Effect of Murine Mast Cell Growth Factor (c-kit Proto-Oncogene Ligand) on Colony Formation by Human Marrow Hematopoietic Progenitor Cells

By Hal E. Broxmeyer, Scott Cooper, Li Lu, Giao Hangoc, Dirk Anderson, David Cosman, Stewart D. Lyman, and Douglas E. Williams

Purified natural (n) and recombinant (r) murine (mu) mast cell growth factor (MGF, a c-kit ligand) were evaluated alone and in combination with r human (hu) erythropoietin (Epo), rhu granulocyte-macrophage colony-stimulating factor (rhuGM-CSF), rhuG-CSF, and/or rhuM-CSF for effects in vitro on colony formation by multipotential colony-forming unit-granulocyte, erythroid, monocyte, megakaryocyte [CUF-GEMM], erythroid (burst-forming unit erythroid [BFU-E]) and granulocyte-macrophage colony-stimulating factor (CFU-GM) progenitor cells from normal human bone marrow. MGF was a potent enhancing cytokine for Epo-dependent CFU-GEMM and BFU-E colony formation, stimulating more colonies and of a larger size than either rhu interleukin-3 (rhulL-3) or rhuGM-CSF. MGF, especially at lower concentrations, also acted with rhulL-3 or rhuGM-CSF to enhance Epo-dependent CFU-GEMM and BFU-E colony formation. MGF had little stimulating activity for CFU-GM colonies by itself, but in combination with suboptimal to optimal amounts of rhuGM-CSF enhanced the numbers and the size of CFU-GM colonies in an additive to greater than additive manner. While we did not detect an effect of MGF on CFU-G colony numbers stimulated by maximal concentrations of rhuGM-CSF, MGF did enhance the size of CFU-G-derived colonies. MGF did not enhance the activity of rhuM-CSF. In a comparative assay, maximal concentrations of rmu and rhuMGF were equally effective in the enhancement of human bone marrow colony formation, but rhuMGF, in contrast to rmuMGF, did not at the concentrations tested enhance colony formation by mouse bone marrow cells. MGF effects on BFU-E, CFU-GM, and CFU-GEMM may be direct acting ones as MGF-enhanced colony formation by these cells in highly enriched progenitor cell populations of CD34++”, HLA-DR” and CD34++”, HLA-DR” CD33- sorted cells in which >1 of 2 cells was a BFU-E plus CFU-GM plus CFU-GEMM. MGF appears to be an early acting cytokine that preferentially stimulates the growth of immature hematopoietic progenitor cells.

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A NUMBER OF CYTOKINES act as positive stimuli for myeloid blood cell production. These include the hematopoietic colony-stimulating factors (CSF): interleukin-3 (IL-3; a multi-CSF), granulocyte-macrophage CSF (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL-5 (an eosinophil-CSF), macrophage CSF (M-CSF or CSF-1), and erythropoietin (Epo). The CSFs have direct acting effects on myeloid progenitor cells (MPC; the multipotential colony-forming unit-granulocyte, erythroid, monocyte, megakaryocyte [CUF-GEMM]), erythroid (burst-forming unit erythroid [BFU-E]), and/or CFU-GM progenitors. IL-3 and GM-CSF are considered to be relatively early acting factors as they stimulate the proliferation of CFU-GEMM, as well as BFU-E and CFU-GM. Other cytokines can enhance the activities of the CSFs by direct actions on MPC, and/or by indirect actions mediated through accessory cells. Enhancing cytokines include IL-1, IL-2, IL-6, IL-9, activin, and the macrophage inflammatory proteins (MIP)-1α, -1β, and -21.

Recently, a number of groups have isolated ligands for the c-kit receptor protein. The c-kit proto-oncogene, whose product has tyrosine kinase activity, is encoded by the “W” locus on murine chromosome 5. These findings link up the abnormalities apparent in the genetically anemic W/W and Sl/SI” mice, which, respectively, have defects in stem cells and the hematopoietic microenvironment, among other disorders. Ligands for the c-kit proto-oncogene product have been isolated as soluble molecules and called mast cell growth factor (MGF), stem cell factor (SCF), and KL by the different groups. Membrane-bound forms have been reported. The soluble ligands (in natural and recombinant forms) from mouse19,27 and rat17,18 have CFU-GM-enhancing and erythroid (BFU-E) potentiating activity on mouse cells, and the rat and human (hu) forms17 have activity on huBFU-E and huCFU-GM. It is not clear if these effects are direct on MPC and there is still a paucity of information on the activities of these molecules on hematopoietic cell proliferation, especially for human cells. Our current study evaluated the actions of the purified natural (n) and recombinant (r) forms of murine MGF on colony formation by normal human bone marrow CFU-GEMM, BFU-E, and CFU-GM, alone and in combination with rhuEpo, rhuIL-3, rhuGM-CSF, rhuG-CSF, and rhuM-CSF, and compared this to the effect of rhuMGF. Our results suggest that MGF is a potent growth factor that enhances/stimulates Epo-dependent CFU-GEMM and BFU-E. MGF activity on these cells is greater than that of IL-3 or GM-CSF, and at low concentrations of MGF, MGF synergises with optimal concentrations of IL-3 or GM-CSF to stimulate Epo-dependent CFU-GEMM and BFU-E. In addition, MGF also acts in combination with IL-3 and GM-CSF to greatly enhance the number and size of colonies derived from CFU-GEMM, BFU-E, and CFU-GM.
GM. In combination with G-CSF, MGF enhances the size of CFU-G colonies. MGF had no effect when placed in combination with M-CSF. Use of highly enriched human bone marrow progenitor cell populations (CD34+HLA-DR+, CD34+HLA-DR-CD33+) with cloning efficiencies of 49% to 67% suggested that the MGF potentiating effects were directly on MPC.

MATERIALS AND METHODS

**Cells.** Bone marrow cells were obtained from normal healthy donors who had given informed consent. Cells were separated by Ficoll-Hypaque (density, 1.077 gm/cm³; Pharmacia Fine Chemicals, Piscataway, NJ) into a low density (LD) fraction, or after adherence to plastic and removal of E-rosette-positive cells into a nonadherent LD T-lymphocyte-depleted (NALDT-) fraction. The NALDT- fraction had ≤ 3% monocytes and T lymphocytes as determined by staining with nonspecific esterase and flow analysis with anti-CD3 antibodies. NALDT- cells were further enriched for hematopoietic progenitor cell fractions by fluorescence activated cell sorting using two-color staining into a CD34+HLA-DR- population and using three-color staining into a CD34+HLA-DR-CD33+ population with a Coulter 753 Flow Cytometry System (Hialeah, FL). For three-color staining, cells were incubated with anti-CD34 (Becton Dickinson Immunocytochemistry Systems, San Jose, CA), followed by an isotype-specific Texas Red conjugated goat-antimouse IgG, antibody (Southern Biotechnology, Birmingham, AL). Mouse serum was added to block free binding sites for the second step reagent. Then fluorescein isothiocyanate (FITC)-conjugated anti-CD33 (Coulter Corp) and phycoerythrin-conjugated anti-CD33 were added (Becton Dickinson). Unseparated bone marrow from BDF, mice (Cumberland View Farms, Clinton, TN) were also used.

**Cytokines.** Natural MGF was isolated from a murine +/- cell line²⁹ and purified as described. Units of MGF activity (amount required to stimulate half-maximal tritiated thymidine incorporation) were calculated using a murine MC-6 factor-dependent cell line proliferation assay.²⁹²⁰ rmuMGF was expressed in yeast and purified.²⁹²⁰³⁰ rhuGM-CSF, rhuGM-CSF, and rhuIL-3 (each 10⁶ U/mg) as assessed by half-maximal tritiated thymidine incorporation into normal human or mouse bone marrow cell proliferation assays, respectively, for human or murine cytokines) were from Immunex Corporation (Seattle, WA), rhu-CSF (≥ 5 × 10⁶ U/mg) and rhu-G-CSF (95% pure, ≥ 5 × 10⁶ U/mg), both assayed by mouse bone marrow colony formation, were kind gifts from Drs Peter Ralph and Robert Drummond (Cetus Corp, Emeryville, CA). Purified rhuEpo was purchased from Amgen Corp (Thousand Oaks, CA). Medium conditioned by the 5637 human urinary bladder carcinoma cell line (5637CM) was prepared as before.²¹ CSF activities of rhuGM-CSF, rhuIL-3, rhuGM-CSF, rhuG-CSF, and rhuEpo, and 5637CM have been reported.²⁹³²

**Analysis of hematopoietic progenitor cells.** LD, NALDT-, CD34+HLA-DR-, and CD34+HLA-DR-CD33- cells were plated for CFU-GM assay in 0.4% agarose culture medium or 0.3% agar culture medium, and for BFU-E and CFU-GEMM assay in 0.9% methylcellulose culture medium (except when sorted cells were plated and 1.3% methylcellulose was used).²⁹³²²⁹³⁴ Cells were incubated at 5% CO₂ in lowered (5%) O₂; and colonies scored after 12 to 14 days of incubation. Relative colony size was estimated by the area of the plate (in microns) occupied and the compact nature of the colony. Colony morphology in agarose was assessed in whole plates (100 colonies scored from each of three dishes per point) stained with α-naphthyl acetate esterase and fast blue and counterstained with hematoxylin.²² CFU-GEMM colonies contained granulocytes, erythroid cells, monocytes/macrophages, and, in most cases, megakaryocytes.

**Statistics.** Three plates were scored for each CFU-GM point and four plates were scored for each BFU-E/CFU-GEMM sample. Results are expressed as the mean ± 1 SEM. Levels of significance between samples were determined using Student’s t distribution and greater than additive effects of cytokines were evaluated using χ² tests arising from a regression procedure assuming a Poisson distribution, with a modified Bonferroni procedure as previously described.²³

**RESULTS**

**Influence of MGF on CFU-GEMM and BFU-E.** rmuMGF and rmuMFG were evaluated in the absence and presence of rhuEpo, with or without rhuIL-3 or rhuGM-CSF, for effects on colony formation by normal human bone marrow CFU-GEMM and BFU-E. rmuMGF and rmuMFG were assayed, respectively, at 5, 20, and 50 U/mL and 5, 50, 100, and 200 ng/mL. When MGF was used in the absence of rhuEpo, no CFU-GEMM or BFU-E colonies formed. In the presence of rhuEpo, both the n and r preparations of rhuMGF had potent colony enhancing activity for CFU-GEMM (Fig 1) and BFU-E (Fig 2). Maximal effects were obtained with 20 to 50 U/mL rmuMGF and 50 to 200 ng/mL MGF and the results shown are for a low (n = 5 U/mL and r = 5 ng/mL) and plateau dose (n = 20 U/mL and r = 50 ng/mL) of MGF. The enhancing effects of MGF were apparent when LD BMC were used (Figs 1A and 2A), and also when NALDT- cells were used (Figs 1C and 2C). In the presence of plateau concentrations of MGF, threefold to fourfold and threefold to eightfold more CFU-GEMM colonies were formed, respectively, than with optimal concentrations (100 U/mL) of either rhuIL-3 or rhuGM-CSF (Fig 1). BFU-E colonies were also stimulated in greater numbers with MGF than with IL-3 or GM-CSF (Fig 2) although the differences were not as great as for CFU-GEMM. In addition to effects on colony numbers, BFU-E colonies grown in the presence of 20 U/mL rmuMGF or 50 ng/mL rmuMGF were much larger in size than those stimulated with optimal concentrations of rhuEpo (1 U/mL) alone. Epo (1 U/mL)-dependent CFU-GEMM and BFU-E colonies grown in the presence of MGF were also larger by about twofold to fivefold than those stimulated in the presence of 100 U/mL rhuIL-3 or rhuGM-CSF. The potent enhancing activity of rmuMGF for Epo-stimulated BFU-E and CFU-GEMM has been noted using marrows from over 15 different normal donors. Representative results for five such experiments are shown in Table 1.

When 5 U/mL rmuMGF or 5 ng/mL rmuMFG was added in combination with 100 U/mL rhuIL-3 or rhuGM-CSF (in the presence of 1 U/mL rhuEpo), the effects of MGF plus IL-3 or GM-CSF were additive or greater than additive for numbers of CFU-GEMM colonies (Fig 1). The number of BFU-E with MGF plus GM-CSF or MGF plus IL-3 increased in a less than additive fashion although the number of BFU-E with either combination was greater than that seen with any factor alone (Fig 2). When 20 U/mL rmuMGF or 50 ng/mL rmuMFG were used with 100 U/mL
Cytokines Epo, IL-3, G-CSF, M-CSF, and GM-CSF were added to human bone marrow cultures to study their effects on colony formation. Figure 1 illustrates the influence of 

- muMGF and rhuEpo, alone and in combination with rhuIL-3 or rhuGM-CSF, on colony formation by human bone marrow CFU-GEMM. Experiments A and B were performed with different marrows, but B and C were performed with the same marrow sample. Differences between groups are given as significant increases (P at least < 0.05) compared with the following treatment points: #1, #2, #4 or #7, #2 plus #4, #3 or #4, #2 plus #7. #3 or #7. These results are comparable with those noted in two other similar experiments not shown.

- Influence of MGF, alone and plus CSF, on CFU-GM. nmuMGF (5 and 20 Ul/mL) and rmuMGF (5 and 50 ng/mL) were evaluated alone and in combination with either 10 or 100 U/mL rhuGM-CSF, 10 or 100 U/mL rhuG-CSF, or 100 or 1,000 U/mL rhuM-CSF, for effects on colony formation by normal human bone marrow CFU-GM (Fig 3). nmuMGF and rmuMGF were able to stimulate some CFU-GM colonies (> 90% contained both granulocytes and macrophages) when added to LD and NALD T- BMC. The size of MGF-stimulated colonies (slightly above 40 to 50 cells/aggregate) was small compared with those stimulated by GM-CSF. The effects of MGF were more apparent when used in combination with other CSFs. MGF (n and r) had additive, or greater than additive, effects on colony numbers in the presence of 10 to 100 U/mL rhuGM-CSF, but not in the presence of 10 to 100 U/mL rhuG-CSF or 100 to 1,000 U/mL rhuM-CSF (Fig 3). When used alone, 100 U/mL rhuGM-CSF stimulated 16% ± 2% GM, 28% ± 2% G, 11% ± 1% Eos, and 45% ± 2% M colonies as assessed by histochemical staining of agarose cultures. The addition of 20 U/mL nmuMGF or 50 ng/mL rmuMGF with 100 U/mL rhuGM-CSF resulted in a fourfold increase in number of GM colonies and a twofold increase in number of G colonies with no change in the number of M colonies. The size of GM and G colonies grown in the presence of MGF plus rhuGM-CSF was about twofold to fourfold greater than those formed in the presence of either molecule alone. The synergistic effects of rmuMGF with rhuGM-CSF has been seen in over 15 experiments, each using marrow from different normal donors. Five such representative experiments are shown in Table 1. rhuG-CSF alone stimulated 77% ± 2% G colonies (Fig 3) and while the numbers of these colonies did not increase with MGF, the size of these colonies did increase by about twofold to fourfold. rhuM-CSF alone stimulated greater than 94% M colonies, but MGF did not appear to influence the number or size of M colonies stimulated with rhuM-CSF. In some cases, when LD BMC were used, MGF plus either rhuG-CSF or rhuM-CSF yielded a small increase in colony numbers, but this was always due to an increase in large-sized GM colonies.

Comparative influences of rmu and rhuMGF. When concentrations of 50 ng/mL rmu or rhuMGF were compared for activity on human bone marrow cells (Table 2) they were found to be equally active. Higher concentrations of each (up to 100 ng/mL) had no greater effect (data not shown). In contrast, while rmuMGF synergized with rmuGM-CSF to enhance mouse marrow CFU-GM colony formation, and synergized with Epo to enhance mouse BFU-E and CFU-GEMM colony formation, as reported elsewhere, rhuMGF (50 ng/mL) did not stimulate or enhance the effects of rmuMG-CSF on mouse CFU-GM colonies.
MGF AND HUMAN HEMATOPOIETIC PROGENITORS

Cytokines (Epo, 1U) Plus

A) nMGF

B) rMGF

C) rMGF

Colony Formation (27 ± 1 and 27 ± 3 colonies with 50 U/mL rmuGM-CSF without or with 50 ng rmuMGF per 7.5 × 10⁴ BDF₁ cells plated) or enhance the effect of Epo on mouse marrow BFU-E or CFU-GEMM (10 ± 1 and 9 ± 1 BFU-E and 0 and 0 CFU-GEMM with 1 U Epo, respectively, without or with 50 ng rhuMGF per 7.5 × 10⁴ BDF₁ cells).

Influence of MGF on highly enriched BFU-E and CFU-GM. To evaluate the effects of muMGF and rmuMGF on a population of cells more highly purified for hematopoietic progenitors than in the NALDT⁻ fraction of cells, NALDT⁻ cells were sorted into a CD34⁺ HLA-DR⁻ population. The cumulative cloning efficiency for CFU-GM plus BFU-E when 125 cells were plated in the presence of 1 U/mL rhuEpo and 5% vol/vol 5637CM was 49% (Table 3, part A). Both rmuMGF (20 U/mL) and rmuMGF (50 ng/mL) were able to significantly (P < .0001) increase colony numbers by BFU-E above the levels noted with Epo alone. CFU-GM numbers were also increased in the

Table 1. Influence of rmuMGF on Colony Formation by Human Bone Marrow Cells From Five Different Donors

<table>
<thead>
<tr>
<th>Cell Populations</th>
<th>2.5 × 10⁴ NALDT</th>
<th>5 × 10⁴ Unsep</th>
<th>1 × 10⁵ LD NALDT</th>
<th>2.5 × 10⁴ NALDT</th>
<th>1 × 10⁵ LD NALDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rhuGM-CSF (100 U/mL)</td>
<td>18 ± 1</td>
<td>18 ± 2</td>
<td>33 ± 2</td>
<td>26 ± 3</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>rmuMGF (50 ng/mL)</td>
<td>3 ± 1</td>
<td>5 ± 1</td>
<td>2 ± 1</td>
<td>4 ± 0.3</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>rmuGM-CSF (100 U/mL) + rmuMGF (50 ng/mL)</td>
<td>45 ± 4*</td>
<td>77 ± 3*</td>
<td>93 ± 7*</td>
<td>88 ± 1*</td>
<td>119 ± 4*</td>
</tr>
<tr>
<td>BFU-E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rhuEpo (1 U/mL)</td>
<td>22 ± 3</td>
<td>30 ± 1</td>
<td>48 ± 2</td>
<td>22 ± 3</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>rhuEpo (1 U/mL) + rmuMGF (50 ng/mL)</td>
<td>119 ± 4†</td>
<td>101 ± 9†</td>
<td>159 ± 6†</td>
<td>162 ± 7†</td>
<td>107 ± 5†</td>
</tr>
<tr>
<td>rhuEpo (1 U/mL) + rhulL-3 (200 U/mL)</td>
<td>48 ± 3†</td>
<td>66 ± 3†</td>
<td>98 ± 4†</td>
<td>42 ± 3†</td>
<td>70 ± 3†</td>
</tr>
<tr>
<td>CFU-GEMM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rhu Epo (1 U/mL)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 ± 0.3</td>
</tr>
<tr>
<td>rhuEpo (1 U/mL) + rmuMGF (50 ng/mL)</td>
<td>12 ± 1†</td>
<td>39 ± 2†</td>
<td>45 ± 3†</td>
<td>28 ± 2†</td>
<td>22 ± 1†</td>
</tr>
<tr>
<td>rhuEpo (1 U/mL) + rhulL-3 (200 U/mL)</td>
<td>2 ± 0.4†</td>
<td>2 ± 1†</td>
<td>11 ± 1†</td>
<td>2 ± 0.3†</td>
<td>3 ± 1†</td>
</tr>
</tbody>
</table>

Assays were set up in 0.3% agar culture medium.

*Significant increase compared with the separate effects of GM-CSF and MGF, P < .01.
†Significant increase compared with Epo alone, P < .05 to .01.

Fig 2. Influence of muMGF and rhuEpo, alone and in combination with rhulL-3 or rhuGM-CSF, on colony formation by human bone marrow BFU-E. Experiments A and B were performed with different marrows, but B and C were performed with the same marrow sample. Significant increases (P at least < .05) compared with the following treatment points: #1, #2, #4 or #7, #2 or #4, #2 or #7. Comparable results were seen in two other similar experiments not shown.

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Fig 3. Influence of muMGF, alone and in combination with rhuGM-CSF, rhuG-CSF, or rhuM-CSF, on colony formation by human bone marrow CFU-GM. Experiments A and B were performed with different marrows, but B and C were performed with the same marrow sample. Assays were in 0.4% agarose culture medium. (a) Colony numbers are additive or greater than additive compared with individual effects of cytokines; all other colony numbers are less than additive and equal to effects of only the more active cytokine. Greater than additive effects, $P < .05$. These results are comparable with those seen in three other similar experiments.

Table 2. Comparative Influence of muMGF and rhu MGF on Colony Formation by Hematopoietic Progenitor Cells

<table>
<thead>
<tr>
<th>Colony Formation by $2.5 \times 10^6$ NALDT- Human Bone Marrow Cells</th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-GEMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (McCoy’s) medium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>rhuGM-CSF (50 U/mL)</td>
<td>33 ± 2</td>
<td>28 ± 3</td>
<td>101 ± 6*</td>
</tr>
<tr>
<td>muMGF (50 ng/mL)</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>rhuMG-CSF (50 ng/mL)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>muMGF (50 ng/mL)</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>rhuMG-CSF (50 ng/mL)</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
</tr>
</tbody>
</table>

CFU-GM assay was set up in 0.3% agar culture medium.
*Significant increase compared with the separate effects of GM-CSF and MGF, $P < .01$.
†Significant increase compared with Epo alone, $P < .01$.

Table 3. Influence of muMGF (c-kit Ligand) and Other Cytokines on Colony Formation by Highly Enriched CFU-GM and BFU-E in CD34+++ HLA-DR- and CD34+++ HLA-DR+ CD33- Sorted Human Bone Marrow Cells

<table>
<thead>
<tr>
<th>Colony and Cluster Formation by CD34+++ HLA-DR- CD33- Cells</th>
<th>125 Cells</th>
<th>250 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A: Epo (1 U) plus:</td>
<td>CFU-GM</td>
<td>BFU-E</td>
</tr>
<tr>
<td>Control medium</td>
<td>5 ± 0.6</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>5% vol/vol 5637 CM</td>
<td>33 ± 2*</td>
<td>28 ± 3*</td>
</tr>
<tr>
<td>rhuMG-CSF (50 ng)</td>
<td>27 ± 5*</td>
<td>17 ± 2*</td>
</tr>
<tr>
<td>muMGF (20 U)</td>
<td>18 ± 1*</td>
<td>23 ± 2*</td>
</tr>
<tr>
<td>rhuL-3 (200 U)</td>
<td>11 ± 2*</td>
<td>15 ± 2*</td>
</tr>
<tr>
<td>rhuGM-CSF (200 U)</td>
<td>17 ± 2*</td>
<td>15 ± 1*</td>
</tr>
</tbody>
</table>

These assays were set up in 1.3% methylcellulose culture medium.
*Significant increase compared with control medium, $P < .01$. For personal use only.
presence of MGF using purified target cells. This enhance-
ment in colony numbers was equal to or slightly greater
than that seen with 200 U/mL of either rhuIL-3 or rhuGM-
SF. It has been reported that a major subset of BFU-E
expresses CD34 and HLA-DR, but not CD33, antigens. Thus
we also evaluated cytokine activity on CD34++*HLA-
DR*CD33− cells using another normal human bone mar-
row (Table 3, part B). rmuMGF plated in the presence of
Epo enhanced colony formation by CFU-GM, BFU-E, and
CFU-GEMM, resulting in a total cloning efficiency of 67%.
In both experiments, the size of Epo-dependent BFU-E
colonies stimulated by MGF was at least threefold to
fourfold greater than that stimulated by IL-3 or GM-CSF,
although the size of CFU-GM colonies was smaller than
that stimulated by GM-CSF. The CFU-GEMM colonies
forming in the presence of Epo plus MGF were as large as
the similarly stimulated colonies seen in NALDT− cells.

DISCUSSION

A growing number of cytokines have been implicated in
the complex network of biomolecule-cell interactions regu-
ating blood cell production. These cytokines can stimu-
late, enhance, and/or suppress hematopoietic progenitor
cell proliferation in vitro. Evidence for the in vivo action of
a number of these cytokines is also available. The variously
named ligand(s) for the c-kit proto-oncogene, can now be
added to the list of early acting cytokines such as IL-3,
GM-CSF, IL-1, and IL-6. In the present study we
have confirmed, using normal human BFU-E, the potent
costimulating activity for human marrow BFU-E and
CFU-GM noted by others using rat and mouse SCF.
Interestingly, maximal amounts of mru and mruMGF were
equally active enhancing agents for human bone marrow
colonies (Table 2), although when titrated on a weight to weight basis the huMGF appears more active
than rmuMGF on human cells (D.E. Williams, unpublished
observations). The rhuMGF, when used at a concentration
of 50 ng/mL, had no effect on mouse cells. Additionally, our
results have produced the following new information. First,
MGF is a potent stimulating/enhancing activity for CFU-
GEMM from human marrow growth in the presence of
Epo. A distinguishing feature of MGF is that it has much
greater proliferative activity than maximal amounts of IL-3
or GM-CSF for Epo-dependent CFU-GEMM and BFU-E
in terms of numbers and size of colonies stimulated. Second, while MGF by itself is a potent cofactor, at lowered
centers and in combination with maximally acting
centers of IL-3 or GM-CSF it has greater than additive activity on CFU-GEMM. Third, MGF does not
appear to influence the growth of CFU-M colonies stimu-
lated by rhuM-CSF. However, MGF is a potent costimula-
tor of human marrow CFU-GM in terms of numbers and
size of colonies formed in the presence of rhuGM-CSF, and
MGF enhances the size, but not the numbers, of CFU-G
colonies stimulated by plateau concentrations of rhuG-
CSF. Others have noted that rhuSCF enhances rhuG-CSF–
stimulated colony numbers. Our inability to detect en-
chanced numbers of G-CSF–stimulated colonies with the
rmuMGF used could be due to the species of MGF we
used (we used mruMGF for our studies), or possibly to the
fact that we used plateau stimulating concentrations of
rhuG-CSF. Fourth, the above effects were noted in the
absence (using NALDT− cells) or presence (using LD cells)
of T lymphocytes and monocytes, two known populations
of accessory cells. Fifth, MGF appears to be a direct
acting cytokine at the level of responsive hematopoietic
progenitor cells as suggested by its stimulating/enhancing
activity for BFU-E, CFU-GM, and CFU-GEMM in the
populations of NALDT− CD34++*HLA-DR− and
NALDT− CD34++*HLA-DR−CD33− cells in which greater
than or equal to half of the cells were BFU-E, CFU-GM,
and CFU-GEMM. Even in this highly purified population,
the size of Epo-dependent BFU-E colonies growing in the
presence of MGF was larger than that grown in the
presence of IL-3 or GM-CSF and the size of Epo-
dependent CFU-GEMM colonies was very large. This
result suggests that MGF may be an earlier acting cytokine
than IL-3 and GM-CSF, a possibility strengthened by the
ability of MGF to enhance the number and proliferative
capacity of CFU-GEMM, BFU-E, and CFU-GM respond-
ing to IL-3 or GM-CSF. MGF has no detectable activity on
CFU-GEMM or BFU-E in the absence of Epo, and its
activity for CFU-GM is minimal in terms of size and
number of colonies stimulated compared with GM-CSF.
Thus, a main function of MGF may be to enhance the
proliferative capacity of progenitor cells responding to
other cytokines. The MGF used in the present studies was
a soluble form, but analysis of the structure of the MGF
protein suggested that it was membrane bound and con-
tained extracellular, transmembrane, and cytoplasmic do-
 mains. In fact, transfection of MGF cDNA into either
COS-7 cells or an SI/Si4-1 stromal cell line resulted in the
expression of a membrane-bound, biologically active form
of MGF when the transfected cells were cocultured with
cells responsive to purified soluble MGF. Thus, MGF may
be active in a local setting within the microenvironment and
its action manifest through cell-cell contact and interaction.
These data and other observations indicate that the primary
hematopoietic target cells for MGF are primitive
precursor cells, particularly those with erythroid differenti-
ation potential (BFU-E and CFU-GEMM). Later pheno-
type cells, such as those responsive to the lineage-specific
regulators M-CSF and G-CSF, appear to be considerably
less or unresponsive to the actions of MGF than their
predecessors in the hematopoietic hierarchy. The profound
effect of MGF on in vitro erythropoiesis is particularly
interesting in view of the defect of this lineage in S1 and W
mice. The observed increase in the size of normal CFU-
GEMM and BFU-E colonies stimulated by MGF plus Epo
and CFU-GM colonies stimulated by GM-CSF or G-CSF
plus MGF suggests that MGF might serve to promote
renewal and expansion of responsive progenitor cells within
individual colonies. Serial replating studies of CFU-
GEMM, BFU-E, and CFU-GM will be required to address
this issue. It is tempting to speculate that the nature of the
severe anemia of S1 and W mutant mice results from an
inability to expand the Epo-responsive intermediate progenitor cell pools due to either absence or reduction of the ligand (MGF) or the receptor for this ligand (c-kit). Such a hypothesis is consistent with the apparent refractoriness of these mutant mice to Epo therapy and the partially corrective effects of pharmacologic dosages of SCF on S1/S1β mice. Preclinical studies are needed to assess the potential of the c-kit ligand(s) (MGF, SCF, KL) for application to disease and treatment-related hematopoietically suppressed states.

ACKNOWLEDGMENT

We thank Linda Cheung and Rebecca Robling for typing the manuscript, and Dr E. Sour and T. Leemhuis for help with the cell sorting.

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Effect of murine mast cell growth factor (c-kit proto-oncogene ligand) on colony formation by human marrow hematopoietic progenitor cells

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