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Experimental Hematology

Mast cell growth factor enhances multilineage hematopoietic recovery in vivo following radiation-induced aplasia

M.L. Patchen,[1] R. Fischer,[1] E.A. Schmauder-Chock,[1] D.E. Williams[2]

[1]Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD; [2]Immunex Corporation, Seattle, WA

Offprint requests to: Dr. Myra L. Patchen, Department of Experimental Hematology, Armed Forces Radiobiology Research Institute, 8901 Wisconsin Avenue, Bethesda, MD 20889-5603

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Abstract

Based on in vitro studies, mast cell growth factor (MGF; also known as steel factor, stem cell factor, and c-*kit* ligand) has been implicated as an important hematopoietic regulator, especially in the presence of additional hematopoietic cytokines. Since hematopoietic regeneration follows sublethal radiation-induced hematopoietic injury and is thought to be mediated by endogenously produced cytokines, the ability to accelerate recovery from radiation-induced hematopoietic hypoplasia was used to evaluate in vivo effects of MGF administration. Female $B_6D_2F_1$ mice were exposed to a sublethal 7.75-Gy dose of ^{60}Co radiation followed by subcutaneous administration of either saline or 100, 200, or 400 μg/kg/d recombinant murine MGF on days 1 to 17 postirradiation. Recoveries of bone marrow and splenic spleen colony-forming units (CFU-S), granulocyte-macrophage colony-forming cells (GM-CFC), and peripheral white blood cells (WBC), red blood cells (RBC), and platelets (PLT) were determined on days 14 and 17 during the postirradiation recovery period. MGF accelerated hematopoietic recovery at the 100 and 200 μg/kg/d doses. The 100 μg/kg/d dose accelerated recovery of only GM-CFC, while the 200 μg/kg/d dose accelerated CFU-S, GM-CFC, WBC, and PLT recoveries. In contrast, hematopoietic recovery was delayed in mice receiving the 400 μg/kg/d dose. These studies demonstrate the in vivo dose-dependent ability of MGF to accelerate multilineage hematopoietic regeneration following radiation-induced hematopoietic hypoplasia. They also document detrimental effects of providing "supraoptimal" doses of this growth factor and suggest caution in dose-escalation trials in humans.

Key words: MGF—Irradiation—Aplasia—c-*kit* ligand—Therapy—Stem cells—Stem cell factor

Introduction

Neutropenia and thrombocytopenia are major factors contributing to morbidity and mortality associated with hematopoietic injury. Agents capable of enhancing regeneration of cellular elements necessary for efficient host defense mechanisms and facilitating hematopoietic hemostasis would be useful in treating hematopoietic hypoplasia caused by accidental radiation exposures, radiotherapy, and chemotherapy.

Hematopoietic proliferation and differentiation are known to be regulated by a variety of colony-stimulating factors and interleukins [1,2]. Mast cell growth factor (MGF), also known as steel factor (SLF), stem cell factor (SCF), and c-*kit* ligand, is the most recent cytokine implicated in hematopoietic regulation [3–5]. This factor was initially identified and purified based on its ability to stimulate mast cell growth; however, it has subsequently been ascribed numerous hematopoietic and nonhematopoietic effects [4–7].

In vitro, c-*kit* ligand has been shown to synergize with numerous hematopoietic cytokines, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), IL-3, IL-6, IL-7, and erythropoietin (Epo) [5–19]. The observations that c-*kit* ligand in combination with other cytokines appears to generate large numbers of both committed colony-forming cells (CFC) and pre-CFC suggest that this factor may act earlier than other hematopoietic factors described to date.

c-*kit* ligand has also been implicated in hematopoietic regulation in vivo. Most notably, mice with mutations at the *Steel* (*Sl*) locus, which encodes c-*kit* ligand, are defective in hematopoietic cell development, exhibiting severe macrocytic anemia that is resistant to erythropoietin treatment [20], profound deficiencies in tissue mast cells [21], abnormalities in megakaryocytopoiesis [22], and reduced granulocytopoiesis [23]. The hematopoietic defects in *Steel* mice can be partially corrected by the administration of c-*kit* ligand [24]. In addition to the data accumulated on *Steel* mice, a limited number of studies have recently reported the ability of c-*kit* ligand to alter hematopoiesis in normal mice, rats, and nonhuman primates [25–27]. In rats, a single intravenous (IV) injection of recombinant rat (rr) c-*kit* ligand induced a rapid and transient neutrophilia and lymphocytosis; prolonged (14-day) administration resulted in bone marrow mast cell hyperplasia but erythroid and lymphoid hypoplasia [25]. When rr c-*kit* ligand was administered daily for 21 days in mice, it was found to be only a modest stimulator of peripheral blood neutrophil production but a potent stimulator of splenic CFU-S production [26]. In baboons, continuous infusion of recombinant human (rh) c-*kit* ligand caused an increase in peripheral blood erythrocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil numbers, as well as an increase in bone marrow cellularity, GM-CFC, and erythroid burst-forming units (BFU-E) [27].

In contrast to previous studies performed with c-*kit* ligand in normal animals, we have evaluated the ability of this factor to stimulate hematopoiesis in the more clinically relevant condition of hematopoietic hypoplasia. Because hematopoietic recovery in the sublethal murine radiation model used in our studies is presumed to be mediated by endogenously produced hematopoietic cytokines [28,29], and because c-*kit* ligand has been shown to synergize with other cytokines, we hypothesized that c-*kit* ligand may synergize

with endogenous cytokines in irradiated mice and accelerate hematopoietic recovery.

Materials and methods

MGF

Recombinant murine c-*kit* ligand, henceforth referred to as MGF, was provided by Immunex (Seattle, WA). It was expressed in yeast and purified to homogeneity as previously described [12]. Endotoxin contamination was below the limit of detection using the limulus amebocyte lysate assay. MGF was administered subcutaneously (s.c.) in a 0.1-mL volume at doses of 100, 200, or 400 μg/kg. Injections were initiated 1 day following irradiation and continued daily for 17 days. Control mice were injected with an equal volume of sterile saline.

Mice

$B_6D_2F_1$ female mice (~20 g) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were maintained in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) in Micro-Isolator cages on hardwood-chip contact bedding and were provided with commercial rodent chow and acidified water (pH 2.5) ad libitum. Animal rooms were equipped with full-spectrum light from 6 a.m. to 6 p.m. and were maintained at 21 ± 1°C 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.

Irradiation

The ^{60}Co source at the Armed Forces Radiobiology Research Institute was used to administer bilateral total-body gamma radiation. Mice were placed in ventilated Plexiglas containers and irradiated with 7.75 Gy at a dose rate of 0.4 Gy/min. Dosimetry was performed using ionization chambers [30] with calibration factors traceable to the National Institute of Standards and Technology. The tissue-to-air ratio was 0.96. Dose variation within the exposure field was <3%.

Peripheral blood cell counts

Blood was obtained from halothane-anesthetized mice by cardiac puncture using a heparinized syringe attached to a 20-gauge needle. WBC, RBC, and PLT counts were performed using a Coulter counter.

Cell suspensions

Cell suspensions for each assay represented tissues from three normal, irradiated, or irradiated-plus-MGF-treated mice at each time point. Cells were flushed from femurs with 3 mL 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 cells were washed from the screen with 6 mL medium. The number of nucleated cells in the suspensions was determined by Coulter counter. Femurs and spleens were removed from mice killed by cervical dislocation.

GM-CFC assay

Hematopoietic progenitor cells committed to granulocyte and/or macrophage development were assayed using a double-layer agar GM-CFC assay in which mouse endotoxin serum (5% vol/vol) was added to feeder layers as a source of colony-stimulating factors [31]. 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.

CFU-S assay

Exogenous CFU-S were evaluated by the method of Till and McCulloch [32]. Recipient mice were exposed to 9 Gy total-body radiation to reduce endogenous hematopoietic stem cells. Three to 5 hours later, bone marrow, spleen, or peripheral blood cells were injected IV into the irradiated recipients. Twelve days after transplantation, the recipients were killed by cervical dislocation, their spleens were removed and fixed in Bouin's solution, and grossly visible spleen colonies were counted. Each treatment group consisted of five mice.

Survival assay

Recipient mice were exposed to 9.5 Gy total-body radiation, and various numbers of bone marrow or spleen cells were injected IV. Animal survival was recorded daily for 60 days.

Histopathology

Mice were killed by cervical dislocation, and the spleen, bone marrow, and proximal small intestine were removed and immersion-fixed for 2 hours in a modified Karnovsky's fixative consisting of 2% paraformaldehyde, 2.5% glutaraldehyde, and 4 mM $MgCl_2$ in 100 mM cacodylate buffer (pH 7.3). Specimens were postfixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in Epon 812. To enhance the visualization of mast cells, sections were stained with methylene blue-azure II, a metachromatic stain. With this stain, the granules of nonsecreted connective tissue mast cells stain dark purple while secreted granules appear pink. Sections were examined and photographed with a Zeiss Ultraphot microscope.

Statistical analysis

Results of replicate experiments were pooled and are represented as the mean ± standard error (SE) of pooled data. Bone marrow and splenic hematopoietic colony and blood cell data were analyzed by Student's *t*-test, survival data were analyzed by Fisher's exact test, and peripheral blood CFU-S data were analyzed by a two-way analysis of variance (ANOVA). Significance level was set at $p<0.05$.

Experimental design

The ability to accelerate hematopoietic regeneration in a murine model of radiation-induced hematopoietic hypoplasia was used to evaluate the in vivo effects of MGF. Mice were exposed to a sublethal (7.75 Gy) dose of ^{60}Co radiation to induce severe hematopoietic hypoplasia. MGF was administered s.c. daily on days 1 to 17 postexposure. On days 14 and 17 during the postirradiation recovery period, three mice from each treatment group were randomly selected and bone marrow and splenic cellularity; CFU-S and GM-CFC recoveries; peripheral blood CFU-S, WBC, RBC, and PLT recoveries; and tissue histopathological changes were evaluated. The day-14 and day-17 assay points were chosen to bracket the most dynamic period of hematopoietic recovery expected following the 7.75-Gy radiation exposure, and were based on our previous knowledge regarding the kinetics of hematopoietic recovery in this murine radiation model [33].

Results

Compared to saline-treated irradiated mice, some irradiated mice given daily injections of MGF exhibited enhanced

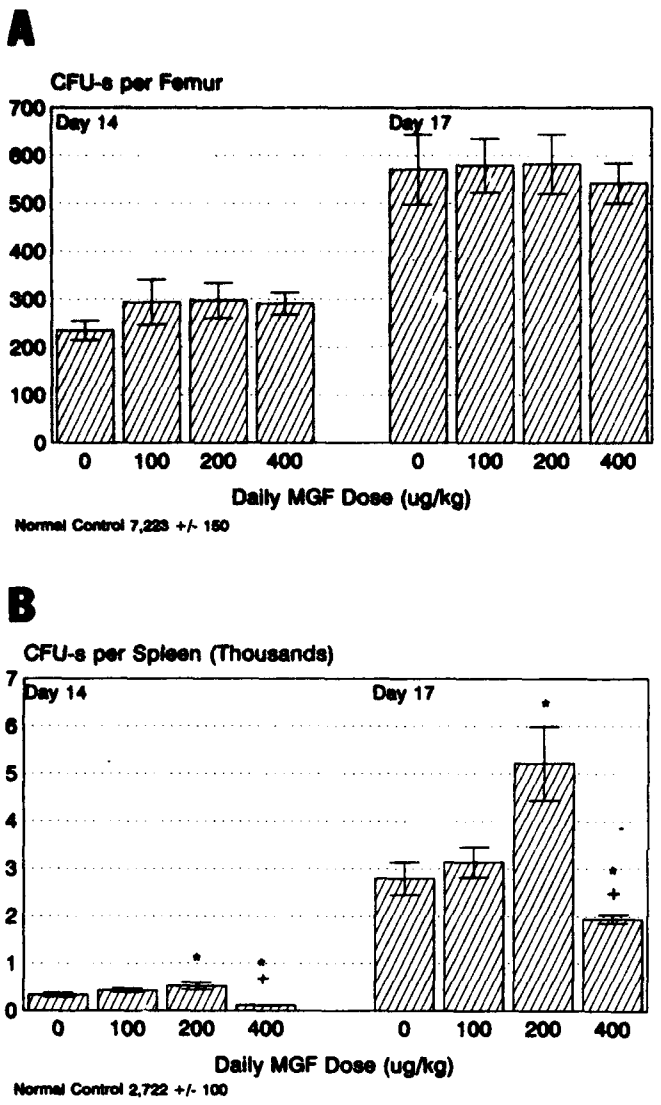

Fig. 1. Effect of MGF (100, 200, or 400 μg/kg/d, s.c.) on postirradiation recovery of bone marrow (A) and splenic (B) CFU-S in sublethally irradiated (7.75 Gy) $B_6D_2F_1$ mice. Data represent the mean ± SE of values obtained from three replicate experiments. *$p<0.05$ with respect to saline controls; †$p<0.05$ with respect to 200 μg/kg/d MGF. Average background CFU-S number in recipient mice not injected with cells was 0.2 ± 0.2.

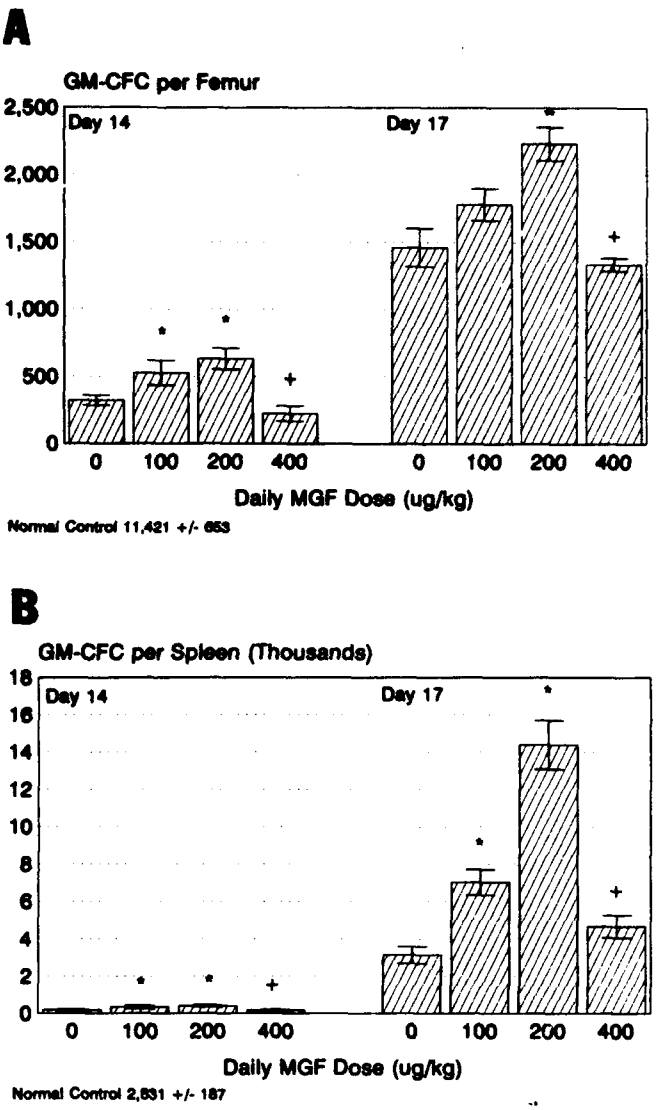

Fig. 2. Effect of MGF (100, 200, or 400 μg/kg/d, s.c.) on postirradiation recovery of bone marrow (A) and splenic (B) GM-CFC in sublethally irradiated (7.75 Gy) $B_6D_2F_1$ mice. Data represent the mean ± SE of values obtained from three replicate experiments. *$p<0.05$ with respect to saline controls; †$p<0.05$ with respect to 200 μg/kg/d MGF.

regeneration of all hematopoietic parameters evaluated, with the exception of bone marrow CFU-S and peripheral RBC (Figs. 1, 2, and 3). Effects were clearly dose-dependent, with the most significant stimulatory effects observed in mice receiving 200 μg/kg/d MGF. In these mice, hematopoietic parameters increased more, and in some instances were observed earlier, than in mice treated with only 100 μg/kg/d MGF. Surprisingly, the 400 μg/kg/d-MGF dose induced less hematopoietic recovery than the 200 μg/kg/d dose; furthermore, splenic CFU-S and peripheral RBC recoveries in these mice were even less than in saline-treated irradiated mice.

Because c-*kit* ligand has been shown capable of mobilizing primitive marrow cells into the circulation, peripheral blood CFU-S levels were evaluated to determine whether CFU-S effects in the bone marrow of MGF-treated mice may be masked by mobilization. Table 1 illustrates that CFU-S mobilization occurred in all MGF-treated mice. This phenomenon was directly dose-dependent, the most significant mobilization being observed following administration of the highest (400 μg/kg/d) MGF dose. Furthermore, in all treatment groups, CFU-S mobilization was more pronounced at day 17 postirradiation than at day 14 postirradiation.

Additional studies were performed to determine the effects of MGF on the subsequent reconstitutional potential of regenerated bone marrow and spleen cells from the sublethally irradiated mice. Recipient mice were irradiated with 9.5 Gy ^{60}Co and transplanted with various doses of bone marrow or spleen cells obtained from regenerating, sublethally irradiated

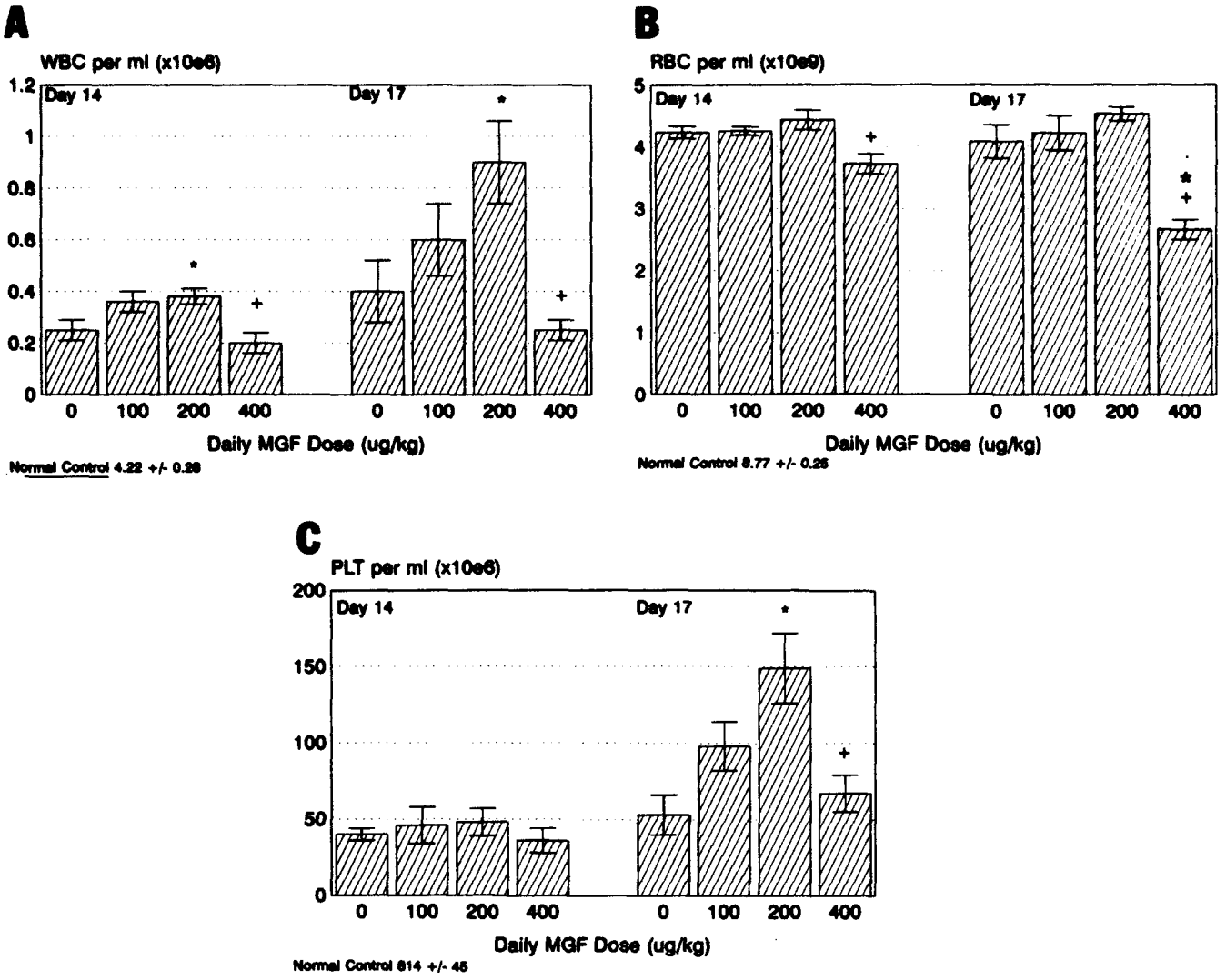

Fig. 3. Effect of MGF (100, 200, or 400 µg/kg/d, s.c.) on postirradiation recovery of peripheral white blood cells (A), red blood cells (B), and platelets (C) in sublethally irradiated (7.75 Gy) $B_6D_2F_1$ mice. Data represent the mean ± SE of values obtained from three replicate experiments. *$p<0.05$ with respect to saline controls; †$p<0.05$ with respect to 200 µg/kg/d MGF.

mice that had been treated for 17 days with either saline or MGF. Transplanted mice were then monitored for survival over a 60-day posttransplant period (Table 2). The survival-enhancing ability of bone marrow cells obtained from irradiated mice treated with either 100 or 200 µg/kg/d MGF was superior to that obtained from saline-treated mice; effects were more dramatic in mice transplanted with cells obtained from mice receiving 200 µg/kg/d MGF. For example, compared to 0% and 5% survival provided by 5×10^4 or 10×10^4 bone marrow cells obtained from saline-treated mice, 60% survival and 100% survival, respectively, were obtained with these cell numbers obtained from the 200 µg/kg/d MGF–treated mice. Survival-enhancing effects were less obvious in mice transplanted with spleen cells, yet spleen cells from mice treated with 200 µg/kg/d MGF did increase survival. In contrast, bone marrow and spleen cells obtained from mice treated with 400 µg/kg/d MGF exhibited a reduced ability to reconstitute irradiated mice.

No adverse effects were observed in mice treated with the 100 µg/kg/d-MGF dose; however, the higher doses induced some lethality in otherwise sublethally irradiated mice. Twelve percent of mice receiving 200 µg/kg/d and 22% of mice receiving 400 µg/kg/d died before day 17, the final assay point. Most of these animals died between days 14 and 17 postirradiation. Mice receiving 400 µg/kg/d MGF also appeared to become progressively emaciated and lost significantly more weight than mice in the other treatment groups; at day 14 postirradiation, body weight had decreased ~15% in these mice compared to only a 5% decrease in saline-treated mice and a 7 to 8% decrease in the 100 or 200 µg/kg/d MGF–treated mice (Table 3).

Because of the possibility that the detrimental effects observed in mice treated with the higher MGF doses may be related to MGF-induced mast cell proliferation and/or degranulation, the bone marrow, spleen, and small intestine (murine tissues that typically exhibit easily detectable mast cells in

Table 1. MGF-induced mobilization of CFU-S into the peripheral blood of irradiated mice

Daily MGF dose (μg/kg)	Day 14 postirradiation CFU-S/mL	Day 14 postirradiation CFU-S/10^5 mononuclear cells	Day 17 postirradiation CFU-S/mL	Day 17 postirradiation CFU-S/10^5 mononuclear cells
0 (saline)	3.50±0.83	0.82±0.31	4.65±0.84	1.34±0.25
100	3.65±0.35	0.99±0.09	5.77±1.37	1.38±0.23
200	5.48±0.98	1.09±0.19	6.10±1.25	2.23±0.55
400	6.67±1.85	1.46±0.40	10.09±2.08	2.73±0.57

Five irradiated (9.0 Gy) $B_6D_2F_1$ recipient mice were injected with peripheral blood obtained from mice on days 14 and 17 after irradiation (7.75 Gy) and daily treatment with saline or the indicated doses of MGF. A two-way ANOVA was performed using the endpoints CFU-S/mL and CFU-S/10^5 mononuclear cells with MGF dose and postirradiation days as factors. For the endpoint CFU-S/mL, a dose effect significant at p=0.0127 and a day effect significant at p=0.0517 were found. For the endpoint CFU-S/10^5 mononuclear cells, a dose effect significant at p=0.0311 and a day effect significant at p=0.0027 were found. The average number of background CFU-S in recipient mice not injected with cells was 0.15 ± 0.15.

Table 2. Survival of lethally irradiated mice transplanted with bone marrow or spleen cells

	Number of cells injected 5×10^4	10×10^4	5×10^5	10×10^5	20×10^5
Bone marrow					
Normal	100%	100%	—	—	—
Saline	0%	5%	—	—	—
MGF 100 μg/kg/d	30%[a]	70%[a]	—	—	—
MGF 200 μg/kg/d	60%[a]	100%[a]	—	—	—
MGF 400 μg/kg/d	0%[b]	0%[b]	—	—	—
Spleen					
Normal	—	—	50%	100%	100%
Saline	—	—	20%	60%	95%
MGF 100 μg/kg/d	—	—	20%	70%	90%
MGF 200 μg/kg/d	—	—	40%	100%[a]	100%
MGF 400 μg/kg/d	—	—	0%[b]	40%[b]	60%[a,b]

Ten to 20 irradiated (9.5 Gy) recipient $B_6D_2F_1$ mice were transplanted with the indicated number of bone marrow or spleen cells from nonirradiated normal control mice, saline-treated mice 17 days after sublethal 7.75-Gy irradiation, or MGF-treated mice 17 days after sublethal 7.75-Gy irradiation. Survival was monitored for 60 days posttransplant. [a]p<0.05 with respect to saline values; [b]p<0.05 with respect to 200 μg/kg/d-MGF values.

Table 3. Weight loss in irradiated mice treated with MGF

	Daily MGF dose (μg/kg) 0	100	200	400
Preirradiation (g)	21.8±0.4	21.0±0.6	21.5±0.5	21.6±0.6
Day 14 postirradiation (g)	20.7±0.4	19.5±1.1	19.7±0.9	18.4±1.1[a]
Loss (g)	1.1	1.5	1.8	3.2
Percent loss	5.0%	7.1%	8.4%	14.8%

Twelve mice in each group were irradiated with 7.75 Gy ^{60}Co and daily injected s.c. with either saline or the indicated dose of MGF. [a]p<0.05 with respect to preirradiation values.

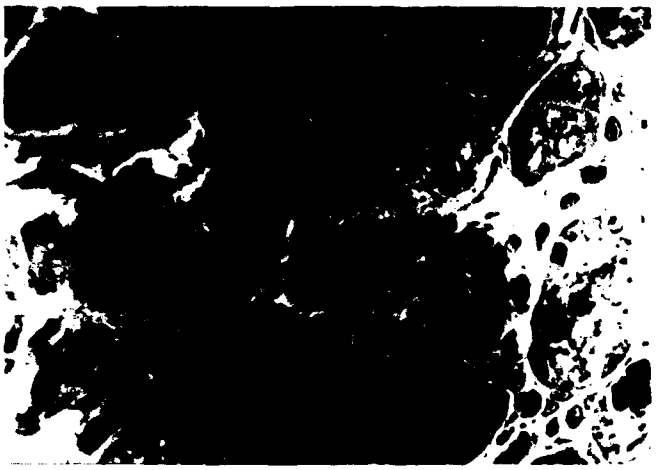

Fig. 4. Light micrograph of connective tissue mast cells (mc) in the gut of $B_6D_2F_1$ mice, demonstrating these easily detectable cells using metachromatic stain.

connective tissue areas as illustrated in Fig. 4) were histologically evaluated for the presence of mast cells and mast-cell degranulation. Almost no mast cells were detected in any tissue of irradiated saline- or MGF-treated mice at either day 14 or day 17 postirradiation. Furthermore, no other obvious histological differences were observed in MGF-treated mice compared to saline-treated mice. The gut, spleen, and bone marrow in both treatment groups appeared similar in terms of cell numbers and composition (Fig. 5).

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 hematopoietic growth factors have been shown to stimulate hematopoietic regeneration following radiation- or chemotherapy-induced myelosuppression, most notably G-CSF, GM-CSF, IL-6, and the GM-CSF/IL-3 fusion protein, PIXY321 [33–37]. Following sublethal radiation exposure, hematopoietic recovery gradually occurs and appears to be mediated by endogenously produced hematopoietic cytokines [28,29]. Because c-*kit* ligand has been shown to have little hematopoietic effect alone, but rather to synergize with a variety of hematopoietic cytokines to enhance their effects, we hypothesized that sublethally irradiated mice should provide a good model in which to evaluate the potential in vivo effects of MGF.

Our results demonstrate that a 17-day treatment course of MGF can alter multiple-lineage hematopoietic regeneration following radiation injury. Effects were dose-dependent, 100 or 200 μg/kg/d stimulating recovery and 400 μg/kg/d appearing to inhibit recovery. The hematopoietic stimulatory effects observed in our studies at the 200 μg/kg/d MGF dose confirmed results published by Scheuning et al. in which 200 μg/kg/d recombinant canine SCF administered for 21 days postirradiation in dogs was shown to enhance recovery from otherwise lethal hematopoietic injury [38]. Furthermore, our studies at the 100 μg/kg MGF dose expand observations of Zsebo et al. in which even a single 100-μg/kg injection of rrSCF administered to lethally irradiated mice 4 hours postex-

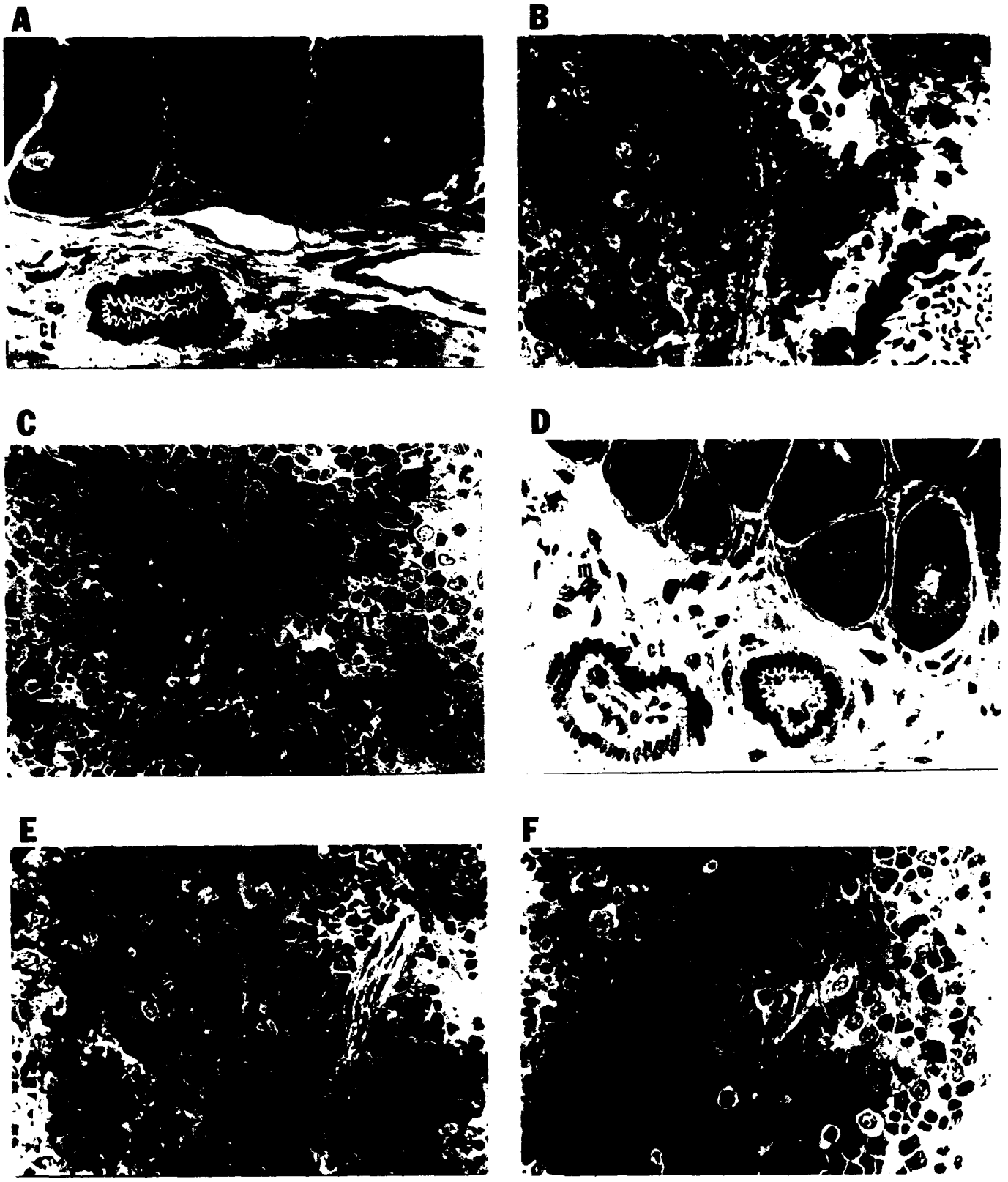

Fig. 5. Light micrograph of gut **(A, D)**, spleen **(B, E)** and bone marrow **(C, F)** from irradiated (7.75 Gy) $B_6D_2F_1$ mice treated for 17 days with 400 μg/kg/d of MGF **(A–C)** or saline **(D–F)**. Gut endothelial cells (e) are hypertrophied. Both spleen and bone marrow are cellular, and normoblasts (n), megakaryocytes (mkc), and neutrophils (pmn) are prominent. The cell in **D** adjacent to the arteriole is a macrophage (m). If present, mast cells would be visible in connective tissue (ct) areas as in Figure 4.

posure extended mean survival time, presumably through enhancement of hematopoietic recovery [39].

An initial enigma in our studies was the observation that, while increased numbers of both CFU-S and GM-CFC were observed in the spleens of MGF-treated mice, only GM-CFC numbers were increased in the bone marrow. Since recent studies in normal mice have demonstrated the ability of c-*kit* ligand to mobilize into the peripheral circulation large numbers of cells capable of engrafting irradiated animals [40,41], we suspected that the apparent lack of CFU-S proliferation in the bone marrow of our MGF-treated mice may be due to the mobilization of these cells out of the marrow as rapidly as they were being produced. Studies presented in Table 1 verify that CFU-S mobilization did occur in the MGF-treated mice, suggesting that CFU-S proliferation in the marrow most likely was occurring but was not apparent due to this mobilization phenomenon.

As described, the 400 μg/kg/d MGF dose appeared to inhibit hematopoiesis. This effect did not appear to be due merely to "toxicity" since, although 22% of mice treated with the 400 μg/kg/d MGF dose died before evaluation, 12% of mice receiving 200 μg/kg/d MGF and exhibiting the greatest hematopoietic stimulation also died before evaluation. The cause of the lethality and weight loss observed in otherwise sublethally irradiated mice given high-dose MGF remains unknown, but mast cells do not appear to be involved. Although high doses of MGF have previously been shown to induce extensive mast-cell degranulation associated with respiratory distress [42], histopathological evaluation of tissues typically exhibiting mast cells in the mouse revealed almost no mast cells in any of the irradiated mice. Since mast cells are ultimately generated from the radiosensitive hematopoietic stem cells [43,44], apparently recovery of these cells to detectable levels had not yet occurred in our irradiated mice.

c-*kit* ligand in combination with additional cytokines has been demonstrated to stimulate the proliferation and differentiation of primitive hematopoietic precursors [11,15]. Because of this, the possibility that stem cell exhaustion may occur following prolonged MGF administration cannot be excluded as a possible explanation for the observed MGF-induced inhibition of hematopoiesis. Indeed, our results in Table 2 indicate that as the MGF dose was increased from 100 to 200 μg/kg/d, the reconstitutional potential of bone marrow and spleen cells increased; however, at 400 μg/kg/d such reconstitutional potential decreased. Thus, following the 400 μg/kg/d MGF dose, primitive cells may have been stimulated to proliferate to the point of exhaustion. Had this occurred, however, an increase in downstream progenitor populations would have been expected; this was not observed in the 400 μg/kg/d MGF–treated mice.

An alternate explanation for the apparent hematopoietic inhibitory effects observed following administration of the highest MGF dose may relate to the nature of the MGF used in our studies and receptor-ligand interactions. c-*kit* ligand is known to exist in two biologically active forms: (1) an integral membrane protein with an extracellular domain, transmembrane domain, and intracytoplasmic domain, and (2) a soluble protein produced by proteolytic cleavage of the membrane-associated form [12]. Membrane-associated c-*kit* ligand has been shown to be prevalent in bone marrow stromal cells [45,46]. Since primitive hematopoietic cells possess c-*kit* (that is, the receptor for c-*kit* ligand [47]), under in situ conditions it can be envisioned that, via c-*kit*, hematopoietic cells bind cell-associated c-*kit* ligand on stromal cells, positioning themselves in proximity to respond to additional stromal-derived hematopoietic cytokines. Since the MGF used in our studies was a soluble c-*kit* ligand, high doses of MGF may have saturated c-*kit* on hematopoietic cells, preventing the binding of these receptors with stromal-associated c-*kit* ligand and therefore preventing subsequent stromal–hematopoietic cell interactions that lead to proliferation and differentiation, resulting in reduced hematopoietic recovery in irradiated mice. The fact that mice treated with the 400 μg/kg/d MGF dose exhibited decreased splenic CFU-S numbers concurrent with the greatest CFU-S mobilization may suggest an inability of these cells to attach to the splenic microenvironment.

In conclusion, these studies demonstrate a dose-dependent ability of MGF to stimulate multilineage hematopoietic regeneration following radiation-induced hematopoietic injury. Detrimental effects observed following high-dose MGF treatment do not appear to be mast cell related, and their cause remains to be determined. However, the effects observed with "supraoptimal" doses of MGF suggest that caution should be taken in dose-escalation trials in humans.

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