In Vivo Administration of Recombinant Human Interleukin-1 and Macrophage Colony-Stimulating Factor (M-CSF) Induce a Rapid Loss of M-CSF Receptors in Mouse Bone Marrow Cells and Peritoneal Macrophages: Effect of Administration Route

By Ben D.-M. Chen

Earlier studies suggested the existence of a blood-bone marrow barrier that significantly inhibits the transfer of plasma macrophage colony-stimulating factor (M-CSF) to responsive hematopoietic cells in vivo as indicated by its failure to induce a receptor downregulation in bone marrow cells. In this study, the effect of recombinant human interleukin-1 (rhIL-1) was investigated. In vivo administration of rhIL-1, either intraperitoneally (IP) or intravenously (IV), induced a rapid transient loss of M-CSF receptor binding activity in bone marrow cells, with a nadir occurring between 2 to 4 hours while loss of M-CSF receptors by cells in the peritoneal cavity occurred only in animals receiving rhIL-1 via IP administration. The loss of M-CSF receptor activity after rhIL-1 treatment was correlated with an elevated level of circulating M-CSF. However, the loss of M-CSF receptors in marrow cells was prevented by dexamethasone (Dex) treatment before rhIL-1 administration. The fact that Dex treatment also reduced the level of circulating M-CSF after rhIL-1 administration suggests that the inhibitory effects of IL-1 are mediated through locally produced M-CSF. Administration of rhM-CSF at higher doses, either IV or IP, also induced a loss of M-CSF receptor of lesser degree in the marrow cells. However, the loss of M-CSF receptors by the peritoneal cells was induced only in mice receiving rhM-CSF through IP administration. Taken together, these results indicate the existence of a unidirectional barrier that prevents the transfer of blood M-CSF and IL-1 to peritoneal cavity but not vice versa.

THE PRODUCTION OF mature macrophages is regulated by a group of hematopoietic growth factors referred to as colony-stimulating factors (CSFs). CSFs are a heterogeneous group of at least four CSF subclasses, which have been described in terms of the type of mature cells produced by their presence in semisolid cultures of bone marrow cells. Three of these CSFs are involved in the direct control of macrophage production: multi-CSF (interleukin-3 [IL-3]), which induces colonies composed of granulocytes, macrophages, megakaryocytes, eosinophils, and erythroid cells; granulocyte-macrophage CSF (GM-CSF), which induces a mixture of granulocyte and macrophage colonies; and macrophage CSF (M-CSF), which induces primarily macrophage colonies.

In vitro, M-CSF stimulates the proliferation of murine macrophage precursors obtained from bone marrow and various other tissues, as well as the differentiation of these precursor cells into mature macrophages. In vivo, M-CSF has been implicated in the regulation of monocyte/macrophage production. However, it has been difficult to demonstrate a sustained effect of M-CSF on bone marrow precursors when exogenous factor is given to mice. Indeed, in vivo treatment of IL-1 has been shown to stimulate hematopoietic regeneration and protect animals from lethal bacterial infection, radiation, and/or chemotherapy, presumably through the production of CSFs. One of the earlier events associated with CSF responsiveness is a rapid loss of surface receptors in which the bound CSF molecule, along with its receptor, is internalized and degraded by the responding cells. Thus, the loss of a specific type of receptor can in some instances be viewed as an indication of cellular responses to that specific factor. In this study, the in vivo effect of recombinant human IL-1 (rhIL-1) on the expression of M-CSF receptors by bone marrow and peritoneal cells was examined. We now report that in vivo treatment with rhIL-1 induced a rapid cellular response to M-CSF by both marrow and peritoneal cells, as evidenced by a complete but transient loss of M-CSF binding activity by these cells.

MATERIALS AND METHODS

Mice. Female C3H/HeJ mice 8 to 12 weeks of age were obtained from the Jackson Laboratory (Bar Harbor, ME). Cells obtained from this strain of mice are refractory to stimulation by endotoxin in vitro. In some studies, B6D2F1 mice were used with

From the Division of Hematology/Oncology, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI. Submitted August 16, 1990; accepted January 8, 1991.

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Address reprint requests to Ben Chen, PhD, Division of Hematology/Oncology, Department of Internal Medicine, PO Box 02188, Wayne State University School of Medicine, Detroit, MI 48201.

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similar results. All mice were fed standard lab chow and water ad libitum.

**CSF and reagents.** rhu IL-1a and β (specific activity, 10⁷ U/mg protein) were kindly provided by Biogen Co (Geneva, Switzerland). Highly purified rhuM-CSF (specific activity: 5 × 10⁷ U/mg protein) was a generous gift from Cetus Co (Emeryville, CA). Mouse L-cell M-CSF was purified by a five-step procedure described previously. The biologic activity of both human and murine M-CSF was determined from the linear portion of the dose-response curve, assigning 50 U/mL to that concentration causing the formation of 50% of maximal colony numbers when 5 × 10⁶ bone marrow cells were cultured in 1 mL of soft agar.

**Cells.** Bone marrow cells were obtained from femoral shafts by flushing with 3 mL cold α-minimal essential medium (MEM) containing 10 mmol/L MOPS and 10% fetal calf serum (FCS; Sterile Systems, Inc, Logan, UT) (α-MOPS-10). The cell suspensions were passed up and down three times through an 18-gauge needle in the same medium to disperse cell clumps. To increase the number of M-CSF receptor-bearing cells in the peritoneal cavity, mice were administered one single interperitoneal (IP) injection of 0.5 mL of thioglycollate medium 6 days before IL-1 treatment. Peritoneal macrophages were harvested by a procedure described elsewhere.

Iodination of M-CSF and receptor binding assay. The iodination of purified L-cell M-CSF and human M-CSF has been described in detail previously. The binding assay for M-CSF receptors in bone marrow cells was performed in 0.5 mL α-MOPS-10 containing 1 to 6 × 10⁶ cells and an appropriate amount of iodinated M-CSF at 4°C for 3 hours. The cell mixtures were then layered over 3.5 mL cold FCS and centrifuged at 300g for 10 minutes to separate free [¹²⁵I]M-CSF from bound [¹²⁵I]M-CSF. Non-specific binding, determined as the amount of radioactivity in the presence of 500-fold excess of unlabeled M-CSF, was subtracted from the total binding to give the specific binding.

**Radioreceptor assay.** The levels of plasma M-CSF were determined by a radioreceptor assay. Briefly, peritoneal macrophages (10⁶/well) were cultured in 24-well culture plates. Twenty-four hours later, cells were chilled, washed once with cold phosphate-buffered saline (PBS) and then replenished with 0.3 mL of cold α-MOPS-10. Appropriately diluted samples along with rhuM-CSF standards in 0.1 mL of α-MOPS-10 were then added to each well. Thereafter, iodinated rhuM-CSF (usually 50,000 cpm/well) in 0.1 mL was added to the cell mixture. After an additional incubation of 3 hours at 4°C, cells were washed two times with cold PBS and lysed from the plate for gamma counting with two washes of 0.75 mL of 0.1 N NaOH containing 1% sodium dodecyl sulfate (SDS). This method measures both human and murine M-CSF without distinguishing them.

**RESULTS**

The presence of M-CSF receptor binding activity in bone marrow cells can be readily detected by using radiolabelled [¹²⁵I]M-CSF despite the fact that only a small fraction of total marrow cells bear M-CSF receptors. The total binding activity in the bone marrow cells appeared to be quite consistent from animal to animal with a variance of less than 20%. In vivo administration of rhuIL-1 induced a rapid but transient loss of M-CSF binding activity in marrow cells, with a nadir occurring between 2 and 4 hours. Within 24 hours the total M-CSF receptor binding activity was restored to a level moderately higher than that of the controls (Fig 1A). The downregulation was dose-dependent with as little as 10 ng/mouse being sufficient to induce nearly a 50% loss of total M-CSF receptor activity in bone marrow cells (Fig 1B). Downregulation of the receptor activity in bone marrow cells was detected regardless of whether rhuIL-1 was administered IV or IP (Fig 2). On the other hand, treatment with comparable doses of rhuM-CSF (0.1 to 0.5 μg/mouse), either IP or IV, caused only a moderate or no reduction in total marrow cell binding for [¹²⁵I]M-CSF (Fig 2). However, marked decline in total bone marrow M-CSF receptor binding was detected at higher doses (1 μg/mouse), in agreement with that reported by Shadduck et al. A similar inhibitory effect of IL-1 was observed in peritoneal macrophages, but was dependent on the route of administration. The loss of M-CSF receptor binding activity in peritoneal macrophages was detected only in animals receiving rhuIL-1 by IP injection (Table 1). Similarly, administration of rhuM-CSF (1 μg/mouse) IP but not IV resulted in over a 90% loss of M-CSF receptor activity in this cell population.

To determine whether IL-1 treatment affects the number of M-CSF binding sites or receptor affinity, we performed binding assays with increasing concentrations of [¹²⁵I]M-CSF...
EFFECT OF IL-1 TREATMENT ON M-CSF RECEPTORS

Fig 2. Effect of rhuM-CSF and rhulL-10 on the M-CSF receptor binding activity of marrow cells. C3H/HeJ mice were injected various doses (μg) of rhuM-CSF either IP or IV. Two hours later, bone marrow cells were removed and the CSF receptor binding activity determined. Data are the mean of three to six mice ± SD.

(Fig 3). It can be inferred directly from the binding curves that the inhibition of M-CSF binding activity is due to a loss of receptor number rather than changes in receptor affinity. This observation prompted us to investigate the possibility that the loss of M-CSF receptors following rhulL-1 treatment was due to direct receptor occupancy by exogenous M-CSF. Groups of mice were administered either rhuM-CSF or rhulL-1 for various time periods, after which serum M-CSF levels were determined by a radioreceptor assay using human M-CSF as standards. As shown in Fig 4, an elevated level of circulating M-CSF was detected following one single IP injection of rhuM-CSF (1 μg), reaching a maximum level of 45 to 55 ng/mL within 1 hour. The level of plasma M-CSF decreased gradually thereafter and returned to a level slightly higher than that of the controls after 4 hours. A maximum level of 156 ng/mL of circulating M-CSF was detected 5 minutes after receiving 1 μg rhuM-CSF via IV administration. After the maximum concentration of M-CSF was attained, levels declined sharply approaching a value of 30 to 40 ng/mL within 60 minutes. The changes seen in the blood M-CSF level after one IV injection of rhulL-1 exhibited a totally different profile, both in magnitude and duration. The appearance of circulating M-CSF was not detected until approximately 30 minutes after rhulL-1 treatment, reaching a maximum level of 180 to 200 ng/mL 2 to 4 hours later. Although subsiding, an elevated level of circulating M-CSF remained detectable 24 hours after rhulL-1 treatment. The fact that IL-1 acts via induction of M-CSF synthesis was further implied by the findings that, unlike M-CSF, both IL-1α and β failed to induce downregulation of M-CSF receptor levels in cultured peritoneal exudate macrophages (data not shown).

Because dexamethasone (Dex) has been shown to inhibit the production of various cytokines in vitro and in vivo, we asked whether treatment of the animals with Dex before rhulL-1 administration would reverse the inhibitory effects induced by rhulL-1. Groups of animals were treated with 1 mg of Dex subcutaneously daily for four doses with the last injection administered 1 hour before rhulL-1 treatment. As shown in Fig 5, Dex treatment reduced the effect of

Table 1  Effect of IP and IV Administration of rhulL-1 on the Number of M-CSF Receptors on Bone Marrow and Peritoneal Macrophages

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Administration Route</th>
<th>rhulL-1β (10^6) CPM</th>
<th>rhuM-CSF (10^6) CPM</th>
<th>Saline (10^6) CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal macrophages</td>
<td>IP</td>
<td>110 ± 75</td>
<td>90 ± 107</td>
<td>5,731 ± 1,150</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>6,198 ± 62</td>
<td>6,519 ± 557</td>
<td>5,847 ± 914</td>
</tr>
<tr>
<td>Bone marrow cells</td>
<td>IP</td>
<td>64 ± 65</td>
<td>1,810 ± 259</td>
<td>3,035 ± 143</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>38 ± 28</td>
<td>1,003 ± 435</td>
<td>2,988 ± 1,082</td>
</tr>
</tbody>
</table>

C3H/HeJ mice were administered with rhulL-1β (0.1 μg) or rhuM-CSF (1 μg) either IP or IV. Two hours later, bone marrow cells and peritoneal macrophages were removed, washed, and labeled with iodinated CSF to determine M-CSF binding activity. Data are means ± SD from three to five determinations.
IL-1-induced receptor loss in marrow cells from over 90% inhibition to less than 40% in treated mice. The elevation of circulating M-CSF induced by IL-1 was also suppressed in animals previously treated with Dex. Multiple injections of Dex appear to be necessary to reverse the inhibitory effect of rhIL-1 because a single injection of Dex either 1 or 24 hours before IL-1 treatment either partially or completely failed to prevent the loss of M-CSF receptors induced by IL-1 (data not shown). Basal levels of circulating M-CSF ranging from 5 to 20 ng/mL were consistently detected in serum obtained from normal animals, but were completely suppressed in mice pretreated with Dex (Fig 5). Compared with that of normal mice, receptor binding activity in the marrow cells of Dex treated mice was slightly lower than that of controls.

Discussion

In vitro studies have clearly established a role for M-CSF in the regulation of macrophage proliferation and differentiation. The physiologic role of M-CSF and the mechanism by which it interacts with the target cells in vivo remain unclear. Earlier studies indicated that only a very small fraction of injected CSF was found to be associated with total marrow cells. In fact, most of the blood M-CSF following in vivo administration was rapidly cleared and metabolized. These findings raised the concern about the physiologic role of circulating M-CSF in the regulation of macrophage progenitor cells of the marrow. It has been proposed that the transfer of plasma M-CSF into responsive precursor cells in the marrow is prevented by a “blood bone-marrow barrier”. The existence of such a “barrier” implies that the in vivo action of M-CSF is accomplished by a “paracrine mechanism” in which M-CSF, once produced, is transferred to and acts directly on the CSF-responsive cells nearby as opposed to the classical endocrine mechanism in which hormones are transferred by way of the circulation from the producer cells to the target cells at some distant location.

At 37°C, the binding of M-CSF to its receptor is an irreversible process resulting in the internalization and degradation of ligand-receptor complex by the responsive cells. Hence, the loss of M-CSF receptors can be viewed as an indirect evidence of ligand-receptor interaction. Taking advantage of this unique method, we found that in vivo administration of rhuIL-1 induced a rapid but transient loss of M-CSF receptors in marrow cells. Treatment with as low as 10 ng rhuIL-1 per mouse was sufficient to cause a substantial decrease in marrow M-CSF receptor binding. By comparison, in vivo administration of M-CSF induced a lesser degree of receptor loss in bone marrow even at the highest dose of rhuM-CSF tested (1 μg/mouse). Because IL-1 does not compete directly for M-CSF binding, the loss of receptor binding activity in marrow cells is most likely caused by direct receptor occupancy by M-CSF released from nearby cells that produce it. In vivo administration of rhuIL-1 led to a steady increase in circulating M-CSF levels, with concomitant loss of M-CSF receptors in marrow cells. Furthermore, Dex, a known inhibitor of cytokine production, when administered before IL-1 administration markedly prevented both the elevation of blood M-CSF levels and the loss of M-CSF receptors in marrow cells.
The cell of origin of the circulating M-CSF following rhuIL-1 injection is not clear at present. One possible candidate cell population is the endothelium, particularly those endothelial cells lining the sinusoids that are in close contact with marrow hematopoietic cells. Other cell types of the hematopoietic stroma, such as fibroblasts, are also likely to be involved in this process. Because of their location, these cells may have a greater opportunity to interact with circulating IL-1 and release growth factors that can affect hematopoietic cells in the vicinity. M-CSF produced from other tissue sources may also contribute, to a lesser degree, to this process via circulation. Indeed, when the mice were administered one single IP injection of rhuIL-1, the level of M-CSF receptors on peritoneal macrophages also declined sharply in a manner similar to that of marrow cells. Interestingly, the loss of M-CSF receptors by peritoneal cells did not occur if rhuIL-1 was administered by the IV route. Unlike peritoneal cells, the loss of M-CSF receptor in marrow cells induced by rhuIL-1 is not dependent on the administration route. Furthermore, the IV injection of rhuM-CSF at 1 µg/mouse caused a substantial loss of M-CSF receptors in marrow cells but not in peritoneal cells, indicating a lesser barrier between the blood and bone marrow. Taken together, these findings suggest that (1) a substantial barrier also exists between the blood and the peritoneal cavity that prevents the transfer of not only M-CSF but also IL-1 from the circulation into the peritoneal cavity; (2) blood-borne IL-1 has greater access to the responsive cells in the marrow than does M-CSF; and (3) excessive M-CSF produced at local levels must be continually released into circulation, where it is rapidly cleared and metabolized, with limited access to interact with marrow progenitor cells and other tissue macrophages. The rapid removal of tissue-derived and circulating M-CSF may explain, at least in part, the relative ineffectiveness of M-CSF in vivo to cause extensive proliferation and differentiation of marrow macrophage precursors. It is proposed that the initiation of cellular responses by marrow precursor cells may depend primarily on locally produced M-CSF induced by IL-1 and, for that matter, circulating M-CSF may be of no physiologic importance.

This study raises some concern about the clinical application of M-CSF. It implies that frequent repetitive injections or continuous infusion of large doses of M-CSF may be needed to achieve a satisfactory biologic activity in vivo. The maximal effect of CSF therapy may depend on the combined treatment with two or more cytokines and, most importantly, the route by which the cytokines are administered. Clearly, more information about the molecular interaction between the various cytokines and their specific target cells in vivo is needed to assure a successful clinical application of recombinant growth factors.

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REFERENCES


In vivo administration of recombinant human interleukin-1 and macrophage colony-stimulating factor (M-CSF) induce a rapid loss of M-CSF receptors in mouse bone marrow cells and peritoneal macrophages: effect of administration route

BD Chen