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Administration of Interleukin-6 Stimulates Multilineage Hematopoiesis and Accelerates Recovery From Radiation-Induced Hematopoietic Depression

By M.L. Patchen, T.J. MacVittie, J.L. Williams, G.N. Schwartz, and L.M. Souza

Hematopoietic depression and subsequent susceptibility to potentially lethal opportunistic infections are well-documented phenomena following radiotherapy. Methods to therapeutically mitigate radiation-induced myelosuppression could offer great clinical value. In vivo studies in our laboratory have demonstrated that interleukin-6 (IL-6) stimulates pluripotent hematopoietic stem cell (CFU-s), granulocyte-macrophage progenitor cell (GM-CFC), and erythroid progenitor cell (CFU-e) proliferation in normal mice. Based on these results, the ability of IL-6 to stimulate hematopoietic regeneration following radiation-induced hematopoietic injury was also evaluated. C3H/HeN female mice were exposed to 6.5 Gy ^{60}Co radiation and subcutaneously administered either saline or IL-6 (1,000 μg/kg) on days 1 through 3 or 1 through 6 postexposure. On days 7, 10, 14, 17, and 22, femoral and splenic CFU-s, GM-CFC, and CFU-e contents and peripheral blood white cell, red cell, and platelet counts were determined. Compared with saline treatment, both 3-day and 6-day IL-6 treatments accelerated hematopoietic recovery; 6-day treatment produced the greater effects. For example, compared with normal control values (N), femoral and splenic CFU-s numbers in IL-6–treated mice 17 days postirradiation were 27% N and 136% N versus 2% N and 10% N in saline-treated mice. At the same time, bone marrow and splenic GM-CFC values were 58% N and 473% N versus 6% N and 196% N in saline-treated mice; bone marrow and splenic CFU-e numbers were 91% N and 250% N versus 31% N and 130% N in saline-treated mice; and peripheral blood white cell, red cell, and platelet values were 210% N, 60% N, and 24% N versus 18% N, 39% N, and 7% N in saline-treated mice. These studies demonstrate that therapeutically administered IL-6 can effectively accelerate multilineage hematopoietic recovery following radiation-induced hematopoietic injury.

NEUTROPENIA and thrombocytopenia are major factors contributing to morbidity and mortality after radiation exposure. Agents capable of enhancing host resistance to infection and/or regenerating hematopoietic elements necessary for efficient host defense mechanisms and hematopoietic hemostasis could be useful in treating myelosuppression caused by radiotherapy or accidental radiation exposures, such as those occurring recently in Chernobyl (USSR), Goiania (Brazil), and El Salvador (San Salvador).

Hematopoietic proliferation and differentiation are regulated by a variety of hematopoietic colony-stimulating factors (CSFs) and interleukins (ILs).[1,2] IL-6 is a pleiotropic cytokine that has been ascribed a variety of biologic activities including antiviral activity,[3,4] ability to stimulate B-cell differentiation and Ig secretion,[5,6] ability to stimulate hybridoma/plasmacytoma growth,[7] ability to activate T cells[8,9] and induce cytolytic T-cell differentiation,[10] and the ability to induce the production of acute-phase proteins.[11] In addition, IL-6 has recently been demonstrated to play a role in hematopoiesis.

Hematopoietic effects of IL-6 were first described by Ikebuchi et al[12] who reported that, in vitro, IL-6 acted synergistically with IL-3 to hasten the appearance of multilineage blast cell colonies grown from murine spleen cells. A similar synergy between IL-6 and IL-3 was shown using purified human bone marrow progenitors.[13] IL-6 has also been demonstrated to augment IL-3–induced megakaryocytopoiesis in vitro.[14] Furthermore, additional murine studies have demonstrated that incubating marrow cells in liquid cultures supplemented with IL-6 and IL-3 increases exogenous spleen colony-forming units (CFU-s) numbers and enhances the ability of the cultured cells to rescue lethally irradiated recipient mice.[15] Ikebuchi et al[12] proposed that IL-6 shifted hematopoietic stem cells from the G_0 to the G_1 stage of the cell cycle where they became more responsive to the effects of additional hematopoietic factors. This hypothesis has recently been substantiated by data of Rennick et al,[16] who demonstrated the ability of IL-6 to interact with IL-4, granulocyte-CSF (G-CSF), macrophage CSF (M-CSF), and GM-CSF to selectively enhance the clonal growth of progenitor cells at specific stages of lineage commitment and maturation. When used alone, IL-6 has been shown to directly support the in vitro proliferation of murine GM progenitors,[17,18] as well as to directly promote megakaryocyte maturation in vitro.[19] Furthermore, IL-6 can enhance the function of mature neutrophils.[20]

Compared with in vitro studies, in vivo experience with IL-6 has been rather limited. Suzuki et al[21] showed that continuous perfusion of IL-6 into normal mice increased splenic CFU-s numbers. Additionally, Okano et al[22] demonstrated that bone marrow transplanted mice which were subsequently treated with IL-6 exhibited both enhanced hematopoietic repopulation and enhanced survival. Dose-dependent increases in platelet counts have also been demonstrated in mice[23] and primates[24] receiving in vivo treatment with IL-6.

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We have further evaluated the in vivo effects of IL-6. In this article we report that IL-6 is capable of stimulating the proliferation of multiple lineages of hematopoietic progenitor cells in normal mice, and is also capable of accelerating multiple lineage hematopoietic regeneration following radiation-induced hematopoietic depression.

MATERIALS AND METHODS

Mice. C3H/HeN female mice (~20 g) were purchased from Charles River Laboratories (Raleigh, NC). Mice were maintained in an AAALAC (American Association for Accreditation of Laboratory Animal Care) accredited facility in Micro-Isolator cages (Lab Products, Maywood, IL) on hardwood-chip, contact bedding and were provided commercial rodent chow and acidified water (pH 2.5) ad libitum. Animal rooms were equipped with full-spectrum light from 6 AM to 6 PM and were maintained at 70°F ± 2°F with 50% ± 10% relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. On arrival, all mice were tested for Pseudomonas and quarantined until test results were obtained. Only healthy mice were released for experimentation. All animal experiments were approved by the Institute Animal Care and Use Committee before performance.

IL-6. IL-6 was provided by AMGen (Thousand Oaks, CA). This IL-6 (lot no. 012789) had a specific activity of 1.52×10^7 U/mg. One unit of IL-6 was defined as the amount required to stimulate the production of IgM by the SKW6.4 cell line to half maximal level. Endotoxin contamination was less than 0.5 ng/mg protein based on the limulus amebocyte lysate assay. IL-6 was administered subcutaneously (s.c.) in a 0.1-mL vol at the doses and times specified for individual experiments. Control mice were injected with an equal volume of sterile saline.

Irradiation. The Theratron-80 source at the Armed Forces Radiobiology Research Institute was used to administer unilateral total-body ^{60}Co γ radiation. Mice were placed in ventilated Plexiglas containers and irradiated at a dose rate of 0.4 Gy/min. Dosimetry was performed using ionization chambers with calibration factors traceable to the National Institute of Standards and Technology.

Cell suspensions. The cell suspensions used for each assay represented tissues from three normal, irradiated, or treated and irradiated mice at each time point. Cells were flushed from femurs with 3 mL of McCoy's 5A medium (Flow Labs, McLean, VA) containing 10% heat-inactivated fetal bovine serum (Hyclone Labs, Logan, UT). Spleens were pressed through a stainless steel mesh screen, and the cells were washed from the screen with 6 mL medium. The number of nucleated cells in the suspensions was determined by Coulter counter (Coulter, Hialeah, FL). Femurs and spleens were removed from mice killed by cervical dislocation.

Spleen colony-forming unit (CFU) assays. Spleen CFU have been shown to arise from the clonal proliferation of pluripotent hematopoietic stem cells. Exogenous CFU (CFU-s) were evaluated by the method of Till and McCulloch.[25] Recipient mice were exposed to 9 Gy of total body radiation to completely eradicate endogenous hematopoietic stem cells. Three to 5 hours later, 5×10^4 bone marrow or 5×10^4 spleen cells were intravenously (i.v.) injected into the irradiated recipients. Twelve days after transplantation, the recipients were killed by cervical dislocation and their spleens were removed. The spleens were fixed in Bouin's solution, and the number of grossly visible spleen colonies was counted. Endogenous spleen colony-forming units (E-CFU) were also evaluated by a method of Till and McCulloch.[26] Mice were exposed to 6.5 Gy of total body radiation to only partially ablate endogenous hematopoietic stem cells. Twelve days after irradiation, the spleens were removed, fixed in Bouin's solution, and the spleen colonies formed by the proliferation of surviving endogenous hematopoietic cells were counted. Each treatment group consisted of five mice and experiments were repeated twice.

Granulocyte-macrophage colony-forming cell (GM-CFC) assay. Hematopoietic progenitor cells committed to granulocyte and/or macrophage development were assayed using a double-layer agar GM-CFC assay.[27] Mouse endotoxin serum (5% vol/vol) was added to feeder layers as a source of CSF. Colonies (>50 cells) were counted after 10 days of incubation in a 37°C humidified environment containing 5% CO_2. Triplicate plates were cultured for each cell suspension, and experiments were repeated twice.

Erythroid colony-forming unit (CFU-e) assay. Bone marrow and splenic CFU-e were assayed by a modification[28] of the original plasma clot technique described by Stephenson et al.[29] Cells were plated in 0.4 mL plasma clots in 4-well Nunclon (Roskilde, Denmark) culture dishes with step III anemic sheep plasma (Connaught Labs, Swiftwater, PA) as the erythropoietin (Ep) source. Bone marrow and splenic CFU-e clot suspensions contained 0.25 and 0.50 U of Ep per milliliter, respectively. After incubation at 37°C in a humidified atmosphere containing 5% CO_2 in air for 2.5 days, plasma clots were harvested, fixed with 5% glutaraldehyde, and stained with benzidine and Giemsa. A CFU-e was defined as an individual aggregate of eight or more benzidine-positive cells.

Peripheral blood cell counts. Blood was obtained from halothane-anesthetized mice by cardiac puncture using a heparinized syringe attached to a 20-gauge needle. White blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts were performed using a Coulter counter. In addition, blood smears were prepared and stained with Diff-Quik (Bayer Healthcare Corp, McGaw Park, IL) to perform WBC differential counts.

Statistics. Results of replicate experiments were pooled and are represented as the mean ± standard deviation of pooled data. The Student's *t*-test was used to determine statistical differences. Significance level was set at $P < .05$.

Experimental design. Cytokine responses can vary significantly in different strains of mice.[30,31] Because of this, the first phase of this study was to identify an IL-6 dose capable of maximally stimulating hematopoietic proliferation in the C3H/HeN mouse strain used in our studies. IL-6 doses bracketing those previously reported to stimulate murine hematopoiesis in vivo[21,23,31] were evaluated using stimulation of endogenous spleen colony formation as a hematopoietic indicator. The second phase of this study was to determine the spectrum of hematopoietic progenitors (eg, CFU-s, GM-CFC, CFU-e) and mature peripheral blood cells (eg, WBC, RBC, PLT) capable of responding to IL-6, as well as to evaluate the duration of IL-6–induced hematopoietic responses. These studies were performed in normal (ie, nonirradiated) mice. Based on the multilineage hematopoietic effects induced by IL-6 in normal mice, the third phase of this study was initiated to evaluate the ability of IL-6 to stimulate multilineage hematopoiesis and to accelerate hematopoietic regeneration following radiation-induced hematopoietic injury. Because of the apparent ability of IL-6 to enhance stem cell lineage commitment and maturation,[12-14,16-19] we were concerned that prolonged in vivo IL-6 administration may induce stem cell "burn-out." For this reason, both long (6-day) and short (3-day) IL-6 treatment modalities were evaluated.

RESULTS

Hematopoietic stimulation is IL-6 dose dependent. The endogenous spleen colony assay was used to determine the dose of IL-6 required to obtain optimal hematopoietic stimulation in C3H/HeN mice. In these studies, IL-6 in doses of 50 μg/kg/d, 500 μg/kg/d, or 1,000 μg/kg/d was

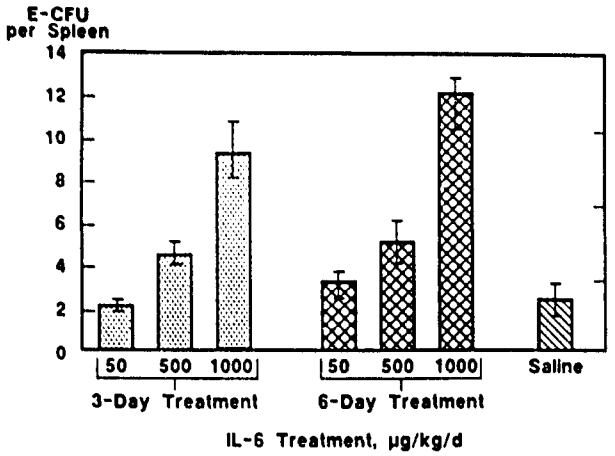

Fig 1. Effect of IL-6 dose and injection schedule on endogenous spleen colony formation in C3H/HeN mice exposed to 6.5 Gy ^{60}Co radiation. Data represent mean the ± standard deviation of values obtained from the spleens of 10 mice. A direct dose-dependent increase in E-CFU numbers was observed following both 3-day and 6-day IL-6 treatment. In both treatment groups IL-6 doses of 500 μg/kg/d and 1,000 μg/kg/d significantly increased ($P < .05$) E-CFU numbers with respect to saline control values. In addition, in both treatment groups the 1,000 μg/kg/d IL-6 dose increased E-CFU numbers to a significantly ($P < .05$) greater extent than the 500 μg/kg/d IL-6 dose.

injected s.c. into mice for either 3 days or 6 days. IL-6 doses were split such that one half of the dose was administered at 6 AM and one half of the dose at 6 PM. Twelve hours after the final IL-6 injection, mice were exposed to 6.5 Gy ^{60}Co and E-CFU numbers were determined 12 days later. Figure 1 illustrates that IL-6 produced a direct dose-dependent increase in E-CFU numbers and that the 6-day treatment was slightly more effective than the 3-day treatment. Based on these results, the 1,000 μg/kg/d IL-6 dose was chosen for subsequent experiments.

IL-6 stimulates multilineage hematopoiesis in normal mice. Normal mice were used to further characterize the hematopoietic response induced following a 3-day or 6-day treatment with IL-6 at the 1,000 μg/kg/d dose. On days 1, 4, 7, 10, 14, 17, and 22 after initiation of IL-6 treatment, bone marrow and splenic cellularity, CFU-s, GM-CFC and CFU-e numbers, as well as peripheral blood WBC, RBC, and PLT numbers were evaluated. Both 3-day and 6-day IL-6 treatment induced multilineage hematopoiesis as evidenced by changes in bone marrow (Table 1) and splenic (Table 2) stem and progenitor cell contents. Increases in splenic stem and progenitor cell numbers became evident as early as 4 days after initiation of IL-6 treatment (Table 2), while increases in bone marrow stem and progenitor cell numbers did not become evident until 10 days after initiation of IL-6 treatment (Table 1). The 6-day IL-6 treatment generally produced more dramatic and prolonged effects than the 3-day treatment. Interestingly, marrow contents on days 1, 4, and 7 following initiation of IL-6 treatment actually decreased, suggesting that IL-6 may induce bone marrow cell mobilization. Compared with the significant IL-6–induced responses observed at the stem and progenitor cell levels, peripheral blood WBC and RBC values remained relatively unchanged (Table 3). However, peripheral blood PLT values did slightly increase after both 3-day and 6-day IL-6 treatment (Table 3).

IL-6 therapy accelerates multilineage hematopoietic recovery following radiation injury. To evaluate the therapeutic potential of IL-6 in treating radiation-induced hematopoietic damage, mice were exposed to 6.5 Gy ^{60}Co radiation and subsequently administered IL-6 (1,000 μg/kg/d) for either 3 days or 6 days. On days 7, 10, 14, 17, and 22 following radiation exposure, bone marrow and splenic cellularity and CFU-s, GM-CFC, and CFU-e contents were evaluated. As shown in Table 4, both 3-day and 6-day IL-6 treatment accelerated recovery of femoral and splenic cellularity compared with irradiated saline controls. Recovery of femoral CFU-s (Fig 2), GM-CFC (Fig 3), and CFU-e (Fig 4) was also accelerated in irradiated mice by both 3-day and 6-day IL-6 treatment. Greater recovery was observed after 6-day IL-6 treatment; however, even with this treatment, femoral CFU-s numbers were only ~30% normal, GM-

Table 1. Effects of IL-6 on Bone Marrow Cellularity, CFU-s, GM-CFC, and CFU-e Contents in Normal Mice

	Treatment								
	Saline*	IL-6† Day After Initiation of IL-6 Injection							
			1	4	7	10	14	17	22
Cells per femur (× 10⁶)	5.4 ± 0.2	IL-6 × 3 d	4.7 ± 0.2	4.1 ± 0.3§	3.9 ± 0.2§	4.8 ± 0.4	6.3 ± 0.3§	6.3 ± 0.5	5.9 ± 0.5
		IL-6 × 6 d			4.1 ± 0.2§	4.3 ± 0.4§	6.8 ± 0.3§	5.8 ± 0.4	5.7 ± 0.3
CFU-s per femur (× 10⁴)	2.1 ± 0.3	IL-6 × 3 d	1.4 ± 0.1§	1.7 ± 0.1	2.3 ± 0.3	2.4 ± 0.2	3.2 ± 0.3§	1.7 ± 0.2	1.6 ± 0.1§
		IL-6 × 6 d			2.1 ± 0.1	3.1 ± 0.1§‖	4.7 ± 0.3§‖	3.1 ± 0.1§‖	1.7 ± 0.1
GM-GFC per femur (× 10³)	3.4 ± 0.3	IL-6 × 3 d	2.7 ± 0.2§	2.2 ± 0.1§	2.5 ± 0.1§	4.2 ± 0.1§	4.6 ± 0.2§	2.9 ± 0.2	3.5 ± 0.2
		IL-6 × 6 d			3.2 ± 0.1‖	4.5 ± 0.1§	5.5 ± 0.2§‖	3.9 ± 0.1‖	3.6 ± 0.1
CFU-e per femur (× 10³)‡	9.8 ± 1.3	IL-6 × 3 d	8.9 ± 0.5	8.0 ± 0.2	6.6 ± 0.5§	13.2 ± 1.0	18.5 ± 2.2§	15.2 ± 2.0§	11.6 ± 1.3
		IL-6 × 6 d			7.8 ± 0.8	8.7 ± 1.4‖	15.0 ± 1.8§	12.4 ± 0.5	14.3 ± 1.5§

Pooled results from two experiments.

*Values from mice treated for 3 days did not differ from values from mice treated for 6 days with saline. Therefore, all saline data were pooled.

†1,000 μg/kg/d of IL-6 administered s.c.

‡Mean results obtained from only one experiment.

§$P < .05$, with respect to saline values.

‖$P < .05$, with respect to values obtained from mice receiving IL-6 for 3 days.

Table 2. Effects of IL-6 on Splenic Cellularity, CFU-s, GM-CFC, and CFU-e Contents in Normal Mice

	Treatment								
	Saline*	IL-6† Day After Initiation of IL-6 Injection							
			1	4	7	10	14	17	22
Cells per spleen ($\times 10^6$)	1.1 ± 0.1	IL-6 × 3 d	1.5 ± 0.1§	2.2 ± 0.2§	1.4 ± 0.1§	1.2 ± 0.1	1.0 ± 0.1	1.5 ± 0.2§	1.8 ± 0.1§
		IL-6 × 6 d			1.6 ± 0.1§	1.2 ± 0.1	0.9 ± 0.1	1.5 ± 0.1§	2.0 ± 0.2§
CFU-s per spleen ($\times 10^3$)	7.3 ± 0.7	IL-6 × 3 d	6.1 ± 0.6	26.1 ± 1.8§	12.8 ± 0.9§	11.6 ± 1.2§	9.7 ± 0.7§	8.1 ± 0.7	7.9 ± 0.8
		IL-6 × 6 d			21.8 ± 0.4§‖	20.7 ± 1.0§‖	13.4 ± 0.4§‖	11.9 ± 1.3§‖	7.8 ± 0.8
GM-GFC per spleen ($\times 10^3$)	4.4 ± 0.5	IL-6 × 3 d	3.4 ± 0.4	31.6 ± 2.2§	8.7 ± 0.3§	6.8 ± 0.3§	6.5 ± 0.4§	6.3 ± 0.1§	4.0 ± 0.4
		IL-6 × 6 d			14.7 ± 0.6§‖	10.2 ± 0.4§‖	9.6 ± 0.5§‖	6.4 ± 0.3§	5.4 ± 0.2§‖
CFU-e per spleen ($\times 10^5$)‡	1.0 ± 0.1	IL-6 × 3 d	1.0 ± 0.2	2.3 ± 0.7	1.3 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	1.1 ± 0.1
		IL-6 × 6 d			3.3 ± 0.2§‖	1.5 ± 0.1§‖	0.9 ± 0.1	1.2 ± 0.1‖	1.3 ± 0.2

Pooled results from two experiments.

*Values from mice treated for 3 days did not differ from values from mice treated for 6 days with saline. Therefore, all saline data were pooled.

†1,000 μg/kg/d of IL-6 administered s.c.

‡Mean results obtained from only one experiment.

§$P < .05$, with respect to saline values.

‖$P < .05$, with respect to values obtained from mice receiving IL-6 for 3 days.

CFC numbers were only ~40% normal, and CFU-e numbers were ~80% normal at 22 days postirradiation. In the spleen, a much more dramatic hematopoietic response was observed (Figs 5 through 7). Even in saline-treated mice, explosive splenic hematopoietic recovery occurred approximately 2 weeks postirradiation, with progenitor cell numbers often overshooting control values before normalizing. Both 3-day and 6-day IL-6 treatment accelerated splenic CFU-s (Fig 5), GM-CFC (Fig 6), and CFU-e (Fig 7) recovery in irradiated mice. GM-CFC recovery was enhanced to a significantly greater extent than either CFU-s or CFU-e recovery. In mice treated with IL-6 for 6 days, peak splenic GM-CFC numbers reached 630% of normal values (Fig 6), compared with peak CFU-s numbers that reached only 210% of normal values (Fig 5), and peak CFU-e numbers that reached only 250% of normal values (Fig 7). In addition to intensifying the magnitude of the GM-CFC recovery, IL-6 treatment also intensified the duration of the overshoot phenomenon (Fig 6). The stimulation induced by IL-6 at the bone marrow and splenic stem and progenitor cell levels also ultimately influenced the mature blood cell levels as indicated by an accelerated reappearance of mature WBC (Fig 8), RBC (Fig 9), and PLT (Fig 10) in the peripheral blood. With regard to WBC, not only did IL-6 accelerate the recovery of total WBC numbers, but WBC differentials also returned to normal more rapidly in IL-6–treated mice. WBC in nonirradiated mice typically consisted of 27% ± 4% neutrophils, 69% ± 6% lymphocytes, and 4% ± 1% monocytes. On day 17 postirradiation, WBC in IL-6–treated mice consisted of 25% ± 5% neutrophils, 73% ± 4% lymphocytes, and 3% ± 1% monocytes, compared with 9% ± 2% neutrophils, 86% ± 5% lymphocytes, and 5% ± 2% monocytes in saline-treated mice. It was also noted that although 6-day versus 3-day IL-6 treatment produced different effects on bone marrow and splenic stem and progenitor cell recovery, very little difference between these two treatments was observed at the peripheral blood cell level.

DISCUSSION

Morbidity and mortality associated with high-level radiation exposures can be directly attributed to infectious and hemorrhagic complications resulting from radiation-induced neutropenia and thrombocytopenia. In recent years, several immunomodulators and hematopoietic growth factors have been evaluated for the ability to stimulate hematopoietic regeneration after radiation- or chemotherapy-induced myelosuppression. Of these, the immunomod-

Table 3. Effects of IL-6 on Peripheral Blood Cellularity in Normal Mice

	Treatment								
	Saline*	IL-6† Day After Initiation of IL-6 Injection							
			1	4	7	10	14	17	22
WBC per [illegible]	7.3 ± 0.3	IL-6 × 3 d	6.5 ± 0.5	6.6 ± 0.5	6.7 ± 0.6	6.9 ± 0.6	7.3 ± 0.5	7.4 ± 0.6	6.8 ± 0.6
[illegible]		IL-6 × 6 d			7.4 ± 0.6	7.5 ± 0.7	7.9 ± 0.8	7.7 ± 0.6	7.9 ± 0.6
RBC per [illegible]	6.5 ± 0.2	IL-6 × 3 d	6.6 ± 0.6	6.7 ± 0.5	6.9 ± 0.6	7.2 ± 0.5	7.4 ± 0.7	6.4 ± 0.4	6.9 ± 0.5
[illegible]		IL-6 × 6 d			7.2 ± 0.5	7.3 ± 0.5	7.5 ± 0.6	6.9 ± 0.6	6.6 ± 0.5
PLT per [illegible]	11.5 ± 0.6	IL-6 × 3 d	9.9 ± 0.6‡	13.7 ± 0.8‡	14.6 ± 1.0‡	10.7 ± 0.9	10.4 ± 0.9	10.0 ± 0.9	10.0 ± 0.7
[illegible]		IL-6 × 6 d			13.6 ± 1.0‡	11.0 ± 0.6	10.3 ± 0.7	10.0 ± 0.4	10.0 ± 0.5

Pooled results from two experiments.

*Values from mice treated for 3 days did not differ from values from mice treated for 6 days with saline. Therefore, all saline data were pooled.

†1,000 μg/kg/d of IL-6 administered s.c.

‡$P < .05$, with respect to values

Table 4. Effects of IL-6 on Bone Marrow and Splenic Cellularity in Irradiated Mice

	Day Postirradiation				
	7	10	14	17	22
Cells per femur ($\times 10^6$)*					
Saline†	1.8 ± 0.2	1.3 ± 0.3	1.8 ± 0.2	2.0 ± 0.1	2.5 ± 0.2
IL-6 × 3 d‡	1.2 ± 0.1‖	1.2 ± 0.2	1.9 ± 0.2	3.3 ± 0.2‖	3.3 ± 0.2‖
IL-6 × 6 d‡	1.1 ± 0.1‖	0.9 ± 0.3	1.5 ± 0.2	1.9 ± 0.2¶	3.7 ± 0.4‖
Cells per spleen ($\times 10^7$)§					
Saline†	1.3 ± 0.1	1.3 ± 0.1	1.9 ± 0.2	8.9 ± 0.9	20.7 ± 0.9
IL-6 × 3 d‡	1.1 ± 0.1	1.5 ± 0.1	2.4 ± 0.2	20.6 ± 0.9‖	8.7 ± 0.6‖
IL-6 × 6 d‡	1.2 ± 0.1	1.3 ± 0.1	4.6 ± 0.5‖¶	26.4 ± 0.9‖¶	14.8 ± 0.8‖¶

C3H/HeN mice were exposed to 6.5 Gy ^{60}Co radiation on day 0; pooled results from two experiments.

*Cellularity per femur in nonirradiated control mice was 5.4 ± 0.2 × 10^6.

†Values from mice treated for 3 days did not differ from values from mice treated for 6 days with saline. Therefore, all saline data were pooled.

‡1,000 μg/kg/d of IL-6 administered s.c. beginning 1 day after irradiation.

§Cellularity per spleen in nonirradiated control mice was 11.0 ± 1.0 × 10^7.

‖$P < .05$, with respect to saline values.

¶$P < .05$, with respect to values obtained from mice receiving IL-6 for 3 days.

ulator glucan[27,32] and the hematopoietic growth factors granulocyte CSF (G-CSF;[33-36]) and granulocyte-macrophage CSF (GM-CSF;[34,37,38]) have shown promise. G-CSF and GM-CSF selectively enhance granulocyte regeneration through their ability to both amplify GM-CFC progenitor cell pools and accelerate granulocyte maturation.[39,40] As a result of accelerating granulocyte reconstitution, these cytokines enhance survival in irradiated animals by reducing susceptibility to life-threatening opportunistic infections. However, preclinical studies involving large animals have demonstrated that, even with enhanced granulocyte regeneration, hemorrhage due to radiation-induced loss of platelets remains a life-threatening problem.[36,38] In addition, hemorrhage exacerbates anemia, which also occurs following radiation-induced hematopoietic injury. Currently these problems are only controlled by PLT and RBC transfusions.[36,37]

In view of these complications, agents capable of stimulating multiple lineage (especially granuloid, platelet, and erythroid) hematopoietic reconstitution would be extremely useful for the treatment of radiation-induced hematopoietic injury. Our studies in normal mice confirmed the ability of IL-6 to enhance CFU-s, GM-CFC, and PLT production.[21,23] In addition, we demonstrated the ability of IL-6 to increase CFU-e numbers. Because of these multilineage hematopoietic effects, IL-6 appeared to be an especially appropriate cytokine to evaluate for usefulness in the treatment of radiation-induced hematopoietic depression. Results obtained from our murine model of radiation-

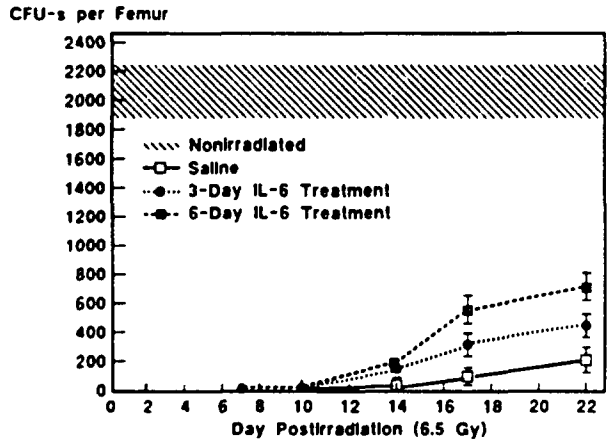

Fig 2. Effect of IL-6 on bone marrow CFU-s recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, CFU-s numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14, 17 and 22; 6-day IL-6 treatment produced a greater response, with 6-day IL-6 values being significantly ($P < .05$) increased above 3-day IL-6 values on days 17 and 22.

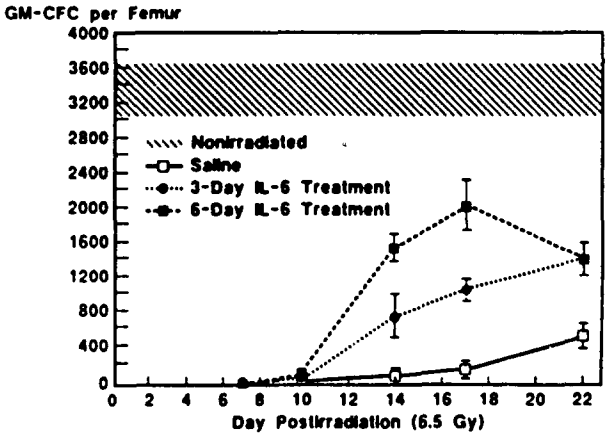

Fig 3. Effect of IL-6 on bone marrow GM-CFC recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, GM-CFC numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 10, 14, 17, and 22; 6-day IL-6 treatment produced a greater response, with 6-day IL-6 values being significantly ($P < .05$) increased over 3-day IL-6 values on days 14 and 17.

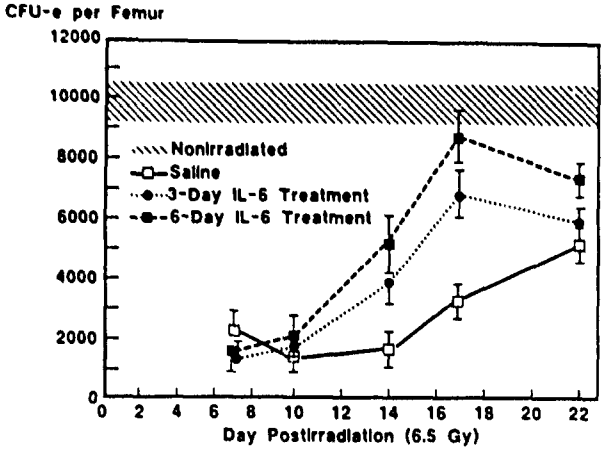

Fig 4. Effect of IL-6 on bone marrow CFU-e recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for 3 days or 6 days. Data for 6-day IL-6 treatment represent the mean ± standard deviation of pooled values obtained from two separate experiments. Data for 3-day IL-6 treatment represent the mean ± standard deviation of values obtained from one experiment. In comparison with saline controls, CFU-e numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14 and 17. On day 22, CFU-e numbers in 6-day IL-6–treated mice were also significantly ($P < .05$) higher than either saline or 3-day IL-6 values.

induced hematopoietic depression clearly showed that IL-6 also stimulates multiple lineage hematopoietic regeneration after radiation injury. IL-6–treated mice exhibited accelerated bone marrow and splenic CFU-s, GM-CFC, and CFU-e regeneration, as well as accelerated recovery of mature peripheral WBC, RBC, and PLT. The 6-day IL-6

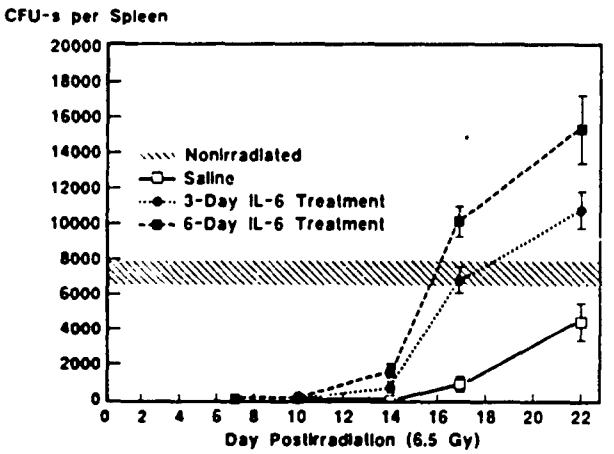

Fig 5. Effect of IL-6 on splenic CFU-s recovery in irradiated C3H HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, CFU-s numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14, 17, and 22. In addition, 6-day IL-6 treatment produced a greater response, with 6-day IL-6 values being significantly ($P < .05$) increased above 3-day IL-6 values on days 10, 14, 17, and 22.

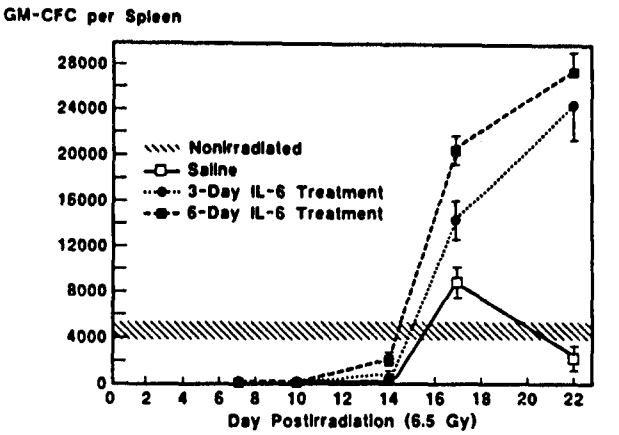

Fig 6. Effect of IL-6 on splenic GM-CFC recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained form two separate experiments. In comparison with saline controls, GM-CFC numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14, 17, and 22. Six-day IL-6 treatment produced a greater response, with 6-day IL-6 values being significantly ($P < .05$) increased above 3-day IL-6 values on days 14 and 17.

treatment induced the greatest recovery, with no evidence of stem cell "burn out." Recently Takatsuki et al[41] have also reported the ability of IL-6 to accelerate CFU-s, GM-CFC, and PLT recovery following chemotherapy-induced hematopoietic depression.

The IL-6 dose used (1,000 μg/kg/d) to produce our reported hematopoietic effects may seem high with respect to doses of cytokines such as G-CSF or GM-CSF. However, to obtain good hematopoietic stimulation with these cyto-

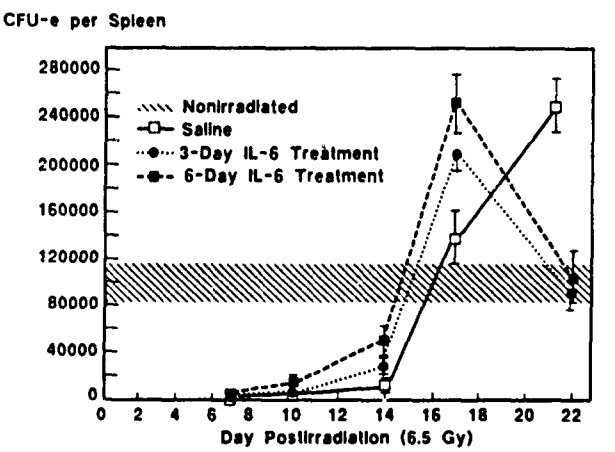

Fig 7. Effect of IL-6 on splenic CFU-e recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for 3 days or 6 days. Data for 6-day IL-6 treatment represent the mean ± standard deviation of pooled values obtained from two separate experiments. Data for 3-day IL-6 treatment represent the mean ± standard deviation of values obtained from one experiment. In comparison with saline controls, CFU-e numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 10, 14, and 17.

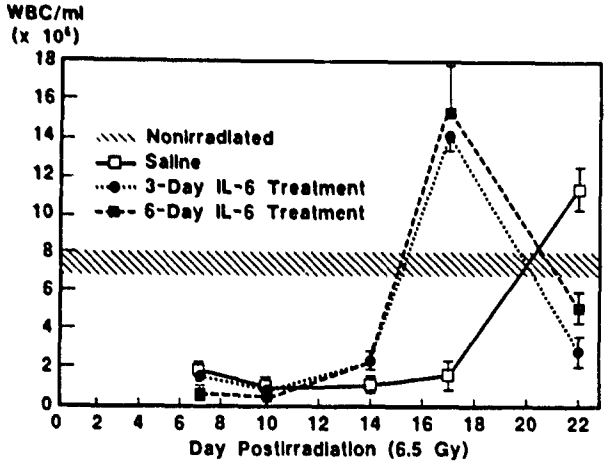

Fig 8. Effect of IL-6 on WBC recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, WBC numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14 and 17.

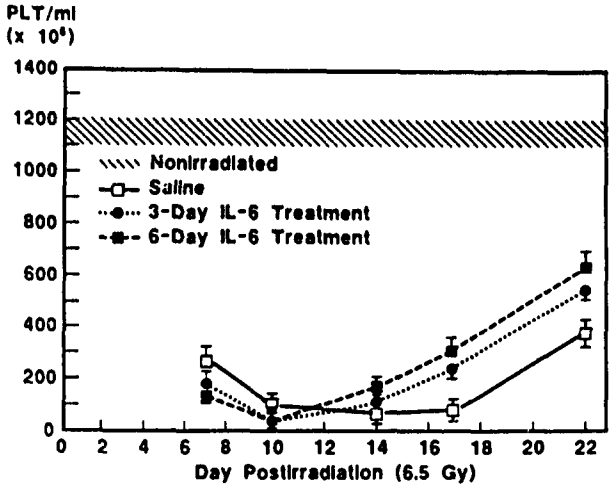

Fig 10. Effect of IL-6 on peripheral blood platelet recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, PLT numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 14, 17, and 22.

kines, extended therapy is generally required and we observed good hematopoietic stimulation with as few as 3 days of IL-6 treatment. In reality, valid comparisons of cytokine effectiveness are difficult to make because of differences in cytokine-specific activities, as well as routes of cytokine administration (s.c., i.p., i.v.), administration schedules (continuous infusion, once a day, twice a day, etc), and durations of treatment (ranging from 1 to 22 days) used in various published studies.

IL-6 has been hypothesized to mediate its multilineage hematopoietic effects by shifting stem cells from the G_0 to the G_1 stage of the cell cycle where they become more responsive to additional hematopoietic growth factors such as IL-3, IL-4, G-CSF, M-CSF, or GM-CSF.[12-16] The effect of radiation exposure alone on the endogenous production of such cytokines has not been fully determined. However, ultraviolet (UV) radiation has been shown to increase the synthesis and release of IL-1 and GM-CSF by epidermal cells,[42,43] and sublethal γ radiation has been shown to increase IL-1 and tumor necrosis factor (TNF) production by peritoneal macrophages.[44] Thus, in the sublethal radiation model used in our studies, it seems likely that some macrophages and accessory cells capable of producing cytokines would survive radiation exposure, and that these cytokines would be present to interact with IL-6 in influencing stem cell proliferation and commitment.

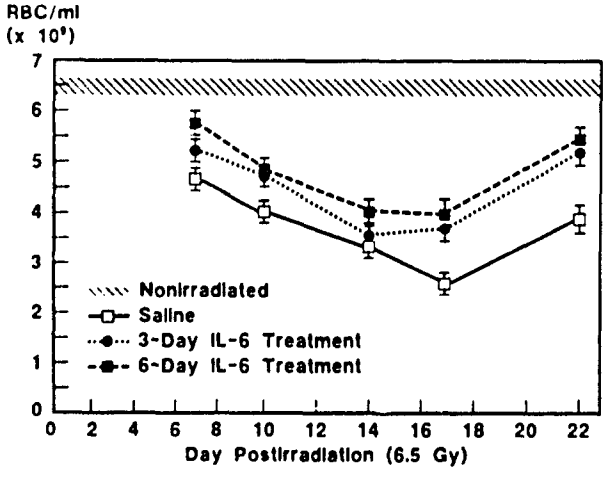

Fig 9. Effect of IL-6 on RBC recovery in irradiated C3H/HeN mice. Mice were exposed to 6.5 Gy ^{60}Co and administered IL-6 (1,000 μg/kg/d, s.c.) for either 3 days or 6 days. Data represent the mean ± standard deviation of pooled values obtained from two separate experiments. In comparison with saline controls, RBC numbers in both 3-day and 6-day IL-6–treated mice were significantly ($P < .05$) increased on days 7, 10, 17, and 22; 6-day IL-6 values were also significantly ($P < .05$) increased on day 14.

The fact that IL-1 and TNF have been reported to increase in mice after a sublethal radiation exposure such as that used in our studies is especially interesting.[44] Both IL-1 and TNF have been demonstrated to be potent inducers of IL-6.[45,46] Hence, the hematopoietic regeneration that ultimately does occur following sublethal radiation injury (as illustrated in Figs 2 through 10 by our data obtained from saline-treated mice) may be partially mediated by the induction of endogenous IL-6 by endogenous IL-1 and TNF released after radiation exposure. Clearly, however, our studies show that augmenting endogenous IL-6 levels further accelerates hematopoietic regeneration. Exogenous administration of IL-1 to myelosuppressed mice has also been shown to enhance hematopoietic regeneration[47-49]; induction of IL-6 may likewise play a role in this phenomenon. Interestingly, as observed with IL-1,[47] administration of IL-6 to sublethally irradiated mice delays splenic and thymic lymphoid recovery (Williams JL, Patchen ML, Darden J: unpublished results, August 1990). These prelim-

inary results further suggest that many of IL-1's effects on lymphopoiesis may involve the induction of IL-6.

In conclusion, we have demonstrated the ability of IL-6 to induce multilineage hematopoietic stimulation in vivo capable of accelerating the regeneration of mature WBC, PLT, and RBC in radiation-injured mice. Whether these effects are directly mediated by IL-6 or mediated by secondary hematopoietic growth factors induced following in vivo IL-6 administration remains to be determined. Nevertheless, these results suggest that IL-6 may be therapeutically useful in the treatment of radiation- or chemotherapy-induced myelosuppression requiring multilineage repopulation.

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