Growth of human hemopoietic colonies in response to recombinant gibbon interleukin 3: Comparison with human recombinant granulocyte and granulocyte–macrophage colony-stimulating factor

(recombinant growth factors/human hemopoietic colony)

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ABSTRACT Supernatants of COS-1 cells transfected with gibbon cDNA encoding interleukin 3 (IL-3) with homology to sequences for human IL-3 were tested for ability to promote growth of various human hemopoietic progenitors. The effect of these supernatants as a source of recombinant IL-3 was compared to that of recombinant human granulocyte–macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) as well as to that of medium conditioned by phytohemagglutinin-stimulated leukocytes. The frequency of multilineage colonies, erythroid bursts, and megakaryocyte colonies in cultures containing the COS-1 cell supernatant was equivalent to the frequency observed in the controls and significantly higher than found in cultures plated with recombinant GM-CSF. G-CSF did not support the formation of multilineage colonies, erythroid bursts, and megakaryocyte colonies. In contrast, growth of granulocyte–macrophage colonies was best supported with GM-CSF, while recombinant IL-3 yielded colonies at lower or at best equivalent frequency. The simultaneous addition of higher concentrations of GM-CSF to cultures containing IL-3 in optimal amounts did not enhance the formation of multilineage colonies, erythroid bursts, and megakaryocyte colonies. However, the frequency of such colonies and bursts increased with GM-CSF when cultures were plated with suboptimal concentrations of IL-3. Growth of colonies within the granulocyte–macrophage lineage is optimally supported by GM-CSF and does not increase with further addition of IL-3.

Recent advances in molecular genetics have led to the cloning of human genes for granulocyte–macrophage colony-stimulating factor (GM-CSF) (1–3), granulocyte colony-stimulating factor (G-CSF) (4, 5), and macrophage-specific colony-stimulating factor (CSF-1) (6). Transfection of these genes into monkey COS cells results in the production of proteins having growth factor activity. G-CSF and CSF-1 promote growth of granulocyte and macrophage colonies, respectively, when tested in appropriate culture systems (4–6). In contrast, GM-CSF displays stimulatory activities for multiple precursors. These include progenitors committed to granulocyte and macrophage production (1–3, 7–9), erythropoiesis (3, 7–9), and megakaryocytopoiesis (3), as well as pluripotent precursors (7–9). However, the frequency of erythroid bursts and of megakaryocyte and multilineage colonies was consistently lower than that observed in cultures stimulated by crude conditioned media as a source of multiple growth factors (3, 9). This observation raises the question whether or not GM-CSF is the only multipotential human CSF or whether other molecules equivalent to murine interleukin 3 (IL-3) exist.

Recently, cDNA sequences were isolated in a gibbon T-cell line that demonstrated homology to the gene encoding murine IL-3 (10). Supernatants of COS-1 cells transfected with this gene promote activity for the growth of erythroid and nonerythroid colonies. By using the same probe, homology was observed with human cDNA sequences for IL-3.

In a series of experiments, we have tested IL-3-containing COS-1 cell supernatants on human bone marrow cells and compared their stimulatory effect for various hemopoietic precursors to that of medium conditioned by phytohemagglutinin-stimulated leukocytes (PHA-LCM) and to those of recombinant GM-CSF and G-CSF.

MATERIALS AND METHODS

Patient Material. Bone marrow cells were obtained from consenting normal volunteers. T-lymphocyte-depleted, non-adherent, mononuclear cell suspensions were prepared as described previously (11, 12).

Sources of Recombinant Growth Factors. Recombinant human GM-CSF and G-CSF as well as gibbon IL-3 were obtained as supernatants from Chinese hamster ovary cells (GM-CSF) or COS-1 cells (G-CSF, IL-3) transfected with the respective genes (Genetics Institute, Cambridge, MA). The supernatants contained 0.5–1.0 μg of protein per ml. They were serially diluted and used at concentrations ranging from 1:10² to 1:10⁶.

Recombinant erythropoietin (Epo) was purchased from Amgen Biologicals (Thousand Oaks, CA) (13).

Culture Conditions. Human bone marrow target cells (10³) were plated in Iscove's modified Dulbecco's medium (GIBCO) supplemented with 30% normal human plasma/50 μM 2-mercaptoethanol (Sigma)/0.9% methylcellulose (Dow) (11, 14). Each target cell suspension was evaluated with and without previously tested PHA-LCM at a concentration of 10% to determine maximal and background colony forma-


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Table 1. Stimulation of human hemopoietic colony formation by recombinant gibbon IL-3

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>IL-3 dilution</th>
<th>Colonies, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU-GEMM</td>
<td>BFU-E</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epo</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Epo/PHA-LCM</td>
<td>24</td>
<td>108</td>
</tr>
<tr>
<td>Epo/IL-3</td>
<td>1:10⁵</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5:10⁴</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1:10⁴</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5:10³</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1:10³</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>5:10²</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1:10²</td>
<td>14</td>
</tr>
</tbody>
</table>

Epo was used at 2 units per culture and PHA-LCM was used at a concentration of 10%. Colonies were counted per 10⁵ T-cell-depleted, nonadherent, normal bone marrow cells.

RESULTS

Influence of Recombinant IL-3 on Human Hemopoietic Colony Formation. Recombinant IL-3 was tested in four experiments on normal bone marrow target cells that were extensively depleted of cell populations capable of producing hemopoietic growth factors. The frequency of erythrocyte-rossette forming T cells was less than 1 in 2000 mononuclear cells. The material was tested at dilutions ranging from 1:10⁵ to 1:10⁶ of the COS-1 cell supernatant to determine the optimal activity. The frequency of each colony type was compared to the numbers of equivalent colonies observed in control cultures that contained optimal concentrations of PHA-LCM. Detailed data for a typical experiment are given in Table 1. Colonies were not observed in controls plated with human plasma in the absence of growth factors. Control plates with PHA-LCM and Epo showed various colony types at the expected frequency. Colony formation stimulated by IL-3 was dose dependent. Occasional multilineage colonies were observed in cultures containing IL-3 at a dilution of 1:10⁵ of the COS-1 cell supernatant. Their highest frequency, seen at a concentration of 1:10², was equivalent to that of PHA-LCM controls. The individual multilineage colonies were smaller and less compact than colonies grown with PHA-LCM and they contained fewer megakaryocytes.

The dose response of BFU-E to IL-3 was equivalent to that of CFU-GEMM. Erythroid bursts were observed when COS-1 cell supernatants were added at a dilution of 1:10⁴. Maximum burst formation was seen at a dilution of 1:10².

Maximum megakaryocyte colony formation was also obtained at a dilution of 1:10³. The frequency of colonies observed at this dilution was again equivalent to that of PHA-LCM controls. The morphological appearance of megakaryocyte colonies in IL-3-containing cultures differed from that observed with PHA-LCM. Colonies remained small. The number of megakaryocytes per colony ranged from 5 to maximally 50, while PHA-LCM controls showed megakaryocyte colonies ranging from 20 to 200 cells. The frequency of megakaryocyte colonies in IL-3-stimulated cultures reached a maximum between day 8 and day 10 and was subsequently sustained at least to day 14. As early as day 8 or 10, colonies usually contained only large, mature megakaryocytes in a loose arrangement. In contrast, the frequency of megakaryocyte colonies in PHA-LCM-containing control plates was maximal at day 14. Furthermore,
The plating efficiency of CFU-GEMM was optimal and comparable to that of PHA-LCM controls in plates containing IL-3 at a dilution of 1:10^{5}. The frequency of multilineage colonies was not increased by further addition of GM-CSF (Fig. 2A) or G-CSF (data not shown). In contrast, when IL-3 was used at suboptimal concentrations (dilutions of 1:10^{5} and 1:10^{6}), addition of GM-CSF resulted in a dose-dependent increase in multilineage colonies (Fig. 2A). However, the frequency of these colonies remained lower in comparison to results obtained at the optimal concentration of IL-3. The effect of IL-3 and GM-CSF was not additive. The frequency of multilineage colonies in cultures grown with various concentrations of GM-CSF increased and reached PHA-LCM control values when IL-3 was added at 1:10^{7} (Fig. 2B).

As shown in Fig. 2 C-F, BFU-E and CFU-Meg behaved similarly. The frequency of erythroid bursts and megakaryocyte colonies in cultures plated with IL-3 at a dilution of 1:10^{5} improved with GM-CSF but did not approximate that of PHA-LCM controls.

The response pattern of clonogenic precursors belonging to the granulocyte–macrophage series was different (Fig. 3). Optimal colony formation by CFU-G, CFU-Eo, CFU-GM, and CFU-M was achieved with GM-CSF. Addition of IL-3 at higher concentrations appeared to augment colony formation by CFU-Eo beyond values observed with optimal concentrations of GM-CSF. The frequency of the other granulocyte–macrophage progenitors was increased by addition of IL-3

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Formation of multilineage colonies, erythroid bursts, and megakaryocyte colonies in cultures containing various dilutions of IL-3 and GM-CSF. (A, C, and E) IL-3 was used at dilutions of 1:10^{6} (○), 1:10^{5} (△), and 1:10^{4} (◆) together with various concentrations of GM-CSF. (B, D, and F) GM-CSF was used at dilutions of 1:10^{6} (○), 1:10^{5} (△), and 1:10^{4} (◆) together with various concentrations of IL-3.

Colonies scored at that time showed a tight configuration and were composed of large mature as well as small immature megakaryocytes.

Colonies of the granulocyte–macrophage series appeared to grow at optimal frequency when higher concentrations of IL-3 were used. In particular, eosinophil colonies were predominant in cultures containing the highest concentration of IL-3 tested (1:10^{7} dilution). At high IL-3 concentration, basophil colonies could be recognized at low frequency (17, 18).

Colony formation promoted by the gibbon recombinant IL-3 was compared in four experiments to that of human recombinant GM-CSF and G-CSF (Fig. 1). The data are expressed as percentage of the PHA-LCM control.

GM-CSF at a dilution of 1:10^{5} yielded multilineage colonies in three experiments and erythroid bursts as well as megakaryocyte colonies in all four studies. The frequency was significantly lower when compared to IL-3 used at a dilution of 1:10^{5}. The formation of neutrophil, eosinophil, and granulocyte–macrophage colonies was equivalent to or better than that of PHA-LCM controls. Macrophage colony formation varied widely in these three experiments.

G-CSF stimulated the formation of neutrophil and a small number of eosinophil colonies without promoting growth of granulocyte–macrophage colonies, erythroid bursts, and megakaryocyte and multilineage colonies.

**Interaction Between Various Recombinant Growth Factors.**

Mixing experiments were performed to study the effect on colony formation when IL-3 was added to cultures simultaneously with GM-CSF or G-CSF. Based on previous experiments, IL-3 was used at dilutions of 1:10^{5}, 1:10^{6} and 1:10^{7}. GM- and G-CSF were added at dilutions covering the same range. Each culture was assessed for the presence of seven different colony types.

![Diagram](https://example.com/diagram.png)

**Fig. 3.** Formation of colonies of the granulocyte–macrophage lineage in cultures containing various dilutions of IL-3 and GM-CSF. (A, C, E, and G) IL-3 was used at dilutions of 1:10^{6} (○), 1:10^{5} (△), and 1:10^{4} (◆) together with various concentrations of GM-CSF. (B, D, F, and H) GM-CSF was used at dilutions of 1:10^{6} (○), 1:10^{5} (△), and 1:10^{4} (◆) together with various concentrations of IL-3.
only when GM-CSF was present at suboptimal concentrations.

G-CSF gave maximal growth support of CFU-G without affecting CFU-Eo, CFU-GM, and CFU-M (Fig. 4).

**Comparison of the Growth-Promoting Effects of Human Plasma and Fetal Calf Serum in Cultures Containing Recombinant IL-3.** Human plasma supports the growth of hemopoietic colonies in general, and megakaryocyte colonies in particular, more effectively than fetal calf serum (11). We have compared the effect of IL-3 with that of PHA-LCM using human plasma and fetal calf serum in three experiments. Human plasma promoted the formation of every colony type more efficiently than fetal calf serum independent of whether PHA-LCM or IL-3 was used, as shown by a representative experiment (Table 2). In particular, the frequency of megakaryocyte colonies was substantially higher with human plasma than with fetal calf serum.

**DISCUSSION.**

A series of human recombinant hemopoietic growth factors is available to investigate the influence of these factors on human hemopoietic precursors (1–6). The recently described gibbon IL-3 (10) promoted growth of human multi- and single lineage progenitors, including BFU-E and CFU-Meg, resulting in the formation of colonies at a frequency comparable to that of PHA-LCM-containing cultures. The results presented here are similar to those obtained by Steff et al. (19) and Leary et al. (20). In addition, Leary et al. have shown the growth of blast cell colonies composed of cells with reconstituting activity. The material was active at dilutions in COS-1 cell supernatants as low as 1:10³. However, when compared to PHA-LCM controls, colonies grown in the presence of recombinant IL-3 differed in size and cohesiveness and possibility also in maturation status. The latter was particularly apparent for megakaryocyte colonies. The predominance of large mature megakaryocytes by day 8 of culture is consistent with the production of mainly acetycholinesterase-positive mature megakaryocytes in murine IL-3-stimulated mouse bone marrow cultures (21).

IL-3 was more effective than GM-CSF in promoting growth of CU-GEMM, BFU-E, and CFU-Meg. Further addition of GM-CSF to cultures containing IL-3 at optimal concentration did not increase the frequency of multilineage colonies, erythroid bursts, or megakaryocyte colonies. The plating efficiency of all three types of precursors improved with GM-CSF when IL-3 was present at suboptimal concentrations. The maximal effect, however, was less than additive. This observation is consistent with the view that GM-CSF may promote growth of a subpopulation of IL-3-responsive CFU-GEMM, BFU-E, and CFU-Meg.

In contrast, GM-CSF promotes optimal growth of granulocyte-macrophage precursors while IL-3 stimulates only a subpopulation of GM-CSF-sensitive cells.

As previously found for PHA-LCM-stimulated cultures, colony formation by all hemopoietic progenitors but especially by megakaryocyte precursors is enhanced with human plasma as compared to fetal calf serum (11). This observation was reproduced when IL-3 was used instead of PHA-LCM. It is not known whether plasma provides only some essential nutrients or whether it may also serve as a source of as-yet-undefined growth factors. The development of a plasma- and serum-free culture system for hemopoietic progenitors may help to answer this question.

IL-3 may interact with clonogenic target cells directly rather than through signals produced by auxiliary cells as convincingly shown in cultures of single genetically separated murine pluripotent precursors (22). This view is supported by our observation that target cells extensively depleted of auxiliary cell populations were able to form colonies in response to recombinant IL-3.

We would like to acknowledge Julie Luker for her generous gift of normal plasma as well as Maureen Phelan and Miss Lorraine Dwyer for their contribution in the preparation of the manuscript. This work

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**Table 2.** Support of hemopoietic colony formation by human plasma or fetal calf serum in cultures stimulated by PHA-LCM or recombinant gibbon IL-3

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Addition</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-Meg</th>
<th>CFU-G</th>
<th>CFU-Eo</th>
<th>CFU-GM</th>
<th>CFU-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA-LCM</td>
<td>Human plasma</td>
<td>12</td>
<td>105</td>
<td>50</td>
<td>168</td>
<td>52</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Fetal calf serum</td>
<td>6</td>
<td>69</td>
<td>3</td>
<td>110</td>
<td>15</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>IL-3</td>
<td>Human plasma</td>
<td>11</td>
<td>102</td>
<td>47</td>
<td>50</td>
<td>14</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Fetal calf serum</td>
<td>6</td>
<td>84</td>
<td>2</td>
<td>66</td>
<td>23</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

In all experiments, Epo was used at 2 units per culture. Colonies were counted per 10⁵ T-cell-depleted, nonadherent, normal bone marrow cells.
was supported by a grant from the Medical Research Council of Canada.


