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Interleukin 3 activates human blood basophils via high-affinity binding sites

(cytokines/basophil histamine release/surface membrane antigens)

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ABSTRACT **Pure populations of human basophilic granulocytes were obtained from chronic myeloid leukemia (CML) blood by negative selection using a mixture of monoclonal antibodies and complement. ^{125}I-radiolabeled recombinant human interleukin 3 (rhIL-3) bound to purified basophils in a specific manner. Quantitative binding studies and Scatchard plot analyses performed on samples from two donors revealed the presence of a single class of high-affinity IL-3 binding sites (500 and 2100 sites per cell; dissociation constant at equilibrium, 230 and 160 pmol/liter, respectively). Purified CML basophils maintained in suspension in the presence of rhIL-3 (100 units/ml) incorporated up to 12 times more [^{3}H]thymidine than basophils in control cultures. Furthermore, after preincubation *in vitro* with rhIL-3 (100 units/ml) for 30 min, normal blood basophils released 2- to 3-fold more histamine than basophils pretreated with control medium when exposed to various concentrations of an anti-IgE antibody. Together, these results show that rhIL-3 binds to a specific receptor on blood basophils and is a regulator of basophil function.**

Blood basophils are circulating effector cells of allergic reactions (1). They store mediators of allergy, which are released into the extracellular space under certain conditions (2–5). Factors regulating histamine release from basophils include a variety of bioactive compounds (for review, see ref. 6). Most significantly, IgE molecules mediate basophil histamine release via specific surface membrane receptors (2–5). Recent data suggest that T-cell activation products regulate growth and function of human basophils (7–16). Interleukin 3 (IL-3), a multipotential lymphokine, has been shown to support differentiation of human basophils from their precursors in bone marrow and cord blood (14–16). Reports on a potential role for IL-3 in regulating histamine release from human blood basophils have been controversial (12–14). Whereas Haak-Frendscho *et al.* (12) described histamine release from blood basophils in direct response to recombinant human IL-3 (rhIL-3) as well as recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF), no direct histamine-releasing activity of these cytokines was found in our own experiments (14). More recently, Hirai *et al.* (13) reported that the histamine release from normal blood basophils induced by various stimuli such as anti-IgE antibodies could be enhanced by preincubation of basophils with rhIL-3 or rhGM-CSF. However, the mechanisms by which rhIL-3 and rhGM-CSF stimulate human blood basophils remain unclear.

So far, little has been reported about the expression of cytokine receptors on human basophils. The immunological phenotype of blood basophils suggests that they express receptors for IL-2 (17). In this study, we investigated whether purified chronic myeloid leukemia (CML) basophils express receptors for IL-3 and GM-CSF and analyzed the effect of rhIL-2, rhIL-3, and rhGM-CSF on the functional properties of human basophils.

MATERIALS AND METHODS

Recombinant Cytokines. Bacterially (*Escherichia coli*) derived rhIL-3 and rhGM-CSF were provided by the Genetics Institute (Cambridge, MA). Purified rhIL-3 showed a specific activity of 4.6×10^6 and rhGM-CSF had an activity of 4×10^7 units per mg of protein as determined by a myeloblast bioassay described by Griffin *et al.* (18). rhIL-2 was provided by Sandoz. The endotoxin content of growth factor preparations was evaluated by a chromogenic *Limulus* amebocyte lysate test assay (Whittaker, M.A. Bioproducts). rhGM-CSF contained 0.0125 ng, rhIL-3 contained 0.02 ng, and rhIL-2 contained 4.1 ng of endotoxin per mg of protein.

Radiolabeling of Recombinant Human Cytokines. rhIL-3 and rhGM-CSF were radiolabeled with Bolton–Hunter reagent (Amersham) essentially as described by the manufacturer. Briefly, aliquots of 5 μg of rhIL-3 or rhGM-CSF were incubated in 20 μl of reagent at 0°C for 30 min and the reaction was terminated by addition of 200 μl of 0.2 M glycine for 5 min. Radiolabeled growth factors were purified by a single gel filtration step on Pharmacia PD 10 G25 M columns in phosphate-buffered saline (PBS) with 0.25% gelatin. Fractions of 500 μl were collected. Radioactive growth factors from fractions 6–8 were pooled and stored without any further additions at 4°C. The specific radioactivity of iodinated cytokines was measured by self-displacement analysis as described by Calvo *et al.* (19). Bioactivity of iodinated cytokines did not differ significantly from the bioactivity of unlabeled cytokines.

Monoclonal Antibodies (mAbs). A number of mAbs directed against well-defined structures [identified either by molecular mass (kDa) or by a cluster of differentiation (CD)] were used in this study. Commercial mAbs: Leu1 (CD5), Leu7 (anti-natural killer cell mAb), and anti-HLA-DR (Becton Dickinson); mAbs MY-7 (CD13) and MY-9 (CD33) (Coulter); BA-2 (CD9) (Hybritech); mAb BMA-022 (anti-HLA-DR) (Behringwerke); and mAb E-124-2-8 (anti-IgE) (Immunotech, Marseille, France). A number of mAbs were produced at the Institute of Immunology (University of Vienna): VIT-3b (CD3) (20), VID-1 (anti-HLA-DR) (21), VIM-12 (CD11b) (22), VIE-G4 (anti-glycophorin A) (23), VIM-D5 (CD15) (24), and VIB-C5 (CD24) (25). mAb anti-TAC (CD25) (26) was a kind gift from T. A. Waldman, LICR LON R10 (anti-glycophorin A) (27) was from D. J. Anstee, and CLB-ERY3 (anti-blood group H) was from P. T. Tetteroo. Anti-mast cell mAb YB5B8 (anti-150 kDa) (28) was

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Abbreviations: mAb, monoclonal antibody; CML, chronic myeloid leukemia; rhIL, recombinant human interleukin; rhGM-CSF, recombinant human granulocyte–macrophage colony-stimulating factor.

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kindly provided by L. K. Ashman and MAX1 (anti-65 kDa) (29, 30) was provided by R. Andreesen.

Purification of CML Basophils. CML basophils of three donors (7%, 13%, and 19% basophils, respectively) were purified as described (17) after informed consent was given. In brief, blood mononuclear cells were obtained by Ficoll (Pharmacia) gradient density (1.077) centrifugation (30 min, 350 × *g*, 20°C), washed twice in PBS, and 5 × 10^8 mononuclear cells were incubated with 100 μg of VIM-D5 antibody for 30 min. Thereafter, cells were washed and incubated with 5 ml of rabbit complement (Behringwerke) at 37°C for 90 min. Remaining cells were depleted of monocytes by scrubbed nylon wool (37°C, 45 min) and then exposed to a mixture of mAbs: VIT-3, VIB-C5, Leu1, Leu7, VIE-G4, anti-HLA-DR, CLB-ERY3, and VIM-D5 (25 μg of each mAb). After incubation for 45 min at 4°C, cells were washed and exposed to 4 ml of complement (37°C, 90 min), again washed, and layered over Ficoll to remove cell ghosts. Neutrophils for receptor analysis were enriched from fresh blood of two healthy donors by centrifugation (300 × *g*, 45 min, 20°C) through Mono-Poly Resolving medium (Flow Laboratories). Basophils were not detected in these cell preparations.

Binding Assay. Purified basophils (2 × 10^6 cells) were incubated with ^{125}I-labeled rhIL-3 or rhGM-CSF in 100 μl of RPMI 1640 medium containing 20 mM Hepes buffer, 10% fetal calf serum, and 0.2% sodium azide (pH 7.2) (binding medium). The cell suspensions were maintained for 1 hr at 37°C in 1.5-ml Eppendorf tubes. Thereafter, the incubation mixture was transferred to 400-μl Beckman tubes containing 200 μl of precooled fetal calf serum. Cells were centrifuged for 2 min (Eppendorf centrifuge model 5413). After aspiration of medium and serum, radioactivity of the cell sediment was determined in a γ counter. Nonspecific binding of iodinated growth factors was quantified in the presence of a 20- to 100-fold excess of unlabeled growth factor.

Culture Studies. Purified CML basophils were cultured in suspension in RPMI 1640 medium and 10% fetal calf serum in the presence or absence of cytokines in a humidified atmosphere of 5% CO_2/95% air at 37°C.

Immunological Examination. Purified CML basophils were cultured in the presence of rhIL-2, rhIL-3, and rhGM-CSF (100 units/ml, respectively) or control medium for 9 days. On days 0, 2, 5, and 9, cultured basophils were analyzed by indirect immunofluorescence and flow cytometry (FACS 440, Becton Dickinson). Expression of IgE receptors was quantified by preincubation of basophils with myeloma-derived IgE (myeloma cell line U266, kindly provided by K. Nillsson) for 5 hr and staining with mAb specific for IgE.

[^{3}H]Thymidine Incorporation Experiments. Incorporation of [^{3}H]thymidine into purified basophils was carried out as described (17). In brief, 10^5 basophils per microtiter well (microtiter plates, Costar) were incubated with cytokines (see above). On days 0, 2, 5, and 9, 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) (New England Nuclear) was added. Twelve hours later, cells were harvested and the incorporated radioactivity was collected on glass-fiber filters and counted in a liquid scintillation counter (Union Packard).

Histamine Release Assay for Normal Blood Basophils. Granulocytes of four nonallergic individuals were isolated from whole blood as described by Marone *et al.* (31). Peripheral blood was fractionated by incubation in 1.1% dextran 70/0.008 M EDTA for 90 min at room temperature. Cells of the granulocyte-rich upper layer were washed twice in Ca-free Pipes buffer (25 mM Pipes/110 mM NaCl/5 mM KCl, pH 7.35) and resuspended in Pipes buffer containing 2.0 mM $CaCl_2$ and adjusted to a final concentration of 5 × 10^6 cells per ml. Cell aliquots were exposed to rhIL-2 (100 units/ml), rhGM-CSF (100 units/ml), or rhIL-3 (1, 10, and 100 units/ml) at 37°C for 30 min. Cells (100 μl per well) were then distributed on microculture plates (Costar) and 50 μl of anti-IgE was added at appropriate concentrations. After incubation at 37°C for 45 min, cells were centrifuged (4°C, 8 min, 350 × *g*) to recover cell-free supernatant. Histamine was measured by a commercial radioimmunoassay (Immunotech) as described (14). Total histamine (extracellular plus intracellular histamine) of cell suspensions was quantified after cell lysis in distilled water and freeze–thawing both before and after incubation with cytokines. Extracellular histamine was measured in cell-free supernatants.

RESULTS

Purification of Human Blood Basophils. CML basophils from three donors could be isolated by mAbs and complement lysis to a purity of 99%, 95%, and 97%, respectively. Purified basophils were found to be viable cells as determined

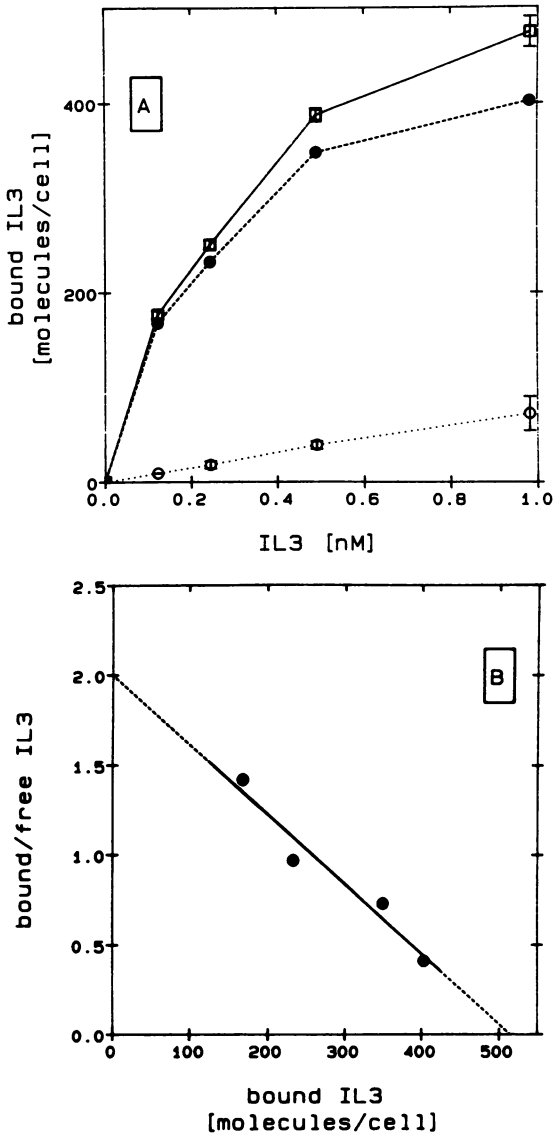

FIG. 1. Binding isotherm and Scatchard plot of ^{125}I-labeled rhIL-3 incubated with purified human CML basophils. Blood basophils were purified from CML blood by mAbs and complement-mediated cell lysis. Cells were incubated with increasing concentrations of rhIL-3 at 37°C in the presence of 0.2% sodium azide. (*A*) Total (□), nonspecific (○), and specific (●) binding of ^{125}I-labeled rhIL-3 to human CML basophils at equilibrium. (*B*) Scatchard transformation of the specific binding data depicted in *A*. Bound/free IL-3 is expressed as molecules per cell per pM. The results obtained with one of two donors are shown.

Table 1. [^{3}H]Thymidine incorporation into enriched CML basophils cultured in the presence of recombinant human cytokines

Cytokine	Patient	[^{3}H]Thymidine incorporation, cpm × 10^{-3}			
		Day 0	Day 2	Day 5	Day 9
None	1	0.13 ± 0.01	0.23 ± 0.02	0.79 ± 0.22	0.34 ± 0.10
	2	0.41 ± 0.13	0.79 ± 0.07	0.24 ± 0.04	0.28 ± 0.28
rhIL-2	1	0.10 ± 0.01	0.31 ± 0.03	0.65 ± 0.31	0.52 ± 0.15
(100 units/ml)	2	0.41 ± 0.07	0.71 ± 0.12	0.44 ± 0.09	0.29 ± 0.13
rhIL-3	1	0.12 ± 0.01	1.56 ± 0.08	2.72 ± 1.20	1.42 ± 0.10
(100 units/ml)	2	0.49 ± 0.10	1.94 ± 0.72	3.07 ± 0.33	1.72 ± 0.98
rhGM-CSF	1	0.19 ± 0.04	0.34 ± 0.07	0.85 ± 0.10	0.51 ± 0.10
(100 units/ml)	2	0.49 ± 0.03	1.22 ± 0.16	0.75 ± 0.05	0.37 ± 0.03

Purified basophils of two CML donors were cultured in the presence or absence of rhIL-2 (100 units/ml), rhGM-CSF (100 units/ml), or rhIL-3 (100 units/ml) for 9 days. On days 0, 2, 5, and 9, cells were exposed to 1 μCi of [^{3}H]thymidine for 12 hr. Incorporation of [^{3}H]thymidine into CML basophils was measured in a liquid scintillation counter. Results represent mean ± SD of triplicate cultures.

by trypan blue exclusion and could be maintained in culture for at least 9 days without substantial loss of cell viability.

Binding of Radiolabeled IL-3 and GM-CSF to Purified CML Basophils. Purified CML basophils of two donors were investigated for expression of specific IL-3 binding sites. Cells were incubated with increasing concentrations of iodinated rhIL-3 or rhGM-CSF. Fig. 1*A* shows specific binding of radiolabeled rhIL-3 to basophils of donor 1. A linear Scatchard transformation curve suggests a single class of IL-3 binding sites. As calculated from Scatchard plots a single CML basophil was found to express ≈500 IL-3 binding sites in donor 1 (Fig. 1*B*) and ≈2100 IL-3 binding sites in donor 2 (data not shown). The dissociation constants were ≈230 pM (donor 1) and ≈160 pM (donor 2). CML basophils failed to specifically bind significant amounts of ^{125}I-labeled rhGM-CSF. In contrast, neutrophils enriched from two healthy volunteers were found to bind ≈2500 and ≈3100 ^{125}I-labeled rhGM-CSF molecules per cell, whereas they did not bind significant amounts of ^{125}I-labeled rhIL-3 (data not shown).

Effect of Cytokines on Thymidine Uptake by Enriched CML Basophils. CML basophils were found to incorporate higher amounts of [^{3}H]thymidine when exposed to rhIL-3 (100 units/ml) in suspension compared to exposure to control medium (Table 1). In contrast, no effect of rhIL-2 and rhGM-CSF on thymidine uptake by CML basophils was observed. IL-3-induced [^{3}H]thymidine uptake by basophils reached maximum levels by day 5 and declined between days 5 and 9 (see Table 1). The effect of rhIL-3 on [^{3}H]thymidine uptake by basophils was found to be dose dependent (data not shown).

Effect of Cytokines on Surface Marker Expression of Enriched CML Basophils. Purified CML basophils of three donors were cultured in the presence of rhIL-2, rhIL-3, or rhGM-CSF (each at 100 units/ml) or control medium. In all cultures, enhanced expression of IL-2 receptor (CD25) and HLA-DR antigen was detected by flow cytometry on days 2 and 5 compared to day 0 (Table 2). Basophils stimulated by rhIL-3 were found to bind more anti-IgE antibody (mAb E-124-2-8) than basophils maintained in control medium (see Fig. 2). No substantial change in reaction of CML basophils with mAbs BA-2 (CD9), MY-7 (CD13), VIM12 (CD11b), VIMD5 (CD15), MAX1, and YB5B8 was observed under any culture condition.

Effect of Cytokines on Anti-IgE-Induced Histamine Release from Basophils. Isolated granulocytes of four healthy individuals contained 5%, 6%, 2%, and 3% basophils. Isolated granulocytes were preincubated with (*i*) control medium, (*ii*) rhIL-3 (100 units/ml), (*iii*) rhIL-2 (100 units/ml), or (*iv*) rhGM-CSF (100 units/ml) for 30 min and then exposed for 30 min to control medium or to various concentrations of mAb specific for IgE. Granulocytes of one donor were, in addition, exposed to 1 and 10 units of rhIL-3 per ml. Anti-IgE-induced histamine release from cells that were preincubated with control medium showed a high response (maximum release, 60.6% of total histamine) in donor 1, an intermediate response (28.2%) in donor 2, a low response (8.1%) in donor 3, and an intermediate response (29.5%) in donor 4. In all donors, preincubation with 100 units of rhIL-3 per ml resulted in a 2- to 3-fold enhanced histamine release compared to control release (Figs. 3 and 4). As shown in Fig. 4, the effect of rhIL-3 on IgE-mediated histamine release was dose dependent. rhGM-CSF (100 units/ml) enhanced anti-IgE-induced histamine release, however, to a lesser extent than 100 units of rhIL-3 per ml (Fig. 3). No effect of rhIL-2 on IgE-mediated histamine release was observed. No substantial histamine release (i.e., >5% of total histamine) was observed after exposure to any cytokine and maintenance in control medium. No change in total histamine values during preincubation of basophils with rhIL-3 was observed.

DISCUSSION

IL-3 is a T-cell-derived multipotential cytokine. The stimulating spectrum of human IL-3 includes different stages of

Table 2. Immunological surface marker profile of purified CML basophils after exposure to recombinant human cytokines in suspension

mAb	CD/reactive structure	Day 0	Day 2				Day 5				Day 9			
			Control	IL-2	IL-3	GM-CSF	Control	IL-2	IL-3	GM-CSF	Control	IL-2	IL-3	GM-CSF
BMA-022	HLA-DR	3	5	7	10	9	17	22	25	20	11	12	8	9
Anti-TAC	CD25	23	43	41	49	37	36	41	59	43	21	26	34	18
MAX1	65 kDa	4	8	4	7	5	4	6	3	2	6	9	4	7
YB5B8	145–150 kDa	3	3	2	3	1	3	4	2	1	3	4	2	5
Control medium		1	1	2	3	2	1	1	2	2	2	2	1	1

Purified CML basophils were maintained in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum and recombinant cytokines or control medium. Cytokines (rhIL-2, rhIL-3, and rhGM-CSF) were added at a final concentration of 100 units/ml. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2/95% air. On days 0, 2, 5, and 9, cells were removed from cultures, washed twice, incubated with various mAbs, and then stained with a fluorescein isothiocyanate-labeled second step mAb. The percentage of reactive cells was evaluated by flow cytometry. One representative experiment is shown.

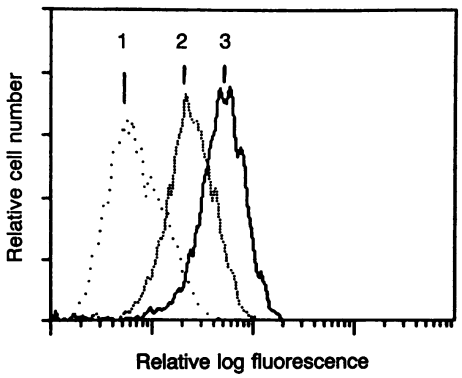

FIG. 2. Increase in binding of IgE to pure CML basophils after preculture with rhIL-3. Enriched basophils were stimulated with 100 units of rhIL-3 per ml or control medium for 48 hr. Basophils were preloaded with U266 myeloma cell line-derived IgE for 5 hr, washed thrice, and exposed to anti-IgE mAb E-124-2-8 for 30 min. Cells were washed again and treated with a fluorescein isothiocyanate-conjugated $F(ab')_2$ of anti-mouse immunoglobulin. The relative logarithm of fluorescence determined by FACS (FACS 440, Beckton Dickinson) is shown. 1, Staining of CML basophils preincubated in control medium with control mAb LICR LON R10 (IgG1 subclass) specific for glycophorin A. The cells preincubated with IL-3 gave an identical result with this mAb. 2, Staining with anti-IgE mAb E-124-2-8 after preincubation with control medium. 3, Staining with mAb E-124-2-8 after preincubation with rhIL-3.

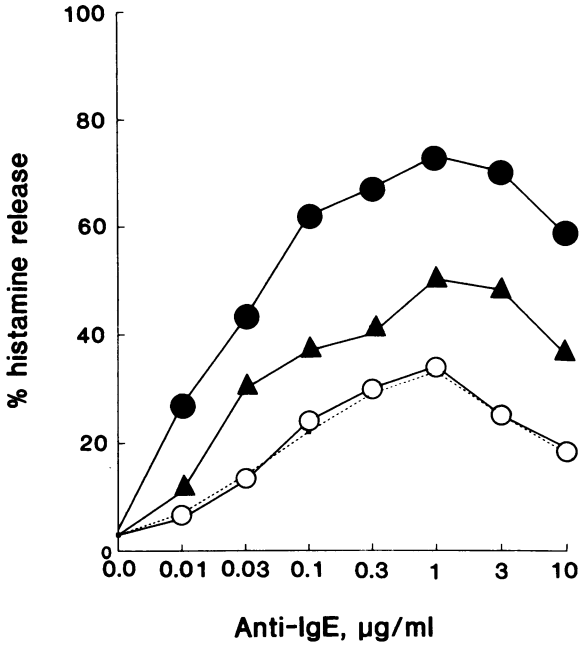

FIG. 4. Dose dependency of enhancement of IgE-mediated histamine release by rhIL-3. Dextran-isolated granulocytes of one donor were exposed to various doses of rhIL-3 (○, 1 unit/ml; ▲, 10 units/ml; ●, 100 units/ml) or to control medium (■) for 30 min and thereafter to various concentrations of anti-IgE mAb E-124-2-8. Histamine release is expressed as percentage of total histamine.

hemopoietic maturation as well as multiple hemopoietic lineages (14–16, 32–34). However, little is known so far about the distribution of specific receptors for IL-3 on the various human leukocyte subsets (35). The present study provides evidence for the existence of specific IL-3 binding sites on the surface membrane of human basophils. In particular, CML basophils purified from two donors by mAbs and complement-mediated cell lysis were found to express a single class of about 500 and 2100 high-affinity IL-3 binding sites per cell. In approximation, these data agree with reported binding properties of murine IL-3 to its target cells (36, 37). The difference in the number of IL-3 binding sites expressed on pure basophils enriched from the two CML donors might be explained by a different stage of cell maturation, since we found a more immature morphology of CML basophils expressing the lower number (i.e., 500) of IL-3 receptors (data not shown).

Functional significance of expression of IL-3 binding sites on the basophil surface membrane was provided by the demonstration that purified CML basophils responded to rhIL-3 in a specific manner. First, rhIL-3 increased the [^{3}H]thymidine uptake of CML basophils compared to control. Furthermore, after preloading with IgE, CML basophils pretreated with rhIL-3 bound more anti-IgE antibodies than CML basophils maintained in control medium. These

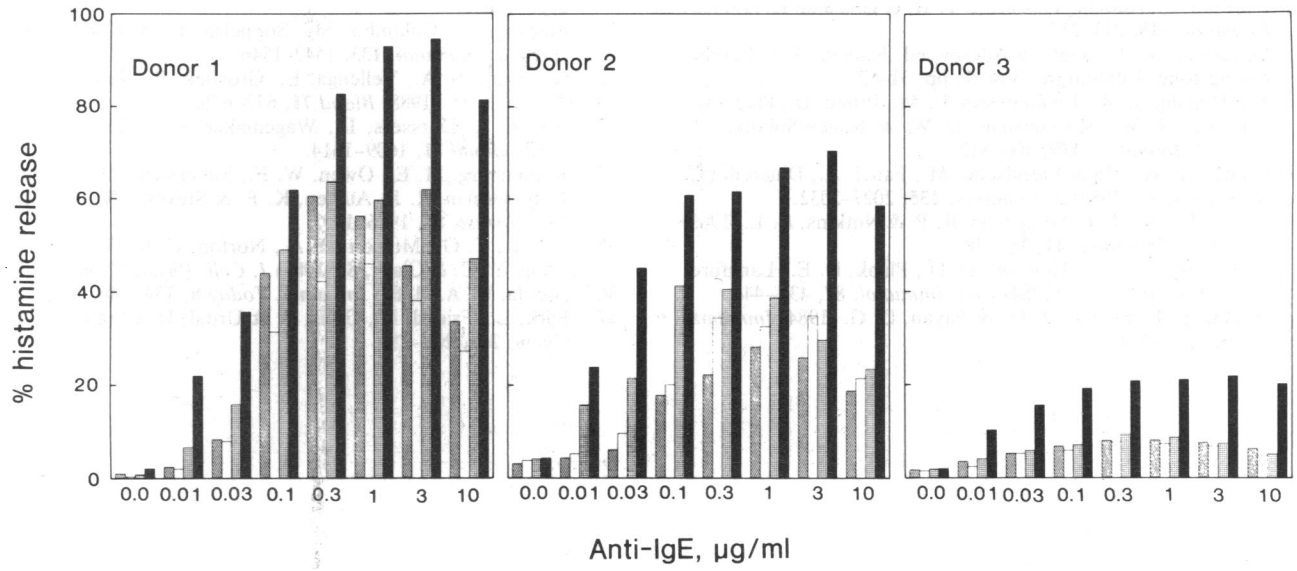

FIG. 3. IgE-mediated histamine release after preincubation with recombinant human cytokines and control medium. Normal granulocytes of three healthy donors (donors 1, 2, and 3) were enriched by dextran sedimentation. Cells were preincubated with rhIL-2 (100 units/ml), rhIL-3 (100 units/ml), rhGM-CSF (100 units/ml), or control medium and then exposed to various concentrations of anti-IgE mAb E-124-2-8. Preincubation with various cytokines is indicated as follows: hatched bars, medium control; open bars, rhIL-2; stippled bars, rhGM-CSF; solid bars, rhIL-3. Histamine release is expressed as percentage of total histamine.

changes observed in CML basophils probably reflect a terminal phase of differentiation in those cell populations induced by rhIL-3. In contrast, no significant effect of rhGM-CSF or rhIL-2 on purified CML basophils was observed in this study.

We also examined the effect of rhIL-2, rhIL-3, and rhGM-CSF on IgE-mediated histamine release from normal donors' blood basophils. Our results show that basophils are activated by rhIL-3 and rhGM-CSF (but not rhIL-2) for increased histamine releasability, which confirms the data obtained by Hirai *et al.* (13). The exact mechanism for this increased response of normal blood basophils to anti-IgE antibodies caused by rhIL-3 and rhGM-CSF remains unknown. However, it might be deduced from our results that the IL-3 effect on normal human blood basophils is mediated through specific IL-3 binding sites.

In contrast to normal neutrophils, CML basophils were found to lack receptors for GM-CSF. This finding was unexpected because (*i*) basophils express a number of myeloid-associated surface membrane antigens (17) and (*ii*) because an effect of rhGM-CSF on IgE-induced basophil histamine release was seen by us as well as by Hirai and his colleagues. One possibility could be that normal donors' basophils express significant numbers of GM-CSF receptors, whereas CML basophils do not. Alternatively, GM-CSF molecules could have influenced basophil functions by indirect effects, possibly mediated by accessory cells present in granulocytic cell fractions. Another possibility could be that blood basophils express very low numbers of GM-CSF receptors, which were not detected in the present study but could mediate the observed effect.

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