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

LBGFs 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. 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 x 10⁴ and 4 x 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.

RESULTS

Titrination 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 100 ng/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.
EFFECT OF GM-CSF AND G-CSF ON BLAST COLONIES

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>
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<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>45 ± 7</td>
<td>0</td>
<td>53 ± 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>M6</td>
<td>0</td>
<td>276 ± 15 (93%)</td>
<td>192 ± 20 (9)</td>
<td>313 ± 10 (130)</td>
<td>308 ± 16 (125)</td>
</tr>
<tr>
<td>7</td>
<td>M7</td>
<td>183 ± 17</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>M8</td>
<td>112 ± 15</td>
<td>592 ± 0</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.

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.

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.

REFERENCES

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