Granulocyte-Macrophage Colony-Stimulating Factor Requires Interaction With Accessory Cells or Granulocyte-Colony Stimulating Factor for Full Stimulation of Human Myeloid Progenitors

By D. Ferrero, C. Tarella, R. Badoni, D. Caracciolo, G. Bellone, A. Pileri, and E. Gallo

Human recombinant GM-CSF (rGM-CSF) was tested on highly purified and fractionated CFU-GM subsets. The fractionation was performed with the DS1-1 monoclonal antibody (MoAb), which distinguishes early and late CFU-GM. On whole bone marrow cells, rGM-CSF had a colony-stimulating activity comparable to that of known sources of CSFs, i.e., the supernatant (SN) of TPA 30-1 or 5637 cell lines, used as control. A greatly reduced activity was observed when CFU-GM were depleted of phagocytizing and E rosetting cells (colony growth of 27% as compared with control). On fractionated CFU-GM, the rGM-CSF activity was even more reduced on both early and late progenitors (18% and 6% of colony growth, respectively). However, when rGM-CSF was used together with rG-CSF at suboptimal concentrations, the colony growth reached values analogous to that of control cultures. A synergistic interaction between rGM-CSF and rG-CSF in stimulating either early or late myeloid progenitors was observed. The results suggest that the activity of rGM-CSF on CFU-GM is mainly exerted through cooperation with accessory cells. r-G-CSF is one of the factors that can synergistically cooperate with r-GM-CSF in the myelopoietic stimulation.

THE granulocyte-macrophage colony-stimulating factor (GM-CSF) has been suggested to stimulate myelopoiesis directly, with a preferential activity on early myeloid progenitors.\(^1\)\(^2\) In most of the studies, however, the activity of GM-CSF has been tested on the whole CFU-GM population, often after partial removal of accessory cells. In the present study, myeloid progenitors were highly enriched by removing phagocytizing and E rosetting cells. In addition, early and late CFU-GM were physically separated on the basis of their antigenic phenotype.\(^3\) Their in vitro proliferation was evaluated in response to recombinant GM-CSF (rGM-CSF), alone and in combination with recombinant granulocyte colony-stimulating factor (rG-CSF).\(^1\)

MATERIALS AND METHODS

Reagents. Human recombinant GM-CSF and G-CSF were provided by Dr S. Clark (Genetic Institute, Cambridge, MA). rGM-CSF was supplied purified at homogeneity (3 \(\times\) 4 \(\times\) 10\(^7\) U/mg), whereas G-CSF preparations were crude conditioned media from transfected Chinese hamster ovary (CHO) cells. They were titrated on low-density human bone marrow cells assuming as 1 U the amount able to induce the growth of 1 colony/7 \(\times\) 10\(^4\) cells. In some experiments, commercially available purified GM and G-CSF were used (Genzyme). Control cultures were seeded either with TPA 30-1 or 5637 cell line supernatant (SN)\(^4\) used at optimal concentration (10% vol/vol).

DS1-1 (provided by Dr G. Rovera, The Wistar Institute, Philadelphia) is a IgM mouse monoclonal antibody (MoAb), which reacts to granulopoietic cells, from late CFU-GM to mature granulocytes, but does not recognize early myeloid progenitors.\(^4\) Indeed, colonies appearing after seven to nine days of agar cultures mainly derive from DS1-1+ progenitors, whereas most stem cells forming colonies in 12 to 14 days belong to the DS1-1+ population.

Cell preparations. During diagnostic procedures, bone marrow was collected, from patients whose disease did not involve the myeloid lineage and from normal volunteer donors. Informed consent was obtained.

Low-density (LD) cells (density 1.077 g/L) were depleted of adherent and phagocytizing (mature myelomonocytic) cells by a previously described technique.\(^4\) Nonphagocytizing cells were depleted of T lymphocytes by rosetting with AET-treated sheep erythrocytes. Residual phagocytizing and E rosetting cells always constituted <2% of the depleted population that included early erythroblasts, early myeloid cells, and non-T lymphocytes. CFU-GM recovered after depletion of phagocytizing and E rosetting cells were 80% to 90% of those in the unseparated BM.

CFU-GM separation and colony assay. Early and late CFU-GM were separated on the basis of their different reactivity to DS1-1 MoAb, either by cell sorting or immune rosetting or complement-mediated cytoxicity.\(^4\) Nonphagocytizing, DS1-1+ cells consisted of myeloblasts, promyelocytes, and some myelocytes, whereas the DS1-1− population contained erythroblasts, lymphocytes, and some undifferentiated blasts, in agreement to previous results.\(^5\) Based on the amount of colonies obtained from whole bone marrow cells, we could estimate that our immunologic separation allowed recovery of 50% to 60% of DS1-1+ and 70% to 80% of DS1-1− progenitors. BM LD cells were cultured at 7 \(\times\) 10\(^4\)/mL. Nonphagocytizing nonrosetting cells were plated at 1 \(\times\) 10\(^5\)/mL, whereas DS1-1+ and DS1-1− cells were seeded at 1.5 \(\times\) 10\(^4\) and 7 \(\times\) 10\(^4\)/mL, respectively.

CFU-C assay was performed as previously described.\(^4\) Aggregates >40 cells were scored as colonies after 7 and 14 days of culture at 37°C. Statistical analysis was performed with Student’s t test.

RESULTS

Effects of rGM-CSF on unfractionated BM CFU-GM. Unseparated LD BM cells gave rise to few colonies in seven days of culture in the presence of rGM-CSF. After 14 days, the plating efficiency (mean of eight experiments) was 62% ± 26% of that achieved in control cultures grown in the presence of either TPA 30-1 or 5637 SN. Colony size in cultures stimulated by rGM-CSF was smaller (40 to 200 cells) than in control cultures (200 to 1,000 cells). Two representative experiments comparing colony growth with
GM-CSF and G-CSF Synergism on Human CFU-GM

Table 1. Representative Experiments Showing Stimulatory Activity of TPA 30-1 SN and rGM-CSF on BM CFU-GM With and Without Accessory Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Stimulator Added*</th>
<th>Whole BM†</th>
<th>PhE ‡</th>
<th>With</th>
<th>Without</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7 Colonies</td>
<td>Day 14 Colonies</td>
<td>Day 7 Colonies</td>
<td>Day 14 Colonies</td>
</tr>
<tr>
<td>1</td>
<td>TPA 30-1</td>
<td>98 ± 2§</td>
<td>53 ± 6</td>
<td>114 ± 10</td>
<td>64 ± 8</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>4 ± 1</td>
<td>51 ± 3</td>
<td>0</td>
<td>22 ± 3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>1 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>TPA 30-1</td>
<td>108 ± 8</td>
<td>78 ± 5</td>
<td>68 ± 8</td>
<td>51 ± 7</td>
</tr>
<tr>
<td></td>
<td>GM-CSF</td>
<td>12 ± 4</td>
<td>37 ± 7</td>
<td>2 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The SN of the TPA 30-1 cell line was used at 10% vol/vol. rGM-CSF was used in the range of maximum activity (1 to 20 ng/mL).
†Values refer to colony number/7 × 10⁴ LD BM cells.
‡Values refer to colony number/1 × 10⁴ LD BM cells depleted of phagocytizing and E rosetting cells.
§Mean ± SD of triplicate cultures.

Of 10 to 30 cells arose from both types of progenitors in response to rGM-CSF.

Synergistic interaction between GM and G-CSF. Recombinant G-CSF stimulated both early and late CFU-GM, and the maximum colony growth was 40% to 60% of that achieved in control cultures supported by the cell-line SNs, in agreement with recently published results. This was observed at rG-CSF concentrations of 300 U/mL (300 pg/mL). At suboptimum concentrations (3 to 100 U/mL), colony growth was significantly reduced in cultures of either CFU-GM subtype (Table 2). However, when rGM-CSF and rG-CSF were used in combination, at 10 ng/mL and 30 U/mL, respectively, a definite increase in cell growth was observed, with values of colony number and colony size comparable (75% to 100%) to those obtained with TPA 30-1 SN (Table 2). Cytochemical stains showed the presence of neutrophilic (26%), eosinophils (20%), granulomacrophagic (38%), and macrophagic (16%) colonies. Indeed, at all concentrations of GM-CSF and G-CSF tested (0.1 to 10 ng/mL and 3 to 100 U/mL, respectively), the number of colonies was always at least twofold higher than expected from the activity of either factor when tested alone. This observation is consistent with a synergistic interaction between rGM-CSF and rG-CSF in stimulating CFU-GM growth. The synergism is also shown by isobologram analysis in Fig 1A and B.

Table 2. Representative Experiments Showing Synergistic Interaction Between rGM-CSF and rG-CSF in Stimulating Early (DS1-1') and Late (DS1-1') CFU-GM Subpopulations

<table>
<thead>
<tr>
<th>Stimulator Added*</th>
<th>Colony Growth From DS1-1' CFU-GM</th>
<th>Colony Growth From DS1-1' CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
</tr>
<tr>
<td>TPA 30-1</td>
<td>103 ± 6†</td>
<td>156 ± 4</td>
</tr>
<tr>
<td>GM 1 ng</td>
<td>1 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>GM 10 ng</td>
<td>9 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>G 30 U</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>G 100 U</td>
<td>5 ± 3</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>G 300-1,000 U</td>
<td>41 ± 4</td>
<td>54 ± 6</td>
</tr>
<tr>
<td>GM 10 ng + G 100 U</td>
<td>65 ± 4</td>
<td>130 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>79 ± 2</td>
<td>45 ± 2</td>
</tr>
</tbody>
</table>

*Values indicate the amount of rGM and rG-CSF added to 1 mL culture medium. TPA 30-1 SN was used at 10% vol/vol.
†Colony/1.5 × 10⁴ plated DS1-1' cells. Values are peak colony number observed at days 7 through 9.
‡Colony/7 × 10⁴ plated DS1-1' cells. Values are peak colony number observed at days 10 through 14.
In this study, we compared the activity of rGM-CSF to that of TPA 30-1 and 5637 SNs, which are likely to induce maximal CFU-GM proliferation. The relatively low colony growth observed with rGM-CSF in cultures depleted of phagocytizing cells indicates that this population is necessary for rGM-CSF to stimulate CFU-GM proliferation optimally. Indeed, phagocytosis is more efficient than adherence in removing accessory cells. In our system, the further removal of T lymphocytes did not appear to affect CFU-GM growth significantly. The low activity of rGM-CSF was even more evident on fractionated CFU-GM subpopulations, with early progenitors showing a slightly higher sensitivity to this growth factor. The high recovery of both early and late CFU-GM rules out the possibility of a selective loss of GM-CSF-sensitive progenitors during the fractionation procedures.

The activity of rGM-CSF on highly purified bone marrow CFU-GM was already shown to be greatly reduced as compared with that of crude Mo cell line SN, in agreement with our results. Conversely, Metcalf et al. and Burgess et al. demonstrated the ability of GM-CSF to support colony formation from cell clusters transferred in cell-free medium after four to five days of culture. The proportion of clusters that reached colony size in those cultures may correspond to CFU-GM that in our experiments formed colonies in the presence of rGM-CSF alone. However, the possibility of some interactions between rGM-CSF and accessory cells during the early days of mass culture must also be considered.

Optimum growth of CFU-GM was restored when rGM-CSF was used in association with rG-CSF. Indeed, rG-CSF at suboptimum concentration synergized with rGM-CSF on both early and late progenitors. Production of G-CSF could possibly account for the ability of BM phagocytizing cells to enhance GM-CSF-induced colony formation. Indeed, monocytes-macrophages can produce G and M-CSF, and their secretory activity is stimulated by GM-CSF. Because our culture assays were seeded at a very low cell concentration, the possibility that interactions between GM and G-CSF occur through residual accessory cells is unlikely. GM-CSF and G-CSF could synergize by giving two separate proliferative stimuli to target cells or by reciprocally inducing the upregulation of their receptors. A similar mechanism has been demonstrated to account for synergism between interleukin 1 (IL-1) and M-CSF and, more recently, between human GM-CSF and M-CSF. In conclusion, our studies support the emerging pattern that many of the hematopoietins interact synergistically at physiologic concentrations in vivo to support myelopoiesis.

ACKNOWLEDGMENT

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