Interleukin-3 Is a Differentiation Factor for Human Basophils

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The effect of recombinant human (rh) cytokines, interleukin-1 α (IL-1α), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), granulocyte/macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte/macrophage colony stimulating factor (M-CSF), interferon-α (IF-α), interferon-γ (IF-γ), and the tumor necrosis factor-α (TNF-α) on differentiation and function of metachromatic cells (MCS) was studied. Among all cytokines tested, rh interleukin-3 (rhIL-3) selectively induced a significant formation of MCS (IL-3: 1.1 ± 0.6 × 10^5 *v* control: 0.02 ± 0.15 × 10^5 MCS/mL suspension) and dose dependent increase in formation of intracellular histamine (IL-3, 100 U/mL: 95 ± 23 ng/mL *v* control: 1.8 ± 0.8 ng/mL) in a bone marrow suspension culture system (analyzed on day 14 of culture). Besides MCS, formation of eosinophils was observed in this culture system in the continuous presence of rhIL-3, whereas IL-3 pulse-stimulation for three hours and subsequent exposure to control medium induced growth of MCS but not of eosinophils. By combined immunofluorescence/toluidine blue staining, MCS were found to express a cell surface marker profile that corresponds to the immunological phenotype of peripheral blood basophils (MY-7(CD13)$^+$, VIM12(CD11b)$^+$, VIM2$^+$, MAX1$^-$, MAX24$^-$ and YB5B8$^-$). Furthermore, cultured MCS expressed surface membrane receptors for IgE and could be triggered for nontoxic histamine release by a monoclonal anti-IgE antibody. To evaluate a possible influence of IL-3 on basophil function, studies were extended to freshly obtained blood basophils (healthy volunteers, n = 3). However, like all other cytokines tested, rhIL-3 failed to induce basophil histamine release. Taken together, our studies demonstrate that IL-3 is a differentiation factor for human basophils.

GROWTH AND DIFFERENTIATION of metachromatic cells is thought to be regulated by distinct lymphokines.[1-7] In mice, interleukin-3 (IL-3), a T cell derived multipotent hematopoietic growth factor, has recently been shown to induce the production of histamine in murine hematopoietic precursor cells[8] and to support growth of murine mast cells.[2,3]

More recently, a cDNA for human IL-3 has also been cloned.[9] Recombinant human (rh) IL-3 is shown here to induce growth of metachromatic cells and production of histamine in a bone marow suspension culture system. However, in contrast to the situation in mice, human metachromatic cells cultured under the influence of rhIL-3 from hematopoietic precursors in this study could be classified as basophilic granulocytes as defined by their immunological phenotype.

MATERIALS AND METHODS

Bone marrow culture system. Bone marrow cells were obtained from normal donors (n = 9) after informed consent was given. Heparinized bone marrow samples were diluted in RPMI 1640 and layered over Ficoll (1.077 density) to separate mononuclear cells (MNC). 0.5 × 10^6 MNC were placed in each well of 24-well microculture plates (Costar, Cambridge, MA) in 1 mL RPMI supplemented with 10% fetal calf serum (FCS). Cultures were maintained at 5% CO_2 and 37°C for 14 days. The following rh cytokines were added to various final concentrations, ie, 10, 50, 100, 500, and 1,000 U/mL: interleukin-1α (IL-1α), interleukin-4 (IL-4), macrophage-colony stimulated factor (M-CSF) (Genzyme, Boston), interleukin-2 (IL-2) (Sandoz Research Institute, Vienna, Austria), IL-3, granulocyte/macrophage colony stimulating factor (GM-CSF) (Genetic Inst, Boston), granulocyte colony stimulating factor (G-CSF) (Amersham, Buckinghamshire UK), interferon-α (IF-α), interferon γ (IF-γ), and tumor necrosis factor-α (TNF-α) (Boehringer, Ingelheim, FRG). The entire panel of cytokines was tested on cells of three different donors. On days 0 and 14, unless otherwise stated, cells were examined by cytospin preparation and Giemsa staining to evaluate the percentage counts of the various hematopoietic cell types. The percentage of viable cells was determined by trypan blue exclusion. Cell counts were determined using a hematocytometer (Coulter Immunology, Hialeah, FL), and the absolute number of cells of the various hematopoietic lineages calculated from total cell numbers and differential counts.

In two seperate experiments, bone marrow cells were precultured with rhIL-3 (100 U/mL) or with control medium for three hours, then washed three times and adjusted to standard (final) cell concentration (0.5 × 10^6 cells/mL). Cells were cultured for a further 14 days in the presence or absence of rhIL-3 as described above. In particular, the following modes of IL-3 application were established (as triplicate cultures): preincubation with control medium, maintainance with control medium; preincubation with control medium, maintainance with rhIL-3 (100 U/mL); preincubation with rhIL-3 (100 U/mL), maintainance with control medium; preincubation with rhIL-3 (100 U/mL), maintainance with rhIL-3 (100 U/mL).

Histamine measurement. Histamine was measured by a commercial radioimmunoassay (Histamine RIA; Immunotech, Marseille, France) as described previously.[10] In brief, standards and samples were prepared by exposure to acylating reagent and mixing with ^{125}-I radiolabeled histamine. This solution was placed into tubes coated with monoclonal antihistamine antibodies. After 18 hours of incubation at 4°C, nonbound iodinated histamine was removed from the tubes by aspiration and surface bound tracer counted in a gamma counter. Histamine values were calculated by direct comparison with standard curve values. Total histamine (extracellular + intracellular histamine) in cell suspensions was quantified after cell lysis in aqua dest. Extracellular histamine was measured in cell-free

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Submitted July 21, 1988; accepted December 19, 1988.

Supported by the 'Fonds zur Förderung der wissenschaftlichen Forschung in Österreich' and by the Österreichische Nationalbank.

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0006-4971/89/7307-0137$3.00/0

supernatants after centrifugation at 4°C. Intracellular histamine was calculated by subtraction of extracellular from total histamine.

Monoclonal antibodies. The following monoclonal antibodies (MoAbs) were used: E124-2-8 (anti-IgE; Immunotech) MY7 (CD13; Coulter Immunology) YB5B8[11] (a kind gift from Dr L.K. Ashman, Department of Microbiology and Immunology, University of Adelaide, Australia), MAX1 and MAX24[12] (kindly sent by Dr R. Andreesen, Medical Clinic, Albert-Ludwigs-University, Freiburg, FRG) and the myeloid MoAbs VIM-2[13] and VIM12 (CD11b),[14] both produced at the Institute of Immunology, University of Vienna, Austria and kindly provided by Drs O. Majdic and W. Knapp. Anti-IL-3 MoAb F13-267-6 (Sandoz, Basel, Switzerland), was produced by immunizing BALB-c mice with CHO derived glycosylated rhIL-3 (400 μg) as described previously.[15] Spleen cells were fused with the myeloma cell line PAI-O and clones screened for antibody production by solid phase ELISA. Anti-IL-3 activity was measured in a standard myeloblast bioassay as described by Griffin et al.[16] Anti-IL-3 MoAb F13-267-6 (IgGl kappa subclass) was purified by ammonium sulphate precipitation and protein A column chromatography and showed a 50% inhibition of IL-3 growth response.

Determination of cell surface membrane receptors for IgE. Cultured cells obtained from three different donors were preincubated for six hours with IgE containing culture supernatant of myeloma cell line U266 (kindly provided by Professor K. Nillsson, Department of Pathology, University of Uppsala, Sweden). Cells were then incubated with MoAb E124-2-8 (specific for IgE) for 30 minutes. Thereafter, cells were washed twice and labeled with a fluorescein-labeled $F(ab')_2$ goat anti-mouse antibody for 30 minutes. Cells were again washed and subjected to either FACS analysis (FACS 440; Becton Dickinson, Sunnyvale, CA) or combined toluidine blue/immunofluorescence staining (see below).

Combined immunofluorescence/toluidine blue staining procedure. The combined immunofluorescence/toluidine blue staining procedure was performed as described previously.[17] Cells were incubated with MoAbs at 4°C for 30 minutes, washed twice, and incubated with fluorescein labeled goat $F(ab')_2$ anti-mouse IgG + IgM antibodies. Then, cells were washed and fixed in glutaraldehyde (0.025%) for one minute and stained with toluidine blue (0.0125%) for eight minutes at room temperature. Fifty toluidine blue positive cells were examined under an immunofluorescence microscope (Dialux, Leitz, Austria) for reactivity with each individual MoAb. Cultured bone marrow cells derived from three different donors were examined.

Histamine release assay for peripheral blood basophils. Histamine release from blood basophils of nonallergic individuals, after informed consent was given, was carried out as described previously.[18] Peripheral blood cells were fractionated by incubation in 1.1% dextran 70 and 0.008 mol/L EDTA for 90 minutes at room temperature. Cells of the granulocyte-rich upper layer were then centrifuged (200 g, +4°C, for eight minutes) and washed twice in Ca-free PIPES buffer (25 mmol/L PIPES, 110 mmol/L NaCl, and 5 mmol/L KCl, pH 7.35). Granulocytes were resuspended in PIPES buffer containing 2.0 mmol/L $CaCl_2$ and adjusted to a final concentration of 2.5×10^6/mL. Cytokines were diluted with PIPES buffer to various final concentrations (1,000 U/mL, 100 U/mL, 10 U/mL, and 1 U/mL) and distributed as 50 μL aliquots into the wells of microtiter plates (Costar). Cytokines and cells were separately prewarmed for ten minutes at 37°C, and then 100 μL of cell suspension was added to cytokines in each well and incubation maintained at 37°C for 45 minutes. Cells were then centrifuged at 4°C for eight minutes (1,000 g), and the cell free supernatant examined for histamine content. The release of histamine was calculated as percentage of total histamine, determined as described above. In each experiment, a monoclonal anti-IgE MoAb at appropriate concentrations (3×10^{-2} μg/mL, 10^{-1} μg/mL, 3×10^{-1} μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, and 8 μg/mL) was used as positive control. Nonspecific histamine release was quantified using control medium (instead of a cytokine) and was always lower than 4% of total histamine. The entire panel of cytokines was tested on cells of three different donors.

Histamine release assay for cultured metachromatic cells. On day 14 of culture, cells were washed twice in RPMI medium and incubated with myeloma IgE (myeloma cell line U266) for four

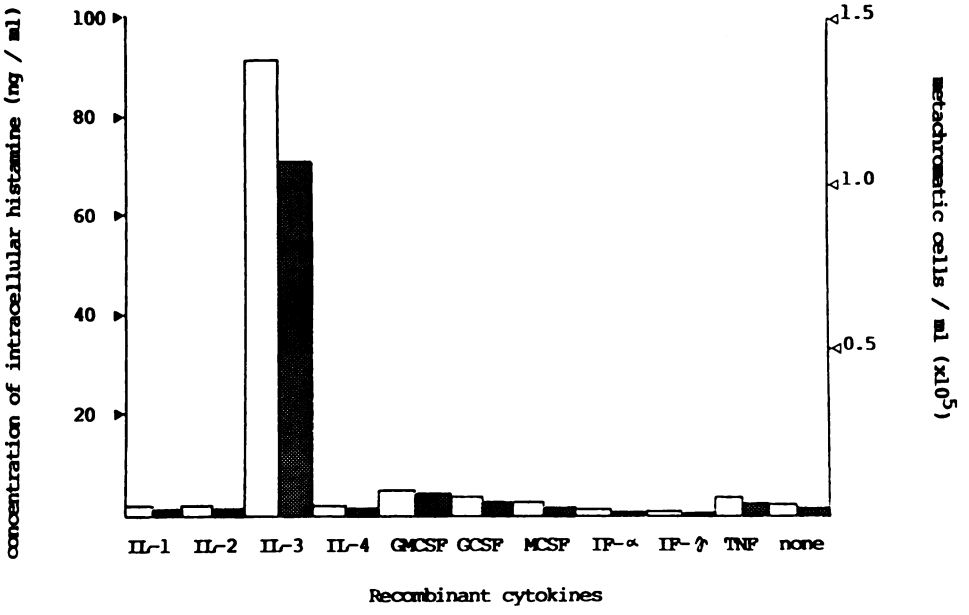

Fig 1. Effect of rh cytokines (100 U/mL of each cytokin) on production of histamine and formation of MCS in a bone marrow suspension culture system. Open bars indicate intracellular histamine per milliliter suspension, shaded bars represent MCS per milliliter suspension. Mean values of three independent experiments are depicted.

hours. Then, cells were washed twice and exposed to appropriate concentrations of anti-IgE MoAb E124-2-8 as described above. After incubation at 37°C in 5% CO_2 for 45 minutes, cells were centrifuged (1,000 g) at 4°C for eight minutes and the supernatant collected and examined for the presence of histamine. Histamine release was calculated as percentage of total histamine (see above).

RESULTS

Effect of rh cytokines on formation of metachromatic cells and intracellular histamine content in a bone marrow suspension culture system. We quantified the effect of ten rh hematopoietic cytokines on growth and differentiation of metachromatic cells (MCS) in a bone marrow suspension culture system. Intracellular histamine was measured in this bioassay as a nonsubjective marker of formation of MCS. Of all cytokines tested, rhIL-3 selectively induced a significant increase in formation of MCS as well as a significant increase in formation of intracellular (stored) histamine compared with control cultures (Fig 1). None of the other cytokines, tested in various concentrations, were found to mimic the effect of rhIL-3. Extracellular histamine was <4% of total histamine in all cultures tested. A time course suggests that rhIL-3 stimulated continuous production and intracellular accumulation of histamine in human bone marrow cells during a 14-day culture period (Fig 2A). As shown in Fig 2B, rhIL-3 induced production of histamine in a dose dependent manner. The calculated histamine content per metachromatic cell on day 14 of culture ranged from 0.4 to 1.1 pg. The effect of rhIL-3 on formation of MCS and synthesis of histamine was significantly reduced by the monoclonal anti-IL-3 antibody F13-267-6 (Fig 3).

Effect of rhIL-3 on growth of various hematopoietic lineages in a bone marrow suspension culture system. In cultures supplemented with rhIL-3 the total cell number per milliliter on day 14 was found to be higher (two- to threefold) than in control cultures. As shown in Table 1, rhIL-3 provided a striking growth advantage to MCS and to eosinophils compared with all other hematopoietic lineages.

Effect of continuous v *short-term stimulation of bone marrow cells with rhIL-3.* As shown in Table 2, pulse-stimulation with rhIL-3 (100 U/mL for three hours) without further exposure to rhIL-3, was effective in inducing growth of basophils from bone marrow progenitor cells. In one experiment, formation of basophils after rhIL-3–pulse stimulation was even more pronounced than in cultures maintained in the presence of rhIL-3 (see Table 2). The intracellular histamine content was almost identical in cultures maintained in control medium after IL-3 pulse stimulation or maintained with rhIL-3 (IL-3 pulse: 136.1 and 71.5 ng/mL, respectively; IL-3 maintenance: 147.2 and 92.3 ng/mL, respectively; and control: 4.4 and 20.3 ng/mL, respectively). In contrast, significant formation of eosinophils was dependent on prolonged exposure to rhIL-3.

Immunologic phenotype of cultured metachromatic cells. The morphology of MCS cultured in the presence of rhIL-3 is depicted in Fig 4. Although most of these cells were classified as basophils, a substantial number of cultured MCS could not be classified as either basophils or mast cells by morphological means alone. Therefore, MCS were analyzed with MoAbs using a combined toluidine blue/immunofluorescence staining procedure. MoAbs were selected according to the unique surface membrane phenotype of basophils[17] and mast cells (Valent et al, manuscript submitted) by which both cell types in humans can definitively be distinguished from each other. MCS cultured in the presence of rhIL-3 were recognized by myeloid MoAbs MY7 (CD13), VIM12 (CD11b), and VIM-2 but failed to react with macrophage associated MoAbs MAX1 and MAX24 (Table 3). Furthermore, cultured MCS also failed to react with MoAb YB5B8, which selectively recognizes mast cells

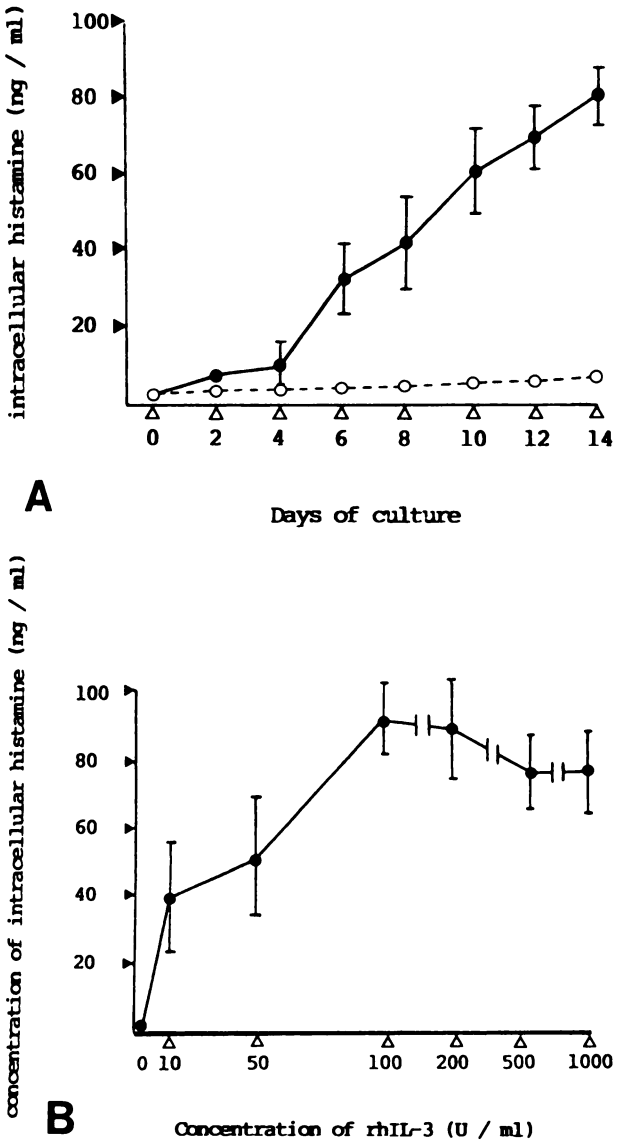

Fig 2. Dose and time dependent effect of rhIL-3 on synthesis of histamine in cultured human bone marrow cells. (A) Time course of IL-3 action during a 14-day culture period. Symbols indicate medium control (O--O) and response to rhIL-3, 100 U/mL (●—●). Results represent the mean ± SD of three independent experiments. (B) Effect of various concentrations of rhIL-3 on the intracellular content of histamine per milliliter of suspension on day 14 of culture. Mean values ± SD of three independent experiments are given.

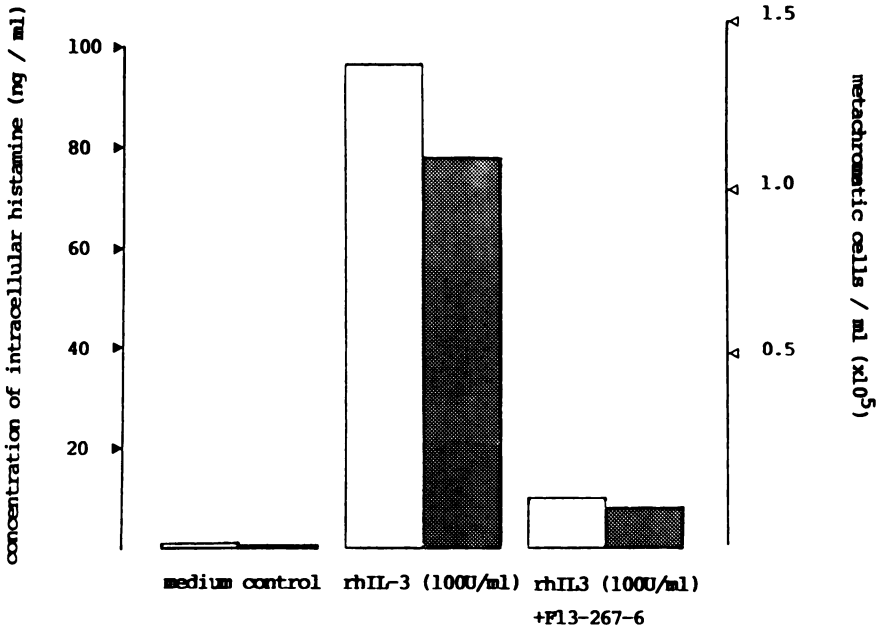

Fig 3. Inhibition of IL-3 action by anti-IL-3 MoAb F13-267-6. rhIL-3 was preincubated for two hours at room temperature with MoAb F13-267-6 (100 μg/mL) specific to IL-3. After preincubation, rhIL-3 (together with MoAb F13-267-6) was added to bone marrow MNC at a final concentration of 100 U rhIL-3/mL. Open bars indicate intracellular histamine content and shaded bars the number of MCS per milliliter suspension on day 14 of culture.

among mature hematopoietic cells.[11] Identical results were obtained from immunological phenotyping of metachromatic cells derived from cultures supplemented with different concentrations of rhIL-3.

Expression of cell surface membrane receptors for IgE. To further characterize human MCS cultured under the influence of rhIL-3, we analyzed formation and function of surface membrane receptors for IgE using a monoclonal anti-IgE antibody. FACS analyses, performed on day 14 of culture, revealed expression of surface IgE binding sites on 24 ± 8% of bone marrow cells cultured in the presence of rhIL-3. No significant formation of IgE receptor (<5% positive cells) was recognized in control cultures on all days examined and before day 10 in all cultures tested (Fig 5). By combined toluidine blue/immunofluorescence staining it could be shown that >95% of IgE receptor positive cells generated by rhIL-3 contained metachromatically stained granules (Table 3). Functional significance of IgE binding to

Table 1. Absolute Number of Cells (×10⁵/mL) and Percentages of the Hematopoietic Lineages Cultured From Normal Donors Bone Marrow MNC in the Presence or Absence of rh IL-3 for a 14 Day Culture Period

	Neutrophils	Eosinophils	Basophils	Monocytes-Macrophages	Lymphocytes	Megakaryocytes	Blasts	Total Cell Number
Experiment 1								
rhIL-3 (100 U/mL)	0.48 (12%)	0.80 (20%)	1.120 (28%)	0.72 (18%)	0.48 (12%)	0.0 (0%)	0.4 (10%)	4.0 (100%)
Control medium	0.75 (30%)	0.15 (6%)	0.025 (1%)	1.12 (45%)	0.45 (18%)	0.0 (0%)	0.0 (0%)	2.5 (100%)
Experiment 2								
rhIL-3 (100 U/mL)	0.50 (11%)	1.15 (25%)	1.330 (29%)	0.73 (16%)	0.73 (16%)	0.0 (0%)	0.14 (3%)	4.6 (100%)
Control medium	0.66 (30%)	0.09 (4%)	0.040 (2%)	1.01 (45%)	0.37 (17%)	0.0 (0%)	0.02 (1%)	2.2 (100%)
Experiment 3								
rhIL-3 (100 U/mL)	0.35 (7%)	0.97 (19%)	1.220 (24%)	0.97 (19%)	1.22 (24%)	0.1 (2%)	0.22 (5%)	5.1 (100%)
Control medium	0.42 (21%)	0.04 (2%)	0.020 (1%)	0.90 (40%)	0.72 (36%)	0.0 (0%)	0.0 (0%)	2.0 (100%)

Table 2. Effect of Continuous- Versus Pulse-Stimulation With rhIL-3 (100 U/mL) on Absolute (×10⁵/mL) and Relative Cell Number of the Various Hematopoietic Lineages Cultured From Normal Donor's Bone Marrow MNC in Suspension for 14 Days

		Neutrophils	Eosinophils	Basophils	Monocytes-Macrophage	Lymphocytes	Megakaryocytes	Blasts	Total Cell Number
Preincubation, control medium; maintenance, control medium	Exp 1:	0.63 (16%)	0.12 (3%)	0.08 (2%)	2.09 (53%)	0.99 (25%)	0.00 (0%)	0.04 (1%)	3.96 (100%)
	Exp 2:	1.27 (31%)	0.29 (7%)	0.20 (5%)	1.52 (37%)	0.82 (20%)	0.00 (0%)	0.00 (0%)	4.10 (100%)
Preincubation, control medium; maintenance, rhIL-3 (100 U/mL)	Exp 1:	0.85 (9%)	2.65 (28%)	2.08 (22%)	1.79 (19%)	0.94 (19%)	0.09 (1%)	1.04 (11%)	9.46 (100%)
	Exp 2:	1.66 (13%)	5.37 (42%)	2.17 (17%)	1.40 (11%)	1.56 (12%)	0.26 (2%)	0.38 (3%)	12.80 (100%)
Preincubation, rhIL-3 (100 U/mL); maintenance, control medium	Exp 1:	0.20 (3%)	0.48 (7%)	3.15 (46%)	1.10 (16%)	1.37 (20%)	0.07 (1%)	0.48 (7%)	6.85 (100%)
	Exp 2:	0.50 (10%)	0.15 (3%)	2.00 (40%)	1.10 (22%)	1.00 (20%)	0.10 (2%)	0.15 (3%)	5.00 (100%)
Preincubation, rhIL-3 (100 U/mL); maintenance, rhIL-3 (100 U/mL)	Exp 1:	1.02 (9%)	3.08 (27%)	2.39 (21%)	2.51 (22%)	1.48 (13%)	0.11 (1%)	0.79 (7%)	11.41 (100%)
	Exp 2:	1.51 (11%)	4.80 (35%)	3.28 (24%)	1.64 (12%)	1.37 (10%)	0.96 (7%)	0.14 (1%)	13.70 (100%)

Abbreviation: Exp, experiment.
Maintenance refers to day 0 to day 14.

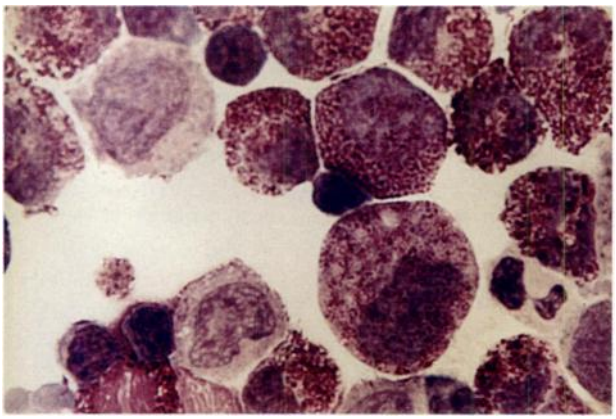

Fig 4. Cytospin preparation of human MCS cultured from bone marrow precursors in the presence of rhIL-3 (100 U/mL). Giemsa staining.

cultured MCS could be demonstrated by nontoxic histamine release after challenge with appropriate concentrations of anti-IgE MoAb E124-2-8 (Fig 6).

Effect of rh cytokines on histamine release from peripheral blood basophils. RhIL-3 as well as all other cytokines tested failed to induce release of a significant amount of histamine (>5% of total histamine) from peripheral blood basophils of three healthy volunteers (Table 4).

DISCUSSION

Among rh human cytokines, IL-3 selectively induced growth of MCS as well as continuous production and intracellular accumulation of histamine in a bone marrow suspension culture system. The effect of rhIL-3 was found to be dose-dependent and time-dependent and could be significantly reduced by a monoclonal anti-IL-3 antibody.

MCS cultured under the influence of rhIL-3 were classified as mature hematopoietic effector cells because (*a*) they stored relatively large amounts of histamine (0.4 to 1.1 pg/cell) corresponding to the histamine content of mature peripheral blood basophils and (*b*) because they expressed surface membrane receptors for IgE, which could be cross-linked for histamine release using a monoclonal anti-IgE antibody. Two MCS types, basophils and mast cells, exist in most mammalian species. In mice, MCS cultured from hematopoietic precursors in the presence of murine IL-3 were identified as mast cells by several investigators.[2,3]

Table 3. The Immunologic Surface Marker Profile of Metachromatic Cells Cultured in the Presence of rhIL-3 in Comparison to the Immunologic Phenotype of Normal Human Basophils and Normal Human Mast Cells

MoAb	CD/Reactive Structure	Reactivity of Cultured Metachromatic Cells	Basophil Phenotype	Mast Cell Phenotype
BA-2	CD9	+	+	+
MY-9	CD33	+	+	+
MY-7	CD13	+	+	−
VIM12	CD11b	+	+	−
VIM-2	NK	+	+	−
YB5B8	145-150kD	−	−	+
MAX1	65kD	−	−	+
MAX24	65kD	−	−	+
EI24-2-8	IgE	+	+	+

Abbreviation: NK, not known; +, >95% of cells reactive; −, <5% of cells reactive.

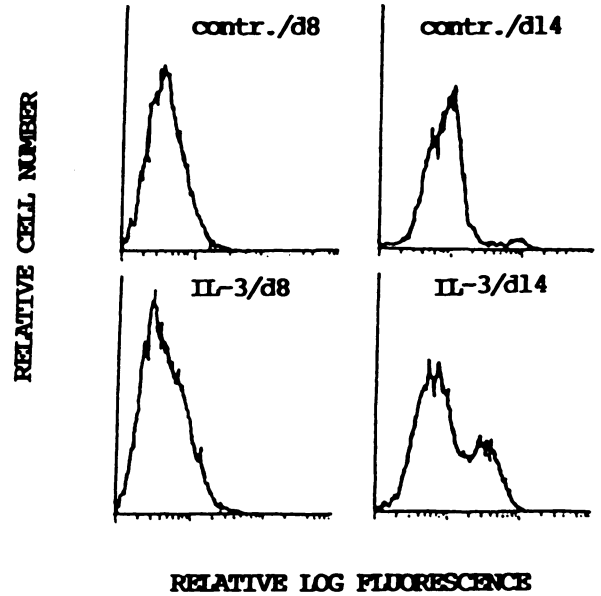

Fig 5. Induction of surface membrane receptors for IgE by rhIL-3 on cultured human bone marrow cells. Cells were maintained under the influence of 100 U/mL of rhIL-3 and analyzed on day 14 of culture. After preincubation with U266-myeloma derived IgE and subsequent staining with MoAb E124-2-8, cells were analyzed by FACS 440 (Becton Dickinson, Sunnyvale, CA). FACS analyses were performed on days 0 (not shown), eight, and 14 of culture. The result of a representative experiment is depicted.

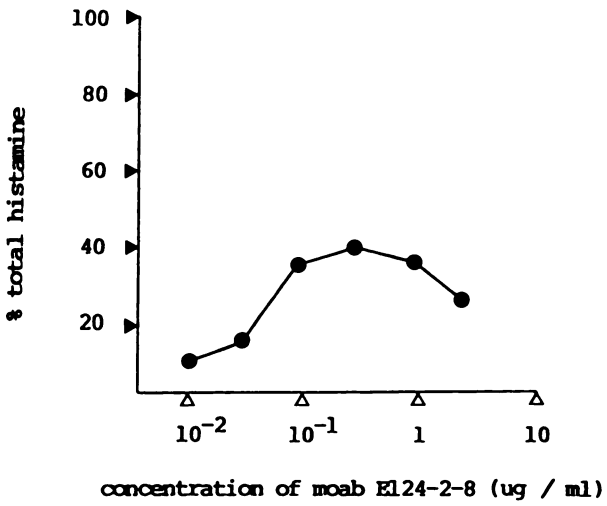

Fig 6. Demonstration of nontoxic histamine release from cultured MCS generated under the influence of rhIL-3 (100 U/mL). Cells were obtained on day 14 of culture and preincubated with myeloma IgE. Cells were then exposed to various concentrations of MoAb E124-2-8. After 30 minutes of incubation at 37°C, cells were centrifuged and histamine measured in cell free supernatant. Release is expressed as a percentage of total histamine (one representative experiment is depicted).

Table 4. Histamine Release From Dextran Isolated Granulocytes

Factor	Concentration	% of Total Histamine Released		
		Donor 1	Donor 2	Donor 3
EI24-2-8 (anti-IgE)	3×10^{-1} μg/mL	23.4	56.3	47.7
	1 μg/mL	33.8	52.6	44.9
Control Medium		2.2	3.5	3.1
rhIL-1				
	10 U/mL	1.3	2.4	2.9
	100 U/mL	1.8	3.2	2.6
	1,000 U/mL	1.7	2.7	2.4
rhIL-2				
	10 U/mL	1.4	2.3	1.7
	100 U/mL	1.4	3.8	2.6
	1,000 U/mL	1.5	4.8	2.7
rhIL-3				
	10 U/mL	1.4	5.0	3.5
	100 U/mL	1.4	4.3	2.6
	1,000 U/mL	1.2	4.6	2.7
rhIL-4				
	10 U/mL	1.3	1.9	2.3
	100 U/mL	1.0	2.2	2.5
	1,000 U/mL	1.2	2.4	2.5
rhGM-CSF				
	10 U/mL	3.4	4.1	2.7
	100 U/mL	3.2	3.4	2.7
	1,000 U/mL	2.7	4.6	3.6
rhG-CSF				
	10 U/mL	2.3	1.5	1.9
	100 U/mL	2.5	1.2	1.8
	1,000 U/mL	2.8	1.9	2.4
rhM-CSF				
	10 U/mL	2.5	2.7	1.8
	100 U/mL	NT	3.9	2.2
	1,000 U/mL	2.7	2.5	2.7
IF-α				
	10 U/mL	3.1	3.6	1.7
	100 U/mL	2.4	3.5	NT
	1,000 U/mL	2.5	4.8	2.5
IF-γ				
	10 U/mL	4.0	2.6	3.2
	100 U/mL	3.7	2.5	3.5
	1,000 U/mL	3.3	3.1	NT
TNF-α				
	10 U/mL	2.8	2.4	3.3
	100 U/mL	2.8	2.1	3.7
	1,000 U/mL	3.1	3.2	3.6

Abbreviation: NT, not tested.

Human MCS cultured under the influence of rhIL-3 in this study were classified as basophils by cell typing with MoAbs. In particular, almost all cultured MCS were stained by myeloid MoAbs (MY7, VIM-2, VIM12) known to bind to mature peripheral blood basophils,[17] but not to mast cells. In contrast, cultured MCS failed at all to react with macrophage/mast cell-associated MoAbs MAX1 and MAX24 as well as with MoAb YB5B8, which selectively recognizes mast cells among mature hematopoietic cells.[11] Because the technique applied has a detection limit of <0.5%,[17] a significant formation of mast cells in our culture system could be excluded. One explanation for the failure to detect mature, YB5B8-positive mast cells among cultured MCS could be that mast cells are end stage products of a multi-step differentiation pathway, which would exclude their formation in a more simple short-term culture system. Therefore, if human IL-3 is a growth and/or differentiation factor for human mast cells remains to be shown, probably in an appropriate long-term culture system.

Recently, it has been shown that rhIL-3 regulates growth and differentiation of human hematopoietic cells comparable with the situation in mice.[19-23] In our culture system, growth of basophils, eosinophils, and blasts was stimulated by rhIL-3.

In order to examine whether continuous stimulation of bone marrow cells by rhIL-3 is necessary for differentiation of both basophils and eosinophils, we compared IL-3 pulse-stimulation with the continuous action of rhIL-3. Interestingly, commitment to eosinophils was found to depend on a prolonged exposure to rhIL-3, whereas the basophil promoting effect of rhIL-3 seems to be independent of the continuous presence of this growth factor. The underlying mechanism for this differential effect of rhIL-3 on eosinophil/basophil commitment can at present not readily be explained. One explanation could be that IL-3 effects on eosinophil differentiation in our culture system were mediated by accessory-cells stimulated by the continuous presence of rhIL-3.

Recombinant hematopoietic cytokines have been described to stimulate not only cell differentiation but also the effector systems of mature cells of certain hematopoietic lineages.[23,24] Because rh IL-3 is considered by us and by others for possible use in clinical trials, a role for human IL-3 on basophil function, in particular on allergic response, would be of considerable clinical significance. In our hands, however, rhIL-3 as well as all other rh cytokines tested failed to induce histamine release from blood basophils. In summary, our results demonstrate that IL-3 is a basophil differentiation factor and histamine producing activity in the human system.

ACKNOWLEDGMENT

We wish to thank Roswitha Kaltenbrunner for expert technical assistance. We also want to thank Professor Walter Knapp for expert advice.

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