We studied whether treatment of dogs with recombinant human granulocyte colony-stimulating factor (rhG-CSF), after 820 cGy total body irradiation (TBI) and transplantation of 3.3 ± 1.0 x 10^8 bone marrow cells per kilogram from a DLA-identical littermate, accelerated hematopoietic recovery and influenced the incidence of subsequent marrow graft failure or graft-versus-host disease (GVHD). Ten animals were treated with 100 μg rhG-CSF/kg/d from days 1 through 10 after TBI. Results were compared with those of a historical control of 14 dogs not administered rhG-CSF. Neither group of dogs received GVHD prophylaxis. The median time to recovery of 1,000 neutrophils/mm^3 was 8 days for dogs administered rhG-CSF compared with 14 days in controls (logrank test: P < .03). The median time to reach 100 monocytes/mm^3 was 17 days in G-CSF-treated dogs compared with 49 days in controls (P < .002).

The median time to attain 500 lymphocytes/mm^3 was 15 days versus 31 days, respectively (P < .01). The median time to reach 20,000 platelets/mm^3 was 26 versus 20 days (P = .68). Graft failure occurred in 1 of 10 G-CSF-treated dogs versus 2 of 14 controls (two-tailed Fisher's exact test: P = 1.00). GVHD was seen in 4 of 9 rhG-CSF-treated dogs compared with 1 of 12 controls (P = .12). Two G-CSF-treated dogs died of GVHD versus none of the controls (P = .17). No unusual toxicities were seen in dogs receiving rhG-CSF. In summary, rhG-CSF significantly accelerated recovery of neutrophils, monocytes, and lymphocytes after DLA-identical littermate marrow transplantation without altering platelet recovery. Graft failure was not seen more often than in controls, but there was a trend toward an increased incidence of GVHD.

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Marrow Transplant candidates either have no marrow function to begin with, as in aplastic anemia, or their marrow function is eradicated by the chemoradiotherapy aimed at destroying the underlying hematologic malignancy. Despite prompt infusion of HLA-identical marrow after completion of the conditioning regimen, patients remain pancytopenic for several weeks before the graft achieves full function. Typically, 15 to 44 (median 24) days will go by before granulocyte counts reach 1,000/mm^3, during which time patients are at risk for bacterial or fungal infections with an overall incidence of 20% to 25%, and a case mortality rate of 25% to 50%.1 Shortening the period of neutropenia after marrow grafting is likely to result in a reduction of the risk of infections. A preclinical study in nonhuman primates and two clinical studies using recombinant human granulocyte colony-stimulating factor (rhG-CSF) after autologous marrow transplantation have shown significant acceleration of granulocyte recovery compared with controls.4-6 The usefulness of rhG-CSF after allogeneic human marrow grafts has been investigated in only one nonrandomized study thus far, at least in part because of concerns of side effects, such as an increased risk of graft-versus-host disease (GVHD) or late graft failure. Because rhG-CSF cross-reacts with canine marrow cells,8,9 the present study explored the effect of rhG-CSF administered after transplantation of marrow from DLA-identical littersmates. We found that treatment with rhG-CSF significantly accelerated recovery of neutrophil, monocyte, and lymphocyte counts over those of controls, but not of platelet counts. Graft failure was rare in both groups but there was a trend toward an increase in the incidence of GVHD in dogs administered rhG-CSF.

Materials and Methods

Experimental animals. Twenty-four dogs of both sexes and various breeds (beagles, fox hounds, mongrels) 6 to 13 months old were used in the study. Dogs were either raised at the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, WA) or purchased from commercial US Department of Agriculture licensed dealers. Dogs were quarantined on arrival, screened for evidence of disease, and observed for a minimum of 2 months before use. All dogs were dewormed and vaccinated for rabies, distemper, leptospirosis, hepatitis, and parvovirus. They were housed in an American Association for Accreditation of Laboratory Animal Care approved facility in standard indoor runs, and provided commercial dog chow and chlorinated tap water ad libitum. Animal holding areas were maintained at 70°F ± 2°F with 50% ± 10% relative humidity using at least 15 air changes per hour of 100% conditioned fresh air. The dogs were on a 12-hour light/dark full-spectrum lighting cycle with no twilight. The protocol of this study was approved by the Institutional Animal Care and Use Committee of the FHCRC.

Histocompatibility typing and selection of donor-recipient pairs. The serologically detectable antigens of the canine major histocompatibility complex (DLA) were determined with 62 antisera by a
two-stage technique, and mixed leukocyte cultures were performed as described. The littermate donor-recipient pairs were chosen on the basis of serotypic identity for DLA-A, -B, and -C and mutual nonreactivity of their peripheral blood mononuclear cells in mixed leukocyte culture.

Total body irradiation (TBI), marrow infusion, and postgrafting care. Marrow for transplantation was obtained by needle aspirations from femora and humeri of anesthetized dogs, as described. Marrow recipients were administered a single dose of 920 cGy TBI delivered at a rate of 7 cGy/min from two opposing 60Co sources, as described. They received an intravenous infusion of 3.3 ± 1.0 x 10^8 marrow cells/kg body weight within 4 hours of TBI. The day of TBI and marrow transplantation was designated as "day 0." After TBI and marrow infusion, parenteral fluids, electrolytes, platelet transfusions, and systemic antibiotics were administered as described.* In addition, all dogs received oral antibiotics (polymyxin B and neomycin sulfate) three times daily starting on day 5 through the day on which the granulocyte count reached 500/mm^3 postgrafting. No postgrafting immunosuppression was given. All blood products used for transfusions were irradiated in vitro (1,500 cGy) to inactivate pluripotent hematopoietic stem cells. Hematocrits, reticulocyte, leukocyte, platelet, and differential counts were obtained before and daily after TBI until full hematologic recovery had occurred. Autopsies with histologic examinations were performed on all dogs that died.

Graft failure was defined as the lack of sustained recoveries of granulocyte and platelet counts after the postirradiation decline, extreme marrow hypocellularity (less than 5% of normal) at autopsy as estimated by light microscopy of marrow sections, and the absence of clinical and histologic features of GVHD. Dogs were evaluated as having GVHD when persistent clinical symptoms, including skin rash, diarrhea, and jaundice, were confirmed by histology at autopsy.

rhG-CSF. The rhG-CSF used in this study was prepared and provided by Amgen Corporation (Thousand Oaks, CA). The gene encoding hG-CSF was cloned from the 5637 cell line, expressed in Escherichia coli and purified to homogeneity by reversed-phase high-performance liquid chromatography. The purified rhG-CSF contained less than 0.5 ng of endotoxin per milligram of protein as measured by the Limulus amebocyte lysate assay. The specific activity of rhG-CSF was 1 x 10^8 U/mg of protein when assayed by serial dilution in a colony-forming unit-granulocyte/macrophage (CFU-GM) assay. One hundred micrograms of rhG-CSF/kg/d was administered to the experimental dogs subcutaneously in divided doses in 1 mL 0.9% NaCl containing 0.1% normal heat-inactivated dog serum. This dose of rhG-CSF was chosen based on results of a previous study, which showed that this dose, administered to a normal dog for 14 days, increased the neutrophil counts 10-fold compared with the preinfusion counts or those of control dogs receiving either heat-inactivated rh interleukin-3 or human serum albumin. Dogs were treated from day 1 after TBI and marrow infusion until day 10.

Statistical analysis. The frequencies of GVHD, lethal GVHD, and graft failure were compared between the current experimental and historical control dogs using the two-tailed Fisher's exact test. Time to death and time to attain sufficient counts of neutrophils, platelets, lymphocytes, and monocytes were compared between the two groups using the logrank test. The Kaplan-Meier method was used to estimate the number of days required for 25%, 50%, and 75% of the dogs to achieve specified levels of blood counts. The P value of .05 was considered significant. All analyses were performed using the Statistical Analysis System (SAS).

RESULTS

Table 1 and Fig 1 summarize the results on hematopoietic recovery. Neutrophil counts in the rhG-CSF–treated dogs reached 500/mm^3 at a median of 7 days and 1,000/mm^3 at 8 days after transplantation. This was significantly faster than neutrophil recovery in the controls who reached values of 500 and 1,000/mm^3 at medians of 10 and 14 days, respectively (logrank test: P < .08 and P < .03, respectively). The median time to reach 50 and 100 monocytes/mm^3 in G-CSF–treated dogs was 13 and 17 days compared with 28 and 49 days in the control group (logrank test: P < .0008 and P < .002, respectively). Lymphocyte counts reached 250 and 500/mm^3 by days 9 and 15 in rhG-CSF–treated dogs compared with 15 and 31 days in the control dogs (logrank test: P < .03 and P < .01, respectively). Platelet recovery to 20,000/mm^3 was not significantly different between the two groups (logrank test: P = .68), with the median times being

<table>
<thead>
<tr>
<th>Variable</th>
<th>Peripheral Blood Count</th>
<th>rhG-CSF</th>
<th>25% of Dogs</th>
<th>50% of Dogs</th>
<th>75% of Dogs</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>500/mm^3</td>
<td>-</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>.08</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>6</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>50/mm^3</td>
<td>-</td>
<td>15</td>
<td>28</td>
<td>35</td>
<td>.0008</td>
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<tr>
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<td>+</td>
<td></td>
<td>7</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>250/mm^3</td>
<td>-</td>
<td>13</td>
<td>17</td>
<td>22</td>
<td>.03</td>
</tr>
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<td></td>
<td>+</td>
<td></td>
<td>10</td>
<td>15</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>20,000/mm^3</td>
<td>-</td>
<td>13</td>
<td>20</td>
<td>37</td>
<td>.68</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>22</td>
<td>26</td>
<td>31</td>
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</tbody>
</table>

*Logrank test.

Table 1. Recovery of Peripheral Blood Counts After TBI and Transplantation of Marrow From DLA-Identical Littermate Without and With Subsequent rhG-CSF
No unusual toxicities were seen in dogs receiving rhG-CSF.

DISCUSSION

We found that treatment with rhG-CSF administered after lethal TBI significantly accelerated recovery of neutrophils, monocytes, and lymphocytes, but had no significant effect on platelet recovery after transplantation of marrow from DLA-identical littermates compared with historical control dogs. Graft failure was uncommon, but more G-CSF–treated dogs showed GVHD.

Most studies of rhG-CSF have involved autologous marrow transplantation. The effects of treatment with rhG-CSF after allogeneic marrow transplantation have been described so far in one preclinical and one clinical study. The preclinical study by Blazar et al involved mice administered continuous subcutaneous infusion of rhG-CSF for 14 days after sublethal TBI and T-cell–depleted histoincompatible marrow grafts. The experimental animals had a significantly greater likelihood of 100-day survival than controls. Total leukocyte numbers on days 7 and 14 posttransplant were higher in treated animals due to an increase in both neutrophils and lymphocytes. Platelet counts were not studied and no significant difference in hematocrit levels was observed. No clinical signs of GVHD were seen in either the experimental or control group. The mean percentages of donor and host cells were similar in the two groups of mice, indicating that engraftment was not adversely affected by rhG-CSF treatment. Masaoka et al investigated the usefulness of rhG-CSF after allogeneic human marrow transplantation in a nonrandomized study. Thirty-six patients received marrow transplants from HLA-matched siblings for the treatment of acute leukemia, chronic myelogenous leukemia, malignant lymphoma, or aplastic anemia. After conditioning with chemotherapy and TBI or chemotherapy only, patients were treated with 200 to 800 pg/m² rhG-CSF for 14 days, starting on day 3 or 5 after transplant. Cyclosporine and methotrexate, or cyclosporine only, were used for GVHD prophylaxis. Granulocytes reached a level of 500/mm³ significantly faster in the treated group compared with historical controls. Little effect on lymphocyte, eosinophil, or platelet recovery was reported. The incidence of acute GVHD was 47.1%, but most cases were only grade 1 or 2. The investigators concluded

![Fig 1. Recovery of peripheral blood counts after 9.2 Gy TBI and transplantation of marrow from DLA-identical littermate without and with twice daily subcutaneous injections of 100 μg rhG-CSF/kg/d from days 1 through 10 after TBI.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>rhG-CSF–Treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death before 100 days (all causes)</td>
<td>5/14 (36%)</td>
<td>3/10 (30%)</td>
<td>.80*</td>
</tr>
<tr>
<td>GVHD†</td>
<td>1/12 (8%)</td>
<td>4/9 (44%)</td>
<td>.12‡</td>
</tr>
<tr>
<td>Lethal GVHD†</td>
<td>0/12 (0%)</td>
<td>2/9 (22%)</td>
<td>.17†</td>
</tr>
<tr>
<td>Graft failure</td>
<td>2/14 (14%)</td>
<td>1/10 (10%)</td>
<td>1.00‡</td>
</tr>
</tbody>
</table>

Results indicate the number of dogs developing either death before 100 days, GVHD, lethal GVHD, or graft failure divided by the total number of evaluable dogs and the percentage in parenthesis.

*Logrank test.
†Dogs that died of graft failure were not included in the comparison of incidence of GVHD and lethal GVHD.
‡Two-tailed Fisher's exact test.
that rhG-CSF should be studied further after allogeneic transplantation.

The present study confirms these previous observations and demonstrates that rhG-CSF significantly accelerates neutrophil recovery. In addition, we observed faster recovery of monocytes and lymphocytes in rhG-CSF–treated dogs, a result that differs from those in the two previous transplant studies. Platelet recovery was delayed but not statistically significantly different from the historic control, similar to the results described by Masaoka et al after allogeneic marrow grafts. However, it should be noted that the present study was a preclinical study and differs from the usual human situation in that only TBI was used in the preparative regimen, and no GVHD prophylaxis was used.

Because rhG-CSF has been described in dogs, monkeys, and humans to increase not only neutrophils but also lymphocytes, it is conceivable that rhG-CSF increases the risks of GVHD or graft failure through an effect on donor-derived or radio-resistant host-derived immunocompetent cells involved in these phenomena. The current study failed to show an increase in graft failure in dogs treated with rhG-CSF, consistent with results described by Blazar et al, but we saw a trend toward an increase in acute GVHD. Blazar et al did not observe clinical evidence of GVHD in either the treated or the control group, perhaps related to the use of T-cell–depleted marrow grafts. Given that our animals received no GVHD prophylaxis and that the trend toward increased GVHD did not impact on overall survival, the results of our study would encourage an investigation of rhG-CSF after allogeneic marrow transplantation in humans, but would suggest that a careful assessment of GVHD would be an important endpoint of such a study.

In summary, we found that rhG-CSF after high-dose TBI and transplantation of DLA-identical littermate marrow significantly accelerated recovery of peripheral blood neutrophils, monocytes, and lymphocytes, but had no significant effect on platelet recovery. Graft failure was exceptional, not different from controls, but there was a trend, though not statistically significant, toward an increased incidence of GVHD.

ACKNOWLEDGMENT

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REFERENCES


