Growth of Clonogenic Myeloblastic Leukemic Cells in the Presence of Human Recombinant Erythropoietin in Addition to Various Human Recombinant Hematopoietic Growth Factors

By Yoshinobu Asano, Seiichi Okamura, Tsunefumi Shibuya, Mine Harada, and Yoshiyuki Niho

The effects of human recombinant erythropoietin (rEpo) in the presence of other stimulators on the growth of clonogenic leukemic blast cells from ten Japanese patients with acute myeloblastic leukemia were studied with an in vitro leukemic blast colony assay in methylcellulose culture. With the addition of rEpo alone, no leukemic blast colony formation was stimulated in any of the cases examined. However, when rEpo and phytohemagglutinin lymphocyte-conditioned medium (PHA-LCM) were added to the culture simultaneously, in contrast to results with PHA-LCM alone, the number of leukemic blast colonies formed was significantly increased in two of the ten cases (P < .01). These two cases were classified as M1 according to the French-American-British (FAB) classification. This enhancing effect of rEpo was observed with human recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF) or human recombinant interleukin-3 (rIL-3) but was not observed with human recombinant granulocyte CSF (rG-CSF).

From the First Department of Internal Medicine and Cancer Center, Faculty of Medicine, Kyushu University, Japan. Submitted March 14, 1988; accepted July 11, 1988.


Address reprint requests to Yoshiyuki Niho, MD, the First Department of Internal Medicine, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.

Vol 72, No 5 (November), 1988: pp 1682-1686


From www.bloodjournal.org by guest on November 15, 2017. For personal use only.
TABLE 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis (FAB)*</th>
<th>Leukocytes (per μL)</th>
<th>Blasts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>M</td>
<td>M1</td>
<td>43,800</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>F</td>
<td>M1</td>
<td>12,900</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>F</td>
<td>M2</td>
<td>9,400</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>M</td>
<td>M4</td>
<td>125,500</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>M</td>
<td>M4</td>
<td>31,400</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>F</td>
<td>M2</td>
<td>24,100</td>
<td>94</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>M</td>
<td>M2</td>
<td>20,800</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>M</td>
<td>M2</td>
<td>16,000</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>F</td>
<td>M3</td>
<td>6,800</td>
<td>59</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>F</td>
<td>M5</td>
<td>130,000</td>
<td>98</td>
</tr>
</tbody>
</table>

*FAB classification was determined as previously described.10

RESULTS

We examined the effects of rEpo on the growth of clonogenic leukemic blast cells from ten AML patients with an in vitro leukemic blast colony assay. GPA on the surface of blast cells from nine patients was also examined. In no case were blast cells significantly stained with anti-GPA antibody.

The numbers of blast colonies formed with the addition of each stimulator are shown in Table 2. With the addition of 1 U/mL rEpo alone, no leukemic blast colonies were formed in any case except in cases 4 and 6, in which spontaneous colonies were formed without addition of stimulator and colony growth was not further stimulated by the addition of rEpo. On the other hand, with addition of 10% PHA-LCM in all cases examined, many colonies were observed, although marked variation in responsiveness of clonogenic cells to PHA-LCM was also observed. Spontaneous colony growth was further stimulated by addition of PHA-LCM in cases 4 and 6.

Table 2. Effect of rEpo Addition With PHA-LCM on Leukemic Blast Colony Formation

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Without rEpo</th>
<th>With rEpo (1 U/mL)</th>
<th>PHA-LCM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>253 ± 18</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>623 ± 38</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>395 ± 57</td>
</tr>
<tr>
<td>4</td>
<td>44 ± 7</td>
<td>41 ± 4</td>
<td>219 ± 16</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>6</td>
<td>135 ± 20</td>
<td>110 ± 23</td>
<td>240 ± 31</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>283 ± 22</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>159 ± 11</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>68 ± 6</td>
</tr>
</tbody>
</table>

*Numbers of leukemic colonies are shown as means ± SD/10^6 cells as determined from triplicate plates.
†Significantly different by unpaired t test.
‡P < .01.

Fig 1. Dose–response relationships between concentration of added rEpo with 10% PHA-LCM and enhanced effects on blast colony formation. Values are normalized to the stimulatory effect of 10% PHA-LCM alone of 100. Data shown are means of triplicate cultures. Peripheral blood cells from ten patients with AML were tested. Case nos. correspond to those in Table 1. Raw colony counts corresponding to 100% stimulation are shown in Table 2. Case 1 (O—O), case 2 (C—C), case 3 (■—■), case 4 (V—V), case 5 (A—A), case 6 (x—x), case 7 (□—□), case 8 (■—■), case 9 (V—V), case 10 (A—A).

rEpo and PHA-LCM were then added to cultures simultaneously to detect any synergistic effects between rEpo and PHA-LCM on leukemic blast colony formation. In two of the ten cases (cases 1 and 2), the numbers of leukemic blast colonies stimulated by rEpo plus PHA-LCM were increased significantly in comparison with those treated with PHA-LCM alone (P < .01). The leukemic types of these two cases were both diagnosed as M1. No significant differences in leukemic blast colony size or cell morphology within the colonies, which were similar to the patients’ blast cells, were observed between rEpo plus PHA-LCM and PHA-LCM alone.

We then added various doses of rEpo to the cultures simultaneously with 10% PHA-LCM to examine the correlation between the rEpo concentration and the percentage of enhancement of leukemic blast colony growth (Fig 1). The leukemic blast colony growth was enhanced with increasing rEpo concentration at >0.5 U/mL with 10% PHA-LCM added. The most remarkable enhancing effect was observed between 1 and 2 U/mL rEpo; therefore, all further experiments in this study were performed with 1 U/mL rEpo added.

Next, we examined the types of recombinant hematopoietic growth factors that were able to produce the phenomenon described above. The numbers of blast colonies formed with rEpo added along with other recombinant hematopoietic growth factors are shown in Table 3. A substantial increase in the number of colonies was produced by addition of rEpo with rGM-CSF in five cases (cases 1 through 5) and with rIL-3 in three cases (cases 1, 3, and 4), whereas the stimulatory effect of rG-CSF was not enhanced by addition of rEpo in any case.
Dose–response curves of leukemic blast colonies formed by the addition of rGM-CSF with and without rEpo in two cases are shown in Fig 2. In both cases, the stimulatory effects on colony formation reached a plateau level at a rGM-CSF concentration of 100 U/mL, irrespective of the presence of rEpo. In addition, the number of leukemic blast colonies stimulated by rEpo plus rGM-CSF was greater than that stimulated by rGM-CSF alone at each concentration of rGM-CSF, and the slopes of the dose–response curves did not differ very much, i.e., the doses of rGM-CSF for half-maximum stimulation were almost the same.

**DISCUSSION**

Leukemic blast CSA has been demonstrated in PHA-LCM1 or human bladder carcinoma cell line 5637-conditioned medium.18 With the recent development of genetic engineering, human recombinant hematopoietic growth factors have become available for the study of CSA. Human GM-CSF,23 G-CSF,46 and IL-367 are capable of stimulating clonogenic myeloblastic leukemic blast cells. Recently, rEpo was shown to stimulate proliferation of clonogenic erythroid leukemic blast cells from human erythroleukemia patients,19 and some murine erythroleukemia cell lines have membrane receptors for Epo.2021 Because the effects of rEpo on the growth of clonogenic myeloblastic leukemic cells have not been well documented, however, we examined the effects of rEpo on growth of clonogenic myeloblastic leukemic blast cells to determine whether any synergistic effects occurred with other recombinant hematopoietic growth factors.

We observed that rEpo alone was unable to stimulate myeloblastic leukemic blast colony growth in any of the cases examined. However, when rEpo and PHA-LCM were added to cultures simultaneously, the number of leukemic blast colonies formed was significantly increased in two of ten cases in comparison with PHA-LCM alone. Because PHA-LCM contains several kinds of CSF,22 we examined which factors contributed to these rEpo-enhancing effects, using human recombinant CSFs; an enhancing effect of rEpo was observed with either rGM-CSF or rIL-3 added, but not with rG-CSF added.

Two possibilities for the mechanism of this rEpo-enhancing effect were considered. The first possibility was that rEpo directly or indirectly increased the sensitivity of clonogenic leukemic blast cells to rGM-CSF or rIL-3. The second was the presence of some clonogenic cell populations requiring rEpo and either rGM-CSF or rIL-3 simultaneously for growth. To clarify this, we examined the dose–response
curves of leukemic blast colonies formed by addition of rGM-CSF with and without rEpo, as shown in Fig 2. Colony formation was dependent on the GM-CSF, and the number of colonies formed with rEpo was consistently greater at every examined concentration of GM-CSF. The slopes of the dose–response curves did not differ greatly, and the doses of GM-CSF for half-maximum stimulation were nearly the same. From these findings, we deduced that a population exists requiring both GM-CSF and Epo for blast colony formation in addition to another population that can form colonies responding to GM-CSF alone. A similar phenomenon involving synergism between GM-CSF and G-CSF has been reported in some AML cases, and this was also attributed to the presence of clonogenic cell populations requiring both GM-CSF and G-CSF for growth.

In normal hematopoiesis, Epo acts as a growth factor for erythropoiesis. However, experiments in a mouse model showed that Epo is not required for erythroid burst or, probably, for multipotential colony formation, during the first few days of culture in the presence of IL-3, even though Epo is required for the final stages of differentiation. Our present results showed that rEpo alone was unable to stimulate the growth of clonogenic myeloblastic leukemic cells, suggesting that these cells have almost no receptors for Epo before culture. One possible interpretation is that the expression of Epo receptors occurred during the course of culture.

Recently, many instances of leukemic cells bearing mixed-lineage surface markers have been reported. The mechanism of mixed leukemia has been proposed to be aberrant gene expression in leukemic cells (lineage infidelity), or leukemic change occurring in multipotential progenitors (lineage promiscuity). In the same way, we offer two hypotheses as to why rEpo affected the growth of clonogenic leukemic blast cells in the presence of rGM-CSF or rIL-3: the first hypothesis is aberrant gene expression of the Epo receptor on leukemic blast cells in the presence of rGM-CSF or rIL-3, and the second alternative is leukemic change occurring in multipotential progenitors that have the potential to differentiate into either erythroid or myeloid cells. We ruled out the possibility of leukemia-containing erythroid components in our study using anti-GPA antibody. Our results also showed that enhancing effects of rEpo could be observed with rGM-CSF or rIL-3 added but not with rG-CSF. In regulation of normal granulopoiesis, GM-CSF and IL-3 stimulate growth of immature progenitor cells, whereas G-CSF is considered to stimulate growth of more mature cells. These findings suggest that the enhancing effects of rEpo are caused by leukemic change occurring in multipotential progenitors. Furthermore, two cases, in which the enhancing effects of rEpo were remarkable, were classified as M1, morphologically the most immature type, by FAB classification. Mitjavila et al reported that erythroid clonogenic leukemic blast cells required only Epo for colony formation and that leukemic change was considered to have occurred at the CFU-E level in two cases of acute erythroid leukemia, whereas the clonogenic cells required Epo and GM-CSF simultaneously for colony formation and leukemic change was considered to have occurred at the BFU-E level in three cases of erythroid blast crisis of chronic myeloid leukemia. In our present cases, therefore, leukemic change may have occurred at a more immature level, upon stimulation with GM-CSF plus Epo or GM-CSF alone.

Recently, Epo has been used as a therapeutic agent for anemia, and CSFs have also been considered for use in supportive care of myelosuppression in some patients with AML. Therefore, sufficient studies on the effects of hematopoietic growth factors on clonogenic leukemic blast cells are needed so that these factors can be used clinically in AML patients. Further studies, including analysis of receptors responding to various growth factors to elucidate the definitive mechanism of action of Epo on leukemic blast cells, are also necessary.

ACKNOWLEDGMENT

We are grateful to Drs K. Arai and A. Miyajima (DNAX Research Institute) for providing the recombinant human GM-CSF, to Chugai Pharmaceutical (Tokyo) for providing the recombinant human G-CSF, and to Snow Brand Milk Products (Tokyo) for providing the recombinant human erythropoietin.

REFERENCES


Growth of clonogenic myeloblastic leukemic cells in the presence of human recombinant erythropoietin in addition to various human recombinant hematopoietic growth factors

Y Asano, S Okamura, T Shibuya, M Harada and Y Niho