Effect of Human Recombinant Granulocyte/Macrophage Colony-stimulating Factor and Native Granulocyte Colony-stimulating Factor on Clonogenic Leukemic Blast Cells

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ABSTRACT

The effects of human recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) and human native purified granulocyte colony-stimulating factor (G-CSF) on the growth of clonogenic leukemic blast cells from eight Japanese patients with acute myeloblastic leukemia were studied, using an in vitro leukemic blast colony assay. The results showed that GM-CSF stimulated leukemic blast colony formation in all cases examined, whereas G-CSF stimulated colony formation in four of the eight cases. The maximum stimulating activity of GM-CSF on the growth of clonogenic leukemic blast cells was higher than that of G-CSF in the majority of cases, while sometimes GM-CSF and G-CSF worked synergistically. Thus, the clonogenic leukemic blast cell populations seemed to be heterogeneous with respect to their in vitro response to growth regulators.

INTRODUCTION

LBGFs3 are necessary for the proliferation of clonogenic blast cells from the peripheral blood of the majority of patients with AML, which cells form colonies in cultures made viscous with methylcellulose in vitro (1). Human GM-CSF has been reported to stimulate the proliferation of clonogenic cells in AML (2, 3), and we previously found that conditioned medium of a human bladder cell line, 5637 (HTB9), contains efficient LBGFs including GM-CSF.4 Recently, we also found that human G-CSF was also active as an LBGF at high concentration. In this paper, we describe how the simultaneous addition of GM-CSF and G-CSF to cultures of clonogenic leukemic blast cells from 8 Japanese patients with AML resulted in synergistic action of the LBGFs in some cases.

MATERIALS AND METHODS

Leukemic blast cells from 8 Japanese patients with AML were studied. Leukemic types were diagnosed according to the FAB classification of acute leukemia (4) (Table 1). Mononuclear cells from the peripheral blood were obtained by density gradient centrifugation (5), and T-lymphocytes were removed by erythrocyte rosetting (6). The remaining cells were used for leukemic blast colony assay.

The leukemic blast colony assay was a modification of the technique of Buick et al. (1). Briefly, blast cells were plated in plastic 96-microwell plates in α-medium (GIBCO, Grand Island, NY) containing 0.88% methylcellulose and 10% FCS. Each well, containing between 2 × 10⁴ and 4 × 10⁴ cells in 0.1 ml of medium with appropriate stimulators, was incubated for 6 days in a moist atmosphere of 5% CO₂ in air at 37°C, and colonies that formed containing more than 20 cells were counted using an inverted microscope.

The purified G-CSF (2.5 to 5 × 10⁻¹⁰ units/mg of protein) was a gift from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). This preparation was purified to homogeneity from medium conditioned by a human G-CSF-producing tumor cell line, CHU-2 (7). Details of the recombinant human GM-CSF have been reported previously (8). Activities of colony-stimulating factors were measured using the method of Nicola et al. (9).

The bladder carcinoma cell line, 5637 (10), was provided by Dr. E. A. McCulloch (Ontario Cancer Institute, Toronto, Canada). The cells were grown in α-medium supplemented with 5% FCS, trypsinized at confluency, and expanded 5-fold. After 5 days of culture, the conditioned medium (5637-CM) was harvested and sterilized by filtration (Millipore; 0.22 µm). Maximum blast colony formation was observed following the addition of 50 ng/ml G-CSF or 5% 5637-CM, as previously reported. Therefore the present experiments were done using 100 ng/ml G-CSF or 5% 5637-CM for sufficient stimulation.

RESULTS

Titration curves of leukemic blast colonies formed by the addition of GM-CSF for 6 patients are shown in Fig. 1. Although patient-to-patient variations were evident, a substantial number of colonies were observed when more than 10 units/ml of GM-CSF were added to the culture. In two cases (Nos. 6 and 7), colonies were formed even in the absence of exogenous GM-CSF. The maximum stimulatory effect on colony formation was obtained at a GM-CSF concentration of 1000 units/ml in all cases except for Case 6. All further experiments in this study were therefore performed with the addition of 1000 units/ml GM-CSF.

The numbers of leukemic blast colonies formed following the addition of each factor are shown in Table 1. Marked variation in the responsiveness of clonogenic cells to LBGFs was observed among the cases tested. A substantial number of colonies were observed with the addition of 1000 units/ml GM-CSF in all cases examined, although spontaneous colonies formed in 2 cases. In both of these cases (Nos. 6 and 7), the patients were diagnosed as M4, and clonogenic blast cell growth was further stimulated by the addition of GM-CSF. The maximum stimulating activity of GM-CSF was nearly equal to that of 5637-CM in 3 cases (Nos. 1, 4, and 8), but GM-CSF was less active than 5637-CM in 2 cases (Nos. 3 and 7). On the other hand, a substantial number of colonies were observed with the addition of 100 ng/ml G-CSF in 5 of the 8 cases examined, and spontaneous colonies still formed in 2 cases. In one of the 2 cases showing spontaneous colonies, no further clonogenic blast cell growth was stimulated by the addition of G-CSF. In another case, a substantial stimulating effect of G-CSF on clonogenic blast cells was also observed. G-CSF was a less effective stimulator of clonogenic blast cell growth than GM-CSF in the majority of cases examined.
effects in 5 cases (Nos. 1, 3, 4, 7, and 8), in which 1000 units/
two factors on colony formation. We evaluated synergistic
neously in order to detect any synergistic effects between the
stimulated by GM-CSF and G-CSF in the majority of cases. In
blast colonies stimulated by 5637-CM was nearly equal to that
stimulated by the addition of each factor alone. The number of
ously was greater than the sum of the numbers of colonies
In 2 of these 5 cases (Nos. 3 and 7), the number of blast colonies
Case 8 (• •).
Case 1 (O O); Case 3 (O O); Case 4 (• •); Case 6 (A); Case 7 (•);
ml GM-CSF stimulated maximum colony formation (Fig. 1).
patients with AML were tested. Case numbers correspond to those in Table 1.
centration of recombinant GM-CSF (rGM-CSF). Peripheral blood cells from 5
leukemic blast cells (12). Thus, LBGFs appear to consist of at
presses the GM-CSF gene (11) and that G-CSF, one of the
other factors, since 5637-CM was usually a more active stimu-
this observation, they postulated that 5637-CM might contain
blast colony formation. Although Hoang et al. (3) confirmed
Binect to form blast colonies in methylcellulose culture in
the presence of both GM-CSF and G-CSF. It thus appears that,
in cases where such a synergistic effect is observed, there are
some cell populations which might require the presence of both
GM-CSF and G-CSF simultaneously.

ACKNOWLEDGMENTS
We are grateful to Dr. K. Arai and Dr. A. Miyajima (DNAX Research Institute) for providing the recombinant human GM-CSF, to Dr. E. A. McCulloch (The Ontario Cancer Institute) for providing the HTB9(5637) cell line, and to Chugai Pharmaceutical Co., Ltd., for providing the native purified human G-CSF.

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Platzer, E., Moore, M. A. S., Mertelsmann, R., and Weite, K. Recombinant
human granulocyte colony-stimulating factor: effects on normal and leukemic

Table 1 Effect of recombinant GM-CSF and native G-CSF on leukemic blast colony formation

<table>
<thead>
<tr>
<th>Case</th>
<th>FAB</th>
<th>Medium control</th>
<th>GM-CSF (1000 units/ml)</th>
<th>G-CSF (100 ng/ml)</th>
<th>GM-CSF (1000 units/ml) + G-CSF (100 ng/ml)</th>
<th>5637-CM (10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1</td>
<td>0</td>
<td>400 ± 33*</td>
<td>0</td>
<td>393 ± 42</td>
<td>407 ± 17</td>
</tr>
<tr>
<td>2</td>
<td>M2</td>
<td>0</td>
<td>26 ± 4</td>
<td>0</td>
<td>28 ± 7</td>
<td>153 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>M3</td>
<td>0</td>
<td>420 ± 16</td>
<td>66 ± 9</td>
<td>689 ± 25</td>
<td>644 ± 18</td>
</tr>
<tr>
<td>4</td>
<td>M4</td>
<td>0</td>
<td>47 ± 7</td>
<td>0</td>
<td>33 ± 6</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>M5</td>
<td>0</td>
<td>47 ± 12</td>
<td>7 ± 3</td>
<td>88 ± 4</td>
<td>119 ± 9</td>
</tr>
<tr>
<td>6</td>
<td>M4</td>
<td>183 ± 17</td>
<td>276 ± 15 (93y)</td>
<td>192 ± 20 (9)</td>
<td>313 ± 10 (130)</td>
<td>308 ± 16 (125)</td>
</tr>
<tr>
<td>7</td>
<td>M4</td>
<td>112 ± 15</td>
<td>218 ± 12 (106)</td>
<td>278 ± 36 (166)</td>
<td>424 ± 33 (312)</td>
<td>369 ± 26 (257)</td>
</tr>
<tr>
<td>8</td>
<td>M6</td>
<td>0</td>
<td>592 ± 31</td>
<td>107 ± 14</td>
<td>589 ± 27</td>
<td>569 ± 35</td>
</tr>
</tbody>
</table>

* GM-CSF and G-CSF were mixed in order to detect any synergy between them.
* Mean ± SD of leukemic colonies/10² cells as determined from triplicate plates.
* Numbers in parentheses in Cases 6 and 7, numbers of colonies except spontaneous colonies.

Since no details of synergistic stimulatory effects of GM-CSF and G-CSF on leukemic blast growth have yet been published, we undertook the present experiments in which GM-CSF and G-CSF were added simultaneously to cultures of clonogenic blast cells in order to clarify the interaction between GM-CSF and G-CSF as LBGFs. The results showed that clonogenic leukemic blast cells from some (2 of 5 patients studied) but not all patients with AML were stimulated synergistically to form blast colonies in methylcellulose culture in the presence of both GM-CSF and G-CSF. It thus appears that, in cases where such a synergistic effect is observed, there are some cell populations which might require the presence of both GM-CSF and G-CSF simultaneously.

![Fig. 1. Dose-response relationships between blast colonies formed and concentration of recombinant GM-CSF (rGM-CSF). Peripheral blood cells from 5 patients with AML were tested. Case numbers correspond to those in Table 1. Case 1 (O – – O); Case 3 (O – – O); Case 4 (O – – O); Case 6 (A); Case 7 (•); Case 8 (• •).](image-url)
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