The Effects of Purified Recombinant Murine Interleukin-3 and/or Purified Natural Murine CSF-1 In Vivo on the Proliferation of Murine High- and Low-Proliferative Potential Colony-Forming Cells: Demonstration of In Vivo Synergism

By Douglas E. Williams, Giao Hangoc, Scott Cooper, H. Scott Boswell, Richard K. Shadduck, Steven Gillis, Abdul Waheed, David Urdal, and Hal E. Broxmeyer

Purified natural murine L cell (macrophage) colony-stimulating factor (nCSF-1) and purified recombinant murine interleukin-3 (rIL-3) were administered to normal or lactoferrin-pretreated mice 20 to 24 hours before sacrifice. rIL-3 and nCSF-1 administered separately increased the percentage of macrophage high-proliferative potential colony-forming cells (HPP-CFC) and low-proliferative potential colony-forming cells (LPP-CFC) in active cell cycle. Endotoxin was not detected in the samples of nCSF-1 or rIL-3.

MURINE MACROPHAGE PROGENITOR cells with a high proliferative potential (HPP-CFC) can be detected in normal bone marrow and 5-fluorouracil-treated bone marrow. HPP-CFC represent a developmentally early cell in the hematopoietic stem and progenitor cell hierarchy and form large (0.5 mm in diameter) colonies of macrophages in vitro. Colony formation is dependent upon a source of macrophage colony-stimulating factor (CSF-1) and a synergistic factor (SF). SF has been detected in media from ferin-pretreated mice 20 to 24 hours before sacrifice. rIL-3 and nCSF-1 synergize to effect the proliferation of the same cell populations in vivo. rIL-3 and nCSF-1 can synergize to effect the proliferation of the same cell populations in vivo.

Experimental procedure. Animals received the appropriate concentration of CSF or pyrogen-free saline 20 to 24 hours before sacrifice. Maximal effects of the CSFs occur at this time when given as a single injection. Both femurs were removed, and cells were carefully flushed from the femoral cavity with ice-cold McCoy's 5A medium (GIBCO, Grand Island, NY). Single-cell suspensions were prepared by passage through a 23-gauge needle. Cells from the two femurs of individual mice were counted with a Coulter Electronic counter and the number of nucleated cells per femur determined for each mouse.

Determination of the percentage of cells in S phase. The cycling status of progenitor cells was determined by the high-specific activity, tritiated-thymidine (3HtdR) kill technique as described. Briefly, 2 x 10^6 cells in 0.5 mL of McCoy's 5A medium supplemented with 10% heat-inactivated (56°C, 30 minutes) fetal bovine serum (HIFBS, Hyclone, Logan, UT) were incubated at 37°C for 20 minutes in the presence and absence of 50 μCi of 3HtdR (20 Ci/mmol, New England Nuclear, Boston). Unlabeled thymidine (2.5 mg) was added, the cells were washed twice and plated in a colony-forming assay. The percent reduction in colony number in cultures of cells incubated with 3HtdR, compared with unlabeled thymidine only, was taken as a measure of the percentage of cells in S phase.

MURINE HPP-CFC culture system. HPP-CFC were grown in a

MATERIALS AND METHODS

Animals. Female (C57BL/6J × DBA/2) F1 (BDF1) mice 6 to 8 weeks of age were purchased from Cumberland View Farms, Clinton, TN.

CSFs. nCSF-1 and rIL-3 with specific activities of 2.3 x 10^7 U/mg and 1 x 10^8 U/mg, respectively, were devoid of detectable bacterial endotoxins (ET) as measured by the Limulus amebocyte lysate test (<0.5 ng/mg, Sigma Chemical Co, St Louis).

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of double-layer agar culture system as previously described. Optimal concentrations of prescreened L929 conditioned medium as a source of CSF-1 and WEHI-3B conditioned medium as a source of SF were included in the bottom layer. Cells (25,000 per plate) were incorporated into the agar overlay and incubated in a humidified atmosphere of 5% CO2 in air for 14 days. HPP-CFC were scored at 40x with a dissecting microscope and were defined as tightly packed aggregates of >0.5 mm overall diameter. Loosely arranged aggregates, or those ≤0.5 mm but >50 cells were scored as low-proliferative potential colony-forming cells (LPP-CFC). In some studies, the macrophage composition of HPP-CFC colonies was confirmed by staining with nonspecific esterase.

RESULTS

Single intravenous injections of 2 x 10^4 units of nCSF-1 or 2 x 10^3 units of rIL-3 were administered to untreated BDF1 mice. These concentrations produce maximal effects on CFU-GM, BFU-E, and CFU-GEMM. I-IPP-CFC went from a noncycling state in saline-treated control animals and increased to 50% ± 2% (P < .001) after rIL-3 and 53% ± 1% (P < 0.05) after nCSF-1 treatments.

LF acts in vivo to decrease the percentage of LPP-CFC (CFU-GM) in active cell cycle by increasing the sensitivity of these cells to exogenous CSF. LF does not effect the number of progenitors per femur or total femoral nucleated cellularity were observed when using these concentrations of nCSF-1 or rIL-3 (Table 1).

Table 1. The Effects of nCSF-1 and rIL-3 Administered Separately or Together on Murine HPP-CFC and LPP-CFC

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Nucleated Cells (x 10^5 per Femur)</th>
<th>HPP-CFC (x 10^3 per Femur)</th>
<th>Percent HPP-CFC in Cycle</th>
<th>LPP-CFC (x 10^3 per Femur)</th>
<th>Percent LPP-CFC in Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>17.6 ± 2.8</td>
<td>12.9 ± 2.0</td>
<td>6 ± 6</td>
<td>41.1 ± 6.5</td>
<td>5 ± 7</td>
</tr>
<tr>
<td>CSF-1, 2,000 U</td>
<td>17.3 ± 1.7</td>
<td>12.8 ± 1.2</td>
<td>43 ± 8*</td>
<td>40.7 ± 4.0</td>
<td>47 ± 9*</td>
</tr>
<tr>
<td>CSF-1, 1,000 U</td>
<td>18.2 ± 2.0</td>
<td>13.7 ± 1.5</td>
<td>37 ± 10*</td>
<td>40.4 ± 4.4</td>
<td>36 ± 9*</td>
</tr>
<tr>
<td>CSF-1, 500 U</td>
<td>18.3 ± 2.4</td>
<td>11.1 ± 1.4</td>
<td>4 ± 7</td>
<td>42.3 ± 2.0</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>IL-3, 200 U</td>
<td>17.3 ± 1.3</td>
<td>11.8 ± 0.9</td>
<td>38 ± 4*</td>
<td>44.3 ± 4.2</td>
<td>35 ± 5*</td>
</tr>
<tr>
<td>IL-3, 100 U</td>
<td>16.1 ± 1.4</td>
<td>11.8 ± 3.2</td>
<td>22 ± 8†</td>
<td>40.1 ± 3.5</td>
<td>31 ± 6*</td>
</tr>
<tr>
<td>IL-3, 50 U</td>
<td>17.1 ± 2.6</td>
<td>11.0 ± 1.7</td>
<td>7 ± 6</td>
<td>44.8 ± 6.8</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>500 U CSF-1 + 50 U IL-3</td>
<td>17.4 ± 2.0</td>
<td>11.2 ± 1.3</td>
<td>37 ± 6*</td>
<td>41.3 ± 4.7</td>
<td>41 ± 6*</td>
</tr>
<tr>
<td>100 U CSF-1 + 25 U IL-3</td>
<td>16.3 ± 1.3</td>
<td>11.2 ± 0.8</td>
<td>30 ± 13*</td>
<td>37.5 ± 3.0</td>
<td>35 ± 5*</td>
</tr>
<tr>
<td>50 U CSF-1 + 10 U IL-3</td>
<td>17.5 ± 4.3</td>
<td>12.7 ± 2.4</td>
<td>23 ± 12†</td>
<td>42.3 ± 1.0</td>
<td>22 ± 12†</td>
</tr>
</tbody>
</table>

Mice received pyrogen-free saline or test material IV 20 to 24 hours before death. Three hours earlier, 50 μg of ET-depleted, iron-saturated LF was administered IV. In mice given nCSF-1 plus rIL-3, separate injections were performed for each factor, one immediately after the other. Each mouse was assayed individually, and data are reported as means ± SD. There was a total of six to ten mice per experimental group in two experiments.

*Significantly different from control, P < .001.
†Significantly different from control, P < .05.
proliferation of murine HPP-CFC is well documented.1–5 Recent studies have also demonstrated that these two factors can have a greater than additive or synergistic effect on the in vitro proliferative potential of CFU-GM.6,7 The present studies demonstrated that nCSF-1 and rIL-3 could synergize in vivo to increase the percentage of HPP-CFC and LPP-CFC (CFU-GM) in active cell cycle. Concentrations of either CSF that had no activity when administered alone exhibited strong stimulatory effects on progenitor cell cycling if given together, in accordance with the definition of a synergistic interaction.18 The in vivo stimulatory effects of rIL-3 and nCSF-1 alone or in combination may result from direct effects on the progenitor cells or effects on accessory cells. Synergistic responses to multiple CSF species are probably of relevance physiologically and may be important clinically.

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REFERENCES

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