In Situ Radiation Sensitivity of Recombinant Human Granulocyte Colony-Stimulating Factor–Recruited Murine Circulating Blood and Bone Marrow Progenitors (Colony-Forming Unit [CFU]–Granulocyte-Macrophage and CFU-Megakaryocyte): Evidence for Possible Biologic Differences Between Mobilized Blood and Bone Marrow

By Stefan Scheding, Joseph E. Media, Mark A. KuKuruga, and Alexander Nakeff

Increasing evidence especially stemming from peripheral blood progenitor transplantation studies points to a possible biologic difference between mobilized blood and bone marrow progenitor cells. The objective of this study was to compare the in situ radiation sensitivity of recombinant human granulocyte colony-stimulating factor (rh-G-CSF)–recruited circulating granulopoietic (blood colony-forming unit–granulocyte-macrophage [CFU-GM\textsubscript{blood}]) and megakaryocytopoietic (blood CFU-Meg\textsubscript{blood}) progenitors, with the nonmobilized fraction remaining in the bone marrow (CFU-GM\textsubscript{nonmobilized} and CFU-Meg\textsubscript{nonmobilized}). Splenectomized \textit{B}_\textit{6D}_{2F}_{1} mice received 50 \textmu g/kg/d rh-G-CSF daily for 8 days to induce high levels of circulating progenitors, followed by either total body X-irradiation (TBI) or X-irradiation of the chest (CI) with 62.5, 125, 250, or 500 cGy. Progenitor cells were assayed 24 hours after irradiation. Circulating CFU-GM and CFU-Meg in the blood were decreased in a dose-dependent fashion by both TBI and CI, with TBI causing greater damage than CI. Average \textit{D}_0 values for TBI were 53 cGy for CFU-GM\textsubscript{blood} and 40 cGy for CFU-Meg\textsubscript{blood}. \textit{D}_0 values for CI were 90 cGy for CFU-GM\textsubscript{blood} and 140 cGy for CFU-Meg\textsubscript{blood}. As seen for blood progenitor cells, TBI caused a dose-dependent decrease of both CFU-GM\textsubscript{nonmobilized} \textit{D}_0 (136 cGy) and CFU-Meg\textsubscript{nonmobilized} \textit{D}_0 (148 cGy). However, radiation-induced bone marrow progenitor cell kill was significantly lower when compared with blood progenitors. Despite the fact that circulating blood elements only received a fraction of the total dose administered as CI, the extent of blood progenitor cell kill caused by CI was higher than the effects of identically mobilized TBI doses on bone marrow CFU. The results of this study showed that rh-G-CSF–recruited CFU-Meg\textsubscript{blood} and CFU-Meg\textsubscript{nonmobilized} were considerably more radiosensitive than femoral progenitors, thereby providing novel evidence for a biologic difference between rh-G-CSF–recruited peripheral blood progenitors and the nonrecruited bone marrow CFU.

© 1996 by The American Society of Hematology.

Within the last few years, peripheral blood progenitor cells (PBPC) have been used increasingly for autologous transplantation after high-dose chemotherapy.\textsuperscript{1} Mobilization of PBPC into the PB is accomplished by administration of either chemotherapy, hematopoietic growth factors, or the combination of chemotherapy followed by growth factor application.\textsuperscript{2,3} When compared with bone marrow (BM) transplantation, the most striking advantage of PBPC transplantation is a considerably faster granulopoietic and megakaryocytopoietic recovery after high-dose chemotherapy independently of whether PBPC were used with or without concomitant BM for transplantation.\textsuperscript{4-12}

This observation raises the question of whether there is a biologic difference between PB and BM progenitor cells.\textsuperscript{13} In fact, differences in antigenic profile, clonogenic cells, self-renewal and proliferative capacity of primitive stem cells, rhodamine uptake and \textit{c-kit} expression between BM and PBPC have been reported.\textsuperscript{14-24} One of the earliest reports addressing this question dates back to Micklem et al.,\textsuperscript{25} who showed that murine steady-state blood and BM colony-forming unit-spleen (CFU-S) markedly differed in their self-renewal capacity. Neben et al.\textsuperscript{26} also found that blood collected from cyclophosphamide plus granulocyte colony-stimulating factor (G-CSF)–treated mice produced equivalent numbers of CFU-S as compared with BM, but the self-renewal capacity was reduced. Differences in colony-forming cells, CFU–granulocyte-macrophage (CFU-GM), and their relationship to CD34\textsuperscript{+} expression between BM and mobilized blood were reported by Janssen et al.\textsuperscript{27} Bender et al.\textsuperscript{17} showed that cyclophosphamide-mobilized PBPC from cancer patients in contrast to BM exhibited low levels of CD71 positivity, thus indicating that a majority of circulating CD34\textsuperscript{+} cells is not proliferating. This finding was confirmed by To et al.,\textsuperscript{18} who furthermore showed that mobilized CD34\textsuperscript{+} PBPC expressed less \textit{c-kit} and retained less rhodamine, and by Roberts and Metcalf,\textsuperscript{19} who showed that considerably fewer recombinant human G-CSF (rh-G-CSF)–mobilized blood progenitors are in S phase when compared with BM. Suzuki et al.\textsuperscript{20} reported clear differences in the features of CD34\textsuperscript{+} cells with regard to antigenic profile, burst-forming unit-erythroid (BFU-E) and CFU-GM numbers, \textit{c-kit} expression from BM and PB, and between healthy volunteers and patients after chemotherapy plus rh-G-CSF. Differences in adhesion molecule expression were reported by Mohle et al.,\textsuperscript{21} Derkszen et al.,\textsuperscript{22} and Turner et al.\textsuperscript{23} Furthermore, we have recently shown that, in splenectomized \textit{B}_\textit{6D}_{2F}_{1} mice, rh-G-CSF–recruited peripheral progenitors, CFU-GM and CFU-megakaryocyte (CFU-Meg), were substantially reduced (up to 99%) by 500 cGy X-irradiation of the chest.\textsuperscript{24} When compared with previously published irradiation dose-response curves for BM progenitors, the extent of radiation-induced damage was much higher than expected. Most of the survival curves obtained for murine progenitor cells from unfractonated marrow have been shown to be exponential or nearly exponential, with
D₀ values for CFU-GM ranging from 128 to 190 cGy. Data from our group reported D₀ values for in vivo irradiation of B₆D₂F₁ femoral CFU-Meg of 128 cGy. No data exist for CFU-Meg after in vitro irradiation. Nothdurft et al studied the in vitro radiosensitivity of steady-state canine granulopoietic progenitors. They found that circulating steady-state granulocyte-macrophage–colony-forming cells (GM-CFC; D₀ = 26.1 cGy) were more radiosensitive than steady-state BM GM-CFC (D₀ = 60 cGy). D₀ values for human steady-state blood and BM progenitor cells were reported by Grilli et al after in vitro X-irradiation (BFU-E, Do = 93 and 127 cGy; CFU-C, D₀ = 146 and 136 cGy for blood and BM, respectively).

Our recent observations led to the speculation that rhG-CSF–recruited progenitors were more radiosensitive than their BM counterparts. Furthermore, no data exist with regard to a possible in vivo radiation sensitivity difference between rhG-CSF-mobilized blood and bone progenitors. Therefore, the aim of the present study was to determine the dose-response relationship for BM and PB progenitors, thus permitting a direct comparison of these two cell populations.

Total body irradiation (TBI) and irradiation of the chest (CI), the latter mimicking primarily extramedullary radiotherapy regimens and being included to directly relate to and extend our previously published data, induced a dramatic kill of circulating granulopoietic and megakaryocytic progenitors. Our data show that the in situ radiosensitivity of rhG-CSF–recruited PB progenitors is significantly higher than the radiosensitivity of nonrecruited BM progenitors.

MATERIALS AND METHODS

**Mice**

Male B₆D₂F₁ mice (C57BL/6 × DBA/2), obtained from the National Institutes of Health (Bethesda, MD), were between 10 and 12 weeks of age at the beginning of all experiments. Acidified water (pH = 2.5) and mouse chow were provided ad libitum and cages were covered by a filter hood. Splenectomy was performed under general anesthesia. Mice were allowed to recover for at least 4 weeks after the splenectomy before being included in the subsequent protocols.

**rhG-CSF Treatment**

Dilutions of rhG-CSF, kindly provided by Dr Carol Long (AMGEN, Thousand Oaks, CA) were prepared in Dulbecco’s phosphate-buffered saline (DPBS; Gibco, Grand Island, NY) + 0.1% bovine serum albumin (BSA). Mice received 50 μg/kg rhG-CSF per day, delivered as two daily subcutaneous injections. rhG-CSF treatment was performed on days 1 through 8. The rhG-CSF dose was chosen according to our previously published results, showing that 50 μg/kg rhG-CSF was sufficient to cause a substantial increase of blood circulating progenitors.

**Irradiation**

After 8 days of rhG-CSF treatment (50 μg/kg), groups of 3 mice received either TBI or CI. Irradiation with 62.5, 125, 250, and 500 cGy was delivered at 87 cGy/min using a Siemens Stabiliplan 2 (250 kV x-ray, 15 mA, 1-mm Cu filter, and 50 cm distance; Siemens Medical Systems, Iselin, NJ). The other body parts were shielded with 50 mm lead. During irradiation, mice were slightly anaesthetized with 44 mg/kg Ketamine HCL intramuscularly (AVECO Co, Fort Dodge, IA). rhG-CSF–treated control mice received anesthesia and were sham-irradiated.

**Number of Marrow and Blood Progenitor Cells**

Mice were bled retrobially and killed by cervical dislocation 24 hours after irradiation. For every group, citrated blood was pooled from 3 animals. PB mononuclear cells were isolated after centrifugation on a layer of Histopaque (1.083 g/mL, Sigma, St Louis, MO). The interphase was removed and washed three times with Hanks Balanced Salt Solution (GIBCO) and resuspended in Leibovitz-15 (GIBCO) supplemented with 2% fetal calf serum (GIBCO). Mono-dispersed cell suspension of BM was obtained by flushing of femurs with Leibovitz-15 medium + 2% fetal calf serum at room temperature. The marrow was pooled from groups of 3 donor mice. Total blood and marrow nucleated cell measurements were obtained using a hemacytometer, and fractions of the resulting cell suspensions were used for assaying progenitor cells committed to the granulocyte-macrophage (CFU-GM) and megakaryocyte (CFU-Meg) lineage using a modified plasma culture technique. Briefly, unfractionated femoral or blood nucleated cells were cultured in Leibovitz-15 medium supplemented with 20% fetal calf serum (GIBCO), 56 μg/mL CytC (Sigma), 0.1 U/mL Thrombin (Sigma), 10% L-cell conditioned medium and 5% PWMCM (Terry Fox Cancer Center, Vancouver, British Columbia, Canada) for stimulation of CFU-GM and CFU-Meg growth, respectively. Clotting was induced by the addition of 10% prescreened bovine citrated plasma (Sigma) and 0.5-mL clots were cultured in quadruplicate in Nunclon culture dishes (Nunc, Kamstrup, Denmark) at 37°C, 5% CO₂, 5% O₂ for 7 (CFU-GM) and 5 days (CFU-Meg). Colonies consisting of more than 50 granulomacrophagocytic cells (hematoxylin-stained) were counted as CFU-GM; CFU-Meg were identified by staining for acetylcholinesterase activity, with three or more megakaryocytic cells defining one colony.

**Blood Cell Numbers**

Numbers of total white blood cells and platelets were counted using a hemacytometer. For each group, blood smears were prepared in duplicate and stained with Wright-Giemsa for quantification of neutrophilic granulocytes.

**Flow Cytometric Determination of Blood IgG⁺ Cells**

After hypotonic water lysis and washing with DPBS, 1 × 10⁶ nucleated blood cells were incubated for 15 minutes at 4°C with goat-antimouse IgG antibody conjugated with phycoerythrin (Boehringer Mannheim, Mannheim, Germany). Control samples were stained with an equivalent amount of goat-antirabbit IgG antibody conjugated with phycoerythrin as an isotype control. Flow cytometric analysis was performed using a FACSscan and the PCLYSIS acquisition and analysis software (Becton Dickinson, Mountain View, CA). The percentages of circulating IgG⁺ cells were obtained after gating on lymphocytes (lymphogate) and the total numbers of B cells were calculated by multiplying the total number of cells in the lymphogate by the fraction of IgG⁺ cells.

**Statistical Analysis**

Results are presented as the mean ± standard error of the mean (SEM) of at least two separate experiments. Statistical analysis was performed using Student’s t-test and one-way analysis of variance (ANOVA) where appropriate.

**RESULTS**

**rhG-CSF Effects on PB and BM Cells**

Treatment of spleenectomized B₆D₂F₁ mice with 50 μg/kg rhG-CSF per day for 8 days caused a significant increase of
total nucleated blood cells (ie, 1.3 ± 0.17 × 10^7/mL in cases of untreated mice compared with 3.96 ± 0.53 × 10^7/mL for rhG-CSF–treated mice; *P < .01), with granulocytes being increased about 10-fold, from 1.5 ± 0.3 × 10^7/mL to 14 ± 0.3 × 10^7/mL (*P < .01). However, platelets were found to be slightly decreased by 8 days of rhG-CSF treatment (1.2 ± 0.3 × 10^7/mL for untreated as compared with 0.88 ± 0.1 × 10^7/mL for rhG-CSF–treated animals; *P > .05).

rhG-CSF–induced progenitor recruitment was documented by high levels of peripheral clonogenic cells after 8 days of rhG-CSF administration. Circulating CFU-GM_blood were 8.2 ± 0.5 × 10^7/mL and CFU-Meg_blood were 5.9 ± 1.6 × 10^7/mL (untreated mice, 33 ± 6 CFU-GM/mL and 19 ± 4 CFU-Meg/mL; *P < .01).

rhG-CSF caused a decrease in total nucleated cells per femur from 3.3 ± 0.14 × 10^7/femur to 2.89 ± 0.18 × 10^7/femur (*P > .05). Granulopoietic marrow progenitor cell numbers were not significantly altered by the rhG-CSF treatment (untreated, 6.3 ± 0.3 × 10^7/femur vs rhG-CSF–treated, 6.4 ± 0.23 × 10^7/femur; *P > .05); however, femoral CFU-Meg were decreased from 4.2 ± 0.26 × 10^7/femur to 2.6 ± 0.29 × 10^7/femur by 8 days of rhG-CSF (*P < .01).

**Effects of X-Irradiation on rhG-CSF–Stimulated Hematopoiesis**

**Irradiation response of circulating blood and BM nucleated cells.** The effects of TBI or CI over the dose range of 62.5 to 500 cGy on circulating leukocytes, neutrophilic granulocytes, and platelets are shown in Table 1. TBI caused a dose-dependent decrease of circulating leukocytes. Cell numbers were significantly reduced to 38%, 20%, 14%, and 11% of nonirradiated control values by 62.5, 125, 250, and 500 cGy TBI, respectively. CI caused a maximum reduction of blood leukocytes of approximately 8% at 500 cGy. Neutrophilic granulocytes also were reduced significantly by both TBI and CI. Platelets did not show any statistically significant changes in cell numbers irrespective of dose and application mode (Table 1).

A dose-dependent decrease in femoral total nucleated cells (TNC) by TBI was observed, with a dose of 500 cGy resulting in a decrease of about 53% of nonirradiated controls (Table 1) with the lack of changes in femoral TNC after CI indicating effective shielding of the marrow.

**Irradiation response of circulating progenitors.** Control animals that received 50 μg/kg rhG-CSF daily for 8 days but were not irradiated exhibited high levels of peripheral progenitors, as shown above. The dose-survival curves for CFU-GM_blood and CFU-Meg_blood presented in Fig 1 showed an exponential decrease in colony-forming ability with increasing dose of either TBI or CI, with TBI decreasing CFUs more than CI (Fig 1). The D_0 values were determined from the slopes of the linear regression lines of log_10 (surviving fraction) versus dose. Specifically, D_0 (slope) = (log_10 e -1)/slope, ie, 0.434/slope. Average D_0 values for CFU-GM_blood were 53 cGy and 90 cGy for TBI and CI, respectively; whereas the corresponding D_0 values for CFU-Meg_blood were 40 cGy (TBI) and 140 cGy (CI).

Mean numbers of CFU-GM_blood per milliliter surviving 62.5, 125, 250, and 500 cGy TBI were 1.730 ± 438, 349 ±

### Table 1. Effects of TBI and CI on rhG-CSF–Stimulated Hematopoiesis in BDF, Mice

<table>
<thead>
<tr>
<th>Dose (cGy)</th>
<th>Total Leukocytes/mL (×10^9)</th>
<th>Neutrophils/mL (×10^9)</th>
<th>Platelets/mL (×10^11)</th>
<th>Nucleated Cells/Femur (×10^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhG-CSF control</td>
<td>0</td>
<td>3.96 ± 0.53</td>
<td>1.42 ± 0.3</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>TBI</td>
<td>62.5</td>
<td>1.49 ± 0.05*</td>
<td>0.34 ± 0.14†</td>
<td>9.1 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0.81 ± 0.1*</td>
<td>0.27 ± 0.11*</td>
<td>9.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.54 ± 0.02*</td>
<td>0.41 ± 0.03†</td>
<td>10.6 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.43 ± 0.08*</td>
<td>0.25 ± 0.06*</td>
<td>10.2 ± 2.2</td>
</tr>
<tr>
<td>CI</td>
<td>62.5</td>
<td>1.42 ± 0.09*</td>
<td>0.43 ± 0.21†</td>
<td>10.9 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.51 ± 0.06*</td>
<td>0.46 ± 0.14†</td>
<td>10.8 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.16 ± 0.12*</td>
<td>0.44 ± 0.18†</td>
<td>10.8 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.33 ± 0.02*</td>
<td>0.12 ± 0.04*</td>
<td>8.9 ± 1.7</td>
</tr>
</tbody>
</table>

Mice were treated with 50 μg/kg/d rhG-CSF daily on days 1 through 8 and then received X-irradiation (62.5, 125, 250, or 500 cGy) to the chest on day 8. Assays were performed on day 9. rhG-CSF control mice received rhG-CSF but were sham-irradiated (rhG-CSF control). Data are shown as the mean ± SEM. Significant differences between rhG-CSF control and irradiated groups are indicated. Statistical analysis was performed with ANOVA using the Dunnett multiple comparison of irradiated groups with the control group.

*P < .01.
†P < .05.
RADIATION RESPONSE OF CSF-RECRUITED CFU

**Fig 2.** Fraction of femoral progenitors surviving X-irradiation with 62.5, 125, 250, and 500 cGy. Groups of 3 mice received injections of 50 μg/kg/d rhG-CSF daily for 8 days. Animals were irradiated on day 8 and assayed 24 hours after irradiation. (Θ) TBI; (C) CI. Data are shown as the mean ± SEM.

119, 4.6 ± 2.8, and 0.9 ± 0.9, respectively. The corresponding values for CI were 511 ± 55, 869 ± 267, 77 ± 5.5, and 32 ± 9.7. Mean numbers of surviving CFU-Meg<sub>blood</sub> per milliliter were 45.4 ± 13.6, 11.9 ± 4, 0.91 ± 0.91, and below detection for TBI doses of 62.5, 125, 250, and 500 cGy, respectively. The corresponding values for CI were 167 ± 17, 164 ± 55.3, 27.5 ± 5.8, and 14.6 ± 4.6.

Irradiation response of marrow progenitors. CI did not affect femoral progenitor numbers (Fig 2) at any given dose. The TBI dose-survival curve for femoral CFU-GM and CFU-Meg is given in Fig 2. There was an exponential decrease in CFU with increasing dose and no clear evidence of a shoulder. The average D<sub>0</sub> value after TBI for CFU-GM<sub>femur</sub> was 136 cGy, as compared with 148 cGy for CFU-Meg<sub>femur</sub>.

Femoral CFU-GM<sub>femur</sub> were reduced from 6.4 ± 0.2 × 10<sup>8</sup> to 4.2 ± 0.2 × 10<sup>4</sup>, 3.4 ± 0.4 × 10<sup>4</sup>, 0.9 ± 0.1 × 10<sup>4</sup>, and 0.18 ± 0.02 × 10<sup>4</sup> per femur by TBI doses of 62.5, 125, 250, and 500 cGy TBI, respectively. Mean numbers of CFU-Meg per femur were found to be decreased from 2.6 ± 0.29 × 10<sup>7</sup> to 1.4 ± 0.2 × 10<sup>4</sup>, 0.86 ± 0.07 × 10<sup>4</sup>, 0.39 ± 0.04 × 10<sup>4</sup>, and 0.08 ± 0.01 × 10<sup>4</sup> by 62.5, 125, 250, and 500 cGy TBI, respectively.

Statistical comparison of the effects of the different x-ray dose levels using ANOVA and Bonferroni multiple comparison tests showed that the extent of either the TBI- or CI-induced reduction of circulating CFU-GM<sub>blood</sub> (Fig 1A) was significantly greater than the TBI-induced reduction of CFU-GM<sub>femur</sub> (P < .001 at 62.5 and 125 cGy; P < .01 for 250 cGy; Fig 2A). In case of CFU-Meg, TBI-induced kill of CFU-Meg<sub>blood</sub> (Fig 1B) was significantly higher than that for CFU-Meg<sub>femur</sub> at 62.5 (P < .001), 125 (P < .001), and 250 cGy (P < .05) with 500 cGy TBI decreasing CFU-Meg<sub>blood</sub> below detection, thus obviating any statistical analysis. No statistically significant differences were found between the dose-survival curves of CFU-Meg<sub>blood</sub> after CI versus CFU-Meg<sub>femur</sub> after TBI, except for a dose of 62.5 cGy (P < .05).

**Fig 3.** Fraction of blood IgG<sup>+</sup> cells surviving X-irradiation with 62.5, 125, 250, and 500 cGy. Groups of 3 mice received injections of 50 μg/kg/d rhG-CSF daily for 8 days. Animals were irradiated on day 8 and assayed 24 hours after irradiation. (Θ) TBI; (C) CI. Data are shown as the mean ± SEM. (Insert) Estimation of the effective dose that circulating cells received by CI expressed as the TBI equivalent (TBI<sub>e</sub>) dose.

DISCUSSION

The administration of rhG-CSF has been applied routinely to recruit hematopoietic progenitor cells into the circulation for later use as autologous or allogeneic PBPC transplants after high-dose chemotherapy. Interestingly, PBPC-mediated recovery of granulopoiesis and megakaryocytopoiesis is considerably faster when compared with transplantation of BM, thus leading to the hypothesis that the fraction of recruitable
CFU-GM and CFU-Meg is biologically different from the nonrecruitable fraction of BM progenitors. We have previously shown that rhG-CSF–recruited murine CFU-GM and CFU-Meg were highly susceptible to partial body (chest) irradiation. In the present study, radiation dose-survival curves of rhG-CSF–recruited blood progenitors and BM CFU-GM and CFU-Meg were recorded in the same animals. Our data clearly showed that, in rhG-CSF–pretreated BDF1 mice, TBI resulted in a significantly higher total femoral marrow. To our knowledge, this is the first report showing that the in situ radiosensitivity of rhG-CSF–recruited PB progenitors is significantly higher than that of their nonrecruited BM counterparts. D0 values for marrow progenitors obtained in this study (136 and 148 cGy for CFU-GM<sub>bone</sub> and CFU-Meg<sub>bone</sub>, respectively) are comparable to those reported previously. These values were clearly higher when compared with D0 values of 53 and 40 cGy for CFU-GM<sub>blood</sub> and CFU-Meg<sub>blood</sub>, respectively, after TBI. Furthermore, radiation-induced kill by partial body CI of circulating CFU was significantly higher (CFU-GM at 62.5, 125, and 250 cGy, CFU-Meg at 62.5 cGy) when compared with their TBI-induced kill in the femoral marrow, although with CI, these circulating CFU only received a fraction of the dose administered as TBI.

Several factors are known to affect the in situ radiation response of hematopoietic progenitor cells. First, the radiosensitivity of cells depends on their stage in the cell cycle, with the highest sensitivity being during the mitotic, late G<sub>2</sub>, or early DNA-synthesis phase of the cell cycle. However, in the case of PBPC, a lower fraction of these cells is in DNA synthesis as compared with BM. This has been shown for the steady-state as well as for mobilized blood cells after chemotherapy and/or rhG-CSF treatment. Second, with photons having energies less than 100 kV, there is a significant secondary short-range emission from the high atomic number elements in bone. This effect becomes negligible at higher doses, but even at 250 kV, as used in this study, BM in close proximity to bone will receive higher doses than expected in soft tissue, leading to an approximately 6% effective increase in the dose to the total femoral marrow. Therefore, one can conjecture that the effective dose received by circulating progenitors was lower than that of femoral progenitors. Third, microenvironmental factors influence the radiation response of hematopoietic cells. It is well known that hypoxic conditions protect cells from the lethal effects of ionizing radiation. For reduction of cells to a given survival level, about three times as much radiation is required under hypoxic conditions as under aerobic conditions. Therefore, the difference in oxygen level between BM and PB might contribute to the difference in radiation sensitivity observed in this study. However, studies by Nothdurft et al. and Grilli et al. comparing the in vitro radiosensitivity of nonmobilized blood and BM hematopoietic precursors showed that, under identical conditions with regard to oxygen tension, blood-derived progenitors were more radiosensitive than BM-derived progenitors. Therefore, the in situ radiosensitivity difference observed in our study may not be explainable solely by a difference in oxygen tension. Furthermore, other microenvironmental factors, such as cell interaction, cytokine production of adherent cells, etc, could be responsible for a more efficient protection or repair of radiation damage in the case of BM progenitors. However, the in vitro studies of Nothdurft et al. showed that irradiation of either blood-derived CFU-GM or BM-derived CFU-GM in the presence of either preirradiated BM or blood cells, respectively, led to small changes of the survival curves when compared with those survival curves generated after the irradiation of blood CFU-GM and total marrow cell suspensions alone. Finally, one could speculate that, due to radiation-induced alterations of the vascular structure or the progenitor cell surface, margination of progenitor cells might occur that accounts for the low recovery of progenitor cells from the blood stream. However, irradiation in the dose range and time frame used in our study does not alter the vascular tissue and especially does not induce endothelial cell alterations, which are the prerequisite for cell margination. Furthermore, the cells most likely to exhibit radiation-induced margination by sticking to the vessel walls are platelets. However, our data clearly showed that platelet levels remained unchanged within 24 hours even after 500 cGy TBI (Table 1). Therefore, we think that margination can be ruled out as a possible factor and conclude that the difference in radiation sensitivity between rhG-CSF–mobilized circulating progenitors and nonmobilized femoral CFU observed in this study is due to inherent differences between mobilized and nonmobilized cells rather than to possible differences in microenvironmental or other factors. However, at this point in time, we have not formally confirmed our in situ results by ex vivo experimentation, which is an important consideration. Our data, furthermore, do not permit us to decide whether a preferential recruitment of very radiation-sensitive progenitors is induced by exogenous G-CSF. Additional experiments would have to be performed to investigate the possible differential mobilization of very radiosensitive progenitor cell subpopulations by means of flow cytometric analysis and sorting. Furthermore, mechanisms such as differences in transcriptional activation, gene induction, and regulation after irradiation, being responsible for a difference in inherent radiosensitivity, are important considerations that remain to be investigated.

The response of circulating blood progenitors to partial body irradiation (CI, as described previously) was investigated in addition to the effects of TBI to directly relate to, and extend, our previously published data. After CI, circulating blood elements receive only a fraction of the administered dose because they are not present in the irradiation field during the entire irradiation period. For circulating progenitors, this is reflected in different D0 values after TBI as compared with CI. Because animals were treated with rhG-CSF until shortly before irradiation, it cannot be excluded that the G-CSF–induced migration of progenitor cells from BM into the circulation might have influenced the measurements taken 24 hours after exposure. However, one can easily estimate possible effects of progenitor cell migration on circulating CFU levels, assuming that migration actually took place within 24 hours after irradiation. Assuming a migration rate of 1%, a total of 1 × 10<sup>6</sup> femoral CFU-GM would have been mobilized into the blood, leading to an
increase in circulating CFU-GM\textsubscript{blood} levels of at least $5 \times 10^5$/mL (note that the 8-day G-CSF treatment used in this study led to a mobilization of about 1.6% of total marrow CFU-GM into the PB). However, even in case of the lowest irradiation dose used (62.5 cGy), only $1.7 \times 10^5$ CFU-GM/mL were detected 24 hours after irradiation, leading to the conclusion that a migration rate of 1% is clearly too high. Similarly, the assumption of an extremely low migration rate of only 0.01% (ie, $1 \times 10^5$ CFU-GM would have been recruited from the marrow, leading to an increase of circulating CFU-GM of at least 50/mL) is clearly too high, because of the lower CFU-GM\textsubscript{blood} levels measured experimentally after 500 cGy chest irradiation. Therefore, it is reasonable to assume that, if migration took place at all within 24 hours after irradiation, it was of no relevance to the findings presented in our study.

Nevertheless, to estimate the dose of circulating blood elements received by CI, we determined the radiation-dose survival of IgG\textsuperscript{+} cells (Fig 3), because they were not influenced by possible G-CSF–induced migration phenomena. By plotting the TBI–versus CI-induced IgG\textsuperscript{+} cell kill (Fig 3, insert), the effective fraction of a given dose administered as CI could be expressed as an isoeffect equivalent TBI dose, TBL. Our data showed that a given dose administered as CI was about as half as effective as the same dose delivered whole-body (TBI).

Taken together, these data clearly show an in situ radiation sensitivity difference between rhG-CSF–recruited PB progenitors and those nonrecruited progenitors residing in the marrow that is inherent in these two cell populations, thereby providing novel evidence for a clear biologic difference between rhG-CSF–recruited peripheral progenitor cells and nonrecruited femoral CFU.

ACKNOWLEDGMENT

The authors thank Dr Colin G. Orton for his invaluable help in analyzing the radiation dose-response data presented in this manuscript.

REFERENCES


7. To LB, Haylock DN, Dowse T, Simmons PJ, Trimboli S, Ashman LK, Juttner CA: A comparative study of the phenotype and proliferative capacity of peripheral blood (PB) CD34\textsuperscript{+} cells mobilized by four different protocols and those of steady-phase PB and bone marrow CD34\textsuperscript{+} cells. Blood 84:2930, 1994


15. Neben S, Marcus K, Mauch P: Mobilization of haemopoietic stem and progenitor subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor. Blood 81:1709, 1993


18. To LB, Haylock DN, Dowse T, Simmons PJ, Trimboli S, Ashman LK, Juttner CA: A comparative study of the phenotype and proliferative capacity of peripheral blood (PB) CD34\textsuperscript{+} cells mobilized by four different protocols and those of steady-phase PB and bone marrow CD34\textsuperscript{+} cells. Blood 84:2930, 1994


cules and c-kit on CD34+ hematopoietic progenitor cells: Comparison of cytokine-mobilized blood stem cells with normal bone marrow and peripheral blood. J Hematother 2:483, 1993


In situ radiation sensitivity of recombinant human granulocyte colony-stimulating factor-recruited murine circulating blood and bone marrow progenitors (colony-forming unit [CFU]-granulocyte-macrophage and CFU-megakaryocyte): evidence for possible biologic differences between mobilized blood and bone marrow

S Scheding, JE Media, MA KuKuruga and A Nakeff