In Vitro Differentiation of Human Granulocyte/Macrophage and Erythroid Progenitors: Comparative Analysis of the Influence of Recombinant Human Erythropoietin, G-CSF, GM-CSF, and IL-3 in Serum-Supplemented and Serum-Deprived Cultures

By Giovanni Migliaccio, Anna Rita Migliaccio, and John W. Adamson

The effects of recombinant human erythropoietin (Ep), granulocyte/macrophage (GM) and granulocyte (G) colony-stimulating factors (CSF), and interleukin-3 (IL-3) on erythroid burst and GM colony growth have been studied in fetal bovine serum (FBS)-supplemented and FBS-deprived culture. Sources of progenitor cells were nonadherent or nonadherent T-lymphocyte-depleted marrow or peripheral blood cells from normal humans. G-CSF, in concentrations up to $2.3 \times 10^{-10}$ mol/L, induced only the formation of neutrophil colonies. In contrast, GM-CSF and IL-3 both induced GM colonies and sustained the formation of erythroid bursts in the presence of Ep. However, the activities of these growth factors were affected by the culture conditions. IL-3 induction of GM colonies depended on the presence of FBS, whereas the degree of GM-CSF induction of GM colonies in FBS-deprived cultures depended on the method by which adherent cells were removed. GM-CSF increased colony numbers in a concentration-dependent manner only if the cells had been prepared by overnight adherence. Both GM-CSF and IL-3 exhibited erythroid burst-promoting activity in FBS-deprived cultures. However, some lineage restriction was evident because GM-CSF was two- to threefold more active than IL-3 in inducing GM colonies but IL-3 was two- to threefold more active in promoting erythroid burst growth. Furthermore, in FBS-deprived cultures, the number of both erythroid bursts and GM colonies reached the maximum only when Ep, GM-CSF, and IL-3 or GM-CSF, IL-3, and G-CSF, respectively, were added together. These results suggest that the colonies induced by IL-3, GM-CSF, and G-CSF are derived from different progenitors.

The in vitro biologic activities of G- and GM-CSF and IL-3 appear to overlap. All these factors promote the formation of GM colonies and, in the presence of erythropoietin (Ep), erythroid bursts in serum-supplemented marrow cell cultures.4 However, a detailed comparison of the biologic activities of these growth factors in different culture conditions has not been reported, and some of the overlap in activities seen may be accounted for by the presence of serum in the cultures.

For this reason we have compared the ability of recombinant human G-CSF, GM-CSF, and IL-3 to induce GM colonies and, in the presence of Ep, erythroid bursts (from BFU-E) in FBS-deprived or FBS-supplemented cultures of progenitor cells from adult human marrow or peripheral blood.

MATERIALS AND METHODS

Cell preparation. Marrow cells, obtained from normal volunteers with their informed consent and under a protocol approved by the institution’s human subjects review committee, were aspirated from the posterior iliac crest into heparinized syringes. In some cases, heparinized peripheral blood was also collected. Specimens were then diluted 1:1 with Isscove’s modified Dulbecco’s medium (IMDM; GIBCO, Grand Island, NY) and the marrow cells suspended with a pipette. The light-density cell fraction was separated by Ficoll (Organon-Teknika Corp, Durham, NC) gradient centrifugation (400 g for 20 minutes). Adherent cells were removed by a double incubation of the cell suspension in plastic flasks containing $10^6$ cells/mL of IMDM supplemented with 10% (vol/vol) FBS (HyClone, Logan, UT) at $37^\circ$C and 5% CO₂ in air. The first incubation was either overnight (overnight adherence) or for one hour (one-hour incubation). The second incubation was for one hour in both cases. The nucleated cell recovery was 40% to 60%. The BFU-E and CFU-GM recovery was 97% and 115%, respectively. The final monocyte count was evaluated by AS-D acetate esterase staining and was 3% and 6% for the one-hour or overnight adherence procedures, respectively. The cells were then washed twice with IMDM containing 1% bovine serum albumin (BSA; Sigma Chemical Co, St Louis) before being plated. In some experiments T lymphocytes were also depleted by a modification (P. Simmons, personal communication) of the soybean agglutination method of Reiser et al.1 In this case, the nucleated cell recovery was 15% to 25%, and the final concentration of AS-D acetate-positive or CD2- and CD3-positive cells was 1%. CD2- and CD3-positive cells were evaluated by incubating the cells for half an hour with the murine monoclonal antibodies 35.1 and 64.1, which recognize the CD2 and CD3 epitopes, respectively, on normal human T lymphocytes4 at saturating concentrations. The cells were washed and incubated with goat antimouse IgG-fluorescein isothiocyanate–conjugated (Southern Biotechnology Associates, Birmingham, AL)
HEMATOPOIETIC COLONY GROWTH IN SERUM-DEPRIVED CULTURE

Table 1. GM Colony Growth in FBS-Supplemented or FBS-Deprived Cultures of Marrow Cells Prepared by Different Adherent Cell Removal Procedures

<table>
<thead>
<tr>
<th>Stimulus*</th>
<th>GM Colonies/3 × 10² Cells</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Overnight Adherence</td>
</tr>
<tr>
<td></td>
<td>FBS+</td>
</tr>
<tr>
<td>None</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>PHA-LCM</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>G-CSF</td>
<td>62 ± 24</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>65 ± 14</td>
</tr>
<tr>
<td>IL-3</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>G-CSF + GM-CSF + IL-3</td>
<td>116 ± 13</td>
</tr>
</tbody>
</table>

*The growth factors were added to culture in the following concentrations: G-CSF, 2.3 × 10⁻¹⁰ mol/L; GM-CSF, 4.5 × 10⁻¹⁰ mol/L; IL-3, 2 × 10⁻¹¹ mol/L. PHA-LCM was added at 3%, vol/vol. The concentrations chosen for PHA-LCM and the growth factors had been shown by previous experiments to give optimal colony numbers under these culture conditions. Results are shown as the mean ± SD of six experiments performed in duplicate.

RESULTS

The effects of recombinant growth factors on GM colony formation. CFU-GM growth in FBS-supplemented and FBS-deprived cultures is compared in Table 1. No GM colonies were observed in FBS-deprived cultures in the absence of stimulus, whereas endogenous colonies were detectable in FBS-supplemented cultures.

When marrow cells were subjected to overnight adherence, 33 ± 6 and 132 ± 10 GM colonies were observed in the presence of PHA-LCM in FBS-deprived and FBS-supplemented cultures, respectively. The colonies contained 10² to 10³ cells in both cases. The degree of maturation of the cells in GM colonies was similar in FBS-supplemented and FBS-deprived cultures. Figure 1 presents the GM-CSF concentration/response curves in FBS-supplemented and FBS-deprived cultures. The curves are almost superimposable, and no differences were seen in the maximal number of GM colonies detected.

for half an hour. All the procedures were performed at 4°C. After being washed, the cells were analyzed on a FACS 440 (Becton Dickinson, Mountain View, CA). Cells incubated with an irrelevant antibody were used as a negative control. A quantity of 5 × 10⁴ were analyzed without detection of fluorescence-positive cells.

Hematopoietic growth factors. The purified recombinant human hematopoietic growth factors used included Ep, G-CSF (Amgen Corp., Thousand Oaks, CA), and GM-CSF. IL-3 was present in conditioned medium from COS-1 cells transfected with a plasmid designed for expression of human IL-3 (Genetics Institute, Cambridge, MA). The concentration of IL-3 was 10 U/mL of conditioned medium, and the specific activity was 10⁷ U/µg of protein (S. Clark, personal communication). In control experiments, a volume of sham-transfected COS-1 cell supernatant corresponding to the maximal IL-3 concentration used was added to the cultures. The control supernatant did not induce colony formation and did not reduce the number of colonies detected in cultures to which phytohemagglutinin-stimulated, lymphocyte-conditioned medium (PHA-LCM) was added under FBS-supplemented conditions (data not shown). PHA-LCM, prepared serum free, was used as a positive control.

FBS-supplemented culture. Each 1-mL dish contained the following components in IMDM: methylcellulose (0.8% wt/vol, final concentration), β-mercaptoethanol (7.5 × 10⁻⁴ mol/L), antibiotics (100 units of penicillin, 250 mg of amphotericin, and 100 µg of streptomycin), a selected heat-inactivated lot of FBS (40%, v/v), L-glutamine (2 × 10⁻³ mol/L), and 3 × 10⁶ nonadherent T-depleted marrow cells (unless otherwise stated).

FBS-deprived culture. FBS was substituted by deionized BSA (2 × 10⁻⁴ mol/L), BSA-absorbed cholesterol (4 µg/mL) and soybean lecithin (12 µg/mL), iron-saturated human transferrin (5 × 10⁻⁷ mol/L), insulin (1.7 × 10⁻⁴ mol/L), nucleosides (10 µg/mL each), inorganic salts, sodium pyruvate (10⁻⁴ mol/L), and L-glutamine (2 × 10⁻⁷ mol/L) as described. All the chemicals were obtained from Sigma.

Cultures were established in duplicate. Plates were incubated at 37°C in a fully humidified incubator with 5% CO₂ in air and scored at 12 to 14 days for the presence of erythroid bursts and GM colonies. The concentration/response curves for at least three growth factors were determined simultaneously in FBS-supplemented and FBS-deprived cultures. Mean values (±SD) from at least three separate experiments are presented.

Scoring criteria. Erythroid bursts (containing ≥ 200 cells) were identified on the basis of their orange-red color. Colonies composed of more than 50 nonerythroid cells were scored as GM colonies. In some experiments, single colonies were harvested, cytto centrifuged, and stained with May-Grünwald/Giemsa. In these experiments G and M colonies were counted separately.
When marrow cells were subjected to two one-hour adherence steps, GM-CSF and PHA-LCM were one half and one third less able to induce GM colonies in FBS-deprived cultures when compared with cells prepared by overnight adherence (Table 1). The effects of varying concentrations of GM-CSF on marrow progenitor cells subjected to two one-hour adherence steps are shown in Fig 2A. No significant differences were found in the GM-CSF concentration/response curves in FBS-supplemented cultures with progenitor cells obtained after overnight adherence or two one-hour adherence steps (solid lines and circles, Figs 1 and 2A). However, the number of GM colonies induced by GM-CSF in FBS-deprived cultures was only one third of the colonies detected in similar FBS-supplemented cultures, and no increase in colony numbers was observed with increasing GM-CSF concentrations above $4.5 \times 10^{-11}$ mol/L, the lowest concentration tested. The variability between different donors was more evident in this series of experiments as compared with the experiments with cells prepared by overnight adherence (cf the SD in Figs 1 and 2A).

The effects of G-CSF and IL-3 on GM colony formation were equivalent whether the marrow cells were prepared by overnight adherence or by two one-hour adherence steps (Table 1). Recombinant human G-CSF induced the formation of GM colonies in FBS-supplemented and FBS-deprived cultures in a similar concentration-dependent fashion (Fig 2B). However, the maximal number of colonies induced by G-CSF under FBS-deprived conditions was half the number of colonies induced in FBS-supplemented cultures. The mean cell number per colony was also different in the two culture systems (50 to 200 v 102 to 103 cells/colony in FBS-deprived and FBS-supplemented cultures, respectively). Furthermore, although G, M, and GM colonies were observed in FBS-supplemented cultures, only G colonies were observed in

Fig 2. The effect of increasing concentrations of GM-CSF (A), G-CSF (B), and IL-3 (C) on GM colony growth in FBS-supplemented (■) or FBS-deprived (○) marrow cell cultures. The cells were prepared by two one-hour periods of adherence to plastic. The results are expressed as means ± SD of three separate experiments performed in duplicate.
HEMATopoietic colony growth in serum-deprived culture

FBS-deprived cultures. This was confirmed by removing colonies from the dish with a thin Pasteur pipette and staining the cells. No AS-D acetate esterase-positive cells were detected.

IL-3 induced the formation of GM colonies in FBS-supplemented cultures in a concentration-dependent fashion (Fig 2C). In contrast, almost no colonies were induced by IL-3 under FBS-deprived conditions. The number of colonies detected at the maximal IL-3 concentration used was only 10% of the number of colonies observed with the same concentration in FBS-supplemented cultures.

Effect of combinations of G-CSF, GM-CSF, and IL-3 on the formation of GM colonies. In FBS-supplemented cultures, no single growth factor induced colony numbers comparable to those obtained with PHA-LCM (Table 1). Furthermore, the maximal colony number observed with each growth factor in FBS-deprived cultures was lower than the colony numbers detected in identical FBS-supplemented cultures. For this reason, we studied the effect of optimal concentrations of GM-CSF and/or the highest IL-3 concentration feasible on the numbers of colonies induced by varying concentrations of G-CSF.

In FBS-supplemented cultures, increasing concentrations of G-CSF increased the number of GM colonies detected in the presence of optimal/maximally tested concentrations of GM-CSF or IL-3 or the combination of GM-CSF and IL-3 (Fig 3A). The greatest increase was observed when both GM-CSF and IL-3 were present. The concentration of G-CSF that gave plateau numbers of GM colonies was dependent on which other growth factor was present. In the presence of GM-CSF, the optimally effective concentration of G-CSF was $4.5 \times 10^{-10}$ mol/L. In the presence of IL-3 or IL-3 and GM-CSF, colony numbers continued to increase up to G-CSF concentrations of $4.5 \times 10^{-10}$ mol/L. The number of colonies detected with various combinations of G-CSF, GM-CSF, and IL-3 corresponded approximately to the sum of the number of colonies detected with each growth factor alone.

In FBS-deprived cultures, IL-3 did not increase the number of colonies induced by G-CSF alone (Fig 3B). In contrast, GM-CSF or the combination of GM-CSF plus IL-3 increased the total number of GM colonies detected in the presence of G-CSF. In particular, in cultures containing all three growth factors, the number of colonies observed under FBS-deprived conditions reached the values observed in the FBS-supplemented cultures stimulated with PHA-LCM (Table 1). The increase in colony numbers was associated with a progressive increase in colony size.

Effect of recombinant growth factors on erythroid burst growth. BFU-E growth in FBS-supplemented and FBS-deprived cultures is compared in Table 2. When marrow cells were subjected to overnight adherence, Ep induced up to 50% of the erythroid bursts detected in the presence of PHA-LCM (Table 2) both in FBS-supplemented and FBS-deprived cultures. This result suggested that significant amounts of burst-promoting activity are released by accessory cells in these cultures. GM-CSF and IL-3 increased by 0.5 to 2.5-fold the number of erythroid bursts detected in the presence of Ep.

Fig 3. The effect on GM colony growth of increasing concentrations of G-CSF alone (A) or G-CSF plus IL-3 ($2 \times 10^{-11}$ mol/L) (C), GM-CSF ($4.5 \times 10^{-10}$ mol/L) (E), or IL-3 ($2 \times 10^{-11}$ mol/L) plus GM-CSF ($4.5 \times 10^{-10}$ mol/L) (B) and in FBS-supplemented (A) or FBS-deprived (B) cultures. The cells were prepared by two one-hour periods of adherence to plastic. The results are expressed as the mean percentage ± SD of PHA-LCM-stimulated, FBS-supplemented cultures. Three separate experiments were performed in duplicate (100% = 122 ± 22 GM colonies/3 x 10^6 nonadherent marrow cells).
When marrow cells were subjected to two one-hour adherence steps, almost no erythroid bursts were observed in the presence of Ep alone in FBS-deprived cultures, at least at the cell concentrations used (Table 2 and Fig 4A). However, half-maximal numbers of erythroid bursts were still observed in FBS-supplemented cultures in the presence of Ep alone.

The response to varying concentrations of Ep was determined in FBS-supplemented or FBS-deprived cultures with or without optimal/maximally tested concentrations of GM-CSF (Fig 4B), IL-3 (Fig 4C), and GM-CSF plus IL-3 (Fig 4D). In FBS-supplemented cultures, IL-3 increased by twofold the number of erythroid bursts detected. GM-CSF did not increase the number of erythroid bursts but increased the sensitivity of BFU-E to Ep: half-maximal burst numbers were observed at 0.5 to 1 and ≈0.1 U Ep/mL in cultures not supplemented with GM-CSF and in cultures supplemented with GM-CSF, respectively (Fig 4A and 4B). The simultaneous addition to the cultures of GM-CSF and IL-3 did not further increase the number of bursts detected.

Under FBS-deprived conditions, a linear relationship between the concentration of Ep and the number of erythroid bursts detected was observed either in the presence of GM-CSF or IL-3. In the presence of GM-CSF, the number of erythroid bursts detected was 30% of the PHA-LCM control, 70% in the presence of IL-3, and 110% when both factors were present (Table 2).

The effects of increasing concentrations of GM-CSF and IL-3 on erythroid burst growth in FBS-supplemented and FBS-deprived cultures are compared in Fig 5. Both GM-CSF (Fig 5A) and IL-3 (Fig 5B) induced erythroid burst growth in FBS-deprived cultures. The maximal effect of GM-CSF was observed at ≈4.5 x 10^{-11} mol/L. IL-3 induced erythroid bursts in a concentration-dependent fashion, the plateau effect being reached at 10^{-11} mol/L.

G-CSF slightly increased the number of erythroid bursts detected in FBS-supplemented cultures but failed to induce any erythroid bursts in FBS-deprived cultures (results not presented).

**Effect of recombinant growth factors on the formation of GM colonies and erythroid bursts from marrow cells depleted of adherent cells and T lymphocytes.** In FBS-deprived cultures of nonadherent marrow cells, the combinations of GM-CSF, IL-3, and G-CSF or GM-CSF, IL-3, and

Ep induced GM colonies or erythroid bursts in numbers equivalent to those obtained in FBS-supplemented cultures stimulated with PHA-LCM. The effect of those combinations of recombinant growth factors on colony formation was also investigated with marrow progenitor cells depleted of monocytes and T-lymphocytes by soybean agglutination. The results are summarized in Table 3. In FBS-supplemented cultures stimulated with PHA-LCM or PHA-LCM and Ep, the numbers of GM colonies and erythroid bursts were roughly proportional to the number of cells plated. In contrast, the effect of the combinations of growth factors on GM colony and erythroid burst growth in FBS-deprived cultures was dependent on the number of cells plated. At 10^6 cells/mL, IL-3, GM-CSF, and G-CSF induced 78% of the GM colony growth observed in PHA-LCM-stimulated, FBS-supplemented cultures. However, when 3 x 10^5 cells were plated, these factors did not induce GM colonies. Equivalent results were observed for erythroid bursts. GM-CSF and IL-3 induced erythroid bursts as efficiently as did PHA-LCM in FBS-supplemented cultures at 10^6 cells/mL but had little effect on burst growth at 3 x 10^5 cells/mL (Table 3).

**Effect of recombinant growth factors on the formation of erythroid bursts from peripheral blood cells depleted of adherent cells and T lymphocytes.** The effect of recombinant growth factors on peripheral blood erythroid burst growth is presented in Table 4. When the Ficoll fraction was used as the source of progenitor cells, Ep alone was sufficient to induce maximal numbers of erythroid bursts either in the presence or absence of FBS. The further addition of PHA-LCM, GM-CSF, or IL-3 did not increase the number of erythroid bursts detected. The removal of adherent cells reduced the proportion of erythroid bursts detected in the presence of Ep alone. This reduction was particularly evident in FBS-deprived cultures. As reported for nonadherent marrow progenitors in FBS-supplemented cultures, GM-CSF and IL-3 increased the number of erythroid bursts to a similar degree. The number of erythroid bursts did not increase further when both factors were added together. Under FBS-deprived conditions, the number of erythroid bursts detected in cultures stimulated with IL-3 was threefold greater than the number of colonies stimulated with GM-CSF. The number of colonies detected increased when both growth factors were present. The similarity of these data with those described for nonadherent marrow progenitors was confirmed by experiments in which cultures of blood and marrows of the same donors were compared (data not shown).

In subsequent studies, the effect of varying concentrations of IL-3 on nonadherent, T-cell-depleted peripheral blood cells was studied in FBS-supplemented or FBS-deprived cultures. IL-3 induced the formation of erythroid bursts in a concentration dependent fashion, the plateau being reached both in FBS-supplemented and FBS-deprived cultures at 10^{-11} mol/L (Fig 6).

**DISCUSSION**

We have compared the biologic activities of recombinant human Ep, G-CSF, GM-CSF, and IL-3 on the formation of
Fig 4. The effect of increasing concentrations of Ep alone on erythroid burst growth (A); in the presence of GM-CSF (4.5 x 10^{-10} M) (B), IL-3 (2 x 10^{-11} mol/L) (C), or GM-CSF (4.5 x 10^{-10} mol/L) plus IL-3 (2 x 10^{-11} mol/L) (D); and in FBS-supplemented (◊) or FBS-deprived (○) cultures. The cells were prepared by two one-hour periods of adherence to plastic. The results are expressed as the mean percentage ± SD of PHA-ICM-stimulated, FBS-supplemented cultures. Three separate experiments were performed in duplicate (100% = 50 ± 12 bursts/3 x 10^5 nonadherent marrow cells).

The biologic activities of the factors in FBS-deprived cultures were different and were affected by the method used to purify the progenitor cells, in particular by techniques that might activate accessory cells. In FBS-deprived cultures, Ep and GM-CSF still were able to induce half-maximal numbers of erythroid bursts and GM colonies, respectively, if the adherent cells were removed by overnight incubation. In contrast, almost no erythroid bursts and only 30% of the GM colonies were detected in the presence of Ep or GM-CSF if the adherent cells were removed by two one-hour incubation steps. Because the cloning efficiency of erythroid bursts and GM colonies in FBS-supplemented cultures was not affected
by the procedure used to remove the adherent cells, the different activities of the growth factors under FBS-deprived conditions could not be explained by the loss of a subpopulation of colony-forming cells due to the adherence to the plastic during overnight incubation. Furthermore, the effects of the growth factors were compared directly against target cells prepared by the two different adherence procedures, and the differences observed cannot be attributed to the marrow donor. Rather, it is likely that synergistic factors are released by the overnight incubation of the marrow cells. Although the number of residual AS-D acetate esterase-positive cells was greater in the marrow preparation subjected to overnight adherence, this fact alone does not explain the different amounts of factors released in the two systems. We propose that factors present in the FBS or released by macrophages activate other accessory cells during the overnight incubation. In this regard, it is known that T-lymphocytes must be activated to produce GM-CSF and activated macrophages release IL-1 and tumor necrosis factor α, both capable of activating T-cells.

The biologic activity of G-CSF in FBS-deprived conditions was independent of the procedure used to remove the adherent cells and was restricted to the granulocytic lineage. G-CSF induced the formation of pure G colonies in a concentration-dependent fashion similar to that observed in cultures supplemented with FBS. Similar results have been

Table 3. GM Colony and Erythroid Burst Growth in FBS-Supplemented or FBS-Deprived Cultures

<table>
<thead>
<tr>
<th>Stimulus*</th>
<th>GM Colonies/3 × 10^5 Cells</th>
<th>GM Colonies/10^5 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FBS+ %</td>
<td>FBS- %</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PHA-LCM</td>
<td>29 ± 4</td>
<td>100</td>
</tr>
<tr>
<td>IL-3 + GM-CSF + G-CSF</td>
<td>41 ± 11</td>
<td>141</td>
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</table>

<table>
<thead>
<tr>
<th>Erythroid Bursts/3 × 10^5 Cells</th>
<th>Erythroid Bursts/10^5 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS+ %</td>
<td>FBS- %</td>
</tr>
<tr>
<td>Ep</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>PHA-LCM + Ep</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>IL-3 + GM-CSF + Ep</td>
<td>—</td>
</tr>
</tbody>
</table>

Target cells were nonadherent, T-cell-depleted marrow cells. The results shown are the means ± SD of three separate experiments performed in duplicate.

*The concentrations of the various CSFs and of PHA-LCM used are the same as those given in the footnote to Table 1.

Fig 5. The effect of increasing concentrations of GM-CSF (A) or IL-3 (B) on erythroid burst growth in FBS-supplemented (●) or FBS-deprived (○) marrow cell cultures. The cells were prepared by two one-hour periods of adherence to plastic, and all cultures contained 1.5 U Ep/ml. The results are expressed as means ± SD of three separate experiments performed in duplicate.
HEMATOPOIETIC COLONY GROWTH IN SERUM-DEPRIVED CULTURE

Table 4. Peripheral Blood Erythroid Burst Growth in FBS-Supplemented or FBS-Deprived Cultures

<table>
<thead>
<tr>
<th>Stimulus*</th>
<th>None</th>
<th>GM-CSF</th>
<th>IL-3</th>
<th>G-CSF</th>
<th>GM-CSF + IL-3</th>
<th>+ PHA-LCM</th>
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<tbody>
<tr>
<td>Ficoll fraction†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FBS+</td>
<td>20 ± 8</td>
<td>24 ± 4</td>
<td>28 ± 2</td>
<td>—</td>
<td>—</td>
<td>24 ± 4</td>
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<tr>
<td>FBS−</td>
<td>22 ± 8</td>
<td>25 ± 4</td>
<td>19 ± 6</td>
<td>—</td>
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<td>25 ± 9</td>
</tr>
<tr>
<td>Nonadherent fraction‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS+</td>
<td>27 ± 10</td>
<td>36 ± 16</td>
<td>60 ± 19</td>
<td>—</td>
<td>63 ± 19</td>
<td>—</td>
</tr>
<tr>
<td>FBS−</td>
<td>3 ± 3</td>
<td>13 ± 3</td>
<td>36 ± 5</td>
<td>—</td>
<td>62 ± 18</td>
<td>—</td>
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<tr>
<td>Nonadherent T-cell−depleted fraction§</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FBS+</td>
<td>9 ± 2</td>
<td>66 ± 14</td>
<td>110 ± 15</td>
<td>3 ± 1</td>
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<tr>
<td>FBS−</td>
<td>1 ± 1</td>
<td>50 ± 12</td>
<td>113 ± 14</td>
<td>4 ± 1</td>
<td>—</td>
<td>18 ± 4</td>
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</table>

*All the cultures contained 1.5 units of recombinant human Ep. For the concentrations of the recombinant CSFs and PHA-LCM see the footnote to Table 1.
†A quantity of 3 × 10⁶ cells were plated per milliliter of culture, and the results shown are the means ± SD of five separate experiments.
‡A quantity of 1.5 × 10⁶ cells were plated per milliliter of culture, and the results shown are the means ± SD of seven separate experiments.
§A quantity of 3 × 10⁶ cells were plated per milliliter of culture, and the results shown are the means ± SD of three separate experiments.

reported by Ohara et al. However, the number of colonies induced in FBS-deprived cultures was only half the number observed in FBS-supplemented cultures.

The biologic activities of IL-3 and GM-CSF overlap also in FBS-deprived cultures: both factors require other growth factors to induce GM colonies and induce erythroid bursts in the presence of Ep. However, there is some lineage restriction: IL-3 is two- to threefold more active than is GM-CSF on erythroid burst growth, whereas GM-CSF is two- to threefold more active than is IL-3 on GM colony formation.

The effect of combinations of growth factors on GM colony and erythroid burst formation was also investigated in cultures deprived of FBS. IL-3 failed to increase the number of colonies detected in cultures stimulated with G-CSF, whereas GM-CSF did enhance colony numbers. However, IL-3 increased by one fourth the number of colonies detected in the presence of both GM-CSF and G-CSF. This fact and the fact that erythroid bursts under these conditions reached the values observed in FBS-supplemented cultures when both IL-3 and GM-CSF were present suggest that these factors may act on different progenitors. In particular, in the myeloid lineage IL-3 may act on less differentiated progenitor cells that do not respond directly to GM-CSF and G-CSF. This interpretation would be in agreement with the biologic activity reported for murine IL-3.

To further clarify whether GM-CSF and IL-3 directly stimulate the formation of GM colonies and erythroid bursts, studies were done with nonadherent, T-cell−depleted marrow or peripheral blood cells. IL-3 and GM-CSF induced GM colonies and, in the presence of Ep, erythroid bursts in numbers comparable to those observed in cultures of nonadherent cells. However, in the marrow cell cultures under FBS-deprived conditions, colony growth was also dependent on the number of cells plated. This result raises the possibility of an indirect effect of IL-3 and GM-CSF on the growth of erythroid bursts and GM colonies. However, it is also possible that a still-unidentified growth factor is required for the formation of hematopoietic colonies in culture that was provided by the limited numbers of accessory cells present.

ACKNOWLEDGMENT

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In vitro differentiation of human granulocyte/macrophage and erythroid progenitors: comparative analysis of the influence of recombinant human erythropoietin, G-CSF, GM-CSF, and IL-3 in serum-supplemented and serum-deprived cultures

G Migliaccio, AR Migliaccio and JW Adamson