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Radioprotection of Mice with Interleukin-1: Relationship to the Number of Erythroid and Granulocyte-Macrophage Colony-Forming Cells[1]

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SCHWARTZ, G. N., PATCHEN, M. L., NETA, R., AND MACVITTIE, T. J. Radioprotection of Mice with Interleukin-1: Relationship to the Number of Erythroid and Granulocyte-Macrophage Colony-Forming Cells. *Radiat. Res.* 121, 220–226 (1990).

This report presents the results of an investigation of changes in the number of erythroid and granulocyte-macrophage colony-forming cells (GM-CFC) that had occurred in tissues of normal B6D2F1 mice 20 h after administration of a radioprotective dose (150 ng) of human recombinant interleukin-1 (rIL-1). Neutrophilia in the peripheral blood and changes in the tissue distribution of GM-CFC demonstrated that cells were mobilized from the bone marrow in response to rIL-1 injection. For example, 20 h after rIL-1 injection marrow GM-CFC numbers were 80% of the numbers in bone marrow from saline-injected mice. Associated with this decrease there was a twofold increase in the number of peripheral blood and splenic GM-CFC. Also, as determined by hydroxyurea injection, there was an increase in the number of GM-CFC in S phase of the cell cycle in the spleen, but not in the bone marrow. Data in this report suggest that when compared to the spleen, stimulation of granulopoiesis after rIL-1 injection is delayed in the bone marrow. Also, the earlier recovery of GM-CFC in the bone marrow of irradiated mice is not dependent upon an increase in the number of GM-CFC at the time of irradiation.

INTRODUCTION

An increase in the number of mice surviving lethal doses of radiation was observed when mice had been administered a single injection of recombinant interleukin-1 (rIL-1)[3] 18–24 h prior to their irradiation (*1*). Also, there was an earlier recovery of hematopoietic colony-forming cells (CFC) in the bone marrow and spleens from mice exposed to sublethal doses of radiation after rIL-1 injection (*2–6*). The physiological mechanisms promoting the earlier hematopoietic recovery in mice injected with rIL-1 are not well understood. Previous studies demonstrated that the earlier recovery of CFC was not dependent upon an increase in the number of spleen colony-forming units (CFU-S) or in the percentage of CFU-S in S phase of the cell cycle at the time of irradiation (*3–5, 7*).

An increase in colony-stimulating activity (CSA) for granulocyte-macrophage colony-forming cells (GM-CFC), erythroid burst-forming units (BFU-E), and multipotential colony-forming units has been observed in cultures of fibroblasts, endothelial cells, bone marrow stromal cells, or fetal liver stromal cell lines after stimulation with rIL-1 (*8–12*). The increased levels of CSA were partially due to an rIL-1 dose-dependent increase in the production of granulocyte-macrophage and granulocyte colony-forming factors and interleukin-6 (*10–12*). Elevated levels of CSA for GM-CFC also occurred *in vivo* after administration of rIL-1 (*13–14*). Several studies demonstrated that shortly after inducing the production of CSA, rIL-1 injection stimulated granulopoiesis in the bone marrow of normal mice (*13–16*). Neta *et al.* (*16*) suggested that the radioprotective effects of rIL-1 injection may be associated with an increase in the number of cycling CFC at the time of irradiation.

In previous studies it was found that in mice irradiated after rIL-1 injection there was an earlier recovery of GM-CFC in the bone marrow (*3, 4*). Studies in the present report were performed to investigate rIL-1-induced changes in CFC numbers in tissues of mice prior to their irradiation that might further delineate possible mechanisms for the observed earlier hematopoietic recovery. Specifically, the numbers of GM-CFC, erythroid colony-forming units

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[3] Abbreviations used: rIL-1, recombinant interleukin-1; CFC, colony-forming cells; CFU-S, spleen colony-forming units; CSA, colony-stimulating activity; GM-CFC, granulocyte-macrophage colony-forming cells; BFU-E, erythroid burst-forming units; CFU-E, erythroid colony-forming units.

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(CFU-E), and BFU-E in bone marrow or spleen were determined in mice 20 h after saline or rIL-1 injection. The decrease in GM-CFC, CFU-E, and BFU-E after hydroxyurea injection was used to measure the proportion of CFC in S phase of the cell cycle.

MATERIALS AND METHODS

Mice

B6D2F1, or (C57Bl/6J × DBA/2)F1, female mice were purchased from Jackson Laboratories (Bar Harbor, ME). Upon arrival, mice were maintained in an AAALAC-accredited facility. They were housed 10 per cage in plastic microisolator cages on hardwood-chip contact bedding, and were allowed food (Wayne Rodent Blox) and HCl-acidified water (pH 2.4) *ad libitum*. Animal holding rooms were maintained at 70 ± 2°F and 50 ± 10% relative humidity using at least 10 air changes per hour of 100% conditioned fresh air and exposed to full-spectrum light from 6:00 AM to 6:00 PM. Upon arrival, mice were tested for *Pseudomonas* contamination and quarantined until test results were obtained. Only healthy mice were released for experimentation. Twelve to 16-week-old mice were used for these studies. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

Irradiation

Twenty hours after saline or rIL-1 injection, mice were placed in ventilated Plexiglas boxes and exposed bilaterally to γ radiation from a ^{60}Co radiation source. In radiation studies, mice were exposed to 6.5, 1.0, or 0.5 Gy total-body irradiation at a dose rate of 0.40 Gy/min 20 h after saline or rIL-1 injection.

Treatment with Interleukin-1

Purified human rIL-1 alpha, a generous gift from Dr. Steve Gillis of Immunex (Seattle, WA), was used in these studies. The rIL-1 was supplied in a solution of phosphate-buffered saline at pH 7.2 with a specific activity of 7.5×10^6 U IL-1/mg protein, and aliquots were maintained at −70°C. Immediately before use, stock solutions of rIL-1 were diluted with pyrogen-free saline (McGaw), and 150 ng/0.5 ml was administered to normal mice by intraperitoneal (i.p.) injection. Mean body weight of mice used in these studies was 27 ± 2 g so that the average dose of rIL-1 was approximately 5.6 μg/kg body weight. Control animals were given 0.5 ml saline at the same time. Endotoxin (LPS) contamination in rIL-1 stock solutions was measured by the *Limulus* lysate assay. Based on these results less than 0.2 ng of LPS was administered per injection. The number of mice surviving >30 days after 10.5 Gy irradiation was similar for noninjected mice (1/10), saline-injected mice (1/10), and mice injected with 150 ng heat-inactivated rIL-1 (1/10). Also, data in an earlier report demonstrated that 150 ng rIL-1 from the same stock solutions as used in the present studies increased the number of B6D2F1 mice that survived after 10.5-Gy irradiation from 7 ± 13% to 85 ± 7% (*4*).

Preparation of Cell Suspensions

Peripheral blood for cell differential determinations was obtained from the orbital sinus of nonanesthetized mice using a 75-μl heparinized microhematocrit capillary tube (Curtin Matheson). Smears were made and stained for differential determinations with Hema 3 (Curtin Matheson), and 200 cells per mouse were counted. Different mice were used for each time point.

Mice were sacrificed by cervical dislocation, and the femurs and spleens were excised. Cells were flushed from the tissues with Hanks' balanced salt solution (HBSS) (GIBCO) and dispersed through a 25-gauge needle until a single cell suspension was obtained. All cell concentrations were determined by hemacytometer counts. When blood was collected for colony-forming assays, mice were anesthetized with ether, and blood was obtained from the vena cava with an 18-gauge needle attached to 1 ml heparinized syringe. The blood was diluted with an equal volume of HBSS and layered over Lympholyte-M (Cedarlane Labs) a Ficol-Hypaque solution with a density of 1.09 g/cm^3 to remove red blood cells and granulocytes (*17*). Cells at the interface were removed, washed two times with HBSS, and counted.

Assay for Granulocyte-Macrophage Colony-Forming Cells

The GM-CFC were assayed using the double-layer agar technique basically as described by Hagan *et al.* (*18*). The culture medium was double strength CMRL-1066 culture medium (Connaught Medical Research Laboratory) containing 10% (v/v) fetal calf serum, 5% (v/v) horse serum, 5% trypticase soy broth, 0.02 g/ml L-asparagine, and penicillin–streptomycin. In the bottom layer of 35-mm plastic petri dishes was 1 ml of a 1:1 mixture of culture medium and 1.0% agar (Bactoagar, Difco) containing 10% (v/v) L-929 cell-conditioned medium as a source of colony-stimulating activity. The top layer contained 1 ml of a 1:1 mixture of culture medium and 0.66% agar containing 5×10^4 bone marrow cells, 1×10^6 separated blood cells, or 1×10^6 spleen cells for assay. Cultures were incubated at 37°C in 5% humidified CO_2 in air. After 10 days of culture colonies greater than 50 cells were scored as GM-CFC.

Assays for Erythroid Colony-Forming Cells

Determinations of CFU-E and BFU-E were made using a plasma clot culture system basically as described by Weinberg *et al.* (*19*). Iscove's modified Dulbecco's medium (GIBCO) was substituted for α medium. Cells were plated with 0.25 U/ml (for CFU-E) or 3.0 U/ml (for BFU-E) anemic sheep plasma, step III erythropoietin (Connaught Labs, Inc., Lot No. 3092-2) as 0.4-ml plasma clots in 4-well Nunclone culture dishes (Nunc). Cultures of CFU-E and BFU-E were placed into a humidified 37°C incubator with 5% CO_2 for 2.5 and 8 days, respectively. Cultures were then harvested, fixed with 5% glutaraldehyde, stained with benzidine, and evaluated as described by McLeod *et al.* (*20*).

Determination of Colony-Forming Cells in S Phase of the Cell Cycle

The percentage of CFC in S phase of the cell cycle was determined basically as described by Rickard *et al.* (*21*). Mice were administered 900 mg/kg body wt hydroxyurea (Sigma) in Dulbecco's PBS (GIBCO) by i.p. injection. Control groups of mice were administered DPBS without hydroxyurea at the same time. Two and a half to 3 h later, tissues were assayed for surviving CFC. The number of CFC in tissues from hydroxyurea injected mice was compared to the number in tissues from DPBS-injected mice, and the percentage decrease in CFC after hyroxyurea injection was calculated as the percentage of CFC that were in S phase of the cell cycle.

Statistics

The two-tailed Student's *t* test was used to test for significant differences in cellularity and CFC per tissue between groups of mice.

RESULTS

Effect of rIL-1 on Recovery of Colony-Forming Cells after Irradiation

Two days after exposure to 0.5- or 1.0-Gy doses of radiation, rIL-1-injected mice had more BFU-E/femur and CFU-E/femur and spleen than saline-injected mice (Table

TABLE I
Recovery of Erythroid Colony-Forming Cells from Tissues of Saline or rIL-1-Injected Mice 2 Days after Irradiation[a]

	Recovery after 0.5-Gy injection		Recovery after 1.0-Gy injection	
Assay[b]	Saline	rIL-1	Saline	rIL-1
No./femur				
CFU-E ($\times 10^4$)[c]	1.1 ± 0.3	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.1
BFU-E ($\times 10^2$)[c]	1.3 ± 0.0	3.6 ± 0.8	0 ± 0	0.7 ± 0.3
No./spleen				
CFU-E ($\times 10^4$)[c]	1.4 ± 0.4	6.5 ± 1.0	0.8 ± 0.2	4.7 ± 0.6
BFU-E[c]	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] Female B6D2F1 mice (16 weeks old) were administered 0.5 ml saline or 150 ng rIL-1 by i.p. injection 20 h prior to their exposure to 0.5 or 1.0 Gy ^{60}Co radiation.

[b] Cells were pooled from both femurs and the spleens of two mice per group.

[c] Mean number ± SD of two plasma clots each.

I). When compared to the number of GM-CFC/femur for nonirradiated control mice, the percentage of GM-CFC surviving 24 h after 6.5-Gy radiation was 1.0 ± 0.3% for saline-injected mice and 1.2 ± 0.6% for rIL-1-injected mice (n = two studies). However, previous studies demonstrated that 4 and 8 days after exposure of mice to 6.5 Gy radiation, the number of GM-CFC in the bone marrow of rIL-1-injected mice was approximately 1.7 greater than the number in the bone marrow from saline-injected mice (*4*). These studies demonstrate that administration of rIL-1 to mice 20 h prior to their irradiation induced an earlier recovery of CFC. The following studies were performed to compare tissue cellularity and CFC content in saline-injected mice and rIL-1-injected mice prior to their irradiation.

Changes in Peripheral Blood Cellularity after rIL-1 Injection

The number of nucleated cells/mm^3 whole blood was similar for noninjected mice (10,423 ± 285 for 13 mice) and for mice 2, 6, 24, and 48 h after saline injection (8420 ± 2618 for 15 mice) or rIL-1 injection (9143 ± 1775 for 14 mice). However, compared to noninjected mice, a significant neutrophilia (Fig. 1) and lymphopenia (Fig. 2) were observed within 2 h after saline or rIL-1 injection. The percentage of neutrophils had increased to almost 80% of the nucleated cells in the blood with a significant increase in nonsegmented neutrophils from 12 ± 5% in saline-injected mice to 56 ± 15% in rIL-1-injected mice. By 6 h, the levels of more immature neutrophils had returned to normal values, but a twofold increase in the more mature segmented cells was still evident 48 h after rIL-1 injection. These results demonstrate that soon after injection of a radioprotective dose of rIL-1, mature and immature neutrophils were mobilized from the bone marrow to the blood.

Effect of rIL-1 Injection on the Number of GM-CFC

Twenty hours after saline or rIL-1 injection, there was no significant difference in splenic cellularity from saline and rIL-1-injected mice (Table II). However, total cellularity per

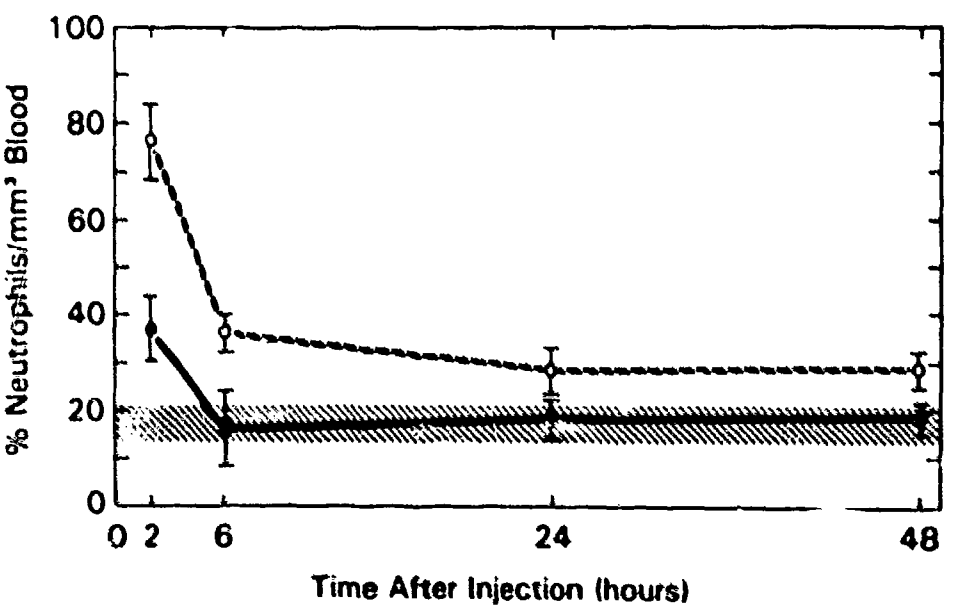

FIG. 1. Effect of rIL-1 injection on neutrophils in peripheral blood. Nucleated cell number and cell differentials were measured in peripheral blood from mice at various times after injection of 150 ng rIL-1 or 0.5 ml pyrogen-free saline. Values represent the mean ± SD of numbers from three to five mice (n = two studies). Separate mice were used at each time point. The number of neutrophils/mm^3 blood from noninjected mice was 1625 ± 573 (n = 10). (●) saline injection, (○) rIL-1 injection, (□) no injection.

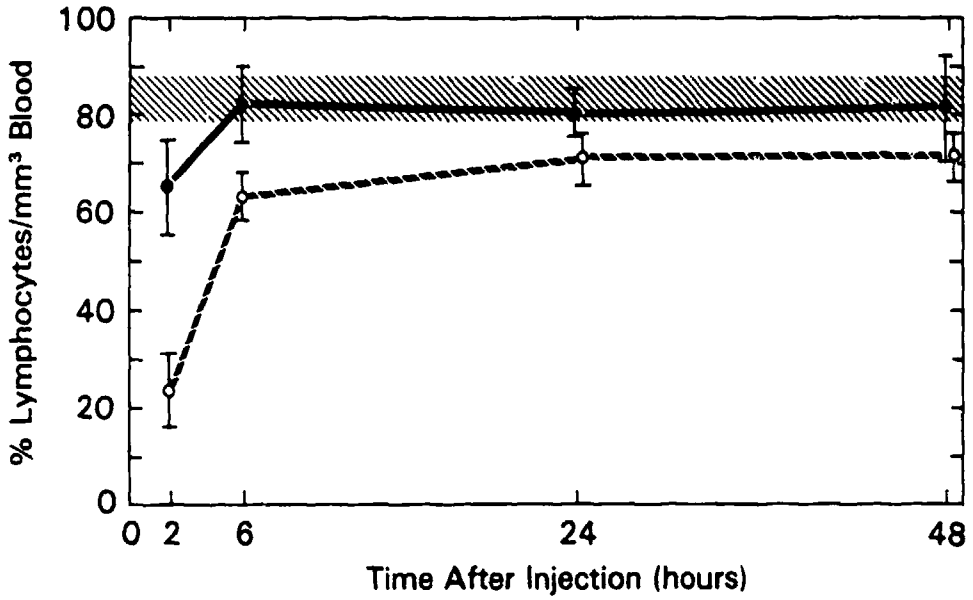

FIG. 2. Effect of rIL-1 injection on lymphocytes in peripheral blood. Nucleated cell number and cell differentials were determined in peripheral blood from mice various times after injection of 150 ng rIL-1 or 0.5 ml pyrogen-free saline. Values represent the mean ± SD of number from three to five mice (n = two studies). Separate mice were used at each time point, and the values were determined from the same samples represented in Fig. 1. Lymphocytes/mm³ blood from noninjected mice were 7870 ± 1705 (n = 10). (●) saline injection; (○) rIL-1 injection; (\) no injection.

femur of rIL-1-injected mice was approximately 79% of bone marrow cellularity from saline-injected mice. Similar to the decrease in marrow cellularity, there were approximately 20% fewer GM-CFC per femur of mice 20 h after rIL-1 injection (Table II). Associated with the reduction in the number of marrow GM-CFC from rIL-1-injected mice, there was an increased number of GM-CFC in the blood and spleen. In both blood and spleen, the number of GM-CFC was approximately twofold greater than the number in these tissues from saline-injected mice. These data suggest that, in addition to neutrophils, rIL-1 injection induced the mobilization of GM-CFC from the bone marrow.

Sensitivity of Granulocyte-Macrophage Colony-Forming Cells to Hydroxyurea

The decrease in the number of GM-CFC after hydroxyurea injection was used to determine the percentage of GM-CFC in S phase of the cell cycle 20 h after saline or rIL-1 injection. The percentage decrease in the number of GM-CFC in the bone marrow and spleen was similar ($P > 0.05$) for saline-injected mice and rIL-1-injected mice (Table III). Due to the increase in total number of GM-CFC per spleen induced by rIL-1 injection, this represented an increase from 0.3 ± 0.1 ($\times 10^3$) GM-CFC/spleen from saline-injected mice to 0.7 ± 0.04 ($\times 10^3$) GM-CFC/spleen from rIL-1 injected mice in S phase of the cell cycle. However, there was not a similar increase in the number of GM-CFC in S phase of the cell cycle in the bone marrow of rIL-1-injected mice. These results suggest that, compared to the spleen, stimulation of granulopoiesis after rIL-1 injection was delayed in the bone marrow.

Effect of rIL-1 Injection on Number of CFU-E and BFU-E

Twenty hours after rIL-1 injection, the number of CFU-E per femur was reduced to 69 ± 8% (n = 7) of the number in bone marrow from saline-injected mice. The number of CFU-E per spleen from rIL-1-injected mice was 101 ± 35% (n = 4) of the number from saline-injected mice. The number of BFU-E per femur was similar (P = 0.5) for noninjected mice [1.3 ± 0.6 ($\times 10^3$) n = 9 studies], mice 20 h after saline injection [1.3 ± 0.4 ($\times 10^3$) n = 6 studies], and mice 20 h after rIL-1 injection [1.1 ± 0.3 ($\times 10^3$) n = 6 studies]. A decrease in the percentage of CFU-E in S phase of the cell cycle was observed in bone marrow of mice 20 h after saline injection (Table III). There was no significant difference in the percentage of BFU-E in S phase of the cell cycle for saline or rIL-1-injected mice. These results demonstrate that rIL-1 injection did not induce an increase in the number of CFU-E or BFU-E in the bone marrow of mice prior to their irradiation.

DISCUSSION

Data in the present report confirm that, similar to previous studies with C57Bl/6J and B6D2F1 mice, rIL-1 injection induced an earlier hematopoietic recovery in mice after their exposure to sublethal doses of radiation (*3–4*). Also, in nonirradiated mice, marrow CFU-E levels 20–24 h after rIL-1 injection were decreased without an apparent compensatory increase in the spleen (*3*). Results in the present report further demonstrate that injection of a radioprotective dose (150 ng) of rIL-1 induced changes in the tissue

TABLE II
Cellularity and Number of GM-CFC in Tissues of Mice 20 h after Saline or rIL-1 Injection[a]

No./tissue[b]	Treatment: Saline injection (n)[c]	Treatment: rIL-1 injection (n)[c]	% rIL-1/saline[d]
Cellularity			
No./femur (×10⁷)	1.4 ± 0.2 (10)	1.1 ± 0.1 (11)[e]	79 ± 11
No./spleen (×10⁸)	1.3 ± 0.2 (9)	1.2 ± 0.2 (10)	95 ± 16
GM-CFC[f]			
No./femur (×10⁴)	1.6 ± 0.5 (10)	1.2 ± 0.4 (10)[e]	77 ± 12
No./spleen (×10⁵)	0.5 ± 0.3 (8)	1.3 ± 0.3 (8)[e]	231 ± 74
No./10⁶ blood	14 ± 5 (2)	33 ± 7 (3)[e]	252 ± 163

[a] Female B6D2F1 mice (12–15 weeks old) were administered 0.5 ml saline or 150 ng rIL-1 by i.p. injection 20 h before assay.

[b] Cells were pooled from both femurs and the spleens of three mice per group in each study.

[c] Number of studies.

[d] Mean ± SD calculated from the percentage of the number from rIL-1-injected mice/saline-injected mice of individual studies.

[e] Significantly different from saline-injected mice at $P < 0.05$.

[f] Values represent the mean ± SD of mean values from three plates per group.

distribution of mature hematopoietic cells and GM-CFC. Neutrophilia and a lymphopenia were observed within 2 h after rIL-1 injection. After 24 h, peripheral blood values were returning to normal. Twenty hours after rIL-1 injection, bone marrow GM-CFC content was 77% of values from saline-injected mice. Also, there was approximately a twofold increase in the number of GM-CFC in the blood and spleens from rIL-1-injected mice. An increase in the number of GM-CFC in S phase of the cell cycle as determined by *in vivo* hydroxyurea treatment was observed in the spleen, but not in the bone marrow of mice 20 h after rIL-1 injection.

Stork *et al.* (*13*) observed a twofold increase in the white blood cell count in mice 6 h after rIL-1 injection. This increase was associated with an increase in the number of neutrophils. Similarly, in the present report, neutrophil counts in the blood were increased in mice after rIL-1 injection. However, there was no increase in total white blood cells due to a comparable decrease in the number of lymphocytes. A lymphopenia has also been observed in rats after rIL-1 injection (*22*).

Others demonstrated that there was a comparable decrease of neutrophils in the bone marrow associated with the increase in the number of neutrophils in the blood after rIL-1 or endotoxin injection (*22–25*). Data in the present report and in an earlier report (*5*) demonstrated that rIL-1 injection induced a decrease in bone marrow cellularity. Neta *et al.* (*16*) observed a 25% increase in the proportion of large or blast cells in the bone marrow of mice 20 h after injection of a radioprotective dose of rIL-1. Metcalf and Wilson (*24*) reported a similar increase in the proportion of large cells such as blasts and myelocytes in the bone marrow of mice after endotoxin injection. In their studies, this was consistent with the capacity of endotoxin to induce mobilization of more mature granulocytes from the bone marrow (*24*).

In the present studies there was a reduction in the number of GM-CFC associated with the decrease in marrow cellularity observed in mice 20 h after rIL-1 injection. Castelli *et al.* (*15*) also observed a decrease in the number of GM-CFC in the bone marrow of mice after rIL-1 injection. In those studies, 6 h after intravenous administration of 0.5 μg/kg body weight, the number of GM-CFC per femur had decreased to 50% of normal values. By 24 h, the number of marrow GM-CFC was still only 75% of normal values. In the present studies and those by Castelli *et al.* (*15*) there was an increase in GM-CFC in blood and spleen associated with the decreased numbers in the bone marrow. Castelli *et al.* (*15*) also observed an early return of GM-CFC levels in peripheral blood to normal. These studies suggest that in addition to neutrophils a radioprotective dose of rIL-1 induced mobilization of GM-CFC from the bone marrow that was still evident 20 h after injection.

Earlier studies suggested that the decrease in marrow GM-CFC content after endotoxin injection is the result of mobilization of GM-CFC from the bone marrow into circulation, as well as differentiation of GM-CFC to replace the decrease in bone marrow neutrophils (*25, 26*). Several reports demonstrated that it is primarily a noncycling population of GM-CFC that is mobilized from the bone marrow

TABLE III
Decrease in the Percentage of Colony-Forming Cells after Administration of Hydroxyurea to Mice 20 h after Saline or rIL-1 Injection

Assay[a]	Treatment[a]: Saline injection (n)[b]	Treatment[a]: rIL-1 injection (n)[b]
% Decrease after hydroxyurea[c]		
GM-CFC/femur	43 ± 13 (4)	47 ± 14 (4)
GM-CFC/spleen	41 ± 14 (3)	56 ± 16 (3)
CFU-E/femur	76 ± 17 (4)	44 ± 15 (5)[d]
BFU-E/femur	46 ± 13 (3)	29 ± 8 (3)

[a] Female B6D2F1 mice (12–16 weeks old) were administered 0.5 ml saline or 150 ng rIL-1 by i.p. injection.

[b] Cells were pooled from both femurs and the spleens of three mice per group in each study.

[c] Number of studies.

[d] Mice were administered DPBS or 900 mg hydroxyurea/kg body weight 20 h after saline or rIL-1 injection, and 2.5–3 h later animals were euthanized for assay.

[e] Significantly different from saline-injected mice ($P < 0.05$).

after endotoxin or dextran injection or extended leukopheresis (*27–29*). Thus an increase in the percentage of GM-CFC in S phase of the cell cycle would be expected. However, in three studies of the present report, as determined by the number of GM-CFC surviving after hydroxyurea treatment, there was no significant increase in the percentage of marrow GM-CFC in S phase of the cell cycle. These studies suggest that the decrease in GM-CFC in bone marrow was a result of rIL-1 injection inducing an early mobilization of GM-CFC from the bone marrow and a differentiation of GM-CFC to replace the decreased marrow neutrophil levels.

Studies in the present report demonstrated that the earlier recovery of GM-CFC in the marrow of mice irradiated 20 h after rIL-1 injection was not dependent upon an increase in GM-CFC in the marrow or stimulation of granulopoiesis at the time of irradiation. However, other studies demonstrated that rIL-1 stimulates granulopoiesis in nonirradiated mice. For example, in studies in which the number of GM-CFC in the marrow from rIL-1-injected mice had returned to values found in saline-injected mice, there was an increase in percentage of GM-CFC in S phase of the cell cycle (*3*). Also, others demonstrated that marrow GM-CFC content increased 1.5- to 2.5-fold above normal values within 48–72 h after rIL-1 injection (*13, 15*). In preliminary studies, 3–5 days after injection of 150 ng rIL-1 (Hoffman La Roche) numbers of GM-CFC in the bone marrow were 2.0 ± 0.7 times greater ($n = 3$ studies) than in marrow from saline-injected mice (data not shown). Thus an increase in marrow GM-CFC content occurred after the optimal time for radioprotection. In a previous report (*3*) and studies by Stork *et al.* (*13*), GM-CFC content of the marrow was similar for mice 24 h after saline or IL-1 injection. Differences in dose of rIL-1 administered per kilogram body weight, timing of assays, batch of rIL-1, and mouse strain are possible reasons for variations in bone marrow GM-CFC content. Other studies demonstrated that the amplitude and time-course of IL-1-induced changes in the cellularity of peripheral blood and bone marrow in mice and rats were dependent upon the source and dose of IL-1 (*6, 13, 15, 22*).

Previous reports demonstrated that the serum CSA levels, which increased after rIL-1 injection, returned to normal within 24 h (*13, 14*). Studies by Francis *et al.* (*28*) also demonstrated that human blood CSA levels did not correlate with granulopoietic activity *in vivo*. However, their studies suggested that CSA produced by bone marrow cells regulated marrow granulopoiesis (*28*). Interleukin-1 has been shown to induce the production of CSA and CSF from a variety of cell types that include cells derived from bone marrow (*8–12*). Also earlier reports demonstrated that the radiosensitivity of GM-CFC could be decreased by an increasing CSA concentration or by changing the source of CSA (*29* and *30*). Thus one possible mechanism for the earlier recovery of GM-CFC in tissues of mice irradiated after rIL-1 injection is the production of hematopoietic growth and synergistic factors from cells that are part of the hematopoietic microenvironment.

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