Granulocyte/macrophage colony-stimulating factor and accessory cells modulate radioprotection by purified hematopoietic cells

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Granulocyte/macrophage colony-stimulating factor (GM-CSF) promotes the survival, proliferation, and differentiation of myeloid lineage cells and regulates chemotaxis and adhesion. However, mice in which the genes encoding GM-CSF (*Gmcsf*) or the β common subunit of the GM-CSF receptor (β*c*) are inactivated display normal steady-state hematopoiesis. Here, we show that host GM-CSF signaling strongly modulates the ability of donor hematopoietic cells to radioprotect lethally irradiated mice. Although bone marrow mononuclear cells efficiently rescue *Gmcsf* mutant recipients, fetal liver cells and Sca1$^+$ lin$^{-/dim}$ marrow cells are markedly impaired. This defect is partially attributable to accessory cells that are more prevalent in bone marrow. In contrast, *Gmcsf*-deficient hematopoietic stem cells demonstrate normal proliferative potentials. Short-term survival is also impaired in irradiated β*c* mutant recipients transplanted with fetal liver or bone marrow. These data demonstrate a nonredundant function of GM-CSF in radioprotection by donor hematopoietic cells that may prove relevant in clinical transplantation.

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Hematopoietic stem cell (HSC) transplantation (HSCT) is a front-line treatment for many hematologic disorders. Most conditioning regimens administer myeloablative doses of radiation and/or chemotherapy; however, how the response of the host microenvironment influences donor cell repopulation remains poorly understood. Transplantation protocols utilize bone marrow, mobilized peripheral blood stem cells (PBSCs), and umbilical cord blood as sources of HSCs, and intrinsic variations in HSCs derived from different sources have been reported previously (1). In clinical practice, the source of HSCs modulates the duration of posttransplant cytopenia with cytokine-mobilized PBSCs inducing the most rapid recovery and umbilical cord blood cells inducing the slowest (2, 3).

GM-CSF promotes the proliferation and differentiation of myeloid progenitors and their progeny (4). Although GM-CSF has not been directly implicated in HSC engraftment, treatment with recombinant GM-CSF accelerates myeloid recovery in patients undergoing autologous marrow transplantation (5). Moreover, some patients with posttransplant graft failure show hematopoietic recovery after GM-CSF treatment (6). The mechanisms underlying these effects are unknown. Primitive hematopoietic cells express low to undetectable levels of the GM-CSF receptor, suggesting that GM-CSF does not act directly on HSCs, but rather on lineage-committed cells (7). The GM-CSF, IL-3, and IL-5 receptors share a common β subunit (βc) that associates with unique α chains to mediate biological responses to these cytokines (8).

Homozygous *Gmcsf* and β*c* mutant mice (*Gmcsf*$^{-/-}$ and β*c*$^{-/-}$) maintain normal blood counts, and bone marrow from these animals restores hematopoiesis in irradiated WT recipients (9–12). The primary abnormality found in both strains is protein accumulation in the pulmonary alveoli due to defective macrophage function (9–12). We previously observed poor survival of irradiated *Gmcsf*$^{-/-}$ mice that were transplanted with fetal liver cells doubly mutant at the *Gmcsf* and *Nf1* loci (13). Here, we show that donor accessory cells and host GM-CSF signaling strongly modulate radioprotection.

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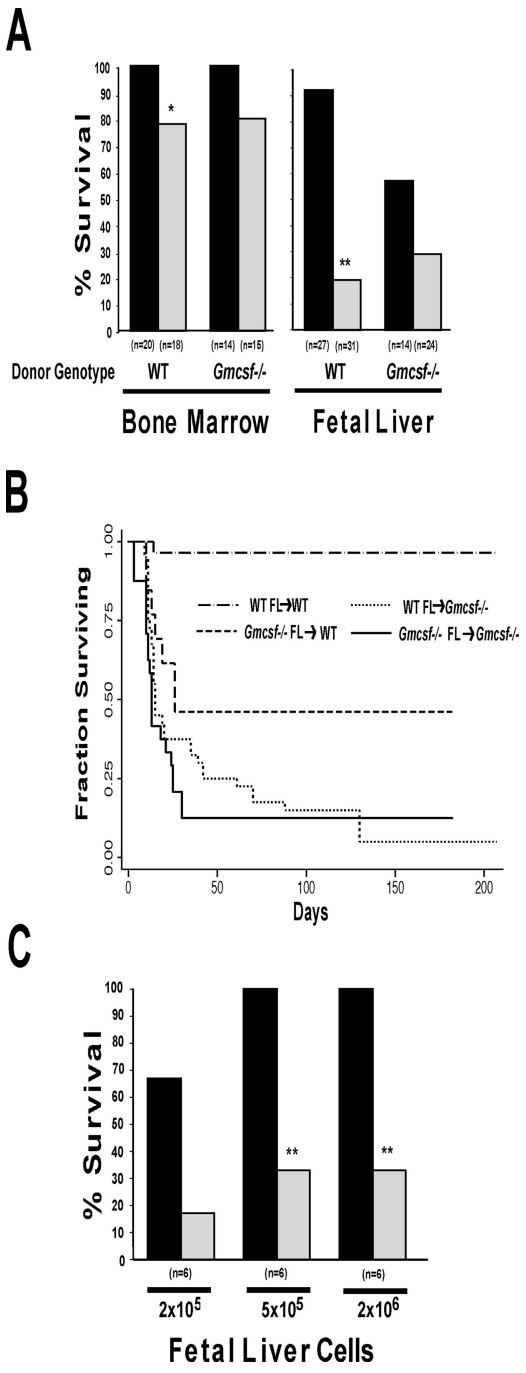

Figure 1. Radioprotection of WT and *Gmcsf*$^{-/-}$ recipients. (A) Survival rates 1 mo after adoptive transfer in WT (black bars) and *Gmcsf*$^{-/-}$ (gray bars) recipients. Statistically significant differences between WT and *Gmcsf*$^{-/-}$ recipients are shown with one ($P < 0.05$) or two ($P < 0.01$) asterisks. Combining the data for all recipients that received bone marrow cells revealed significantly lower survival in *Gmcsf*$^{-/-}$ ($n = 33$) versus WT ($n = 34$) hosts ($P = 0.009$). Similar differences between WT and *Gmcsf*$^{-/-}$ recipients were seen 4 mo after adoptive transfer. (B) Kaplan-Meier analysis of survival in recipients of fetal liver cells. The survival of WT mice transplanted with WT fetal liver cells was greater than *Gmcsf*$^{-/-}$ mice that received WT or *Gmcsf*$^{-/-}$ donor cells ($P < 0.00001$ for both comparisons) and was also significantly different than WT hosts injected with *Gmcsf*$^{-/-}$ fetal liver cells ($P = 0.0001$). (C) Survival at 1 mo in WT and *Gmcsf*$^{-/-}$ mice injected with a range of cell doses. Pooling the data for the 18 animals of each genotype revealed a significant difference in survival ($P = 0.00002$).

These data, which define the first essential role for GM-CSF in hematopoiesis, may prove relevant for enhancing HSCT.

RESULTS AND DISCUSSION

Defective radioprotection of *Gmcsf*$^{-/-}$ mice by fetal liver cells

Consistent with previous observations, 5×10^5 *Gmcsf*$^{-/-}$ bone marrow cells efficiently repopulated irradiated WT hosts (9, 12). However, *Gmcsf*$^{-/-}$ recipients that received either *Gmcsf*$^{-/-}$ or WT bone marrow showed a modest reduction in survival (Figs. 1 A and Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20041504/DC1). In contrast, *Gmcsf*$^{-/-}$ mice that were transplanted with fetal liver cells demonstrated markedly reduced survival (Fig. 1 A). In addition, WT mice that received *Gmcsf*$^{-/-}$ fetal liver cells had reduced survival compared with the recipients of WT cells (Fig. 1 B). Recipient mice succumbed 10–30 d after adoptive transfer with signs of hematopoietic failure (Fig. 1 B). Various numbers of WT fetal liver cells were injected to observe if the cell dose influenced survival rates. All WT mice that received $\geq 5 \times 10^5$ donor cells survived, whereas transferring up to 2×10^6 of the same cells did not rescue additional *Gmcsf*$^{-/-}$ animals (Fig. 1 C). These data reveal defective radioprotection of irradiated *Gmcsf*$^{-/-}$ mice, which is highly dependent on whether donor cells are derived from fetal liver or bone marrow. We also observed an independent effect of donor fetal liver cell genotype on the survival of WT recipients.

WT and *Gmcsf*$^{-/-}$ fetal liver cells show equivalent repopulating potentials

Next, we performed competitive repopulation experiments to assess the short- and long-term repopulating abilities of *Gmcsf*$^{-/-}$ and WT hematopoietic cells in recipients of either genotype. *Gmcsf*$^{-/-}$ and WT bone marrow cells contributed equally to hematopoiesis in irradiated recipients (Fig. 2 A). Because WT fetal liver cells failed to efficiently radioprotect *Gmcsf*$^{-/-}$ recipients (Fig. 1), bone marrow–derived competitors were used to assess the repopulating potential of fetal liver test cells. *Gmcsf*$^{-/-}$ fetal liver cells produced similar levels of chimerism as WT cells in recipients assessed 1 and 4 mo after transfer (Fig. 2 B). To exclude the possibility that GM-CSF production by competitor cells might mask an intrinsic defect, we crossed the *Gmcsf*$^{-/-}$ mutation onto the B6.BoyJ background and used CD45.1$^+$ bone marrow cells from these mice as a source of competitors. The repopulating potentials of *Gmcsf*$^{-/-}$ and WT fetal liver cells were equivalent in the absence of GM-CSF production by either competitor cells or irradiated recipients (Fig. 2 C).

Impaired survival of *Gmcsf*$^{-/-}$ recipients injected with bone marrow–derived Sca1$^+$ lin$^{-/dim}$ cells

The normal short- and long-term repopulating potentials of *Gmcsf*$^{-/-}$ fetal liver cells suggested that the failure to radio-

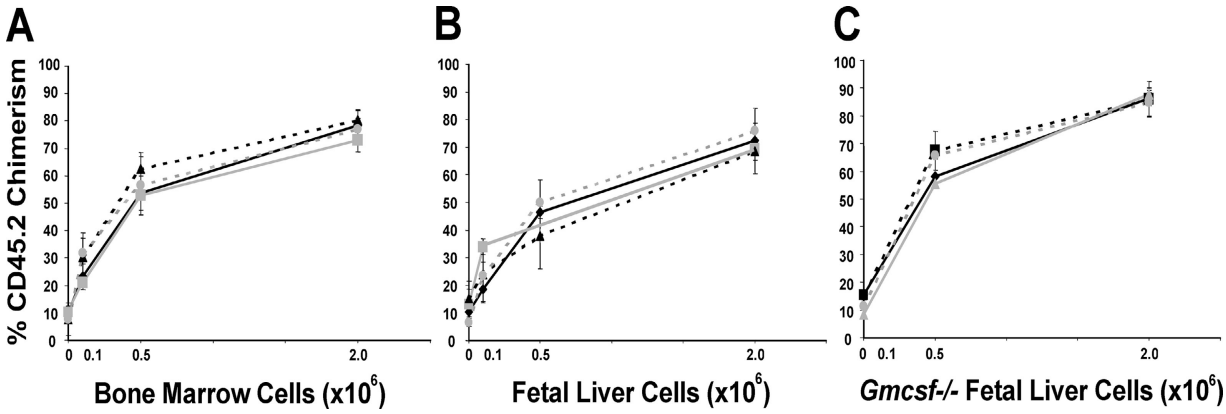

Figure 2. *Gmcsf*$^{-/-}$ and WT HSCs show equivalent repopulating potentials. (A and B) WT test cells transplanted into WT recipients are shown as a solid black line, *Gmcsf*$^{-/-}$ test cells transplanted into WT recipients are shown as a dotted black line, WT test cells transplanted into *Gmcsf*$^{-/-}$ hosts are shown as a solid gray line, and *Gmcsf*$^{-/-}$ test cells transplanted into *Gmcsf*$^{-/-}$ recipients are shown as a dotted gray line. Error bars represent standard deviations. (A) Bone marrow mononuclear cells from WT and *Gmcsf*$^{-/-}$ mice at the designated doses were transplanted in conjunction with 5×10^5 whole bone marrow cells from WT B6.BoyJ (CD45.1$^+$) mice. Levels of donor (CD45.2) chimerism are shown 1 mo after transplant. (B) Levels of CD45.2 chimerism in recipients transplanted with various numbers of WT or *Gmcsf*$^{-/-}$ donor cells that were mixed with 5×10^5 WT CD45.1$^+$ bone marrow competitors. (C) Levels of CD45.2 chimerism in recipients transplanted with various numbers of *Gmcsf*$^{-/-}$ fetal liver cells that were mixed with either WT or *Gmcsf*$^{-/-}$ CD45.1$^+$ bone marrow competitors. The solid black line represents WT hosts receiving WT competitor cells, the dotted black line shows WT hosts receiving *Gmcsf*$^{-/-}$ competitor cells, the solid gray line shows *Gmcsf*$^{-/-}$ hosts receiving WT competitor cells, and the dotted gray line shows *Gmcsf*$^{-/-}$ hosts receiving *Gmcsf*$^{-/-}$ competitor cells. The percentages of donor cell chimerism were stable at 4 mo (not depicted).

protect *Gmcsf*$^{-/-}$ recipients might be due to accessory cells that are present in bone marrow but are deficient in fetal liver. The Sca1$^+$ lin$^{-/dim}$ fraction, which is enriched for hematopoietic cells with high repopulating potential, comprised a similar percentage of cells in *Gmcsf*$^{-/-}$ and WT mice (unpublished data). Sca1$^+$ lin$^{-/dim}$ bone marrow cells were isolated from WT adult mice and injected into irradiated WT and *Gmcsf*$^{-/-}$ recipients (Fig. 3 A). Although 12% of WT recipients that were transplanted with 1,000 Sca1$^+$ lin$^{-/dim}$ cells survived, increasing the number of cells injected to 3,000 or more cells rescued 65–70% of irradiated mice (Fig. 3 B and Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20041504/DC1). In contrast, only 3 out of 32 *Gmcsf*$^{-/-}$ mice that were injected in parallel with 3,000–20,000 of the same Sca1$^+$ lin$^{-/dim}$ donor cells survived for 1 mo ($P < 0.0005$).

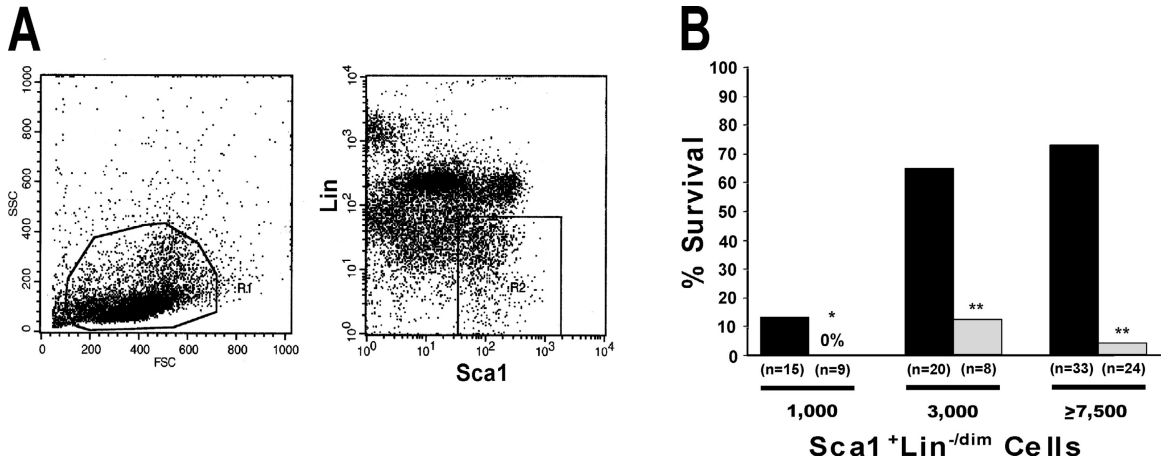

Figure 3. Hematopoietic reconstitution of wild-type and *Gmcsf*$^{-/-}$ recipients transplanted with Sca1$^+$ lin$^{-/dim}$ bone marrow cells. (A, left) A forward and side scatter plot of bone marrow cells after MACS selection of Sca1$^+$ cells and (right) the sorting gate used to isolate the Sca1$^+$ lin$^{-/dim}$ fraction for adoptive transfer (boxed area, 11.35% of total cells). (B) Survival of irradiated WT (black bars) and *Gmcsf*$^{-/-}$ (gray bars) recipients 1 mo after adoptive transfer of the indicated number of WT Sca1$^+$ lin$^{-/dim}$ bone marrow cells is shown. Significant differences between WT and *Gmcsf*$^{-/-}$ recipients are shown with one ($P < 0.05$) or two ($P < 0.01$) asterisks.

Table I. Effect of irradiated *Gmcsf*$^{-/-}$ bone marrow cells on fetal liver cell engraftment

Recipient genotype	WT fetal liver	Irradiated/*Gmcsf*$^{-/-}$ bone marrow	Survival/total	p-value
Gmcsf	+	−	1/11	
WT	+	−	8/9	<0.0005
Gmcsf	+	Lin$^+$	8/11	0.001
Gmcsf	+	Mac1$^+$	10/10	<0.0005

WT C57BL/6 mice received 2 × 10^6 fetal liver cells with or without Lin$^+$ or Mac1$^+$ *Gmcsf*$^{-/-}$ marrow cells that were radiated with 1,800 cGy before injection. The p-values indicate differences in survival at 1 mo between *Gmcsf*$^{-/-}$ recipients that received fetal liver cells only and the other groups (unpaired Student's *t* test).

Irradiated accessory cells and exogenous GM-CSF enhance the survival of *Gmcsf*$^{-/-}$ recipients

Because the percentage of cells expressing the Mac1 integrin complex was much lower in fetal liver versus bone marrow (~5 versus ~45%; unpublished data), we asked if accessory function provided by bone marrow–derived Mac1$^+$ cells contributes to radioprotection. To test this hypothesis, hematopoietic subpopulations were isolated from *Gmcsf*$^{-/-}$ bone marrow by cell sorting, irradiated with 1,800 cGy, mixed with fetal liver cells, and transferred into irradiated recipients. Table I and Fig. S3 (available at http://www.jem.org/cgi/content/full/jem.20041504/DC1) display the results of two independent experiments. Consistent with our previous data, the same WT donor fetal liver cells rescued 8 out of 9 WT recipients, but only 1 out of 11 *Gmcsf*$^{-/-}$ mice. Irradiated Sca1$^-$ Lin$^+$ *Gmcsf*$^{-/-}$ bone marrow cells did not rescue irradiated hosts (unpublished data). However, cotransplanting 2 × 10^5 or 10^6 irradiated Sca1$^-$ Lin$^+$ *Gmcsf*$^{-/-}$ cells with 2 × 10^6 WT fetal liver cells produced durable engraftment in 8 out of 11 *Gmcsf*$^{-/-}$ recipients. Furthermore, coinjecting 10^6 irradiated Mac1$^+$ cells derived from *Gmcsf*$^{-/-}$ bone marrow with fetal liver cells also rescued hematopoiesis in all 10 *Gmcsf*$^{-/-}$ recipients (Table I). These studies identify an accessory function within the differentiated fraction of bone marrow cells that facilitates the ability of fetal liver cells to radioprotect *Gmcsf*$^{-/-}$ mice.

We also asked if irradiated *Gmcsf*$^{-/-}$ accessory cells could restore the ability of bone marrow–derived Sca1$^+$ lin$^{-/dim}$ cells to radioprotect *Gmcsf*$^{-/-}$ recipients. In these studies, irradiated *Gmcsf*$^{-/-}$ bone marrow cells (5 × 10^6 per mouse) were coinjected with 7,500 WT or *Gmcsf*$^{-/-}$ Sca1$^+$ lin$^{-/dim}$ cells. *Gmcsf*$^{-/-}$ recipients that received Sca1$^+$ lin$^{-/dim}$ cells of either genotype died with graft failure within 14 d of transfer (n = 10). In contrast, 6 out of 10 animals that received Sca1$^+$ lin$^{-/dim}$ cells with irradiated bone marrow cells survived for >3 mo (Fig. S2), including 3 out of 5 transplanted with *Gmcsf*$^{-/-}$ Sca1$^+$ lin$^{-/dim}$ cells. These data confirm the existence of accessory cells in murine bone marrow that cooperate with Sca1$^+$ lin$^{-/dim}$ donor cells to radioprotect irradiated *Gmcsf*$^{-/-}$ mice. Furthermore, this accessory cell population achieves rescue through a mechanism that appears to be independent of both proliferation and GM-CSF production.

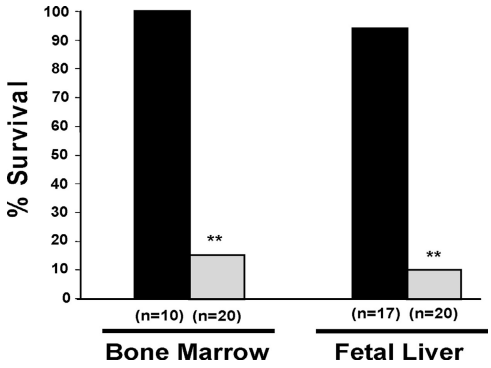

Figure 4. Radioprotection of βc mutant mice. WT bone marrow or fetal liver efficiently repopulated WT recipients (black bars). In contrast, βc$^{-/-}$ recipients (gray bars) that were transplanted with WT donor cells showed reduced survival. Significant differences are shown with two asterisks ($P < 0.01$).

We administered a supernatant prepared from B16 melanoma cells that had been engineered to produce murine GM-CSF (500 μl of supernatant containing ~73 ng/ml of GM-CSF) or a control B16 supernatant to ask if exogenous GM-CSF could improve the survival of *Gmcsf*$^{-/-}$ recipients transplanted with fetal liver cells. All mice were injected intraperitoneally on the day before adoptive transfer, the day they were irradiated and transplanted with 10^6 WT fetal liver cells, and on the day after transfer. In the first experiment, the mice were treated daily for the next week. In the other, a single additional injection of B16/GM-CSF or control parental B16 supernatant was administered 1 wk after adoptive transfer. In these two experiments, only one out of eight *Gmcsf*$^{-/-}$ recipients that were injected with the control supernatant survived for 14 d after adoptive transfer, whereas six out of eight animals that received B16/GM-CSF supernatant were alive after 1 mo.

Defective survival of irradiated βc$^{-/-}$ recipients

Our studies of *Gmcsf*$^{-/-}$ mice suggested that signaling through the βc chain in recipient stromal cells modulates the ability of donor cells to rescue short-term hematopoiesis. To further investigate this idea, we transferred WT bone marrow or fetal liver cells (5 × 10^5 per recipient) into irradiated βc$^{-/-}$ or control mice. βc$^{-/-}$ mice that received WT fetal liver cells showed impaired survival that was similar to *Gmcsf*$^{-/-}$ hosts (Fig. 4 and Fig. S4, available at http://www.jem.org/cgi/content/full/jem.20041504/DC1). However, this deficit was not restricted to donor fetal liver cells, but was also seen in recipients that were transplanted with WT bone marrow.

These studies identify a nonredundant role of GM-CSF signaling in radioprotection that is strongly modulated by donor accessory cells. The high rate of lethality in *Gmcsf*$^{-/-}$ or βc$^{-/-}$ recipients transplanted with fetal liver cells and the ability of exogenous GM-CSF to improve survival in *Gmcsf*$^{-/-}$ mice revealed a major influence of host genotype in radioprotection. Similarly, depleting accessory cells from

donor bone marrow or adding these cells to fetal liver modulated the survival of *Gmcsf*$^{-/-}$ recipients, demonstrating a strong and independent effect of accessory cells in facilitating engraftment. Although we examined short-term radioprotection as the primary endpoint, Kaplan-Meier survival analysis of recipients observed beyond 1 mo gave similar results (Figs. S1–S4). Together, our data indicate a complex interaction between host GM-CSF signaling and donor accessory cells in radioprotection and underscore the importance of both factors in this phenotype.

Myelo-erythroid and common myeloid progenitors are responsible for radioprotection, whereas long-term reconstitution requires engraftment by HSCs (14). However, myelo-erythroid progenitors, common myeloid progenitors, and HSCs do not express the GM-CSF receptor (15) and β*c* mutant cells efficiently repopulate wild-type recipients (10, 11). These data infer that GM-CSF facilitates radioprotection by a mechanism that does not require direct effects on repopulating donor cells. GM-CSF is concentrated in the bone marrow microenvironment where it is membrane anchored or immobilized within the extracellular matrix (16), which allows it to signal through receptors on target cells in a spatially localized manner. Irradiation induces GM-CSF production by cultured bone marrow stromal cells (17) and by stromal and endothelial cell lines (18). Furthermore, whereas GM-CSF mRNA levels are up-regulated within the bone marrow and spleen of mice 2 d after irradiation, there is no increase in serum levels (19). These data support the idea that GM-CSF is produced by stromal cells in response to radiation and acts locally. Although it is not known how GM-CSF signaling in recipient mice enhances radioprotection, GM-CSF has antiapoptotic effects in some cell types (20, 21). Alternatively, the observation that Mac1$^+$ cells from *Gmcsf*$^{-/-}$ mice demonstrate defective phagocytosis of apoptotic cells in vivo (22) suggests that impaired clearance of dead or dying cells within the microenvironment might contribute to the radiation sensitivity of *Gmcsf* and β*c* mutant mice.

Our finding that donor accessory cells facilitate hematopoietic recovery after irradiation is consistent with a report that examined engraftment of nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice by human Lin$^-$CD34$^+$CD38$^-$ cord blood cells (23). Furthermore, CD26 expression on donor hematopoietic cells reduces engraftment efficiency and it has been suggested that accessory cells partially rescue this phenotype (24). Accessory cells may also modulate the effects of other molecules such as CXCR4 on homing and engraftment. In studies using fetal liver donor cells, we found that a relevant accessory population is contained within the Mac1$^+$ fraction. However, we have not formally excluded the possibility that other cell fractions contribute to radioprotection. The ability of *Gmcsf*$^{-/-}$ bone marrow–derived accessory cells to improve the survival of *Gmcsf*$^{-/-}$ mice transplanted with fetal liver or with bone marrow–derived Sca1$^+$ lin$^{-/dim}$ cells is consistent with the observation that these recipients are efficiently rescued by *Gmcsf*$^{-/-}$ bone marrow. However, it is intriguing that this accessory activity is radioresistant, particularly because the same populations are present in recipient bone marrow. The apparent paradox might be explained by differential effects of in vivo versus ex vivo radiation on accessory function, or may be modulated by the number of accessory cells injected. Consistent with the latter possibility, WT donor fetal liver cells provided better radioprotection than *Gmcsf*$^{-/-}$ fetal liver cells but had equivalent long-term repopulating potential. These data suggest that GM-CSF production by donor accessory cells becomes important at reduced cell numbers. Limit dilution experiments comparing WT versus *Gmcsf*$^{-/-}$ accessory cells are required to definitively address this issue. Potential mechanisms through which irradiated donor accessory cells might facilitate radioprotection include guiding cells with short- and long-term repopulating potential to appropriate niches, facilitating adherence, and/or clearing apoptotic cells and debris from the irradiated host microenvironment.

Our data raise the possibility that a relative deficit in accessory cell function contributes to delayed engraftment in patients who are transplanted with umbilical cord blood (2, 3). Recombinant GM-CSF has not proven superior to G-CSF in recipients of mobilized PBSCs and is no longer used in most transplant centers because of a higher incidence of side effects. However, investigating how GM-CSF and other growth factors modulate the responses of host bone marrow microenvironment to irradiation and how cytokines act upon accessory cells that are infused could uncover strategies for enhancing the safety and efficacy of HSCT. Furthermore, manipulating donor cell populations ex vivo to isolate cells with high repopulating potential could paradoxically increase the risk of graft failure if critical accessory cell populations are eliminated during processing.

MATERIALS AND METHODS

Mice. *Gmcsf* and β*c* mutant mice (9, 10) were backcrossed six generations onto the C57BL/6 strain (CD45.2$^+$). WT C57BL/6 mice (CD45.2$^+$) and congenic B6.SJL-PtrcaPep3b/BoyJ (B6.BoyJ) mice (CD45.1$^+$) were purchased from The Jackson Laboratory. The University of California San Franscisco Committee on Animal Research approved the experimental procedures.

Hematopoietic cells. Pregnant WT, *Gmcsf*$^{-/-}$, and β*c*$^{-/-}$ females were killed by CO_2 inhalation at E14 and fetal liver cells were prepared as described previously (13). Bone marrow cells were collected by flushing tibias with IMDM supplemented with 20% FCS (Hyclone Laboratories).

Genotyping. Mice were genotyped at the *Gmcsf* and β*c* loci by Southern blotting as described previously (9, 10). PCR was also used to genotype *Gmcsf* mice (protocol available upon request).

Adoptive transfer and competitive repopulation. Recipients were irradiated with a single fraction of 850 cGy, which was uniformly lethal in the absence of donor cells. Fetal liver or bone marrow cells were injected into the dorsal tail vein of 10–12-wk-old recipients after irradiation. Short-term engraftment was defined as survival for 30 d after adoptive transfer, and radioprotection was defined as survival beyond the 12–18 d critical window of bone marrow failure. Competitive repopulation experiments were performed essentially as described by coinjecting CD45.2$^+$ test cells with CD45.1$^+$ competitors (25). Recipients received prophylactic oral antibiotics for 3 wk after irradiation.

Flow cytometry. Blood leukocytes were analyzed for chimerism using antibodies to CD45.1-PE and CD45.2-FITC. In some experiments, cells were counterstained with the lineage specific antibodies CD3-TC, B220-TC, Gr1-PE, and Mac1-PE (BD Biosciences). CD45 chimerism analysis was performed by flow cytometry with 10,000 events collected using a FACScan (Becton Dickinson) and analyzed using CELLQuest software. For the $Sca1^+$ $lin^{-/dim}$ sort, cells were stained with the following mix of antibodies: Sca-1–FITC, B220-PE, CD3-PE, Mac1-PE, TER119-PE, and Gr-1–PE. The cells were labeled with anti-FITC MACS beads to select for $Sca1^+$ cells on an autoMACS instrument and the Sca-1–enriched product was sorted using a BD FACS Vantage SE cell sorter. A rectangular sorting gate was drawn around the $FITC^+$ $PE^{-/dim}$ population and these cells were collected for injection (Fig. 3 A).

Online supplemental material. Kaplan-Meier analyses of data shown in Figs. 1 A, 3 B, and 4 and in Table I are presented as Figs. S1–S4. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20041504/DC1.

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