CONCISE REPORT

Interleukin-6 Enhances Growth Factor-Dependent Proliferation of the Blast Cells of Acute Myeloblastic Leukemia

By T. Hoang, A. Haman, O. Goncalves, G.G. Wong, and S.C. Clark

The effects of recombinant interleukin-6 (IL-6) on the proliferation of blast precursors present in the peripheral blood of patients with acute myeloblastic leukemia (AML) was investigated. IL-6 had little effect by itself; however, it synergized with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) in the stimulation of AML blast colony formation. Responsiveness of blast progenitors to IL-6 was heterogeneous. On normal bone marrow cells the same synergy was observed on granulocyte and monocyte precursors (GM-CFC), while there was no significant effect on erythroid and multipotential precursors.

MATERIALS AND METHODS

AML blasts were obtained by separating the peripheral blood cells from patients at presentation (Hotel-Dieu Hospital, Montreal) using a Ficoll-Hypaque (FH) gradient, as described elsewhere. Mononuclear cells were stored frozen in 10% dimethylsulfoxide in liquid nitrogen.

A cDNA clone encoding IL-6 (pCSF-309) was originally identified by functional mammalian cell-expression cloning using a murine hematopoietic colony formation assay. The recombinant IL-6 was prepared by transfecting COS-1 cells with pCSF-309, as previously described. The conditioned medium from this transfection stimulated half of the maximal murine GM colony formation obtained with a saturating concentration of IL-6 at a final dilution of 1:10,000. rGM-CSF was purified to 99%, with a specific activity of 8 x 10^8 U/mg. One unit of activity corresponds to a half maximal stimulation of cells from a patient with chronic myelogenous leukemia. Growth factors were stored at -70°C in aliquots. Once thawed the aliquot was kept at 4°C for several months without decline in biological activity. Conditioned medium from the HTB9 cells was harvested from confluent cultures, filtered, and stored at 4°C.

Growth factors were added to methylocellulose cultures of AML blasts at the indicated concentrations. Cells were plated in 96-well dishes (Linbro, Flow Lab, McLean, VA) at 7,000 cells per well in presence of fetal calf serum (FCS, 10%; Gibco, Grand Island, NY) in 100 μL of Iscove’s modified Dulbecco’s medium (IMDM, Gibco) vicosified with methylocellulose (1%, Fluka, Switzerland), as described elsewhere.

Normal bone marrow cells were depleted of plastic adherent cells by an overnight incubation in IMDM supplemented with 10% FCS. Nonadherent cells were plated in 35-mm culture dishes (Lux, Miles Scientific, Naperville, IL) in 1 mL of IMDM supplemented with FCS (10%), iron-saturated transferrin (300 μg/mL, Hoechst, Montreal), deionized and delipidated bovine serum albumin (10 mg/mL, BSA, Hoechst), α-thioglycerol (7.5 x 10^-5 mol/L, Sigma, St Louis), and methylocellulose, as described previously.

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RESULTS

Using the cells from one patient (designated AML 1), neither GM-CSF nor G-CSF was very effective in supporting the proliferation of the clonogenic leukemic blasts; at optimal concentrations of either factor (15 U/mL G-CSF or 50 U/mL GM-CSF), the blast colony count obtained was less than 20% of that obtained with HTB9 CM (Fig 1). In comparable cultures with cells from the same patient, IL-6 alone had no effect on AML blast colony formation, but when combined with GM-CSF, IL-6 resulted in a fourfold enhancement of the GM-CSF-dependent colony formation (Fig 1). This synergistic effect of IL-6 proved to be dose dependent and was still significant (twofold to threefold) at a final dilution of 1:50,000. A similar synergy was observed when IL-6 was tested in combination with G-CSF. However, substantially higher concentrations of IL-6 were required to achieve the same colony count obtained with GM-CSF, and no significant synergy was observed with dilutions of IL-6 of 1:1,000 or greater. With the cells from patient AML 1, IL-6 also enhanced the colony formation when tested in the presence of both G- and GM-CSF, but this combination still did not fully reconstitute the growth-promoting activity found in the HTB9 CM.

As documented with other cytokines, the IL-6 responsiveness of the clonogenic cells from different AML patients has proved to be very heterogeneous. The responses of the cells from five different patients are summarized in Table 1. In two cases (AML 2 and AML 7) the responses were very similar to that described in Fig 1. IL-6 alone had little or no effect on the clonogenic AML blasts but significantly enhanced the response of the cells to GM-CSF. The cells from two patients (AML 3 and AML 5) displayed little or no synergy in responsiveness between IL-6 and GM-CSF, although the cells from both patients could be stimulated to form colonies by other combinations or growth factors: AML 3 with G-CSF and IL-1; AML 5 with G-CSF and GM-CSF.

The synergistic activity of IL-6 with other growth factors was not limited to GM-CSF or G-CSF. It was also observed in the presence of IL-3. Thus the clonogenic cells from patients AML 1 and AML 7 demonstrated a synergistic response to IL-6 either in the presence of GM-CSF or IL-3. In contrast, cells from patient AML 3 and AML 5 did not respond to IL-6, either in combination with GM-CSF or IL-3 (Table 1).

Because the growth factor responsiveness of leukemic cells may or may not reflect those of the normal counterparts, the authors tested bone marrow-derived progenitors from normal donors for the ability to respond to IL-6. As found in three of the five AML cases, IL-6 proved to have little ability by itself to support colony formation by normal progenitors but was capable of significantly enhancing colony formation in the presence of GM-CSF. This effect was due to increased numbers of granulocyte/macrophage colonies (GM-CFC), while no effect was observed with either erythroid (BFU-E) or multipotential progenitors (CFU-GEMM; Table 2).

### Table 1. The Effect of rIL-6 on Different AML Blasts

<table>
<thead>
<tr>
<th>Growth Factor Added</th>
<th>AML 1</th>
<th>AML 2</th>
<th>AML 7</th>
<th>AML 3</th>
<th>AML 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>rIL-6</td>
<td>4 ± 0</td>
<td>13 ± 8</td>
<td>28 ± 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>rGM-CSF</td>
<td>12 ± 5</td>
<td>31 ± 8</td>
<td>135 ± 8</td>
<td>4 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>rIL-6 ± rGM-CSF</td>
<td>46 ± 6</td>
<td>82 ± 16</td>
<td>189 ± 22</td>
<td>15 ± 5</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;.0005</td>
<td>&lt;.005</td>
<td>&lt;.005</td>
<td>&lt;.02</td>
<td>NS</td>
</tr>
<tr>
<td>rIL-3</td>
<td>11 ± 4</td>
<td>nd</td>
<td>30 ± 4</td>
<td>53 ± 8</td>
<td>40 ± 13</td>
</tr>
<tr>
<td>rIL-6 ± rIL-3</td>
<td>41 ± 3</td>
<td>nd</td>
<td>50 ± 4</td>
<td>51 ± 8</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;.0005</td>
<td>—</td>
<td>&lt;.0005</td>
<td>NS</td>
<td>NS (&lt;.10)</td>
</tr>
</tbody>
</table>

- Clinical data
- FAB classification: M2, M4, M2, M5b, M3
- % blasts: 68, 60, 95, 38, 50

Cells were plated at 7,000 per well (AML 1, 4, 5) and 10² per well (AML 2, 3). For each AML sample, four dilutions of growth factors were tested, as represented in Fig 1. Results are shown with the optimal concentrations of growth factors: 50 U/mL (GM-CSF), 1:300 dilution (rIL-6), and 1:600 dilution (rIL-3). The proportion of blasts in the peripheral blood is expressed as % total nucleated cells. Results on AML 5 are typical of five distinct experiments. P values were obtained by the Student's t test. Values were not considered significantly different when P was higher than .05.
DIRECTIONS

The authors' results demonstrate that IL-6 acts in support of both normal and leukemic myeloid cell proliferation. As found in the murine system, IL-6 alone proved to have relatively little ability alone to support colony formation supported by another hematopoietic growth factor, in this case, GM-CSF. In the murine system IL-6 was found to shorten the G0 phase of quiescent stem cells, causing them to enter cycle earlier and become IL-3-dependent. The data presented here indicate that IL-6 may function similarly on GM-CSF and IL-3-dependent blast progenitors in the human system. It will also be important to test for enhancement of IL-3-dependent colony formation using normal targets.

While IL-6 clearly results in enhanced colony formation, it has not yet been possible to distinguish between direct interaction with target progenitor cells and the possibility that IL-6 may induce accessory cell growth factor production in the cultures. Indeed, IL-6 has also proved capable of acting as a second signal in mitogen-dependent-T-cell activation, and it is possible that activated T cells (and possibly other cells) may be triggered in culture to release other direct-acting growth factors. The authors' preparations of leukemic blasts represent a more homogenous cell population than normal human bone marrow cells, and at least some of the effects seen with AML blast cells may be direct. However, the leukemic cells do often make and secrete GM-CSF, G-CSF, and IL-1. To limit interactions of IL-6 with these endogenously produced factors, the authors have kept the cell concentrations at the lowest level possible in the present study. In the murine system IL-6 was found to act directly in support of proliferation of at least some GM-CSF, a finding consistent with the authors' observations with normal human marrow in the presence of GM-CSF that most of the enhanced colony formation is due to stimulation of cells from the granulomonopoietic pathway. Nevertheless, it will be important in analyzing the role of IL-6 in hematopoiesis to develop target cell receptor-binding assays and immunologic reagents to distinguish between direct and indirect actions of the factors.

As has been noted with many different samples of AML blasts, the growth factor responsiveness of the five patients was very heterogeneous. These observations confirm the large heterogeneity of the disease, as documented with other parameters. In three of five cases the authors noted synergy between IL-6 and GM-CSF in support of colony formation. Of these, two patients (AML 1 and AML 2) also demonstrated a synergistic response when tested with IL-1 and either G- or GM-CSF. The clonogenic cells from patient AML 3 did not show synergy between IL-6 and GM-CSF or IL-3, while they responded to the synergistic effects of IL-1α and GM-CSF or G-CSF. The cells from patient AML 5 yielded optimal colony formation in the presence of G- and GM-CSF, and this response could not be enhanced by either IL-1 or IL-6. While it has not been possible to correlate these properties with the French-American-British (FAB) classification of the disease, it will be interesting to see if the observed responses might correlate with properties of normal progenitors. If the cell of origin of the AML blasts is derived from multipotential stem cells as documented by isoenzyme studies, perhaps different clones have acquired different programs of differentiation. Alternatively, the multipotential stem cells from which the disease arises may in different individuals represent different subsets of normal stem cells. In either case, the responsiveness to the different cytokines should provide useful ways for analyzing these possibilities.

REFERENCES

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