In Vivo Hematopoietic Effects of Recombinant Interleukin-1α in Mice: Stimulation of Granulocytic, Monocytic, Megakaryocytic, and Early Erythroid Progenitors, Suppression of Late-Stage Erythropoiesis, and Reversal of Erythroid Suppression With Erythropoietin

By Candace S. Johnson, Douglas J. Keckler, Maureen I. Topper, Paul G. Braunschweiger, and Philip Furmanski

Interleukin-1α (IL-1α) is a macrophage-derived, multifunctional cytokine that broadly potentiates myelopoiesis and induces the synthesis of hematopoietic colony-stimulating factors (CSF) in vitro and in vivo. To evaluate the possibility for use of IL-1α in ameliorating in vivo bone marrow suppression induced by drugs or radiation, we examined the in vivo effects of the cytokine on erythropoiesis and other hematopoietic progenitor cells. Normal mice were treated with a single intraperitoneal (IP) injection of recombinant human IL-1α at varying doses and were assayed at various times post-treatment. By six hours postinjection, a significant suppression of mature erythroid progenitors (CFU-E) was observed in animals treated with IL-1α (0.5 μg/mouse), with maximum suppression of CFU-E and peripheral blood reticulocyte counts occurring at 24 hours.

Hematopoiesis is regulated by colony-stimulating factors (CSF) and other cytokines, which can exhibit either lineage-restricted or multipotent activities. Interleukin-1α (IL-1α) is a multifunctional cytokine that appears capable of broadly potentiating hematopoiesis. Produced mainly by macrophages and monocytes, IL-1α plays a primary role in T- and B-cell activation, stimulates the synthesis of CSF in vitro and in vivo, acts in concert with CSF to stimulate primitive hematopoietic progenitors, enhances myeloid recovery following cyclophosphamide and 5-fluorouracil treatment, and protects mice from the lethal effects of irradiation.

The diverse myelopoietic effects of IL-1, its known synergistic or additive activity with other regulatory molecules, and its ability to enhance recovery from severe hematopoietic insult have led to great interest in this cytokine as a myelopoietic agent for treatment of bone marrow toxicity or insufficiency. Studies of the in vivo hematopoietic effects of IL-1α have focused mainly on cells of the granulocyte-macrophage lineage. We have demonstrated that tumor necrosis factor (TNF), which shares many biological activities with IL-1, significantly increases numbers of macrophage, granulocyte, megakaryocyte, and immature erythroid progenitor cells. However, TNF also markedly suppresses late-stage erythropoiesis, leading eventually to anemia in chronically treated animals.

We report here that, like TNF, IL-1α significantly suppressed late-stage erythropoiesis in normal mice, while the number of more immature erythroid progenitors, as well as granulocytic, monocytic, and megakaryocytic precursors were markedly stimulated. Suppression of erythropoiesis was abrogated by simultaneous administration of the primary regulator of erythropoiesis, erythropoietin (EPO). These results demonstrate that IL-1α has profound effects on hematopoiesis that could be exploited for therapeutic purposes.

MATERIALS AND METHODS

Mice. These experiments were carried out in inbred NIH/PlcR mice that were originally obtained from the Veterinary Resources Branch, NIH, and inbred in our laboratories by brother-sister mating. The colonies were regularly monitored for the absence of adventitious viruses using mouse antibody production tests. Mice were age and sex matched for experimental use. For hematopoietic colony assays, spleens or femurs were removed from three to five animals per treatment group and assayed individually. All experiments reported here were replicated at least three times.

IL-1α. Recombinant human IL-1α was generously provided by Dr Peter Lomedico, Hoffmann-LaRoche, Nutley, NJ. The specific activity for the recombinant IL-1α was 2.5 x 10^6 U/mg protein, as determined using the D10.G4.1 assay. All solutions were made with pyrogen-free reagents; the endotoxin content of the IL-1α used was 0.05 EU/mg.

Reagents. Endotoxin (lipopolysaccharide W Escherichia coli 0127: B8) was obtained from Difco, Detroit. Human recombinant EPO for in vivo use was obtained from Amgen, Thousand Oaks, CA and had a specific activity of 70,000 U/mg of protein. Iron (Imferon, iron dextran, 50 mg/mL) was obtained from Merrell Dow, Cincinnati.

Hematopoietic progenitor assays. The plasma clot method of McLeod et al was used for the culture of mature erythroid...
progenitors (CFU-E) as previously described. A modification of the methyleneblue method of Isocure et al.⁴ was adapted to agar for the culture of immature erythroid burst-forming (BFU-E), granulocytomacrophage (CFU-GM), granulocyte (CFU-G), and megakaryocyte (CFU-meg) progenitors, as follows: 0.25 mL of spleen or bone marrow cells (1 x 10⁴ and 2 x 10⁴ cells/mL, respectively) were mixed with 0.25 mL of 10% deionized bovine serum albumin (BSA; containing 0.17% NaHCO₃), 0.5 mL EPO (10 U/mL; TCEpo, Amgen, Thousand Oaks, CA), 0.4 mL heat-inactivated horse serum, 0.4 mL pokeweed mitogen-stimulated spleen cell-conditioned medium,⁰ 0.5 mL heat-activated fetal calf serum (FCS), 0.020 mL hemin (10 mmol/L), and 1.5 mL of an agar mixture containing 1.0 mL of Bacto agar (1.5%), 1.0 mL 2x NCTC 109 medium, 0.020 mL hemin (10 mmol/L), and 1.5 mL of medium, 0.5 mL heat-activated fetal calf serum (FCS), 0.4 mL heat-inactivated horse serum, and 0.1 mL 0.4 mol/L). Aliquots of 0.5 mL (3 x 10⁵ spleen and 6 x 10⁵ bone marrow cells/well) were plated into four wells of a round-bottom well (2.2 x 1.4 cm) flexible culture plate (Linbro, #76-354-05). After seven to nine days incubation at 37°C in an N₂ atmosphere containing 5% CO₂, the agar plugs were fixed with 5% glutaraldehyde, adhered to glass slides, stained with di-methylbenzidine and hematoxylin, and scored on the basis of morphology. Agar cultures for macrophage (CFU-M) progenitors were performed as described previously using L-cell-conditioned media as a source of CSF.²¹

RESULTS

Effect of IL-1 on erythropoiesis. To determine the in vivo effect of IL-1 on normal erythropoiesis, groups of IL-1-treated and untreated animals were examined for changes in their erythroid progenitor compartments. Normal mice were injected intraperitoneally (IP) with 0.5 µg of recombinant human IL-1α (1.25 x 10⁶ U/mouse), the dose which in vivo maximally stimulates an increase in CFU-GM.⁷ After 48 hours, spleen and bone marrow were removed and examined for absolute numbers of CFU-E and BFU-E (Table 1). Numbers of mature CFU-E were significantly suppressed in the spleen and bone marrow of IL-1α-treated animals. No significant change was observed in spleen weight or total viable numbers of nucleated cells per spleen or femur from animals treated with IL-1α. The effect of IL-1α treatment on late-stage erythropoiesis was also manifest in peripheral blood reticulocyte counts, decreasing the percent reticulocytes from 9.5 ± 0.3 to 2.2 ± 0.7 (P < .001). No significant difference was observed in the hematocrits of normal (49 ± 2) and treated animals (49 ± 1) after a single IL-1α injection. In contrast to CFU-E, the immature erythroid progenitor, BFU-E, was significantly stimulated by in vivo IL-1 treatment (Table 1).

To quantitate the response of normal erythropoiesis to IL-1α, animals were injected IP with various doses of the cytokine and assayed for progenitor colony formation. As shown in Fig 1, CFU-E suppression was observed with a single injection of 0.125 µg/mouse or more. The BFU-E compartment was significantly stimulated in a dose-dependent manner between 0.25 µg and 1.0 µg/mouse, the highest dose analyzed. As previously reported,²² severe side effects (hair ruffling, diarrhea, lethargy) and mortality were observed in animals treated with doses greater than 1.0 µg IL-1 (2.5 x 10⁶ U/mouse).

The time course for effects of IL-1α on erythropoiesis in normal animals was determined following a single injection of 0.5 µg/mouse. As shown in Fig 2, numbers of spleen CFU-E were suppressed as early as six hours after IL-1α (31% ± 9%, decrease, P < .05), reached a nadir at 24 hours, and remained significantly suppressed for 72 hours post-treatment. By four days postinjection, CFU-E numbers returned to normal, and after five days a significant stimulation was observed in the splenic CFU-E compartment. The BFU-E compartment (Fig 2) was significantly stimulated 48

![Figure 1](https://example.com/fig1.jpg)

**Figure 1.** Dose response for IL-1α effects on normal CFU-E (Θ) and BFU-E (Ο). Animals were treated with various doses of IL-1α IP and assayed 48 hours post-treatment for numbers of CFU-E and BFU-E colony-forming cells in the spleen. Points represent mean percent of change ± SD calculated from three to five animals assayed individually (three cultures counted/animal) at each time point. Numbers of colony-forming cells at doses equal to or greater than 0.25 µg (BFU-E) or 0.125 µg (CFU-E) per mouse were significantly different from control without IL-1α (P < .001, Student’s t test).
hours after a single injection of 0.5 μg with numbers of BFU-E returning to normal by 72 hours. The dose and time parameters for the effects of IL-1α on bone marrow CFU-E and BFU-E were identical to those for spleen erythroid progenitors.

To determine the effects on late-stage erythropoiesis of multiple IL-1α injections, normal animals were treated with 0.5 μg of IL-1α every 12 hours for three days. Twelve hours after the last IL-1α injection, spleen CFU-E numbers were markedly decreased in treated mice (70,340 ± 10,220 SD) compared with controls injected with vehicle alone (820,000 ± 54,000, P < .001). Hematocrits were also significantly decreased in animals treated with repeated injections of IL-1α compared with controls [43 ± 2 SD and 48 ± 1, respectively, P < .001].

To establish that the effects on erythropoiesis were not due to contaminating endotoxin animals were injected with either heat-treated preparations of IL-1α (100°C for 60 minutes) or varying doses of purified endotoxin alone. Heat treatment abrogates IL-1α activity but does not affect endotoxin levels. No effect on erythropoiesis was observed when animals were injected with either heat-treated IL-1α or endotoxin at concentrations as high as 0.4 EU (0.05 ng/mL) had no effect on in vitro CFU-E colony formation (data not shown). Similarly, IL-1α had no effect on CFU-E colony formation in the presence of varying concentrations of EPO.

Reversal of in vivo CFU-E suppression with erythropoietin (EPO). Suppression of erythropoiesis by IL-1α was observed in the EPO-dependent CFU-E but not in the EPO-independent BFU-E compartment. To determine whether EPO could thus abrogate the suppressive effect on CFU-E, animals were treated simultaneously with 0.5 μg IL-1α and varying doses of human recombinant EPO (70,000 U/mg). As shown in Table 2, 0.5 U/mouse of EPO were sufficient to fully reverse the suppressive effects of IL-1α on CFU-E; EPO alone at this dose had no significant effect on spleen CFU-E numbers in normal animals (data not shown).

Effect of IL-1α on CFU-M, CFU-GM, CFU-G, and CFU-meg progenitors. In vivo treatment of normal animals with IL-1α has been shown to increase numbers of granulocyte-macrophage progenitors. To extend these observations, the effects of IL-1α on the various hematopoietic progenitor compartments were determined. As shown in Table 3, a single dose of IL-1α (0.5 μg) resulted in significant stimulation of total bone marrow and spleen CFU-M, CFU-GM, CFU-G, and CFU-meg at 48 hours postinjection.

Table 2. Reversal of CFU-E Suppression with In Vivo Treatment with EPO

<table>
<thead>
<tr>
<th>Treatment</th>
<th>rEPO Dose* U/Mouse</th>
<th>Per Spleen (x 10^5)</th>
<th>%C§</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>568.5 ± 86.6</td>
<td>100</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0</td>
<td>234.0 ± 20.05</td>
<td>59</td>
</tr>
<tr>
<td>0.1</td>
<td>279.4 ± 29.15</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>552.1 ± 62.7</td>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>594.8 ± 45.6</td>
<td>+5</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>712.5 ± 94.0</td>
<td>+25</td>
<td></td>
</tr>
</tbody>
</table>

*Human rEPO, 70,000 U/mg protein, was given IP simultaneously with IL-1α.
†Mean ± SD per total spleen was determined in three to five animals that were assayed individually (three cultures counted/animal) 48 hours after IP injection of 0.5 μg of IL-1α.
‡Percent change from control.
§Significantly different than control, no treatment (individual animals), P < .001 (Student’s t test).
¶P < .01.
Table 3. Effect of IL-1α on CFU-M, CFU-GM, CFU-G, and CFU-meg Progenitor Cells in Normal Animals

<table>
<thead>
<tr>
<th>Colony Type</th>
<th>Treatment</th>
<th>Per Spleen (x10^5)</th>
<th>%C†</th>
<th>Per Femur (x10^5)</th>
<th>%C§</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-M</td>
<td>None</td>
<td>14.2 ± 4.9</td>
<td>25.5 ± 1.9</td>
<td>48 hours after IP injection of 0.5 μg of IL-1α.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-1α</td>
<td>55.2 ± 5.6†</td>
<td>43.5 ± 3.1†</td>
<td>+288 (increases of 83% ± 22% and 52% ± 15%, respectively, P &lt; .001) with maximum stimulation obtained with 1.0 μg/mouse.</td>
<td></td>
</tr>
<tr>
<td>CFU-GM</td>
<td>None</td>
<td>18.6 ± 3.2</td>
<td>4.8 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-1α</td>
<td>28.8 ± 3.8†</td>
<td>7.6 ± 0.5†</td>
<td>+58</td>
<td></td>
</tr>
<tr>
<td>CFU-G</td>
<td>None</td>
<td>18.4 ± 5.2</td>
<td>15.6 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-1α</td>
<td>42.1 ± 6.9†</td>
<td>37.2 ± 9.7†</td>
<td>+138</td>
<td></td>
</tr>
<tr>
<td>CFU-meg</td>
<td>None</td>
<td>10.2 ± 5.3</td>
<td>4.4 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-1α</td>
<td>19.0 ± 5.8†</td>
<td>7.5 ± 2.1§</td>
<td>+70</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD per total spleen or femur was determined in three to five animals that were assayed individually (three cultures counted/animal) 48 hours after IP injection of 0.5 μg of IL-1α.
†Percent change from control.
‡Significantly different than control, no treatment (individual animals), P < .001 (Student’s t test).
§P < .01.

FIG 3. Dose response for in vivo IL-1α stimulation of normal CFU-M (○), CFU-G (●), CFU-GM (△), and CFU-meg (▲). Normal animals were treated with 0.5 μg/mouse of IL-1α IP and assayed for colony-forming cells in the spleen at various times post-IL-1α treatment. Points represent mean percent of change ± SD calculated from groups of three to five animals assayed individually (three cultures counted/animal) at each time point. Numbers of colony-forming cells at doses of IL-1α equal to or greater than 0.125 μg (CFU-M and CFU-G), 0.25 μg (CFU-GM), or 0.50 μg (CFU-meg) per mouse were significantly different from control (P < .001, Student’s t test).

FIG 4. Time course for stimulation of CFU-M (○), CFU-G (●), CFU-GM (△), and CFU-meg (▲). Normal animals were treated with 0.5 μg/mouse of IL-1α IP and assayed for colony-forming cells in the spleen at various times post-IL-1α treatment. Points represent mean percent of change ± SD calculated from groups of three to five animals assayed individually (three cultures counted/animal) at each time point. Numbers of colony-forming cells at 6, 12, 24, and 48 hours (CFU-M), 24 and 48 hours (CFU-G and CFU-GM), or 48 and 72 hours (CFU-meg) were significantly different from control without IL-1α (P < .001, Student’s t test).
drugs, could thus greatly enhance the therapeutic index of many cytotoxic drugs.

Recent advances in recombinant DNA technology have made large quantities of highly purified human IL-1 readily available. There are at least two biochemically distinct types of human IL-1, isofocusing at pIs of 5 (IL-1α) and 7 (IL-1β). Although a comparison of the primary structures of IL-1α and IL-1β revealed only 26% homology, they share many activities in vitro and the target cell specificities for both appear to be the same.33 Preliminary data suggest that many activities in vitro and the target cell specificities for many cytotoxic drugs, could thus greatly enhance the therapeutic index of many cytotoxic drugs.

Treatment with IL-1α, however, could result in lowered EPO levels prior to the manifestation of cachexia.

These studies were carried out using recombinant human IL-1α. In vivo activities of IL-1 appear to lack species specificity.46 Using recombinant murine and human IL-1α, however, we have observed quantitative differences in the effects of the cytokines on isologous vs homologous cells (manuscript in preparation).

In summary, IL-1α significantly increases BFU-E, CFU-M, CFU-G, CFU-GM, and CFU-meg numbers in the spleen and bone marrow of normal animals. This stimulation is accompanied by a significant suppression of late-stage erythropoiesis (CFU-E) that can lead to anemia in animals treated with multiple doses of IL-1α. The suppression of CFU-E was reversed when animals were simultaneously treated with EPO. These studies form the basis for gaining an understanding of the hematologic effects of IL-1α and for further evaluation of IL-1α as an agent to mitigate the bone marrow toxicities that occur during treatment of human malignancy. Recent studies in our laboratories have shown that IL-1α causes acute hemorrhagic necrosis and marked decrease in clonogenic cellularity of solid tumors49-50, together with the hematologic effects reported here, the results point to potentially important uses of IL-1 in tumor therapy.

ACKNOWLEDGMENT

We thank Cathrine Allen for preparation of the manuscript.

REFERENCES

12. Stork L, Barczuk L, Kissinger M, Robinson W: Interleukin-1...
IN VIVO EFFECTS OF IL-1α


In vivo hematopoietic effects of recombinant interleukin-1 alpha in mice: stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin

CS Johnson, DJ Keckler, MI Topper, PG Braunschweiger and P Furmanski