Interleukin-3 and Interleukin-1α Allow Earlier Bone Marrow Progenitors To Respond to Human Colony-Stimulating Factor 1


By using human bone marrow cells enriched for early progenitors by selective immunoadsorption and plated at low cell density (10^5 to 10^6 cells/mL/9.6 cm^2) in a semisolid methylcellulose culture, we have analyzed the cooperative effects of human colony-stimulating factor 1 (CSF-1), granulocyte-macrophage-CSF (GM-CSF), interleukin-1α (IL-1α), and gibbon as well as human recombinant IL-3 on the formation of monocytic colonies. CSF-1 alone stimulated mature monocytic colony formation by human CFU-M. However, in the presence of IL-3 and erythropoietin, CSF-1 stimulated maximal immature monocytic colony formation at low concentrations and inhibited the formation of granulomonocytic, erythrocytic, and mixed colonies. Cultures with CSF-1 and IL-3 contained more immature monocytic colonies than did cultures with CSF-1 alone. IL-1α alone had little effect. However, IL-1α in combination with optimal concentrations of either CSF-1, GM-CSF, or IL-3 increased the number of colonies containing immature or mature monocytic colonies.

SIGNIFICANT progress has been made in the last few years in the cloning of the genes for human hematopoietic growth factors. The cloning of the genes has resulted in the availability of pure, recombinant molecules that are otherwise difficult to purify from natural molecules. In addition, their nucleic acid probes have been extremely useful in studies of growth factor gene expression. These probes have been used to map the genes for interleukin-3 (IL-3), colony-stimulating factor (CSF-1), and granulocyte-macrophage CSF (GM-CSF) to the long arm of chromosome 5, a clustering that has clinical and possibly regulatory implications. The receptor for one of these growth factors (CSF-1) has been purified and shown to be a tyrosine kinase whose gene encodes the c-fms protooncogene product. This gene maps on chromosome 5 close to the genes for IL-3, CSF-1, and GM-CSF. These findings have provided impetus for studies of the mechanisms of regulation of normal and abnormal hematopoiesis.

In mice, synergism between hematopoietic growth factors has been shown to play an important role in the proliferation and differentiation of very primitive cells in vitro. For example, CSF-1 and IL-3 have been shown to act on more immature progenitor cells in combination with hemopoietin-1, a factor that is identical to IL-1α. Synergism in vivo has also been reported in mice for CSF-1, IL-3, and GM-CSF. The mechanism(s) underlying these synergisms remains to be elucidated. In this paper, we show that human CSF-1 cooperates at suboptimal concentrations with IL-3 and at optimal concentrations with IL-1α to generate more immature human monocytic colonies in methylcellulose cultures of human bone marrow cells.

MATERIALS AND METHODS

Growth factors. Recombinant GM-CSF was a generous gift from Biogen, Geneva; recombinant IL-1α was from Dr Peter Lo Medico, Hoffman-La Roche, Nutley, NJ; recombinant gibbon IL-3 was obtained by transfecting COS cells with pCSF-MLA and used as a crude supernatant; human recombinant IL-3 was isolated from an Escherichia coli strain in which the synthesis of IL-3 was driven by the bacteriophage λ pl promoter; the intercellularly expressed IL-3 was kindly supplied by J. Seehra, Genetics Institute, Cambridge, UK; and was estimated to have a specific activity of 3 to 5 · 10^6 U/mg in the chronic myelogenous leukemia blast proliferation assay; recombinant human erythropoietin (Epo) was from Integrated Genetics, Framingham, MA. CSF-1 was purified as previously described.

Bone marrow. Twelve specimens of bone marrow from the iliac crest, ribs, or sternum were obtained either from surgically removed normal bones at orthopedic operative procedures or from normal bone marrow transplant donors with their informed consent and under a protocol approved by the Institution's human subjects review committee. All samples were collected into heparin.

Cell preparation. All specimens were gently dispersed in Iscove's modified Dulbecco's medium (IMDM) (Biochrom, Angoulême, France) supplemented with 10% fetal calf serum (FCS) (Flobio, Paris). Mononuclear cells of less than 1.077 g/mL density were isolated by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). They were adjusted to a density of 2 · 10^6 cells/mL and allowed to adhere to the bottom of T-75 tissue culture flasks (CEB, Angers, France) at 37°C overnight. Nonadherent cells were removed and the precursors purified by panning isolation. Briefly, cells were incubated with B1.3CS monoclonal antibody (CD34) (50 μL/10^6 cells) for 20 minutes at room temperature and then washed and incubated 90 minutes at 4°C on bacteriologic Petri dishes previously incubated overnight at 4°C with 60 μg antimouse immunoglobulins in 5 mL 50 mmol/L Tris-HCl, pH 9.2. Nonadherent cells were removed by washing the Petri dishes twice with Ca^2+Mg^2+-free phosphate-buffered saline and 0.2% bovine serum albumin (Sigma Chemical Co, St Louis; Type A 7906); these cells...
constitute the more mature cell population. Adherent cells, ie, enriched progenitor cells, were detached by gentle pipetting, centrifuged, and resuspended in IMDM supplemented with 10% FCS. This procedure enriched the population 40 to 50 times with CFU-Mix.

Colony assays. The enriched population was plated at low cell density (10⁴ to 10⁴ cells/mL) to obtain mixed colonies that were large, easily identified, and well separated from each other. The general procedure used was the mixed colony assay of Messner et al. Cells were plated in 35-mm Petri dishes in IMDM containing 2.5% (vol/vol) purified phytohemagglutinin-stimulated, leucocyte-conditioned medium (PHA-LCM) or various growth factors, 300 µg/mL transferrin, 1 mg/mL deionized bovine serum albumin, conditioned medium (PHA-LCM) or various growth factors, 300 µg/mL transferrin, 1 mg/mL deionized bovine serum albumin, and 10% to 30% FCS, 1 U/mL Epo, and 0.9% (wt/vol) methylcellulose. Cultures were incubated for 14 to 28 days at 37°C in a humidified atmosphere containing 5% CO₂ in air.

Colonies. Colonies ranging from 50 to 16,000 cells were counted at 14 days. Ninety percent of the colonies had between 500 and 5,000 cells. They were classified according to established criteria by direct observation of the dishes with a Diavert Leitz inverted microscope. A mixed colony derived from CFU-Mix is a colony containing erythroid cells together with cells of one or more other hematopoietic cell lineages. These colonies may contain to 16 x 10⁴ cells. Only those colonies in which the various subpopulations were obviously part of the one large colony were included. Doubtful mixed colonies (<10%) were discarded. Independent colony counts were performed by two different investigators.

Colonies of the monocytic lineage were classified on the basis of cell maturity. Immature colonies contained immature cells, monoblasts, immature and mature monocytes, and small macrophages with projections. Mature colonies contained round macrophages with a small nuclear-to-cytoplasmic ratio (<0.3). Cytologic evaluation of each colony was performed by removing single colonies from methylcellulose with a finely drawn Pasteur pipette. The smear obtained from each colony on a glass slide was stained with May-Grünwald-Giemsa stain. To study the response to a given growth factor, for each experiment the number of colonies was expressed as a percentage of the number of colonies of the same lineage counted in the control Petri dishes in the presence of PHA-LCM. The data are presented as the means and SDs for five experiments with different bone marrow samples. The control values were 3.7 ± 1.8 for mixed colonies, 20.1 ± 13.6 for erythroid colonies, 31.8 ± 16.5 for granulocytic colonies, and 40.1 ± 22.6 for monocytic colonies.

Table 1. Effect of CSF-1 and IL-3 on the Degree of Maturity of Macrophage Progenitors

<table>
<thead>
<tr>
<th>CSF-1 Concentration (U/mL)</th>
<th>0</th>
<th>1/2</th>
<th>200</th>
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<tr>
<td>0</td>
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<tr>
<td>55</td>
<td>0.9</td>
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<tr>
<td>100</td>
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<td>1.2</td>
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</tr>
<tr>
<td>550</td>
<td>2.8</td>
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*The ratio obtained with PHA-LCM was 1.1.
CSF-1 alone were rare and mainly of the type containing small macrophages with projections. More immature colonies with monocytes or monoblasts were absent irrespective of the CSF-1 concentration and the time at which the plates were counted and the colonies stained. A modest inhibition of erythroid colony formation was also observed, but this was difficult to assess because of the low colony numbers. This inhibition was better observed when IL-3 was added (Fig 3).

Effect of CSF-1 with IL-3. With an optimal concentration of IL-3 selected for the 100% reference value, the addition of a low concentration of CSF-1 (33 U/mL), which alone would not stimulate macrophage colony formation, stimulated the formation of a larger number of mononuclear colonies (Fig 3). A similar experiment is detailed in Fig 2 with PHA-LCM selected as the 100% reference. Figure 2 compares the effect of CSF-1 with or without IL-3. The number of mononuclear colonies observed with 33 U/mL of CSF-1 was significantly higher with IL-3 than without (P < .02, df = 6). Table 1 shows that in the presence of IL-3 there was a dose-dependent effect of CSF-1 on the degree of maturity of colony cells. The ratio of mature to immature colonies was three times lower when IL-3 was added to the lowest concentration of CSF-1. Even at higher concentrations of CSF-1, the colonies were much more immature when IL-3 was added. However, the majority of the monoblast–early monocyte colonies, which were not observed with CSF-1 alone, were able to mature to macrophages when the highest concentrations of CSF-1 were added to IL-3 (Table 1).

In addition to the development of new types of mononuclear colonies, Fig 3 shows that colonies derived from CFU-Mix, BFU-E, and CFU-G dropped when increasing amounts of CSF-1 were added to an optimal concentration of IL-3. At the two highest concentrations of CSF-1 in the dose-response curve, the inhibitory effects were significant for mixed colonies (P < .02, df = 4) and for erythroid and granulocytic colonies (P < .01, df = 4).

Effect of IL-1α with CSF-1, IL-3, and GM-CSF on immature and mature macrophage colony formation. Figure 4 shows that IL-1α stimulated both immature and mature macrophage progenitors when added to CSF-1, IL-3, or GM-CSF alone. The cooperative effect was also observed when IL-1α was added to any combination of CSF-1, IL-3, and GM-CSF together (data not shown). Figure 4 illustrates a typical experiment; however, analysis of identical experiments with different bone marrow samples showed the same significant differences (P < .01 to .05) when IL-1α was added to CSF-1, IL-3, or GM-CSF.

DISCUSSION

Our results show a cooperative effect in vitro of IL-3 or IL-1α with CSF-1 in stimulating the proliferation of human macrophage precursor cells. These findings confirm and support earlier studies in mice. It was shown, by adding polymixin B to our cultures, that the synergistic effects of IL-3 and IL-1α with CSF-1 on colony formation were not due to traces of lipopolysaccharides present in the preparations or in the serum (data not shown).

The cooperative effect of IL-3 and CSF-1 appears clearly at low CSF-1 concentrations. Indeed, at 33 U/mL, CSF-1 alone has almost no effect in our culture conditions. Added to
IL-3, it allows undifferentiated monoblasts or immature monocyte colonies to grow and/or mature further. These immature colonies cannot be detected at any time in culture with CSF-1 alone. Table 1 and preliminary results (not shown) indicate that, with IL-3, CSF-1 allows both cell division and/or maturation. There is probably more than one mechanism of action of IL-3 and CSF-1, depending on cell types and culture conditions. At optimal CSF-1 concentrations, however, the cooperative effect of IL-3 on the total number of monocyctic colonies disappears. This could reflect either IL-3–induced down-modulation of CSF-1 receptors on the more mature monocyctic progenitors or a different effect of IL-3 and CSF-1 on the same progenitors.

Other investigators have studied the effect of recombinant gibbon or human IL-3 in the mixed colony assay system. Messner et al have observed a smaller difference than we did between the numbers of CFU-Mix obtained with PHA-LCM and IL-3. However, this may have been due to the fact that they plated 10 to 100 times more cells than we did. Nevertheless, the general conclusions were similar, with the demonstration of a major effect of IL-3 on CFU-Mix and BFU-E. Sieff et al have found that IL-3 is most effective in support of BFU-E colony formation using progenitors isolated from fetal liver, a rich source of erythroid lineage progenitors. Our findings of the effects of CSF-1 on IL-3–supported colony formation are qualitatively similar to the results of Sieff et al in the analysis of the effects of G-CSF on IL-3–supported colony growth. In their study, they reported significant enhancement of neutrophilic granulocyte formation when G-CSF and IL-3 were combined relative to either factor alone. This is consistent with our observation that CSF-1 in combination with IL-3 resulted in optimal macrophage colony formation. Thus, the lineage-restricted factors G-CSF and CSF-1 are both able to cooperate with the earlier factor IL-3 in generating colonies of the expected lineage. However, in our studies we observed that the IL-3–stimulated increase in colonies of monocyctic progenitors correlates with a decrease in the number of colonies derived from CFU-G, BFU-E, and CFU-Mix. Such an effect was not evident in the experiments of Sieff et al with G-CSF/IL-3 combinations. CFU-Mix and large BFU-E are the progenitors that exhibit the best response to IL-3 and are considered early progenitors. In our experiments, it is tempting to speculate that CSF-1 treatment of the early IL-3–responsive progenitors diverts the developing cells down the monocyctic pathway.

Commitment to the mononuclear phagocyte lineage could be achieved by several different mechanisms. One possibility would be that macrophages are induced to secrete factors that trigger new progenitors toward the mononuclear phagocyte lineage and inhibit the development of colonies derived from BFU-E and CFU-Mix. In preliminary experiments in which monocyctic colonies were removed as soon as they appeared during the 2 weeks of culture in semisolid medium, depletion did not increase the number of colonies derived from BFU-E and CFU-Mix in the presence of CSF-1. Of course, this result, even with cultures of enriched progenitors plated at a low cell concentration, cannot exclude the possibility that a few remaining macrophages were sufficient to effect stimulation and inhibition of neighboring cells. A second possibility is that CSF-1 down-modulates the receptor for erythropoietin or other factors, thereby decreasing colony formation by BFU-E and CFU-Mix. Hierarchical downmodulation of hematopoietic growth factor receptors has been reported. Although we do not know whether CSF-1 can down-modulate the Epo receptor, a situation where Epo down-regulated the CSF-1 receptor has been described. A third possibility is that IL-3 or IL-1α triggers the expression of the CSF-1 receptor gene on early progenitors that have still the potential to be committed to BFU-E, CFU-Mix, or CFU-M. In the presence of CSF-1, the commitment toward CFU-M would be favored. In the murine system, experiments carried in liquid culture do not favor increased expression of the CSF-1 receptor being directly regulated by either IL-3 or IL-1α. However, these experi-
ments could not provide information about the CFU-GEMM present in the unpurified cell populations. More complex loops of regulation are possible. The intriguing observation that the human gene for IL-3 is at 9 kb of the gene for GM-CSF and on the same part of chromosome 5 as the genes for CSF-1 and its receptor suggests further possible regulatory mechanisms that are currently under investigation.

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REFERENCES

Interleukin-3 and interleukin-1 alpha allow earlier bone marrow progenitors to respond to human colony-stimulating factor 1

YQ Zhou, ER Stanley, SC Clark, JA Hatzfeld, JP Levesque, C Federici, SM Watt and A Hatzfeld