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Synergy of IL-1 and Stem Cell Factor in Radioprotection of Mice Is Associated with IL-1 Up-Regulation of mRNA and Protein Expression for c-*kit* on Bone Marrow Cells[1]

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Administration of IL-1 and stem cell factor (SCF) to mice 18 h before lethal ^{60}Co whole-body irradiation resulted in synergistic radioprotection, as evidenced by increased numbers of mice surviving 1,200 to 1,300 cGy doses of radiation and the recovery of increased numbers of c-*kit*$^+$ bone marrow cells at 1 and 4 days after the lethal dose of 950 cGy. Anti-SCF Ab inhibited IL-1-induced radioprotection, indicating that endogenous production of SCF is necessary for radioprotection by IL-1. Conversely, radioprotection induced by SCF was reduced by anti-IL-1R Ab, indicating that endogenous IL-1 contributes to SCF radioprotection. SCF, unlike IL-1, does not induce hemopoietic CSFs and IL-6 or gene expression of a scavenging mitochondrial enzyme manganese superoxide dismutase in the bone marrow, suggesting that SCF and IL-1 radioprotect by distinct pathways. The mRNA expression for c-*kit* (by Northern blot analysis) and ^{125}I-SCF binding on bone marrow cells was elevated within 2 and 4 h of IL-1 administration respectively. Four days after LD 100/30 radiation the recovery of c-*kit*$^+$ bone marrow cells was increased sixfold in IL-1-treated mice, almost 20-fold in SCF-treated mice, and 40-fold in mice treated with the combination of the two cytokines. Thus, endogenous production of both IL-1 and SCF is required for resistance to lethal irradiation and the synergistic radioprotective effect of the two cytokines may, in part, depend on IL-1 and SCF-induced increases in numbers of c-*kit*$^+$ hemopoietic stem and progenitors cells that survive lethal irradiation. *The Journal of Immunology,* 1994, 153: 1536.

Death from LD 100/30 of ionizing radiation can be prevented by a supply of undamaged bone marrow cells and has therefore been attributed to a lethal hemopoietic syndrome (1). The death of animals receiving LD 100/30 doses of radiation can also be prevented by administration of immunomodulatory agents or proinflammatory cytokines before irradiation, with subsequent recovery of the hemopoietic system (2–5). We have demonstrated that, in the case of immunomodulatory LPS, the radioprotective effect is mediated by endogenously produced proinflammatory cytokines, IL-1 and TNF, because Abs to these cytokines block LPS-induced radioprotection (6). Radioprotection with IL-1 and TNF in turn could also be blocked by anti-IL-6 Ab as well as by anti-TNF and anti-IL-1R Abs (6, 7), providing evidence that obligatory interaction of these three endogenously produced cytokines is required for protection by IL-1 or TNF from lethal hemopoietic syndrome.

Recently, a receptor for hemopoietic cytokine (c-*kit*) and its ligand (SCF)[3] have been identified and cloned (8, 9). Numerous studies determined that SCF synergizes with

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[3] Abbreviations used in this paper: SCF, *kit* ligand; BMC, bone marrow cells; G-CSF, granulocyte CSF; GM-CSF, granulocyte-macrophage CSF; MnSOD, manganese superoxide dismutase; PE, phycoerythrin.

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hemopoietic cytokines (IL-1, IL-3, GM-CSF, G-CSF, and IL-6) to stimulate the growth of hemopoietic progenitor cells in vitro and stimulates hemopoiesis in vivo (10–14). The SCF receptor (c-*kit*) is expressed on hemopoietic stem cells and progenitor cells in the bone marrow (estimated as 0.05% and 5% of the total bone marrow population, respectively), but not on mature neutrophils or nucleated erythroid cells (14–16). Thus, c-*kit* provides a marker for monitoring changes in progenitor populations. Mice treated with Ab to c-*kit* displayed a loss of hemopoietic progenitor cells (13).

We recently demonstrated that Ab to SCF blocks IL-1 and LPS-induced radioprotection and also reduces the resistance of untreated mice to radiation lethality (17). This result suggested that endogenous SCF may be the penultimate mediator required for radioprotection. To test this hypothesis, we further investigated the interaction of IL-1 with SCF in radioprotection. In this report we present results that show that IL-1 contributes to SCF radioprotection, that IL-1 and SCF synergize in radioprotection, and that this synergy may be based in part on IL-1-induced increases in numbers of bone marrow cells (BMC) expressing c-*kit*, which suggests that IL-1 generates more progenitor cells with the capacity to respond more effectively to SCF.

Materials and Methods

Mice

B6D2F1 female mice, 8 to 10 wks old, were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were handled as described previously (6). Adrenalectomized mice were purchased from Charles River Laboratories. The experiments were performed within 2 wk after adrenalectomy.

Abs

Rat monoclonal IgG1, anti-IL-1R Ab (35F5), and anti-IL-6 (20F3) were generous gifts from Dr. Richard Chizzonite (Hoffmann-La Roche, Nutley, NJ) and Dr. John Abrams (DNAX, Palo Alto, CA), respectively. A rat mAb to β-galactosidase (GL113) was used as a control. The polyclonal anti-murine SCF Ab was generously provided by Dr. Douglas Williams (Immunex, Seattle, WA). Chromatographically purified rat IgG (Sigma Chemical Co., St. Louis, MO) was used as an additional control. R-phycoerythrin (R-PE)-conjugated rat anti-mouse c-*kit* mAb 3 C1 (IgG2b), and PE-conjugated rat IgG2b (control) were purchased from PharMingen (San Diego, CA).

Treatment

Human rIL-1 (rHu IL-1α 117–271 Ro 24–5008 lot IL 1 2/88, activity 3×10^{8} U/mg) was kindly provided by Dr. Peter Lomedico (Hoffmann-La Roche).

Rat PEG-SCF was prepared and coupled with polyethylene glycol and was kindly provided by Dr. Ian McNiece, (Amgen, Thousand Oaks, Ca). PEG was used as a control for its nonspecific effect as a radioprotector. G-CSF was provided by Amgen, and IL-6 (SDZ 280–969, Batch PPG 9001; SA 5.2×10^{7} U/mg) was a generous gift from Dr. E. Liehl (Sandoz, Vienna, Austria). The Abs and recombinant cytokines were diluted in pyrogen-free saline on the day of injection. Abs or control Ig were given i.p. 6 to 20 h before i.p. injection of 5 μg/mouse of SCF. Mice were also treated with 1 μg mouse of IL-1, 3 μg/mouse of SCF, or the combination thereof, before or after irradiation.

Irradiation

Mice were randomized, placed in ventilated Plexiglass containers, and bilaterally irradiated using the AFRRI ^{60}Co whole body irradiator. Before irradiating the mice, the midline tissue (MLT) dose rate was measured by placing a 0.5-cc tissue equivalent in the ionization chamber (calibration factor traceable to the National Institute of Standards and Technology) at the center of 2.5-cm diameter, cylindrical acrylic mouse phantom. The tissue-to-air ratio (TAR), dose ratio was 0.96, and the field was uniform to within ± 5%. Exposure time was adjusted so that each animal received the specified dose at a fixed MLT dose rate of 0.4 Gy min. Dosimetric measurements were made in accordance with the American Association of Physics in Medicine protocol for the determination of absorbed dose from high-energy photon and electron beams. The number of surviving mice was recorded daily for 30 days.

FACS Analysis

BMC were obtained by flushing femurs into RPMI media containing 5% FCS. After washing, cells were counted and resuspended in Dulbecco's PBS with 2% FCS at the concentration of 2×10^{7}/ml. Cells were stained for 30 min with 10 μg/ml of either PE-conjugated anti-murine c-*kit* Ab (3C1) or PE-conjugated control IgG2b. The cells were washed twice and resuspended in 1 ml of 2% FCS-Dulbecco PBS. The percentage of c-*kit*⁺ cells were calculated by subtracting the percentage of cells stained with control Ab from percentage of cells stained with c-*kit* Ab. In experiments in which bone marrow cells from irradiated mice were evaluated, each group consisted of a pool from eight femurs at 1 day (D+1) and 14 femurs at 4 days (D+4) after irradiation. Normal, nonirradiated mice were examined individually with pools of cells from both femurs.

Immunofluorescence analysis was performed with an EPICS ELITE flow cytometer (Coulter Cytometry, Miami, FL) using logarithmic amplification. RBCs, platelets, and debris were excluded from the analysis on the basis of light scatter criteria. Twenty-five thousand cells were counted for each histogram.

SCF binding to BMC

Mouse rSCF was iodinated by the chloramine T method, which yielded ^{125}I-SCF preparations with sp. act. of approximately 20 μCi/μg protein. BMC (5×10^{6}) were distributed in duplicate Eppendorf tubes containing 200 μl binding medium (RPMI 1640, 25 mM HEPES, 1% BSA, 0.05% sodium azide) and 0.5 ng ^{125}I-SCF corresponding to approximately 10^{5} cpm. Parallel duplicate tubes contained the 400-fold excess of unlabeled SCF. The cells were incubated at 4°C overnight, under rotation, and centrifuged through a 10% sucrose-PBS cushion. The tips of the tubes with cell pellets were removed, and the radioactivity was measured in a gamma counter (Gamma 400, Beckman Instruments, Fullerton, CA). Nonspecific binding determined in the presence of unlabeled SCF was subtracted from total binding to obtain specific binding.

For steady-state binding, cells were incubated in duplicate with different concentrations of ^{125}I-murine SCF. Matching replicates also contained a 100-fold excess of unlabeled ligand. The radioactivity associated with cell pellets was measured as in standard binding assays. To estimate the binding sites per cell and the K_d values, a nonlinear regression calculation was used (18). In all cases, complete sets of data generated in the assays were used in the analysis. Scatchard plots were reformatted presentations of nonlinear regression.

RNA extraction and Northern blot analysis

BMC collected from six femurs/group were pelleted and total cellular RNA was isolated by acid guanidine thiocyanate-phenol-chloroform extraction according to Chomczynski and Sacchi (19). RNA was separated by electrophoresis on 1% agarose and transferred onto a Hybond-N (Amersham, Oakville, Canada) membrane for Northern blot analysis. Membranes were prehydrated for 4 h in a mixture containing 120 mM Tris, 8 mM EDTA, 0.1% NaPP, 0.2% SDS, and 100 μg/ml heparin. Hybridization was conducted overnight at 68°C in prehybridization buffer containing 625 μg/ml heparin and 10% dextran sulfate. The murine c-*kit* probe was a 3710-bp *EcoR*1-Hind31 insert of the c-*kit* cDNA clone pGEM3 (c-*kit3*) and the human superoxide dismutase probe was a 0.1-Kb *Pst*I insert of the SODI cDNA clone, pSP 64 cSOD. The c-*kit* probe was further fragmented with *Dra*I-*Acc*I and the fragments were labeled using

Table I. *Effect of anti-IL-1R Ab on SCF-induced radioprotection*[a]

Treatment	Dead/Tested	% Survival
Saline	30/30	0
Saline + SCF	11/30	63
Rat Ig + SCF	12/33	64
Anti-IL-1R + SCF	24/32	25*

[a] B6D2F1 mice received 100 μg/mouse anti-IL-1R Ab or rat IgG 6 h before administration of 3 μg/mouse of SCF. Twenty-four hours later, mice were irradiated with 1050 cGy ^{60}Co source. The 30-day survival of mice receiving anti-IL-1R Ab and SCF was significantly lower (* indicates $p < 0.00!$) than that of control saline and rat Ig-pretreated SCF-radioprotected mice.

a multiprime DNA labeling system (Amersham), with [α-^{32}P]dCTP (sp. act ~3000 Ci/mM, Amersham). The membranes were then washed once at room temperature for 20 min in 2X SSC, 0.1% SDS at 68°C for 60 min, and then rinsed at room temperature with 0.1X SSC. The membranes were exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) with intensifying screens at −80°C. Signal intensity was quantified by densitometry using a Pharmacia LKB Ultrascan XL (Pharmacia, Canada). As a control for RNA integrity, blots were rehybridized with a 1-kb *Pst*I cDNA probe (American Tissue Culture Collection, Rockville, MD) of the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH).

CSF, IL-6, and fibrinogen assays

Circulating CSF and IL-6 were determined in the sera of mice bled 2 to 3 h after treatment with IL-1 or SCF, as previously described (20). Fibrinogen was determined in the citrated plasma of such mice bled 18 h after treatment by the rate of conversion of fibrinogen to fibrin in the presence of excess thrombin, using a Sigma Chemical Company Diagnostic Kit.

Statistical analysis

Statistical evaluation of the results was conducted using χ^2 analysis, probit analysis, and analysis of variance followed by a Bonferroni corrected *t*-test.

Results

Effect of anti-IL-1R Ab on SCF radioprotection

To examine whether endogenously produced IL-1 contributes to SCF-induced radioprotection, mice were treated with 100 μg mouse of anti-IL-1R Ab, rat IgG at an equivalent dose or saline, and 6 h later with a single dose of 3 μg mouse PEG-SCF. Whereas at a dose of 1,050 cGy of radiation, 63% of saline pretreated and 64% of IgG pretreated mice were protected from death by SCF, only 25% of mice receiving anti-IL-1R Ab and SCF survived irradiation (Table I). Thus, although anti-IL-1R Ab did not entirely abolish the survival-enhancing effect of SCF, it reversed the protective effect by 40%, indicating that IL-1 contributes to radioprotection of SCF-treated mice.

Effect of anti-IL-1 and anti-IL-6 Abs on survival of anti-SCF Ab-treated mice

Our previous work showed reduced incidence of survival after LD 50/30 (875 cGy) irradiation of mice treated with anti-SCF, anti-IL-1R, or anti-IL-6 Abs (6, 7). To evaluate the contribution of endogenous IL-1 and IL-6 in addition

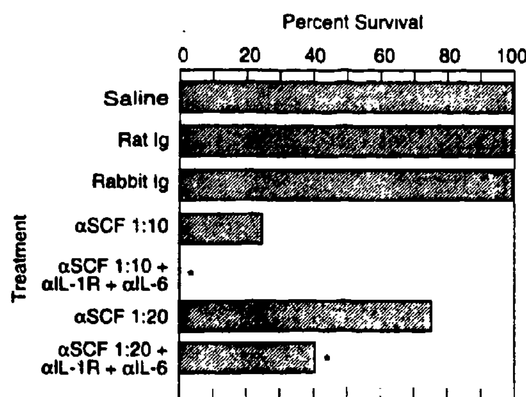

FIGURE 1. Abs to IL-1R and IL-6 further reduce survival of anti-SCF Ab-treated, irradiated mice. Groups of B6D2F1 mice (16 to 26 per group in two to three separate experiments) received rat IgG, 1:10 dilution of rabbit preimmune serum, 1:10 and 1:20 dilution of rabbit anti-SCF Ab, or the combination of anti-SCF Ab with 100 μg/mouse of anti-IL-1R Ab and 600 μg/mouse of anti-IL-6 Ab (as indicated). The mice were exposed a day later to a sublethal 750 cGy dose ^{60}Co irradiation (LD50/30 = 866 cGy). The survival of mice that received the combination of Abs was significantly lower ($p < 0.01$) than that of mice treated with anti-SCF alone.

to SCF, mice were given the Abs in combination. The results in Figure 1 indicate that addition of anti-IL-1R and anti-IL-6 Abs further reduced survival significantly, at nonlethal 750 cGy irradiation of anti-SCF Ab-treated mice suggesting that IL-1, IL-6, and SCF are mutually interdependent in protecting mice from lethal irradiation.

Effect of combinations of IL-1 and SCF in protection from lethal irradiation

The observed codependence of SCF- and IL-1-induced radioprotection led us to examine the effect of their combined administration on survival of irradiated mice. We chose the doses of 3 μg/mouse of SCF and 1 μg/mouse of IL-1, because 5 μg of SCF and 3 μg of IL-1 conferred identical protection. Results in Figure 2 document that a single injection of both cytokines 18 h before irradiation resulted in synergistic radioprotection at 1200 to 1300 cGy doses of radiation. Whereas the control saline-treated mice had an LD50/30 of 866 cGy (95% confidence limits: 828 to 888), IL-1-treated LD50/30 of 1009 cGy (991 to 1034), and SCF-treated mice LD50/30 of 1106 cGy (1048 to 1194), and the combination of the two cytokines resulted in an LD50/30 of 1273 (1248 to 1317) cGy. Administration of both cytokines 48 or 4 h before or 1 h after irradiation (Table II) had either a greatly reduced effect (at 4 h before) or no effect (at 48 h before or 1 h after). As was the case with IL-1, SCF given alone 48 h before or 1 h after irradiation did not afford significant protection.

The radioprotective effect of SCF was not associated with the induction of early or late acute serum proteins

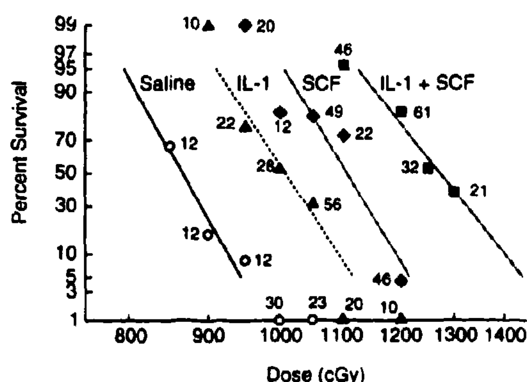

FIGURE 2. Effect of treatment with IL-1, SCF, or their combination on survival of B6D2F1 lethally irradiated mice. Mice received i.p. 1 μg IL-1, 3 μg SCF, their combinations, or saline; and 18 h later received ^{60}Co radiation in doses indicated. Survival was recorded daily for 30 days and plotted using probit analysis. The numbers indicate the number of mice per given treatment.

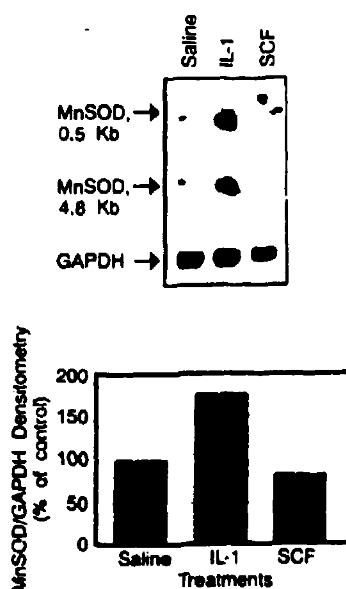

FIGURE 3. IL-1, but not SCF, induces MnSOD gene expression. Groups of mice (4 mice/group) received i.p. 1 μg IL-1, 3 μg SCF, or saline. BMC were obtained 4 h after injections (see *Materials and Methods* for details of procedures).

Table II. *Survival percentage of mice receiving IL-1 and SCF treatment before and after irradiation*

	48 h Before	4 h Before		1 h After
Treatment	1000 cGy	1000 cGy	1050 cGy	1000 cGy
Saline	0	0	0	0
IL-1	0	25*	10	0
SCF	0	19*	0	0
SCF + IL-1	0	75*	0	0

[a] B6D2F1 mice (10 to 16 mice per group) were administered i.p. 1 μg of IL-1, 3 μg of SCF, or their combination, before or after irradiation. Survival of mice was recorded daily for 30 days.
* Significantly lower ($p < 0.01$) than survival obtained with 8-h pretreatment (see Fig. 2).

Table III. *Comparison of IL-1 and SCF for induction of CSF, IL-6, and fibrinogen*[a]

Treatment	Fibrinogen (mg/dl)	CSF (U/ml)	IL-6 (pg/ml)
Saline	203.6 ± 4	<20	<50
IL-1	384.0 ± 16*	2513 ± 388*	1600
SCF	188.0 ± 3	<20	<20

[a] Mice received i.p. 1 μg/mouse of IL-1, 5 μg/mouse SCF, or saline (control) and were bled 2 to 3 h later to determine CSF and IL-6 or 18 h later, for fibrinogen determination.
* $p < 0.01$ compared to control.

Table IV. *The effect of treatment of mice with cytokines on the recovery of c-kit$^+$ BMC after 950 cGy irradiation*[a]

	Day 0	Day +1	Day +4
Treatment	c-*kit*$^+$ cells/femur (×10^5)	c-*kit*$^+$ cells/femur (×10^4)	c-*kit*$^+$ cells/femur (×10[illegible])
Saline	2.6 ± 1.4	2.0 ± 0.1	0.3 ± 0.1
IL-1	3.6 ± 1.8	6.0 ± 1.4*	1.9 ± 0.9[b]
SCF	2.0 ± 0.2	2.5	5.7 ± 1.5*
IL-1 + SCF	2.3 ± 1.3	9.4 ± 0.8**	12.0 ± 2.0**

[a] Mice received i.p. 1 μg/mouse of IL-1, 3 μg/mouse of SCF alone or in combination, or saline and 18 h later, 950 cGy ^{60}Co irradiation. BMC were obtained from unirradiated mice (D 0) and at 1 day and 4 days after irradiation. Each group of mice consisted of a pool of eight femurs (D 0, D + 1) and 14 femurs (D + 4). The results are the mean ± SD of two experiments. The numbers of c-*kit*$^+$ cells were significantly higher than in control saline-treated mice.
[b] $p = 0.063$. * $p < 0.05$. ** $p < 0.01$.

such as IL-6, CSF, or fibrinogen, all of which are induced with IL-1 (Table III). Similarly, SCF treatment, in contrast with IL-1, did not up-regulate the mRNA for MnSOD (Fig. 3).

Determination of changes in c-kit expression by BMC

Both IL-1 and SCF were reported to induce BMC cycling and increases in progenitor compartment (2, 22). Such changes should be reflected in increased numbers of c-*kit*$^+$ BMC, as c-*kit* is expressed on stem cells and all immature progenitors, but not on mature neutrophils, nucleated erythrocytes, or macrophages (13, 14). To determine whether treatment with cytokines before radiation results in an improved survival of progenitor and stem cells, FACS analysis was used to compare the numbers of c-*kit*$^+$ BMC from cytokine and saline control-treated lethally irradiated mice. As shown in Table IV, BMC obtained from IL-1-, SCF- or IL-1 + SCF-treated mice 1 day after lethal (LD 100/30) irradiation (950 cGy) had increased numbers of surviving c-*kit*$^+$ cells compared with control saline-treated mice. At 4 days after irradiation, with progressive loss of total nucleated BMC, these differences became more striking, with IL-1-treated mice showing a sixfold increase, SCF-treated mice,

Table V. *Effect of treatment of mice with IL-1, SCF alone, or the two in combination on specific binding of ^{125}I-SCF on BMC*[a]

Treatment	4 h	10 h	24 h	48 h
IL-1	47%	65%	26.5 ± 0.5%	62 ± 2%
SCF	ND	ND	32.5 ± 4.5%	63 ± 18%
IL-1 + SCF	ND	ND	67 ± 11%[b]	105 ± 30%[b]

[a] Mice received cytokines as specified in Table IV. BMC were treated as specified in *Materials and Methods*. The results are expressed as percentage of specific binding above that of specific binding of control BMC. Each experimental group consisted of four mice. The results for 24 and 48 h are the mean ± SD of two separate experiments.

[b] Statistical comparisons at 24 and 48 h for each of the sum of specific binding for IL-1 and SCF with that for the combination treatment do not reject ($p > 0.1$) the hypothesis that the treatment effects are additive.

Table VI. *The effect of adrenalectomy on specific binding of ^{125}I-SCF on BMC of IL-1- vs saline-treated mice*[a]

	Saline	IL-1 (0.1 μg)	% Increase
ADX I	1030[b]	2867	178
ADX II	879	1472	70
Sham ADX	1092	1455	33

[a] Adrenalectomized (ADX I and ADX II) or sham-adrenalectomized mice (four mice per group) received i.p. saline or IL-1 injections and BMC were obtained 4 h later for the binding assay.

[b] Specific binding (cpm) calculated as indicated in *Materials and Methods*. For comparison, see Table 5.

an approximately 20-fold increase and IL-1- and SCF-treated mice, a 40-fold increase in c-*kit*$^+$ BMC per femur, relative to saline control. Because the nadir in the BMC number occurs at 3 days after irradiation, these results suggest that a substantially greater number of c-*kit*$^+$ BMC survive lethal doses of radiation in mice pretreated with IL-1, SCF, or their combinations.

Repeated attempts to establish that, in normal unirradiated mice, treatment with IL-1, SCF, and their combination results in increased numbers of c-*kit*$^+$ BMC within 24 h, were not successful. This may be a result of an increase in only a small cohort of BMC, i.e., selected populations of early progenitor cells.

Effect of IL-1 on SCF binding by BMC

Therefore, we have examined the changes in c-*kit*$^+$ BMC by evaluating SCF binding to such cells. A binding assay of radiolabeled ligand provides a sensitive means of assessing changes in the numbers and affinity of receptors. Administration of 1 μg/mouse of IL-1, a dose used in a combined IL-1/SCF radioprotective treatment, increased binding of radiolabeled SCF (Table V). In contrast with our previous observation that IL-1-induced up-regulation of IL-1R on BMC was mediated by corticosteroid and G-CSF (23), G-CSF in doses ranging from 0.3 to 5 μg/mouse did not up-regulate SCF binding (results not shown), and adrenalectomized mice did not differ from normal mice in up-regulation of SCF binding in response to IL-1 (Table VI). Furthermore, treatment with dexamethasone at 2 and 10 μg/mouse did not affect SCF binding on normal BMC (data not shown). SCF treatment resulted in similar increases as IL-1 in ^{125}I-SCF binding on BMC, and the combined treatment of IL-1 and SCF resulted in an approximately additive effect (Table V).

Scatchard analysis performed with normal cells and cells from mice treated for 10 and 48 h with IL-1 showed increases of 25 and 50%, respectively, in the number of receptors/cell with no change in affinity (Fig. 4). Previous reports determined two affinities (10^{-11} K_d, and 10^{-9} K_d) for human c-*kit* receptors (24, 25). However, in our study only one affinity (10^{-9} K_d) receptor can be detected on murine BMC. Although the results are expressed as numbers of receptors per cell, we actually determined an increase in total numbers of receptors on populations of BMC. Because less than 5% of BMC express c-*kit* receptors, and the number of receptors decreases with BMC lineage differentiation, we could not distinguish whether such an increase reflects greater numbers of cells with high or low receptor expression or the combination of the two.

Effect of IL-1 treatment on c-kit gene expression

To determine that the up-regulation of SCF binding on the IL-1-treated BMC was associated with increased c-*kit* gene expression, a Northern blot analysis was performed to compare mRNA for c-*kit* in saline with that of IL-1-treated BMC. Results (Fig. 5) demonstrate that IL-1 induced dose-dependent increases in c-*kit* mRNA, which could be detected within 2 h of IL-1 treatment and, which peaked at 6 h. In contrast, treatment with IL-6 did not up-regulate c-*kit* gene expression (Fig. 6).

Discussion

Our previous studies using Abs to SCF and IL-1R suggested that endogenous production of both cytokines is required to protect mice treated with LPS or IL-1 from lethal radiation (17). This study further establishes that radioprotection with SCF requires the participation of IL-1 (Table I) and that IL-1 and SCF protect synergistically at 1200 to 1300 cGy doses of radiation (Fig. 2). This synergistic protection is evidenced by an increase in the percentage of mice surviving doses of radiation over 45% greater than LD 100/30.

There is considerable evidence that administration of IL-1 induces an increase in numbers of progenitor cells. We and others previously observed that IL-1 increased cycling of hemopoietic progenitor cells and progressively increased proliferative expansion of myeloid progenitor cells in the marrow from 6 to 48 h (21, 26, 27). In a more recent study, Hestdal et al. (28) demonstrated that IL-1 treatment of mice results within 24 h in a fivefold up-regulation of HPP-CFC and twofold to threefold up-regulation of CFU-c when grown in the presence of GM-CSF or IL-3. In this study, we found a 50% increase in transcription of c-*kit*, a phenotypic marker of progenitor cells, and a 50% increase in SCF binding as early as 2 and 4 h,

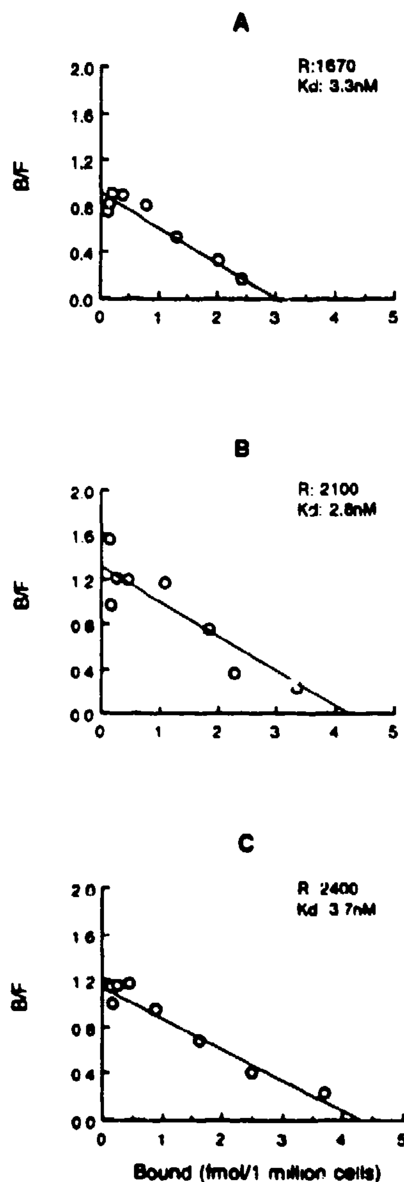

FIGURE 4. Binding of ^{125}I-SCF to murine BMC. Cells (5×10^6) in 200 μl of binding medium were incubated in duplicate with different concentrations of ^{125}I-SCF. Matching replicates also contained 100-fold of unlabeled SCF. Receptor number S(R) and K_d values were analyzed as described in *Materials and Methods*. Scatchard plots from one of two experiments with virtually similar results represent: *Panel a*) BMC obtained from untreated mice; *b*) BMC from mice treated with IL-1 (1 μg/mouse) after 10 h; *c*) BMC from mice treated with IL-1 after 48 h.

respectively, after IL-1 treatment. These results are strengthened by the equilibrium binding studies with ^{125}I-SCF, which show that, within 10 h after IL-1 treatment, BMC express 25% more c-*kit* receptors. Despite such increases in c-*kit* expression, a significant increase in numbers of c-*kit*$^+$ BMC was not observed by FACS analysis. Taken together, our results suggest that the observed increase in binding sites may occur on progenitor cells expressing more receptors.

The stimulating effects of IL-1 on progenitor cells and hemopoiesis may contribute to its radioprotective effects. The hemopoietic effects may depend in part on the ability of IL-1 to induce hemopoietic IL-6 and CSFs (29, 30). In addition, Il-1 induction of the scavenging mitochondrial enzyme, MnSOD in BMC (Ref. 31, and confirmed in our study), and its ability in vivo to induce BMC to enter a relatively radioresistant S phase of cell cycle (21), should contribute to radioprotection. Most recently, Zucali et al. have shown that in vitro treatment with IL-1 of male mouse BMC and irradiation results in greater survival than treatment without IL-1, of short-term and long-term repopulating stem cells transferred to female mice (32). These results clearly document, for the first time, the direct radioprotective effects of IL-1 on hemopoietic stem and progenitor cells. In our study, 1 day after lethal irradiation the total number of c-*kit*$^+$ BMC in the femurs of IL-1-treated and IL-1- and SCF-treated mice were threefold and fivefold greater, respectively, than that in control saline-treated mice. This provides evidence that in vivo IL-1 actually has the capacity to induce increases in numbers of progenitor cells surviving lethal irradiation.

As with IL-1, SCF treatment of mice given in two injections before irradiation, increased the numbers of surviving mice concomitant with hemopoietic recovery evident within 6 to 10 days after irradiation (33). Our work confirms and extends these findings by demonstrating that even a single injection of SCF is highly radioprotective. However, as shown in this report, SCF does not induce production of hemopoietic growth factors (CSF), IL-6, acute phase response, or MnSOD, all believed to contribute to radioprotection by IL-1. Despite this, multiple doses of SCF in several previous reports (22, 34, 35) and a single injection, as shown in this study, induce hemopoietic expansion. Because in multiple reports SCF was shown to require costimulatory addition of CSFs to induce in vitro proliferation of hemopoietic colonies, it is likely that these growth factors are available in sufficient supply in vivo to allow hemopoietic expansion.

In contrast with IL-1 and SCF, well-known hemopoietic cytokines, such as G-CSF and IL-6, promote in vitro expansion of hemopoietic cells, yet their in vivo hemopoietic effects as shown here and elsewhere (36) are much more limited than these of IL-1 or SCF. Possibly this is because IL-6 and G-CSF do not cause an increase in the expression of c-*kit* by BMC. In fact, hemopoietic growth factors IL-3 and GM-CSF are reported to actually down-regulate the expression of mRNA for c-*kit* on mast cells and progenitor cell lines (37), which is consistent with the role of these factors in promoting differentiation of progenitor cells. These contrasting in vivo and in vitro hemopoietic effects support the fundamental importance of IL-1 and SCF in

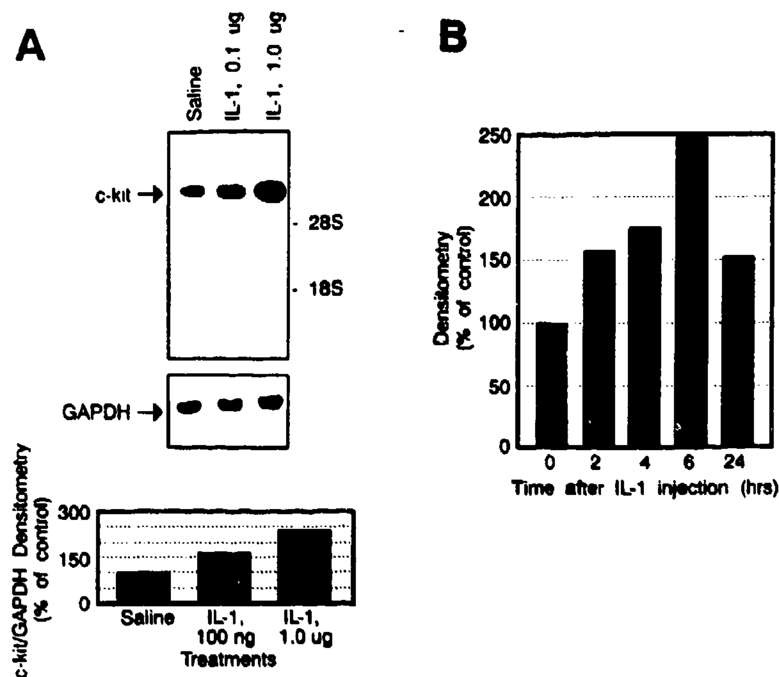

FIGURE 5. Effect of treatment with IL-1 on c-*kit* gene expression. *Panel a)* mice were treated with 0.1 and 1.0 μg/mouse of IL-1 or saline and 4 h later BMC were harvested for Northern blot analysis. *Panel b)* BMC from mice receiving 1.0 μg/mouse of IL-1 were assessed at different times for c-*kit* mRNA expression. Each group represents a pool of BMC from four mice.

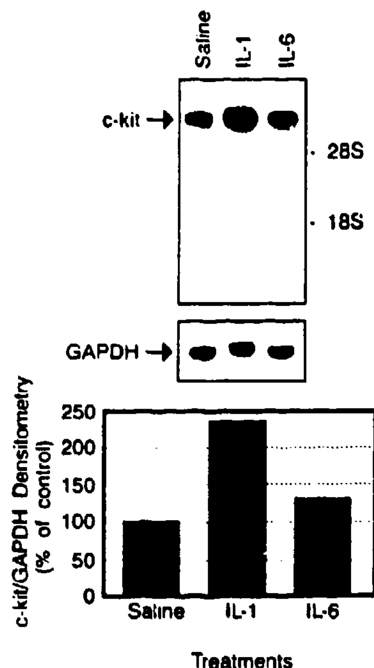

FIGURE 6. Comparison of treatment of IL-1 vs IL-6 on c-*kit* gene expression. Mice were treated with 1 μg of IL-1 or 2 μg of IL-6 or saline and BMC were recovered 4 h later for Northern blot analysis. Each group represents a pool of four mice.

increasing the numbers of c-*kit*$^{+}$ progenitor cells and, thus, facilitating hemopoietic recovery from radiation.

The mechanism of radioprotection with SCF remains to be established. The findings that SCF acts as a most potent comitogen on multilineage cells (10–14) and that Ab to SCF increases lethality of irradiation to mice (17) provide strong evidence that SCF is required for the regeneration of hemopoiesis. If such were the case, however, merely supplying SCF after irradiation should suffice for this effect. Yet SCF administration at 1 h after irradiation (Table II) did not result in significant radioprotection. Thus a lag period similar to that required for IL-1 is also required for radioprotection with SCF. Such results would suggest that the increase in numbers of progenitor cells in the BM before irradiation may account for the observed increase in resistance to radiation conferred by these two cytokines. However, the findings that the radioprotective effect of IL-1, SCF, or their combination cannot be demonstrated 48 h after their administration (Table II), despite a progressive increase in numbers of progenitor cells, suggest that either differentiation of these cells or, as previously suggested (21, 33), a particular phase in their cycle contribute to radioprotection.

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