Walter and Eliza Hall Institute animal facility.

Human Bone Marrow Cells. In accordance with a protocol approved by the Ethics Committee of The Walter and Eliza Hall Institute, human bone marrow cells were obtained from individuals undergoing cardiac surgery who had no hematological disorder. Cells were layered on Ficoll/Hypaque (1.077 g/ml) (Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged for 20 min at 1,000 *g*. The cells were washed and resuspended in Iscove's Modified Dulbecco's Medium (IMDM) (Gibco, Grand Island, NY) containing 10% FCS (Flow Laboratories, North Ryde, New South Wales, Australia).

Bone Marrow Cultures. The colony assay used was as previously described (1), with few modifications. Stimuli (0.1 ml) were placed in 35-mm tissue culture dishes to which murine

or human hematopoietic progenitor cells were added in 1 ml mixtures of double-strength IMDM, FCS, and 0.6% Bacto-agar (Difco Laboratories, Detroit, MI.) in the ratio 3:2:5 vol/vol/vol. C57BL bone marrow cells or CBA 12-d fetal liver cells were cultured at concentrations of 7.5×10^4 cells/ml and 3×10^4 cells/ml, respectively. Human bone marrow cells were cultured at $2–5 \times 10^4$ cells/ml. Cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air for 7–16 d. Colonies (clones containing ≥50 cells) were counted using a dissection microscope and were morphologically identified after glutaraldehyde fixation of the entire culture, followed by staining with Luxol Fast Blue and Meyer's hematoxylin.

Clone Transfer Experiments. Developing clones from cultures initiated in EDF were picked off individually using a fine Pasteur pipette and transferred in groups of five to the surface of agar-medium in a second culture dish containing different stimuli. Transfers were made after 7 d of incubation, and the cells were incubated for a further 9 d. Clone size was recorded at both of these points, and clones that had at least doubled in size were picked off and fixed and stained for morphological identification as described above.

Purification of Human Granulocytes. Peripheral blood from normal volunteers was sedimented on dextran, and the leukocyte-rich supernatant was centrifuged on a discontinuous gradient of hypertonic Metrizamide (Nyegaard, Oslo, Norway) as previously described (19). The eosinophil and neutrophil preparations were always >90 and >95% pure, respectively. Unless otherwise stated, granulocytes were suspended in Eagle's MEM (Flow Laboratories) containing 10% FCS and antibiotics (benzylpenicillin 60 mg/liter and streptomycin 100 mg/liter) (Glaxo, Boronia, Victoria, Australia).

ADCC Assay. This was carried out using as targets ^{51}Cr-labelled P815 cells coupled with TNP as previously described (11). Briefly, 40 μl P815 cells (4×10^3) were incubated in V-bottom microtiter plates (Linbro; Flow Laboratories, McLean, VA) with 24 μl crossreacting rabbit IgG anti-DNP (Miles-Yeda, Rehovot, Israel), 16 μl stimulus, and 80 μl human eosinophils or neutrophils (1.3×10^5) as effector cells. After incubation for 2.5 h at 37°C, 80 μl supernatant was removed and assayed in a γ-counter. Percent cytotoxicity was determined according to the formula: 100 × (test − control)/(total − control), where control was the ^{51}Cr released by P815 in the presence of medium alone, and total the ^{51}Cr released by the addition of 5% Triton X-100. The results shown are the means of triplicate determinations.

Phagocytosis Assay. This assay used heat-killed baker's yeast and was performed as previously described (20) and modified (10). Purified eosinophils or neutrophils (100 μl; 2×10^7 cells/ml in PBS) were incubated with 250 μl yeast organisms, 100 μl fresh autologous serum, and 50 μl EDF, BCM, or medium for 1 h at 37°C. The cells were then centrifuged at 4°C, resuspended in 50 μl cold saline, and smears were made before mixing in methanol and staining with Giemsa. The smears were examined for the number of eosinophils or neutrophils showing different numbers of phagocytosed yeast, and the data are expressed as the percentage of cells containing different numbers of phagocytosed organisms by counting a minimum of 200 cells on each of three replicate slides.

Immunofluorescence Assay. Purified human eosinophils were made up to 2×10^6 cells/ml and 400 μl aliquots (8×10^5 cells) were incubated with 80 μl EDF, CSF-α, FMLP (Sigma Chemical Co., St. Louis, MO), LPS (011:B4, Sigma Chemical Co.) or medium for 1 h at 37°C. The cells were then washed and incubated (2×10^5 cells/tube) with different mAb for 1 h at 4°C. The mAb used were: WEM-G1 (21), WEM-G11 (22), and OKM1 (23) (Ortho-mune Diagnostic, Melbourne, Victoria, Australia) directed against functional antigens on human granulocytes, anti-β_2-microglobulin (β_2m) (IgG2b anti-β_2m, gift from Dr. I. F. C. McKenzie, Dept. of Pathology, Univ. of Melbourne, Australia), and also K7 (IgM anti-TNP, gift from Dr. G. Klauss, National Institute for Medical Research, London) and PB 10 (IgG1 against the chicken θ antigen, gift from Dr. P. Bartlett, Walter and Eliza Hall Institute of Medical Research) as control mAb that do not bind to granulocytes. With the exception of OKM1, the mAb were directly conjugated with FITC (24). In the case of OKM1, eosinophils were further incubated with goat anti-mouse Ig-FITC (Tago Inc., Burlingame, CA). After incubation the cells were washed three times and fixed as described (25).

TABLE I

Morphology of Mouse Colonies Stimulated by EDF

Exp.	Cells	Stimulus	Colonies typed	Colonies per 10^5 cells	Type of colony (%)			
					Eo	G	GM	M
1	Bone marrow	EDF	6	2	100	0	0	0
		SCM	50	180	4	20	41	35
		MLCM	50	201	2	14	51	33
2	Bone marrow	EDF	5	2	100	0	0	0
		SCM	50	180	2	5	44	49
		MLCM	50	177	1	5	37	57
3	Bone marrow	EDF	9	3	100	0	0	0
		SCM	50	180	2	5	47	46
		MLCM	50	160	3	3	40	54
4	Fetal liver	EDF	14	12	100	0	0	0
		SCM	50	280	1	1	36	62
		MLCM	50	149	8	0	38	54
5	Fetal liver	EDF	17	14	100	0	0	0
		SCM	50	337	7	9	43	41
		MLCM	50	175	10	7	40	43
6	Fetal liver	EDF	16	13	84	11	0	5
		SCM	50	274	2	2	36	60
		MLCM	50	152	2	5	43	50

Quadruplicate plates containing 7.5×10^4 C57BL bone marrow or 3×10^4 CBA fetal liver cells were incubated for 7 d with EDF, SCM, or MLCM. After counting the number of colonies on each plate, they were fixed and stained for morphological typing. Where <50 colonies were obtained, all of them were scored. Purified EDF was used in exps. 3, 4, and 5, whereas in the others, EDF-containing NIMP-TH1-conditioned medium was used.

Flow Cytometry. Fixed eosinophils were analyzed in a FACS (FACS II; Becton-Dickinson Immunocytometry Systems, Mountain View, CA) equipped with a three-decade logarithmic amplifier in the fluorescence detection channel and linked to a PDP11 computer data-handling system. In each sample, more than 9,000 cells were analyzed. The coefficient of variation of the analysis by the FACS II was <3%. After computer transformation to a linear scale, the mean and median fluorescence of each sample were calculated. The mean was defined as the sum of the fluorescence intensities of individual cells divided by the number of cells analyzed, and the median was defined as the fluorescence below which 50% of the cells were found.

Results

Selective Stimulation of Mouse Eosinophil Colonies by EDF. To determine whether EDF could stimulate the clonal proliferation and differentiation of murine eosinophil progenitors in semisolid agar cultures, mouse bone marrow and fetal liver cells were cultured in the presence of EDF and other CSFs. It was found (Table I) that EDF generated small numbers of eosinophil colonies containing at least 200 cells after 7 d of culture of both bone marrow and fetal liver cells. In most experiments, EDF stimulated only eosinophil colonies, whereas the expected proportions of neutrophil (G), neutrophil-macrophage (GM), and

macrophage (M) colonies developed in the presence of SCM and of MLCM; a source of G- and GM-CSF (Table I). When the results of the three experiments using bone marrow cells and fetal liver cells were pooled, it was found that all 20 and 44 of 47 colonies stimulated by EDF were eosinophilic. Furthermore, the absolute number of eosinophil colonies stimulated by EDF was similar to those obtained with SCM and MLCM, suggesting that EDF stimulates the proliferation of all responsive eosinophil progenitor cells. At high concentrations EDF also stimulated some G, GM, and M clusters (3–49 cells per clone), suggesting that it may have some activity on these lineages (data not shown). Notwithstanding a degree of crossreactivity, these data indicate that EDF is a murine Eo-CSF, and they are of considerable interest since this had not been observed with the human-active Eo-CSF present in SCM (13).

EDF Selectively Stimulates Growth of Eosinophils from Human Bone Marrow. In experiments examining the ability of EDF to stimulate human bone marrow cells to form colonies in agar, only day 14 colonies were obtained. The absolute number of clones was less than that stimulated by CSF-α (the human analog of murine GM-CSF) or HPCM, but was similar to the numbers of colonies stimulated by murine SCM (Table II). Morphological examination of the day 14 colonies stimulated by EDF showed that these were almost exclusively eosinophil in type (Fig. 1), whereas CSF-α and HPCM also stimulated the formation of G, GM, and M colonies. In exp. 3 (Table II), a few macrophage colonies were seen in cultures stimulated by EDF, but these were also seen in unstimulated cultures (data not shown), suggesting that they arose spontaneously in this particular bone marrow sample. In any one experiment, the total number of eosinophil colonies induced by EDF was similar to that found with EDF, SCM, CSF-α, or HPCM, suggesting that EDF was stimulating all eosinophil progenitor cells and not simply a subset of these, a conclusion supported by the observation that the eosinophil colonies stimulated by EDF and HPCM were of similar size (Fig. 2).

Direct Effect of EDF on Eosinophil Progenitor Cells. The proliferative effect of murine EDF on human cells and the higher frequency of clonogenic eosinophil precursors in human bone marrow enabled further studies on the actions of EDF. To confirm that the stimulation of eosinophil colonies was a direct effect of EDF and not mediated via an accessory cell, two types of experiment were performed. First, human bone marrow cells were cultured at varying concentrations, and it was shown that the relationship between cell concentration and number of eosinophil colonies stimulated by EDF was linear. The concentration of cells cultured ranged from $0.0625–2.0 \times 10^5$ cells/ml, and the numbers of colonies stimulated were between 0.5 ± 0.5 and 16.7 ± 1.2 (mean $\pm$ SD).

The second type of experiment involved clone transfers. Bone marrow cells were initially stimulated by EDF for 7 d in primary culture, and developing clones (mean 15 cells per clone) were then transferred to recipient culture dishes containing either EDF, CSF-α, or medium. This transfer results in a dilution of the initiating CSF by a factor of 1:500, and allows an assessment of the direct effect of CSF in cultures lacking other cell types (26). Following a further 9 d of incubation, a significant proportion of the transplanted clones showed a progressive increase in size in cultures containing EDF and CSF-α, whereas clones decreased in size and died when transferred to cultures lacking CSF (Fig. 3).

TABLE II

Morphology of Human Bone Marrow Colonies Stimulated by EDF

Exp.	Stimulus	Colonies per plate*	Type of colony (%)				Calculated number of Eo colonies
			Eo	G	GM	M	
1	EDF	13	100	0	0	0	13
	CSF-α	36	39	0	44	17	14
	HPCM	23	19	0	45	36	4
2	EDF	10	100	0	0	0	10
	CSF-α	38	33	0	28	39	13
	HPCM	50	12	0	44	44	6
3	EDF	40	94	0	0	6	38
	SCM	38	98	0	0	2	37
	CSF-α	68	18	0	17	65	12
	HPCM	174	16	4	43	37	28
4	EDF	63	100	0	0	0	63
	SCM	67	100	0	0	0	67
	HPCM	220	20	28	22	30	44
5	EDF	12	100	0	0	0	12
	SCM	14	100	0	0	0	14
	CSF-α	78	22	7	17	54	17
	HPCM	108	22	25	19	33	24
6	EDF	6	100	0	0	0	6
	SCM	4	100	0	0	0	4
	HPCM	54	14	41	24	22	8
7	EDF	8	100	0	0	0	8
	SCM	25	81	0	0	19	20
	CSF-α	60	12	17	17	54	7
	HPCM	87	13	21	29	38	11
8	EDF	8	100	0	0	0	8
	SCM	8	100	0	0	0	8
	CSF-α	34	23	7	30	40	8
	HPCM	42	26	21	23	30	11

* Normal human bone marrow cells were incubated for 14 d and stimulated as indicated. The mean absolute number of colonies was determined by scoring four replicate cultures. These cultures were then fixed and stained, and consecutive colonies were examined to determine the percentage of each colony type present. The number of cells cultured was 2–5 × 10^4 cells/ml. Exps. 1–3 used EDF as NIMP-TH1-conditioned medium, and Exps. 4–8 used purified EDF.

The proliferating clones were confirmed to be eosinophilic in type. Three granulocyte-macrophage clones were noted to develop from clones transferred to CSF-α, however, it was not possible to attribute the initiation of these clones to EDF because of the presence of accessory cells in the primary culture.

Selective Activation of Human Eosinophil Function by EDF. Several parameters of functional activation were measured after treatment of mature human eosinophils with EDF. EDF was firstly tested for its ability to stimulate peripheral blood eosinophils and neutrophils in an ADCC assay. Eosinophils showed very

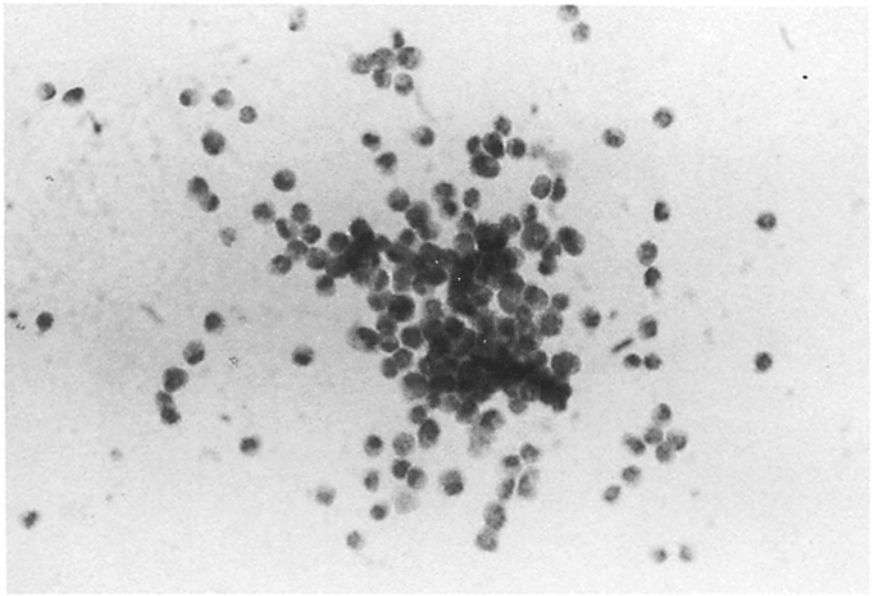

FIGURE 1. Typical appearance of a day 14 eosinophil colony derived from human bone marrow cells stimulated with purified EDF.

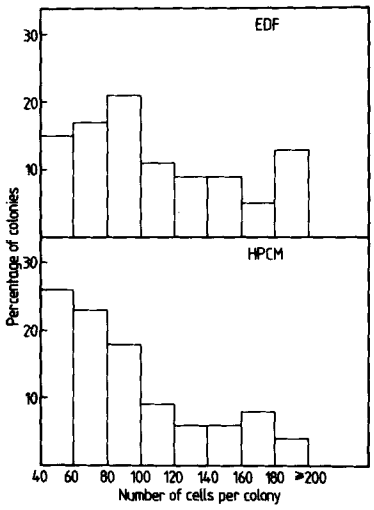

FIGURE 2. Histogram showing a comparison between the size of eosinophil colonies obtained by stimulating human bone marrow cells with purified EDF (*top*) and HPCM (*bottom*). These data were obtained from exps. 4 and 5 shown in Table II. 65 consecutive eosinophil colonies were scored for both stimuli.

low levels of ADCC against target cells in the absence of EDF, however, the addition of EDF resulted in a highly increased capacity to kill target cells by eosinophils from all six individuals tested (Fig. 4). Neutrophils tested in parallel, while showing a higher level of basal ADCC activity, were not activated by EDF.

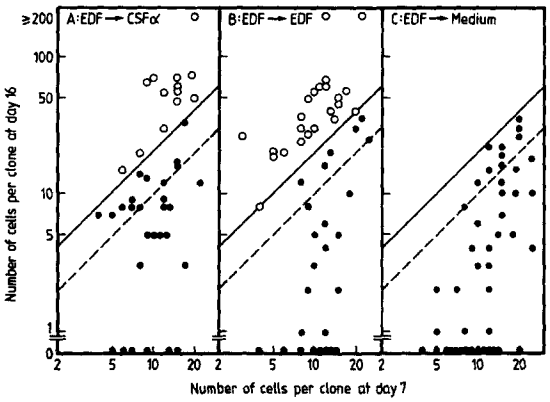

FIGURE 3. Proliferation of eosinophil clones 9 d after transfer of clones to cultures containing CSF-α (*A*), purified EDF (*B*), or medium (*C*). The broken and solid lines indicate maintenance or doubling of initial cell number, respectively. Clones which had at least doubled in size were confirmed to be eosinophilic (open circles).

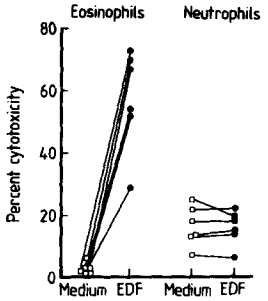

FIGURE 4. Selective stimulation by EDF of human eosinophil-mediated ADCC against target cells. The squares show values obtained in the presence of medium, and the circles show values obtained in the presence of EDF tested at a final dilution of 1:10. Four experiments were performed with EDF as NIMP-TH1-conditioned medium, and two with purified EDF.

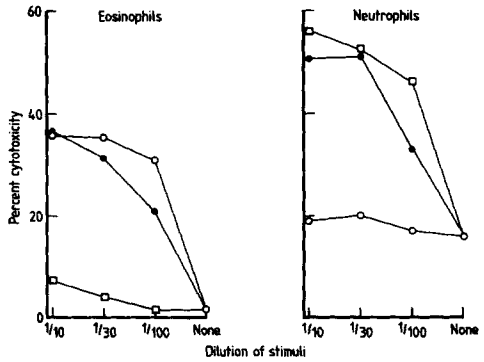

FIGURE 5. Titration of purified EDF (open circles) on human eosinophil- and neutrophil-mediated ADCC. Titrations of CSF-α (closed circles) and CSF-β (open squares) are also shown.

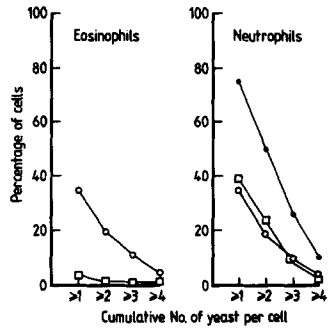

FIGURE 6. Effect of purified EDF (open circles) in stimulating the phagocytosis of serum-opsonized yeast by human eosinophils but not neutrophils. The effects of BCM (closed circles) and medium (open squares) are also shown.

In contrast to EDF, CSF-β did enhance ADCC by neutrophils and CSF-α stimulated both eosinophils and neutrophils (Fig. 5).

Purified EDF was also found (Fig. 6) to selectively enhance eosinophil-mediated phagocytes of serum-opsonized yeast organisms. Very little phagocytosis was seen when eosinophils were incubated with yeast organisms opsonized with 2.5% human serum in the absence of EDF. However, the addition of EDF resulted in an increase in both the number of eosinophils showing internalized yeast particles, and the number of yeast particles internalized per eosinophil. Again, human neutrophils were not stimulated by EDF, although an increase in their phagocytic activity was evident when these cells were incubated with BCM.

Enhancement of Functional Granulocyte Antigen Expression by EDF. The activation of neutrophils has been shown to be associated with an increased expression of membrane antigens such as granulocyte functional antigens 1 and 2 (GFA-1 and GFA-2) (25), and the receptor for C3bi (27) identified by the mAb WEM-G1, WEM-G11, and OKM1 respectively. To determine whether EDF induced similar changes, eosinophils were incubated with or without EDF, and the degree of binding of these mAb was tested.

It was found (Table III) that the binding of WEM-G1, WEM-G11, and OKM1 to eosinophils was greater after the cells had been incubated with EDF than after incubation with medium, LPS, or FMLP. In three different experiments, EDF enhanced the binding of WEM-G1, WEM-G11, and OKM1 from medium control levels by 48 ± 11 (percent mean fluorescence increase ± SE), 61 ± 10 and 94 ± 11%, respectively, much higher than the coefficient of variation of the FACS (<3%). An increase in binding was also observed with eosinophils preincubated with CSF-α, but to a lesser extent than that seen with EDF. Essentially no change in the binding of the control antibodies anti-β_2m, K7, PB10, or goat anti–mouse Ig was observed, suggesting that the effect was a specific event.

To establish the relative amounts of EDF necessary to induce this enhancement of the expression of functional antigens on eosinophils, a titration was carried out. It was found (Fig. 7) that EDF increased the expression of GFA-1, GFA-2, and OKM1 in a dose-dependent manner, and was specific since no increase in the expression of β_2m or in nonspecific binding by mAb PB10 was seen.

TABLE III

Effects of EDF and Other Stimuli on Expression of Functional Antigens on Human Eosinophils

mAb probe	Stimulus*				
	EDF‡	CSF-α	FMLP§	LPS§	Medium
WEM-G1	703	508	421	432	421
WEM-G11	57	48	47	46	44
OKM1 + goat anti-mouse Ig	100	90	58	58	57
K7	20	20	20	20	19
Anti-β_2m	26	24	25	25	26
PB10	24	21	23	22	26
Goat anti-mouse Ig	25	24	25	24	24

* Median fluorescence intensity expressed in arbitrary units. The mean fluorescence values (±SEM) obtained with eosinophils from three different individuals and incubated with and without EDF were 722 ± 95 and 492 ± 63 for WEM-G1 ($P < 0.05$), 88 ± 37 and 54 ± 20 for WEM-G11 (not significant), and 172 ± 22 and 89 ± 11 ($p < 0.05$) for OKM1. Note that the degree of WEM-G1 and WEM-G11 binding is very different between individuals (37), which leads to relatively high variations.

‡ EDF was used as NIM-TH1-conditioned medium.

§ The concentrations of FMLP and LPS used were 10^{-7} M and 10 ng/ml, respectively.

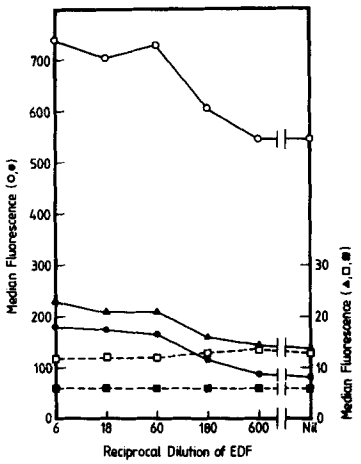

FIGURE 7. Enhancement by purified EDF of the binding of mAb WEM-G1 (open circles), WEM-G11 (triangles), and OKM1 (closed circles), but not of anti-β_2m (open squares) and PB10 (closed squares) to human eosinophils.

Discussion

This paper shows that the murine lymphokine, eosinophil differentiation factor has all the properties of a human CSF with specificity for the eosinophil lineage. These properties include the ability to stimulate the clonal proliferation and differentiation of eosinophil progenitor cells, as well as the capacity to stimulate mature eosinophils. In light of other examples of functional crossreactivity (5, 12, 28) and sequence homology (9) between murine and human CSFs, these findings suggest the existence of a human analog of EDF and should stimulate further efforts towards its characterization. They would also predict the existence of a functionally conserved receptor.

The colony-stimulating activity of EDF on human bone marrow was demon-

strated after 14 d in culture. These colonies contained only eosinophils and were similar in number to those eosinophil colonies resulting from stimulating with HPCM or CSF-α. HPCM and CSF-α also induced the growth of neutrophil, neutrophil-macrophage, and macrophage colonies, as expected. The size distributions of the eosinophil colonies generated by EDF and HPCM were comparable, and hence it is likely that they act on the same responsive cells. That this action is due to the effect of EDF on the progenitor cells was shown by clone transfer experiments, in which continuing clonal proliferation was stimulated by EDF in the absence of accessory cells, and by showing a linear relationship between colony number and number of progenitor cells plated. In other experiments (our unpublished observations), EDF, in contrast to CSF-α and CSF-β, was shown to preferentially induce the growth of small numbers of eosinophil clusters from bone marrow fractions highly enriched for promyelocytes, thus suggesting that eosinophil promyelocytes behave similarly to those of the neutrophil lineage (29) in their ability to divide and in their potential to respond to a lineage-specific CSF.

After differentiation into mature, postmitotic peripheral blood cells, human eosinophils retained their responsiveness to EDF. Assays for antibody-dependent killing and phagocytosis both showed enhancement with EDF. The specificity of this factor for eosinophils was conserved at the mature cell level, since neutrophils were unresponsive to EDF in both assays; neutrophils, however, could be activated by other stimuli such as CSF-α, CSF-β, BCM. In addition, we have found that EDF prolonged the survival in vitro of eosinophils but not neutrophils, while CSF-α increased the survival of both cell types (C. G. Begley, A. F. Lopez, D. J. Warren, C. J. Sandersen, and M. A. Vadas, manuscript in preparation).

The effect of EDF on phagocytosis could be explained in part by its ability to upregulate the C3bi receptor, as shown by an increase in the binding of mAb OKM1. This was accompanied by the augmented expression of two other surface antigens, GFA-1 and GFA-2, previously shown (21, 22) to be involved in several granulocyte functions. The specificity of this effect was indicated by the unaltered expression of β_2m. The C3bi receptor (27), GFA-1 and GFA-2 (25) are selectively upregulated on activated neutrophils. This is the first description of their upregulation on activated eosinophils and their modulation by a growth factor.

The existence of a human-active murine Eo-CSF was suggested in previous experiments, where it was shown that a factor present in conditioned media from mouse EL4 cells (30) and in murine SCM stimulated human bone marrow (13) and mature eosinophils (15). While the human-active Eo-CSF in SCM was not fully characterized, it was biochemically separable from murine GM-CSF and purified multi-CSF (IL-3) also present in SCM (13); both of which are inactive on human bone marrow cells. Purified EDF has also been distinguished from GM-CSF and IL-3 (17). Thus it is possible that the human-active Eo-CSF previously described in SCM and the supernatants of alloreactive T cell clones may be identical to EDF.

Hitherto it was thought that these human-active Eo-CSFs were inactive in the murine semisolid agar culture (13, 14), but in the present study it was found that under optimal culture conditions EDF stimulated the formation of murine colonies, and that the colonies obtained were almost exclusively eosinophilic. At

these concentrations of EDF, however, clusters of granulocytes and/or macrophages were also consistently stimulated in murine cultures, suggesting that EDF may also have some role in initiating other hematopoietic cell types, in keeping with other lineage-specific CSFs (1).

The stimulation of mature granulocyte function by CSFs may have important implications in clinical conditions since such activation may be amenable to exogenous control. At least in vitro, mouse GM-CSF and G-CSF have been shown to stimulate granulocyte function (5, 7). Human monocyte–conditioned media (31, 32), semipurified CSF-α and semipurified CSF-β (11), and purified natural (33, 34) and purified recombinant GM-CSF (9, 10) have been found to enhance several neutrophil and eosinophil functions. Furthermore, the observation that patients with *Schistosoma mansoni* infection develop eosinophilia and enhanced eosinophil-mediated killing suggests that an eosinophil-specific CSF may be at work in vivo (35). Moreover, in experimental situations, it was found that the injection of multi-CSF (IL-3) into mice induced the cell proliferation and activation expected from its action in vitro (36), thus demonstrating the therapeutic potential of these molecules.

The specificity of mouse EDF (Eo-CSF) for the eosinophil lineage in man strongly indicates the presence of a corresponding human molecule and its receptor, thus providing a mechanism for the selective eosinophila seen in helminth infections, allergic conditions and the hypereosinophilic syndrome.

Summary

A purified murine lymphokine, eosinophil differentiation factor (EDF), was found to be a selective stimulus for the clonal proliferation and differentiation of murine eosinophil progenitor cells, establishing it as the murine eosinophil colony–stimulating factor (Eo-CSF). EDF was also active on human eosinophil progenitors and mature blood eosinophils, but had no effect on neutrophil or macrophage precursor cells, nor on blood neutrophils. In culture of human bone marrow cells, EDF stimulated equal numbers and equal sizes of eosinophil colonies to develop when compared with human placental conditioned medium, a source of human CSFs, suggesting that all responsive progenitor cells were stimulated. Clone transfer experiments and the linear relationship between number of bone marrow cells plated and colonies produced confirmed that the action of EDF was directly on eosinophil progenitor cells. EDF increased the capacity of human blood eosinophils, but not neutrophils, to kill antibody-coated tumor cells and to phagocytose serum-opsonized yeast cells. This functional activation was associated with the enhanced expression of functional antigens (GFA-1, GFA-2, and the receptor for C3bi) on eosinophils.

The possession by EDF (Eo-CSF) of all the properties expected of a human eosinophil CSF raises the possibility that a human analog of this molecule exists, and is involved in the regulation of production and function of human eosinophils in vivo.

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