Distribution of Receptors for Granulocyte-Macrophage Colony-Stimulating Factor on Immature CD34+ Bone Marrow Cells, Differentiating Monomyeloid Progenitors, and Mature Blood Cell Subsets

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Biotin-labeled granulocyte-macrophage colony-stimulating factor (GM-CSF), in combination with phycoerythrin-conjugated streptavidin, enabled flow cytometric analysis of specific cell-surface GM-CSF receptors on rhesus monkey bone marrow (BM) and peripheral blood (PB) cells. GM-CSF receptors were readily detected on PB monocytes and neutrophils, but not on lymphocytes. In BM, GM-CSF receptors were identified on monocyte and neutrophil precursors and on subsets of cells that expressed the CD34 antigen. CD34+ cells with high GM-CSF-receptor expression coexpressed high levels of the class II major histocompatibility antigen RhLA-DR, whereas CD34+/RhLA-DR- cells, which represent developmentally earlier cells, were either GM-CSF-receptor negative or expressed GM-CSF receptors at very low levels. The fluorescence histogram of CD34+GM-CSF+RhLA-DR+ cells stained with biotin-GM-CSF showed that at least a fraction of these cells expressed low levels of GM-CSF receptors. CD34+ cells with high GM-CSF-receptor expression, purified by cell sorting, did not form colonies in culture or proliferate in response to GM-CSF. Instead, GM-CSF stimulation resulted in terminal differentiation into adherent cells, showing that these cells represented monocyte precursors. A distinct subset of CD34+ cells expressed GM-CSF receptors at low-to-intermediate levels and proliferated strongly in the presence of GM-CSF during short-term culture, but produced very few erythroid or monomyeloid colonies after longer culture periods. Most colony-forming cells, also those responsive to GM-CSF alone, were recovered in the subset of CD34+ cells on which GM-CSF receptors were virtually undetectable. These cells showed weaker proliferation in short-term proliferation assays than the CD34+/GM-CSF-receptor-intermediate cells, consistent with an immature phenotype. The results show that GM-CSF-receptor expression is initiated in a subset of immature, CD34+/RhLA-DR+ cells and is progressively increased during differentiation into mature granulocytes and monocytes. The method used provides a new way to delineate developmentally early CD34+ cell of differentiating granulocyte and monocyte precursor cells.

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of receptors for GM-CSF on peripheral blood (PB) and BM cells as well as the functional properties of CD34+ immature hematopoietic cells sorted on the basis of their GM-CSF-receptor phenotype.

MATERIALS AND METHODS

Growth factors. Recombinant human GM-CSF free of carrier proteins was a gift from Genetics Institute (Cambridge, MA). GM-CSF was biotin-labeled, essentially as described for IL-6. Briefly, 5 μg of GM-CSF in 0.1 mol/L sodium bicarbonate buffer pH 8.4, containing 0.02% (wt/vol) Tween-20, was incubated with a 1000-fold molar excess of N-hydroxy-succinimidy1-biotin (Pierce, Rockford, IL) for 2 hours at 20°C, after which biotin-labeled GM-CSF molecules were separated from free reagent by size exclusion chromatography on a 1-mL Sephadex G-25 column (Pharmacia, Uppsala, Sweden), equilibrated in phosphate-buffered saline, containing 0.02% (wt/vol) Tween-20. Recovery of biotin-labeled GM-CSF ranged between 10% and 50% for individual preparations, as tested in a 3H-thymidine uptake assay using M07E cells. Only batches of which greater than 99% of biologically active GM-CSF was biotin-labeled, as tested by absorption to streptavidin-agarose beads (Sigma Chemical Co, St Louis, MO), were used.

Procurement of BM and PB cells. PB was obtained by vena puncture. BM was collected from young-adult rhesus monkeys (Macaca mulatta), bred at the TNO Primate Center, Rijswijk, The Netherlands, by piercing the head of the humeral shaft to obtain small samples or by piercing the knee joint into the femoral shaft in case larger amounts of BM were needed. To enrich for progenitor cells, buffy-coat cells were subjected to a discontinuous bovine serum albumin (BSA)-density gradient and low-density cells were collected. Residual red blood cells were removed by osmotic lysis using 10 mmol/L potassium bicarbonate, 155 mmol/L ammonium chloride, pH 7.4, containing 0.1 mmol/L EDTA. CD34+ cells were isolated by immunomagnetic separation, using an IgG2A antibody against CD34 (MoAb 561, kindly provided by T. Egeland, University of Oslo, Norway) that was noncovalently linked to rat-antimouse IgG2A beads (Dynal, Oslo, Norway). CD34+ cells devoid of the anti-CD34 antibody were recovered by competitive elution using a polyclonal antibody against the Fab part of the anti-CD34 antibody (Detachabead, Dynal). Before staining, cells were incubated for 30 to 45 minutes at 37°C in a large volume of HEPES-buffered Hanks' balanced salt solution (HBSS), containing 2% (vol/vol) fetal calf serum (FCS)(HF buffer), followed by washing with ice-cold HF buffer, containing 0.05% (wt/vol) sodium azide (HFN) to remove possible receptor-bound natural GM-CSF and allow reexpression of unoccupied receptors.

Binding of biotin-labeled GM-CSF to cells. One million cells were incubated with biotin-labeled GM-CSF (at a concentration of 1 mmol/L, unless indicated otherwise) in 100 μL HFN for 15 hours on ice. Nonspecific binding of biotin-GM-CSF was evaluated in the presence of a 100-200-fold molar excess of nonlabeled GM-CSF. After incubation, cells were washed twice and incubated with streptavidin-phycocerythrin (SA-RPE, Molecular Probes, Eugene, OR) at a concentration of 7 μg/mL for 1 hour on ice. In most experiments, the fluorescence intensity was amplified by sequential incubation with a biotin-labeled anti-RPE MoAb and a second incubation with SA-RPE, as described. For two-color analysis, cells were stained during the last SA-RPE incubation step with a MoAb against human CD34 that reacts with rhesus monkey CD34 (MoAb 566) and had been fluoresceinated by conjugation with fluorescein isothiocyanate (FITC; Sigma) according to standard procedures. For three-color analysis, cells were simultaneously incubated with SA-RPE, FITC-labeled anti-CD34 and a peridinin chlorophyll (PerCP) conjugated MoAb against human HLA-DR that reacts with rhesus monkey HLA-DR.

Fig 1. Flow cytometric detection of GM-CSF receptors on M07E cells (A), HL60 cells (B), and KG1a cells (C). Cells were sequentially incubated with biotin-labeled GM-CSF and SA-RPE and analyzed by flow cytometry after amplification of the fluorescence signal (- -). Controls for nonspecific staining, cells were also incubated without biotin-GM-CSF (---) or with biotin-GM-CSF in the presence of a 200-fold molar excess of unlabeled GM-CSF (-----). In A and B, the fluorescence intensity of the biotin-GM-CSF-stained cells is higher than for both controls, which have identical low fluorescence profiles, showing specific binding of biotin-GM-CSF and absence of nonspecific binding. In C, the fluorescence intensity of the biotin-GM-CSF stained cells is identical to that of the excess unlabeled GM-CSF control, indicating absence of specific binding and some nonspecific binding of biotin-GM-CSF.
Flow cytometry and cell sorting. Analytical experiments were performed using a FACScan flow cytometer (Becton Dickinson). Cell sorting was performed using a FACS Vantage flow cytometer with the Argon laser at 488 nm (100 mW). Spillover of FITC fluorescence in the RPE and PerCP detectors and of RPE in the PerCP detector was electronically compensated using appropriately stained control cells. List mode data for 10,000 to 20,000 cells were collected either ungated or in an electronic gate distributed. Background fluorescence of cells stained without biotin–GM-CSF (-- - -). Note that the fluorescence profiles of both controls are virtually indistinguishable.

**RESULTS**

Detection of GM-CSF receptors on cell lines and PB cells. The specificity and sensitivity of biotin-labeled GM-CSF for detecting GM-CSF–receptor expressing cells was assessed by flow cytometric analysis of two cell lines known to express GM-CSF receptors, M07E and HL60, and one recep-
FLOW CYTOMETRIC DETECTION OF GM-CSF RECEPTORS

Fig 3. GM-CSF-receptor expression on BM cells. Light-density rhesus monkey BM cells were stained for GM-CSF expression, counterstained with anti-CD34-FITC and analyzed by flow cytometry. Dual-parameter dot plot of GM-CSF receptor versus CD34 expression (A). Nonspecific binding of biotin-GM-CSF was evaluated in the presence of excess unlabeled GM-CSF (B). The rectangular boxes indicate the electronic windows used to sort cells on the basis of CD34 and GM-SF-receptor expression levels. C, D, and F to H: Light-scatter dot plots of the respective sorted populations. For comparison, the light-scatter dot plot of unsorted light-density cells is also shown (E).

Fig 4. RhLA-DR and CD11b expression on sorted CD34+ GM-CSF-receptor+ cells (A,B) and of sorted CD34+ GM-CSF-receptor intermediate cells (C,D) are shown. Aliquots of sorted cells were reanalyzed after staining with PerCP-anti-RhLA-DR, FITC-anti-CD11b antibodies (−−) or a control antibody (···).
GM-CSF-receptor expression as indicated by the boxed areas in Fig 1A. GM-CSF receptors could be detected on M07E cells, which was consistent with the lower GM-CSF-receptor expression reported for HL60 cells, ie, 60 to 120 pmol/L, which is consistent with the presence of receptors that bind GM-CSF with intermediate affinity on these cells.\textsuperscript{14,16,19,24,35}

GM-CSF receptors on CD34\textsuperscript{+} BM cells. The GM-CSF–receptor distribution of unfractionated BM was grossly similar to that of PB cells (not shown). To examine GM-CSF–receptor expression on immature hematopoietic cells, GM-CSF-receptor expression was compared with expression of the CD34 antigen. These experiments were performed using the low-density fraction of BM cells, which generally contains 5\% to 15\% of nucleated cells and 50\% to 95\% of the clonogenic progenitor cells.\textsuperscript{33} As shown in Fig 3A, GM-CSF receptors were detected on at least two subsets of CD34\textsuperscript{+} cells and also on subsets of the \(\approx\)10\% of low-density BM cells that expressed the CD34 antigen. The light-scatter properties of the CD34\textsuperscript{+} cells that expressed high GM-CSF-receptor levels were similar to those observed for mature monocytes in unfractionated BM and in PB (Fig 3C). Purified CD34\textsuperscript{+}/GM-CSF-receptor

Assessment of the proliferation rates of sorted BM cells, which express 300 to 400 high-affinity sites,\textsuperscript{18,20} were stained specifically by biotin-labeled GM-CSF (Fig 1A). GM-CSF receptors could also be detected on HL60 cells, but at lower intensity than on M07E cells, which was consistent with the lower GM-CSF-receptor expression reported for HL60 cells, ie, 50 to 150 sites per cell (Fig 1B).\textsuperscript{21,22} KG1a cells displayed only background fluorescence (Fig 1C), in concordance with the absence of detectable receptor expression as determined with radiolabeled GM-CSF.\textsuperscript{21}

GM-CSF–receptor expression was examined on PB lymphocytes, monocytes and granulocytes, identified by characteristic forward-angle and right-angle light-scatter properties (Fig 2A). GM-CSF–receptor expression could be readily detected on monocytes (Fig 2C) and granulocytes (Fig 2D), whereas lymphocytes did not express detectable levels. (Fig 2B). The fluorescence intensity was twofold to threefold higher on monocytes than on granulocytes, suggesting either more receptors or a greater binding affinity (Fig 2). These experiments were performed using biotin–GM-CSF at concentrations of 1 nmol/L, which is saturating for high-affinity receptors and close to the dissociation constant of low-affinity sites. In titration experiments, GM-CSF–receptor expression on monocytes and M07E cells was still detectable using biotin–GM-CSF concentrations as low as 30 pmol/L, which may indicate that detectable binding to these cells was mainly attributable to high-affinity sites. Specific binding to granulocytes required higher concentrations, ie, 60 to 120 pmol/L, which is consistent with the presence of receptors that bind GM-CSF with intermediate affinity on these cells.\textsuperscript{14,16,19,24,35}

The fluorescence intensity of the CD34\textsuperscript{+}/GM-CSF–receptor intermediate cells was similar to that obtained for mature granulocytes and granulocyte precursors in PB and unfractionated BM (Fig 3A). Sorted CD34\textsuperscript{+}/GM-CSF–receptor intermediate cells were more heterogeneous with respect to morphology, light-scatter properties (Fig 3D), CD11b expression (Fig 4C) and RhLA-DR expression (Fig 4D) than the CD34\textsuperscript{+}/GM-CSF–receptor\textsuperscript{high} cells. Morphologically, most cells resembled differentiated neutrophils with band-shaped and segmented nuclei, whereas a smaller proportion of cells had less-differentiated blast cell morphology (not shown). Approximately 70\% to 75\% of sorted CD34\textsuperscript{+}/GM-CSF–receptor intermediate cells expressed high CD11b levels but were almost negative for RhLA-DR (Fig 4, C and G).
FLOW CYTOMETRIC DETECTION OF GM-CSF RECEPTORS

Fig 6.
In general, these cells displayed high right-angle light-scatter characteristic for relatively mature granulocyte precursors (Fig 3D). The 25% to 30% of CD34+/GM-CSF-receptor intermediate cells that were RhLA-DR bright and CD11b-intermediate had lower right-angle light scatter (Fig 3D), which is characteristic for less-differentiated myeloblasts (Fig 4, C and D). As was expected from their relatively mature stage of differentiation, CD34+/GM-CSF-receptor receptor intermediate cells did not proliferate in cultures stimulated with GM-CSF (Fig 5).

**GM-CSF receptors on CD34**. Staining with biotin–GM-CSF distinguished three subsets of CD34+ cells (Fig 3A). One subset expressed GM-CSF receptors at relatively high levels, similar to those observed on the CD34+/GM-CSF-receptor bright monocyte precursors and had light-scatter properties that are similar to those of monocytes (Fig 3F). GM-CSF–receptor expression appeared to increase with decreasing CD34 expression, which may be taken as evidence that this subset represents differentiating cells (Fig 3A). Sorted CD34+/GM-CSF-receptor bright cells had large round to oval nuclei and had small inclusions in the cytoplasm (Fig 6A). The cells in this subset showed little proliferation during short-term culture (Fig 5). Instead, proliferation was diminished in the presence of GM-CSF relative to the control. After 3 days of stimulation with GM-CSF, most adhered to the bottom of the culture vessel, which indicated maturation into macrophages and thus showed that this fraction contained the immediate precursors of the CD34+/GM-CSF-receptor bright monocytes (Fig 6B).

The second subset of CD34+ cells expressed GM-CSF receptors at lower levels and displayed intermediate-to-high forward-angle light scatter and heterogeneous right-angle light scatter (Fig 3G). The light-scatter properties of these cells were distinct from those of the third subset of CD34+ cells, which had undetectable GM-CSF–receptor expression and displayed intermediate forward-angle light scatter and very low right-angle light scatter (Fig 3H). After cell sorting, CD34+ cells with intermediate and undetectable GM-CSF–receptor expression were morphologically indistinguishable and appeared less mature than the CD34+/GM-CSF-receptor bright cells (Fig 6, C and E). Both populations consisted of blast-like cells with large nuclei and scanty cytoplasm without granules. After 3 days of stimulation with GM-CSF, CD34+/GM-CSF-receptor intermediate cells showed a strong proliferative response to GM-CSF (Fig 5). The cells that were produced during this period resembled differentiating monocyte and granulocyte precursors, which indicated that both lineages were represented in this subset (Fig 6D).

The CD34+ cells with undetectable GM-CSF–receptor expression also responded to GM-CSF in short-term cultures, but thiolutidine incorporation was much less than for the CD34+/GM-CSF–receptor intermediate cells (Fig 5). During this culture period, most CD34+/GM-CSF–receptor-negative cells stayed relatively small and retained a blast-like phenotype (Fig 6F).

**Clonogenic abilities of GM-CSF–receptor-expressing BM cells.** Virtually none of the sorted CD34+ cells with high GM-CSF–receptor expression and less than 5% of sorted CD34+/GM-CSF–receptor intermediate cells could form colonies in culture, in agreement with the finding that most of the cells in both subsets are relatively mature (Table 1). Erythroid and myeloid progenitors were detected in majority in the CD34+ subset with undetectable GM-CSF–receptor expression (Table 1). These cells formed colonies in the presence of IL-3, SCF, and GM-CSF, but also in the presence of GM-CSF alone, with a lower frequency. The high cloning efficiency of these cells indicated that the relatively weak proliferative response of these cells during shorter culture periods (Fig 5) was not caused by loss of viability as a result of the staining and sorting procedure and that these cells represented an earlier stage of differentiation than the CD34+/GM-CSF–receptor intermediate cells.

**Comparison of GM-CSF–receptor expression with RhLA-DR expression on CD34+ cells.** To further characterize the GM-CSF–receptor-positive cells within the CD34+ population, three-color FACScan experiments were performed in which GM-CSF–receptor expression was compared with expression of RhLA-DR on CD34+ cells, purified by immunomagnetic beads separation. CD34+ human BM cells with low HLA-DR expression have been shown to be enriched for immature, multipotent hematopoietic cells whereas high HLA-DR expression is characteristic for activated lineage-committed progenitors representing later stages of differentiation.  

By analogy, we assumed a similar relation between CD34 and RhLA-DR expression for rhesus monkey BM. As shown in Fig 7, all purified CD34+ cells with intermediate-to-high GM-CSF–receptor expression coexpressed RhLA-DR at high levels (Fig 7, C and D). These GM-CSF–receptor expressing cells were found in the CD34bright subset (region 1 in Fig 7A) as well as the subset with lower CD34 expression (region 3). The majority of the cells in the latter population had low forward light scatter (Fig 7B) and undetectable GM-CSF–receptor expression (Fig 7C). The light-scatter properties of these cells were similar to those of lymphocytes and human pre-B cells,34,42,43 which suggested that these GM-CSF–receptor-negative cells represented B-lymphocyte precursors. High-level GM-CSF–receptor expression could not be detected on the small subset of CD34bright cells with low RhLA-DR expression (region 2 in Fig 7A). However, the fluorescence histogram of CD34bright/RhLA-DRnull cells stained with biotin-GM-CSF showed a small shift as compared with control-stained cells in this subset (Fig 7E). Similar low-level specific binding of biotinylated GM-CSF to CD34bright/RhLA-DRnull cells was observed in two additional experiments, which indicated that at least a fraction of the most immature hematopoietic cells identified in normal BM expressed the GM-CSF receptor at low, but detectable levels.

**DISCUSSION**

In this study, the cellular distribution of GM-CSF receptors in PB and BM has been visualized by means of flow cytometry after staining with biologically active biotin-labeled GM-CSF and fluorescently tagged streptavidin. As based on the ability to detect receptor expression on human hematopoietic cell lines expressing low levels of the high-affinity GM-CSF receptor, the method could detect receptor levels as low as ≈50 molecules per cell, very similar to that estimated previously for the detection of Epo and IL-6 receptors using
Table 1. Colony Formation by Rhesus Monkey BM Cells Sorted on CD34 and GM-CSF Receptor Expression

<table>
<thead>
<tr>
<th>Cell Fraction*</th>
<th>TNC (%)</th>
<th>BFU-E Colonies/1,000 Cells</th>
<th>Recovery (%)</th>
<th>CFU-GM (GM-CSF) Colonies/1,000 Cells</th>
<th>Recovery (%)</th>
<th>CFU-GM (GM-CSF/IL-3/SCF) Colonies/1,000 Cells</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cells (open gates)†</td>
<td>100</td>
<td>3.1 ± 0.6</td>
<td>(100 ± 29.9)</td>
<td>10.4 ± 1.2</td>
<td>(100 ± 15.8)</td>
<td>12.4 ± 1.3</td>
<td>(100 ± 14.5)</td>
</tr>
<tr>
<td>CD34+/GM-CSF rec-</td>
<td>2.3</td>
<td>0.5 ± 0.5</td>
<td>(0.4 ± 0.4)</td>
<td>1.5 ± 0.9</td>
<td>(0.3 ± 0.2)</td>
<td>1.0 ± 0.7</td>
<td>(0.2 ± 0.1)</td>
</tr>
<tr>
<td>CD34+/GM-CSF rec-</td>
<td>2.4</td>
<td>9.0 ± 2.1</td>
<td>(6.9 ± 2.2)</td>
<td>38.5 ± 4.4</td>
<td>(8.9 ± 1.4)</td>
<td>44.5 ± 4.7</td>
<td>(8.6 ± 1.3)</td>
</tr>
<tr>
<td>CD34+/GM-CSF rec-</td>
<td>3.0</td>
<td>70.0 ± 5.9</td>
<td>(67.3 ± 14.9)</td>
<td>162.0 ± 9.0</td>
<td>(46.7 ± 5.8)</td>
<td>292.5 ± 12.1</td>
<td>(71.1 ± 7.9)</td>
</tr>
</tbody>
</table>

Abbreviation: TNC, total nucleated cells.

* Light-density BM cells were sorted in the electronic gates indicated by the rectangular boxes in Fig 3A.
† Mean number of colonies ± SEM of duplicate cultures; parentheses: mean percentage recovery ± SEM; recovery of progenitors in sorted fractions was calculated relative to the sort control, which was set at 100%.
‡ Control: Light-density BM cells that were stained for CD34 and GM-CSF receptor expression, but sorted in a gate that included all cells.

bion-labeled ligands. Consistent with the biologic activities of GM-CSF, GM-CSF receptors were detected on mature monocytes and granulocytes, on the immediate BM precursors of these cells and on subsets of immature cells expressing the CD34 antigen. CD34+ cells which expressed the GM-CSF receptor at intermediate-to-high levels expressed high levels of the class II major histocompatibility complex antigen RhLA-DR, whereas GM-CSF receptors on CD34+ cells with low RhLA-DR expression were either absent or expressed at very low levels.

Using a similar approach as described here for GM-CSF, receptors for IL-6, G-CSF, IL-3, and SCF have also been detected on CD34-expressing BM cells. The receptor for SCF, ie, c-kit, has been shown on the most immature, ie, CD34+/HLA-DRlow cells from human BM as well as on long-term repopulating cells in mice. We have recently found that the IL-3 receptor is also expressed on CD34+RhLA-DR cells, whereas GM-CSF receptors on CD34+ cells with low RhLA-DR expression were either absent or expressed at very low levels.

GM-CSF receptors are not expressed on small CD34+/RhLA-DRhigh cells (Fig 7). Although analysis of CD19 expression was not possible because of lack of a suitable anti-
CD34 antibody that reacts with rhesus monkey cells, these GM-CSF-receptor-negative/CD34dim/RhLA-DR−/CD11b− cells (Fig 7C) most likely represented B-lymphocyte precursor cells because of their small size (low forward light scatter) and similarity to the small CD34dim/CD19− B-lymphocyte progenitor cells that have previously been identified in human BM.34,42,43

The inability to detect high levels of GM-CSF receptors on the majority of the clonogenic progenitor cells indicated that GM-CSF receptors are either absent on lineage-committed progenitors or are expressed at levels that are below the detection limit of the flow-cytometric method. The ability of sorted CD34+ cells with undetectable GM-CSF-receptor expression to form colonies in response to GM-CSF in the absence of added other growth factors would favor the latter possibility and may indicate that very low receptor levels are sufficient for cells to respond to the high GM-CSF concentrations used in culture.

Most of the CD34+/GM-CSF-receptor intermediate cells appeared to have differentiated beyond the clonogenic cells stage, but could still respond to proliferative signals supplied by GM-CSF. The CD34+/GM-CSF-receptor intermediate cell fraction appeared to contain precursors for granulocytes and monocytes because cells of these lineages were produced during short-term liquid culture of sorted cells in the presence of GM-CSF. The inability of CD34+ cells with high GM-CSF-receptor expression to proliferate in short-term cultures and in colony assays indicated that these cells represented even later stages of differentiation than the CD34+ cells with low-level expression of the GM-CSF receptor. The phenotypic similarity of the CD34+/GM-CSF-receptorbright cells and the CD34−/GM-CSF-receptorbright monocytes, together with the ability of the CD34+/GM-CSF-receptorbright cells to adhere to plastic in response to GM-CSF, showed that this subset represented relatively differentiated monocyte precursors. The absence of granulocytic cells in the CD34+/GM-CSF-receptorbright fraction indicated that there is a more limited increase in GM-CSF receptor levels when granulocyte progenitors lose CD34 and mature via RhLA-DR−/CD11b−/CD11b+ mature neutrophils. Thus, our findings show that GM-CSF receptors are absent or expressed at very low levels on the earliest stages of hematopoiesis, but are expressed at progressively higher levels during neutrophil and, particularly, monocyte differentiation.

The cellular distribution of GM-CSF receptors thus appears to be different from the expression of the receptor for IL-3, which has been shown to be undetectable on neutrophils and only weakly expressed on (human) monocytes.46 IL-3 receptors are abundantly expressed on human eosinophils (which also express GM-CSF receptors) and on human and rhesus monkey basophils, which express low levels of the GM-CSF receptor.7,17,47 These differences probably reflect independent expression patterns of the α chains of the GM-CSF receptor and the IL-3 receptor, in particular at the developmentally late stages of hematopoietic cell differentiation, which results in differential distribution on mature cells. This is in agreement with the responses observed following administration of IL-3 and GM-CSF to monkeys: GM-CSF predominantly stimulates neutrophil, monocyte, and eosinophil production and IL-3 induces a vigorous basophil response.5,91

Negative selection of CD34+ cells that express high levels of the GM-CSF receptor by using biotin-labeled GM-CSF provides a novel method to deplete developmentally early hematopoietic cells of differentiating monocyte and granulocyte precursors. Biotin-labeled preparations of other HGFs, used separately or in combination, may be similarly used. Such an approach complements conventional cell-separation methods based on depletion of CD34+ cells that coexpress differentiation-specific and/or lineage-associated cell surface antigens, such as HLA-DR, CD71, and CD19, and identifies immature multilineage hematopoietic cells on the basis of their HGF-receptor phenotype.

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Distribution of receptors for granulocyte-macrophage colony-stimulating factor on immature CD34+ bone marrow cells, differentiating monomyeloid progenitors, and mature blood cell subsets

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