Human Recombinant Multilineage Colony Stimulating Factor (Interleukin-3): Stimulator of Acute Myelocytic Leukemia Progenitor Cells In Vitro

By Ruud Delwel, Lambert Dorsers, Ivo Touw, Gerard Wagemaker, and Bob Löwenberg

Acute myeloid leukemia colony forming cells (AML-CFU) require the addition of colony stimulating factors (CSFs) for in vitro proliferation. Recently, we isolated a human recombinant multilineage CSF (hMulti-CSF). We investigated the ability of hMulti-CSF to stimulate AML clonogenic cells in seven patients in direct comparison with the effects of human granulocyte CSF (hG-CSF), human granulocyte-macrophage CSF (hGM-CSF), and feeder leukocytes. We show that hMulti-CSF is an efficient stimulator of AML colony formation in four of seven cases. In these patients, hGM-CSF was also capable of stimulating AML colonies in vitro. In two of seven cases hMulti-CSF appeared to be a weak stimulus of AML-CFU proliferation. In these latter two cases, however, hG-CSF and in one case hGM-CSF effectively stimulated AML-CFU growth. In one patient none of the hCSFs, either alone or in combination, induced AML colony formation, whereas AML colonies consistently appeared in the phytohemagglutinin (PHA) leukocyte assay. This finding suggests that PHA stimulated leukocytes produce components other than the tested hCSFs that may have a role in the proliferation of AML cells in vitro. Multi-CSF, like hGM-CSF, revealed a limited capacity to induce progressive maturation during AML colony growth, ie, not beyond the promyelocytic stage. On the other hand, in one case, hG-CSF stimulated the growth of AML colonies containing (met)myelocytes and granulocytes. We conclude that hMulti-CSF is a regulator of AML-CFU proliferation in a significant number of cases. The patterns of responsiveness of AML precursors to the three hCSFs in different patients show a striking variability, which may indicate that AML-CFU are the neoplastic representatives of normal bone marrow progenitors at different stages of maturation and with distinct CSF requirements.

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hGM-CSF. The effects of hMulti-CSF on AML colony formation as compared with hGM-CSF, hG-CSF, and feeder leukocytes are shown in Table I. Without the addition of any of the stimuli, no colonies were formed. AML colony formation was stimulated efficiently by hMulti-CSF in cases no. 2, 4, 5, and 6. In patients no. 2 and 6, hMulti-CSF alone induced numbers of AML colonies that exceeded the values of the standard PHA l.f. assay. In the other cases the stimulatory effect of the single most efficient stimulator was not enhanced as a consequence of using a mixture of the three hCSFs in culture.

In cases no. 1, 3, 4, and 5 it appeared that AML colonies were induced more efficiently in the PHA l.f. assay than in the cultures with the complete mixture of recombinant hCSFs. Moreover, AML-CFU from patient no. 7 did not respond to the three hCSFs, whereas significant numbers of colonies appeared in the PHA l.f. assay. These data suggest that feeder leukocytes may produce at least one other component regulating AML-CFU proliferation.

The AML colony cells of patients no. 1, 3, and 6 were harvested and analyzed morphologically to assess whether the CSFs differ in their capacity to induce maturation (Table II, Fig 2). In these cases the colony cells from the cultures stimulated by either hMulti-CSF or hGM-CSF showed limited differentiation, ie, not beyond the promyelocytic stage. In contrast, hG-CSF-stimulated colonies from patient no. 6 contained cells that had matured towards (meta)myelocytes and granulocytes (Fig 2).

**DISCUSSION**

We investigated the stimulatory effects of hMulti-CSF, in comparison with the effects of hG-CSF and hGM-CSF and feeder leukocytes on the proliferation and differentiation of AML colony cells of patients no. 1, 3, and 6 were harvested and analyzed morphologically.

The data from Table I indicate that in most cases more than one recombinant hCSF stimulated AML colony formation. To investigate the possibility that the different hCSFs acted on AML-CFU in an additive fashion the preparations were also tested in combination. Only in cases no. 2 and 6 was an indication of additive stimulation by hMulti-CSF and hGM-CSF obtained. In the other cases the stimulatory effect of the single most efficient stimulator was not enhanced as a consequence of using a mixture of the three hCSFs in culture.

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**DISCUSSION**

We investigated the stimulatory effects of hMulti-CSF, in comparison with the effects of hG-CSF and hGM-CSF and feeder leukocytes in the proliferation and differentiation of

**Table 1. Induction of AML Colony Formation**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>hMulti-CSF</th>
<th>hG-CSF</th>
<th>hGM-CSF</th>
<th>hMulti-CSF + hG-CSF + hGM-CSF</th>
<th>PHA l.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0</td>
</tr>
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</table>

The effects of hMulti-CSF, hG-CSF, and hGM-CSF on AML colony formation were studied in a modified PHA l.f. system from which feeder leukocytes were omitted. Standard PHA l.f. cultures for AML colony formation were run in parallel.

*Classification according to the FAB Cooperative Group. 10,11*

†Colonies per 10^5 cells (mean ± SD).
AML-CFU in colony culture. Human Multi-CSF, hG-CSF, and hGM-CSF were all capable of stimulating AML colony formation in vitro, although the responses of AML-CFU of different individuals to these hCSFs were variable.

The results indicate that AML colony formation in only one of the three hCSF tested (case no. 6). Usually two (cases no. 1, 2, 4, and 5) or all three hCSFs (case no. 6) were able to induce AML-CFU proliferation. In most of these patients the presence of the three hCSFs in the cultures simultaneously did not result in an increased colony response as compared with the single most active factor, suggesting that the different hCSFs acted on overlapping AML-CFU target populations. However, exceptions were patients no. 2 and 6 in whom additive effects of hMulti-CSF and hGM-CSF on AML colony formation were observed. Thus, in these cases different AML-CFU subpopulations were apparently stimulated by different growth stimuli. The data in Table 1 do not provide evidence that the combination of the three factors can enhance colony numbers synergistically. To explore further the possibility of a synergistic action of the hCSFs, we set up cultures to which suboptimal concentrations of the different growth stimuli were added simultaneously. These studies also did not indicate synergistic stimulation of AML colony formation by these hCSFs (data not shown).

An interesting observation was that in patient no. 7 AML colony growth could not be induced by the recombinant hCSFs, whereas colonies appeared consistently in the standard PHA l.f. assay. The PHA l.f. colony culture has been an efficient assay for clonogenic AML cells, although the active stimulatory components of the system are probably multiple and not exactly defined. The fact that colony growth in patient no. 7 appeared only in the PHA l.f. assay could imply that components other than these hCSFs have a critical role in stimulating the proliferation of AML precursor cells. This suggestion is also supported by the fact that in

<table>
<thead>
<tr>
<th>Table 2. Differential Counts of MGG-Stained AML Colony Cells</th>
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<tbody>
<tr>
<td>Case No. 1*</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Myeloblasts</td>
</tr>
<tr>
<td>Promyelocytes</td>
</tr>
<tr>
<td>(Meta)myelocytes</td>
</tr>
<tr>
<td>Granulocytes</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Percentages of 250 cells counted.</td>
</tr>
</tbody>
</table>

*The purified AML samples preculture contained 51% (case no. 1), 96% (case no. 3), and 98% (case no. 6) myeloblasts. The AML sample from case no. 1 preculture contained 46% promyelocytes.
4 cases (no. 1, 3, 4, and 5) the PHA l.f. system supported the outgrowth of larger numbers of colonies than was achieved with the mixture of recombinant hCSFs.

Morphologic analysis of the AML colony cells of three patients revealed that progressive maturation occurred occasionally in the hG-CSF stimulated but not in the hMulti-CSF and hGM-CSF stimulated cultures (Table 2). Since the three factors have been reported to induce granulocytic or monocytic differentiation in normal bone marrow cultures, this could indicate that in patient no. 6, the AML blasts respond abnormally to hMulti-CSF and hGM-CSF but normally to hG-CSF, as regards the induction of maturation.3,4 However, at this stage, the possibility cannot be excluded that differentiation towards granulocytic end cells, observed in the normal bone marrow cultures stimulated by hMulti-CSF or hGM-CSF, is not caused by direct effects of these two factors but by endogenously produced G-CSF.

Thus, it remains unclear whether the variations of CSF responses of AML progenitors either represent leukemic characteristics of the cells or reflect the features of normal bone marrow progenitor subsets at different maturation stages with distinct CSF requirements. To discriminate between these two possibilities it will be essential to study in detail the effects of the recombinant hCSFs on highly purified normal bone marrow progenitors.

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