Augmentation of Donor Bone Marrow Engraftment in Histoincompatible Murine Recipients by Granulocyte/Macrophage Colony-Stimulating Factor

By Bruce R. Blazar, Michael B. Widmer, Christine C.B. Soderling, David L. Urdal, Steven Gillis, Leslie L. Robison, and Daniel A. Vallera

T cell depletion of donor bone marrow can prevent graft v host disease (GVHD) in human and murine marrow graft recipients. However, engraftment in the recipient may be compromised as a consequence of donor marrow T cell depletion. The effect of recombinant murine granulocyte/ macrophage colony-stimulating factor (rmu GM-CSF) on engraftment and hematologic reconstitution was evaluated in a murine allogeneic bone marrow transplantation (BMT) model involving T cell depletion of marrow. Before transplantation into irradiated mice differing at major and minor histocompatibility loci, rmu GM-CSF was preincubated with T cell–depleted donor marrow. When low degrees of engraftment were noted in control recipients, treatment of donor marrow with high concentrations of rmu GM-CSF led to enhanced engraftment. Ex vivo donor graft incubation with rmu GM-CSF or a single in vivo injection of GM-CSF were both effective means of promoting engraftment. When the engraftment rate in control recipients was high, rmu-GM-CSF did not have an identifiable effect. Only slight increases in hematologic recovery were detected regardless of the rate of engraftment. Neither host BMT survival nor marrow stem cell capacity was affected by rmu-GM-CSF incubation. Furthermore, growth factor administration did not have a significant effect on the incidence of GVHD in recipients of non–T cell–depleted bone marrow/splenocyte preparations. In vitro natural killer–mediated target cell lysis was not altered by incubation of effector cells with rmu GM-CSF. These results demonstrate the potential of ex vivo rmu GM-CSF treatment of donor marrow to facilitate engraftment across extensive histocompatibility barriers.

MAJOR LIMITATION

In allogeneic bone marrow transplantation (BMT), the occurrence of severe graft–v–host disease (GVHD).1-3 GVHD has proven to be mediated by T lymphocytes in animal models of BMT.4-9 Efforts to eliminate the potential for GVHD in humans include the use of a variety of methodologies to reduce the number of T lymphocytes in donor marrow.10-18 As a result of T cell depletion, however, incomplete donor cell engraftment has emerged as a significant complication.12,14-18

We have developed an allogeneic murine BMT model to study engraftment in the context of T cell–depleted, major histocompatibility complex–disparate donor bone marrow.19 In this model, treatment of donor BALB/c (H-2d) marrow with a pan-T cell monoclonal antibody (anti-Thy 1.2) and complement (C) results in a low incidence of GVHD and a high incidence of engraftment failure in unrelated C57BL/6 (H-2b) recipients that are conditioned for BMT with 7.5 Gy of total-body irradiation (TBI). Because the removal of T cells presumably decreases the quantity of growth and differentiation factors, we hypothesized that the availability of these donor T cell–derived, colony-stimulating factors (CSF) may confer proliferation and differentiation advantages to progenitor cells and/or may activate resting immunocompetent cells in the marrow graft. In this case, the addition of exogenous CSF to T cell–depleted donor marrow before BMT might selectively increase the incidence of subsequent donor graft survival and/or increase the rapidity and/or extent of hematologic recovery.

In this study, we present data demonstrating that exogenous recombinant murine granulocyte/macrophage colony-stimulating factor (rmu GM-CSF) facilitates donor cell engraftment in recipients that would otherwise have a low incidence of engraftment.

MATERIALS AND METHODS

Mice. BALB/c (H-2d) and C57BL/6 (H-2b) mice were bred and maintained at the University of Minnesota mouse colony. Animals were housed in conventional cages with filter lids and fed a fat-supplemented diet and antibiotic-supplemented water for two weeks before pretransplant immunosuppression and for 1 month posttransplant. Transplant recipients were at least 12 weeks old; donors were at least 6 weeks old. In each experiment, 12 to 24 recipients per group received transplants.

Recipient pretransplant conditioning. Our conditioning regimens have been previously described.19 For engraftment experiments, C57BL/6 mice were irradiated to a total dose of 7.5 Gy by using a 220 keV General Electric Maximar-20 x-ray source at a dose rate of 0.45 Gy/min. For GVHD experiments, C57BL/6 mice were irradiated to a total dose of 9.0 Gy.

BMT procedure. Our transplant protocol has been reported.4 BALB/c marrow cells (20 x 10^6/mL) were depleted of T cells with monoclonal anti-Thy 1.2 plus C as previously described.12,20 This procedure routinely results in the lysis of greater than 95% of BALB/c thymocytes as assessed in a microcytotoxicity assay.
rmu GM-CSF. rmu GM-CSF was purified by high-performance liquid chromatography from the supernatant yeast. Studies were performed to test the effect of rmu GM-CSF on murine bone marrow cell proliferation as assessed by tritiated thymidine incorporation. Unfractionated BALB/c or C57BL/6 bone marrow cells were suspended in RPMI/10% inactivated fetal calf serum at a concentration of 1 × 10^6 cells/mL. One hundred microliters of the suspension was placed into microtiter wells to which 100 µL of medium with varying concentrations of rmu GM-CSF was added. After 24 hours of incubation at 37°C and 5% CO₂ in humidified air, 1 µCi of tritiated thymidine was added per well for six hours before harvesting.

Treatment with rmu GM-CSF. T cell-depleted marrow (prepared as described earlier) was washed, resuspended, and incubated with or without rmu GM-CSF to permit receptor saturation (37°C, 60 minutes). Unwashed marrow inoculum, 0.5 mL (ex vivo incubation with in vivo infusion), was injected via the caudal vein into the recipients. Initial dose titration experiments were performed with concentrations of rmu GM-CSF ranging from 1.3 to 13,000 ng/mL. For all subsequent experiments, the concentration of rmu GM-CSF was 13,000 ng/mL. Animals were monitored for survival, evidence ofGVHD, hematologic recovery, and engraftment.

For experiments designed to test the relative efficacy of ex vivo incubation (without in vivo infusion) vs. infusion of a single in vivo dose (without ex vivo incubation) vs. nontreated controls, a different experimental scheme was used: four groups of mice received transplants. T cell-depleted marrow was incubated without (aliquot 1) or with (aliquot 2) exogenous rmu GM-CSF (13,000 ng/mL) as described earlier. The marrow aliquots were washed and resuspended in medium without (aliquot 1, no ex vivo incubation, and half of aliquot 2, ex vivo incubation alone) or with 13,000 ng/mL rmu GM-CSF (half of aliquot 2, ex vivo incubation with in vivo infusion). The mice received 0.5 mL of the final marrow inoculum. Half of the mice receiving T cell–depleted marrow from aliquot 1 that had not been incubated ex vivo with rmu GM-CSF were injected with 6,500 ng rmu GM-CSF (equivalent to the amount that is infused along with the rmu GM-CSF–containing allograft). These latter mice make up the single intravenous administration group.

Induction of GVHD. A quantity of 25 × 10^6 non–T cell–depleted BALB/c or donor marrow cells were mixed with 2.5, 5, or 15 × 10^6 BALB/c spleenocytes. The cell suspension was incubated with or without rmu GM-CSF as described earlier. A total volume of 0.5 mL of the mixture was injected through the caudal vein into irradiated C57BL/6 recipients. The animals were monitored daily for signs of GVHD and survival.

Complement-dependent microcytotoxicity assay to determine engraftment. The percentage of donor- and host-type peripheral blood mononuclear cells in transplant recipients was determined by H-2 typing in a complement-dependent microcytotoxicity assay as described.22 Engraftment was defined as >30% donor cells with <70% host cells. H-2 phenotyping was performed 60 to 69 days post-BMT. After this time, graft failures are rare.23

Assessment of leukocyte and erythroid recovery. One hundred fifty to 250 µL peripheral blood was obtained by retro-orbital venipuncture on days 7, 14, and 28. Leukocyte number and morphology were determined by examination of Wright-Giemsa–stained slides. Reticulocytes were enumerated on slides stained with methylene blue. Hematocrit values were determined by capillary tube volume red cell–to–plasma ratios after centrifugation.

CFU-spleen assay. For day 12 CFU-S formation, 3.0 × 10^4 bone marrow cells from nontransplanted controls or from murine BMT recipients killed 100 days post-BMT were injected into BALB/c recipients that were irradiated one day earlier with 7.5 Gy TBI from a 137Cs source at a dose rate of 68 cGy/min. Spleens to be analyzed for CFU-S formation were removed on day 12, stained with Bouin's solution, and examined for colony number. Mice that had received untreated or rmu GM-CSF treated marrow grafts and had >90% donor cells by H-2 phenotyping 60 to 69 days post-BMT were used as a source of marrow for assessment of CFU-S formation. Day 12 CFU-S assays were all performed in triplicate.

Natural killer assay. NK-mediated cytolyis was performed as previously described.24 Lysis of the NK-sensitive YAC-1, a Moloney virus–induced T lymphoma cell line that is propagated in continuous culture, was used as a measurement of NK activity. The YAC-1 target cells were maintained in RPMI 1640 (GIBCO, Grand Island, NY) with 10% fetal calf serum and 1% penicillin/streptomycin (GIBCO) at 37°C in 5% CO₂/95% air. Cells were labeled with 51Cr for 60 to 90 minutes at 37°C by mixing 200 µCi Na2CrO4 (200 to 900 µCi/mg 51Cr; New England Nuclear, Boston) with 1 × 10⁴ YAC-1 cells in 0.5 mL RPMI 1640/5% fetal bovine serum (FBS). An additional one-hour incubation at 37°C was performed during the washes for 18-hour release assays to reduce spontaneous release.

NK-enriched splenic effector cells were obtained by layering the single-cell suspension of spleenocytes (in RPMI 1640/5% FBS) onto a discontinuous Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient as described by Santoni et al with modifications.25 A 51Cr release assay was performed in 96-well microtiter plates (Linbro, Flow Laboratories, McLean, VA). A graded number of effector cells was mixed with labeled target cells at effector-to-target (E:T) ratios of 100:1 through 12.5:1. Wells contained 1 × 10⁴ targets in a total volume of 200 µL. The cell mixture was incubated for 18 hours and spun. One hundred microliters of supernatant was removed and counted in a gamma counter. Spontaneous release was measured in supernatants from targets incubated in medium alone. Maximal release was measured in supernatants containing targets lysed with detergent (mixed alkytrimethyl ammonium bromide, Sigma Chemical Co., St Louis). The percent cytotoxicity was calculated as follows:

percent specific lysis = cpm experimental release – cpm spontaneous release x 100 – cpm maximal release – cpm spontaneous release

Statistical analyses. Groupwide comparisons of continuous data were made by using Student's t test, and categorical data were assessed by Fisher's exact test. In some experiments, the engraftment rate of the controls was lower (≤30% of recipients), whereas in others, the engraftment rate of the controls was at least 1.8-fold higher. Therefore, engraftment data were analyzed for individual experiments and also as cumulative data and as pooled data from experiments with low engraftment rates or high engraftment rates of control recipients. Survival data were analyzed by life table methods using the Mantel-Peto-Cox summary of chi square.27

RESULTS

The effect of rmu GM-CSF on bone marrow cells. The dose response of unfractionated BALB/c or C57BL/6 bone marrow cells to rmu GM-CSF is shown in Fig 1. Maximal proliferation as measured by tritiated thymidine incorporation was obtained at a concentration of 0.1 to 1 µg/mL. Fifty percent maximal response occurred at approximately 400 pg/mL rmu GM-CSF. The dose response of the GM-
assayed for tritiated thymidine incorporation. Dose-response with rmu GM-CSF. incubated for 24 hours. and then mixed in vitro. BALB/c or C57BL/6 bone marrow cells were depleted BALB/c marrow at concentrations ranging from 1.3 to 13,000 ng/mL, neither the mean percentage of donor cells nor the percentage of animals that showed engraftment were altered between recipients of marrow that was administered in the absence of exogenous GM-CSF (Table 1). In contrast, at 13,000 ng/mL (experiment 2) the percent donor cell number increased from 55% ± 15% in the control group to 73% ± 9% in the GM-CSF–inoculated group. This was also accompanied by a decrease in the percentage of host cells: 43% ± 13% in controls as compared with 16% ± 18% in the GM-CSF–treated group. All mice in the latter group showed engraftment as contrasted to 67% of controls. Based on these findings, four additional experiments were performed that involved a total of 156 mice that received transplants with either untreated marrow or marrow preincubated with 13,000 ng/mL rmu GM-CSF (Table 2). The overall data summary (at the bottom of Table 2) shows that the mean percentage of host cells in the control group of 78 animals was 60% ± 5%, whereas treatment resulted in 44% ± 5%. This reduction in the percentage of host cells was significant at p < .01. Although treatment increased the percentage of donor cells from 39% ± 5% to 49% ± 4% and the overall percentage of animals showing engraftment was increased from 49% to 66%, these differences were not statistically significant. Consideration of the five experiments individually, however, revealed further levels of stratification. When the level of engraftment in mice receiving control marrow was the lowest (experiments 1, 2), augmentation of engraftment by rmu GM-CSF was most pronounced and significantly increased (57% engraftment) as compared with controls (29% engraftment). When the level of engraftment in the controls was higher (experiments 3 to 5), the effect of rmu GM-CSF was less obvious and not statistically significant.

To determine whether the observed engraftment promotion effect occurred as a result of ex vivo incubation or from in vivo infusion of rmu GM-CSF along with the donor graft (because the unwashed donor inoculum was infused), we performed experiments on 15 mice per group with (a) control marrow; (b) control marrow followed by a single intravenous dose of rmu GM-CSF; (c) ex vivo treated, washed marrow; or (d) ex vivo treated, unwashed marrow (ex vivo plus in vivo). Control mice had the lowest donor cell numbers (mean ± 1 SEM, 27% ± 13%) as compared with 42% ± 11%, 53% ± 11%, and 45% ± 14% (mean ± 1 SEM) for groups b, c, and d, respectively. Control mice also had the highest host cell numbers (mean ± 1 SEM, 75% ± 12%) as compared with 62% ± 10%, 49% ± 11%, and 54% ± 11% for groups b, c, and d, respectively. In addition, controls had the lowest degree of engraftment (27%) as compared with 54%, 64% and 56% for groups b, c, and d, respectively.

Survival. Overall, 76% of the recipients of control grafts

### Table 1. The Engraftment Effects of Various Concentrations of rmu GM-CSF in the Ex Vivo Treatment of T Cell–Depleted Donor Marrow Grafts

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>Transplant Recipients</th>
<th>rmu GM-CSF* (ng/mL)</th>
<th>No. H-2† Typed</th>
<th>Donor Cells‡ (%)</th>
<th>Host Cells§ (%)</th>
<th>Engraftment¶ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>0</td>
<td>9</td>
<td>46 ± 15</td>
<td>59 ± 12</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.3</td>
<td>7</td>
<td>40 ± 18</td>
<td>58 ± 17</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>32 ± 16</td>
<td>71 ± 15</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>130</td>
<td>6</td>
<td>48 ± 20</td>
<td>45 ± 19</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0</td>
<td>9</td>
<td>55 ± 16</td>
<td>43 ± 13</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1,300</td>
<td>7</td>
<td>30 ± 14</td>
<td>86 ± 7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13,000</td>
<td>9</td>
<td>73 ± 9</td>
<td>16 ± 18</td>
<td>100</td>
</tr>
</tbody>
</table>

* T cell–depleted BALB/c marrow was incubated (60 minutes, 37°C) with or without varying concentrations of rmu GM-CSF before infusion into irradiated (7.5 Gy) C57BL/6 recipients.
† All recipients alive on day 60 were H-2 typed.
‡ Values represented are expressed as means ± 1 SEM.
§ Percent engraftment is the percentage of recipients that were noted to have >30% donor cells with ≤70% host cells by H-2 typing.
¶ P = .08 by Student’s t test as compared with the 0 ng/mL GM-CSF–treated group.
90 80 70 60 50 40 30 20 10 0
Fig 2. Actuarial survival rates of mice receiving ex vivo rmu GM-CSF treated T cell-depleted histoincompatible bone marrow. T cell-depleted BALB/c marrow was incubated with 13,000 ng/mL GM-CSF and infused into lethally irradiated C57BL/6 recipients. Animals were monitored daily for survival. Data were plotted in an actuarial manner. The $P$ value is shown.

Table 2. Donor Cell Engraftment of BMT Recipients of rmu GM-CSF–Incubated Marrow Grafts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. H-2 Typed</th>
<th>Donor Cells* (%)</th>
<th>Host Cells* (%)</th>
<th>Engraftment† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>27 ± 12</td>
<td>74 ± 12</td>
<td>27</td>
</tr>
<tr>
<td>rmu GM-CSF</td>
<td>9</td>
<td>45 ± 14</td>
<td>54 ± 16</td>
<td>56</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>19 ± 6§</td>
<td>79 ± 6‖</td>
<td>30</td>
</tr>
<tr>
<td>rmu GM-CSF</td>
<td>19</td>
<td>46 ± 9</td>
<td>48 ± 8</td>
<td>58</td>
</tr>
<tr>
<td>Pooled data</td>
<td>Control</td>
<td>31</td>
<td>23 ± 6§</td>
<td>76 ± 6‖</td>
</tr>
<tr>
<td>rmu GM-CSF</td>
<td>28</td>
<td>44 ± 7</td>
<td>48 ± 6</td>
<td>57</td>
</tr>
</tbody>
</table>

Low degree of engraftment in controls‡

Experiment 3
Control 9 55 ± 15 43 ± 13 56
rmu GM-CSF 9 73 ± 8 16 ± 6 89
Experiment 4
Control 13 54 ± 9 44 ± 10 69
rmu GM-CSF 8 54 ± 15 44 ± 15 62
Experiment 5
Control 12 65 ± 12 38 ± 12 75
rmu GM-CSF 14 56 ± 10 35 ± 10 71
Pooled data
Control 34 60 ± 8 41 ± 8 71
rmu GM-CSF 31 55 ± 5 38 ± 8 78

High degree of engraftment in controls

Experiment 3
Control 9 55 ± 15 43 ± 13 56
rmu GM-CSF 9 73 ± 8 16 ± 6 89
Experiment 4
Control 13 54 ± 9 44 ± 10 69
rmu GM-CSF 8 54 ± 15 44 ± 15 62
Experiment 5
Control 12 65 ± 12 38 ± 12 75
rmu GM-CSF 14 56 ± 10 35 ± 10 71
Pooled data
Control 34 60 ± 8 41 ± 8 71
rmu GM-CSF 31 55 ± 5 38 ± 8 78

Combined high and low engraftment in controls

Control 65 39 ± 5 60 ± 5§ 49#
rmu GM-CSF 59 49 ± 4 44 ± 5 66

*Values represented are expressed as means ± 1 SEM.
†Percent engraftment is the percentage of recipients that were noted to have >30% donor cells with ≤70% host cells by H-2 typing.
‡Thirty percent or less of control recipients showed engraftment.
§$P < .05.$
‖$P < .01.$
#>55% of control recipients showed engraftment.

survived the 100-day observation period as compared with 75% of recipients of ex vivo, unwashed, rmu GM-CSF–treated grafts (Fig 2). These differences were not significant when the data were analyzed either in toto or in individual experiments in which rmu GM-CSF had a more profound effect on engraftment rates.

GVHD generation. In the experiments described earlier, donor marrow was administered to histoincompatible recipients as a means of monitoring engraftment. Because the marrow was depleted of T cells, the propensity for these recipients to experience GVHD was significantly reduced. Neither recipients of control marrow grafts nor marrow

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were monitored daily for survival. Data were plotted in an actuarial
infused into lethally irradiated causing splenocytes were incubated with 1
of GVHD. BALB/c marrow cells and GVHD-
were noted to have clinical evidence of GVHD and a 100-day survival rate of
petence of the donor graft is conceivably altered by rmu GM-CSF incubation to establish a baseline of
Differences in actuarial survival rates in the two groups were
Fig 3. The effect of rmu GM-CSF treatment of donor marrow on the development of GVHD. BALB/c marrow cells and GVHD-
engraftment. T cells are not only immunologic effectors but
preventing severe GVHD against the detriment of poor
engraftment. The total number of cells obtained from femurs of control and experimental mice were equivalent, which indicated that the total marrow day 12 CFU-S capacity was similar in each group.

NK assay. As shown in Fig 4, rmu GM-CSF at any of the four concentrations tested did not affect the effector phase of NK cell–mediated YAC-1 lysis.

DISCUSSION
BMT clinicians using T cell depletion for GVHD prophylaxis have recently been forced to weigh the benefit of preventing severe GVHD against the detriment of poor engraftment. T cells are not only immunologic effectors but also play a key role in lymphohematopoiesis, presumably because they are a rich source of hematopoietic growth

Table 3. Hematologic Recovery of BMT Recipients of Nontreated or rmu GM-CSF–Treated Donor Marrow Grafts

<table>
<thead>
<tr>
<th>Day (n=32)</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (× 10⁹/μL)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>3.4 ± 0.4</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>63.8 ± 2.2</td>
<td>78.4 ± 14.3</td>
<td>70.4 ± 6.7</td>
<td>61.7 ± 2.3</td>
<td>45.8 ± 2.6</td>
<td>64.7 ± 15.0</td>
</tr>
<tr>
<td>Bands (%)</td>
<td>0.3 ± 0.11</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0 ± 0</td>
<td>0.1 ± 0.1</td>
<td>2.7 ± 1.4</td>
<td>1.2 ± 0.2</td>
<td>2.9 ± 0.5</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 1.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>35.3 ± 2.2</td>
<td>33.6 ± 2.1</td>
<td>34.4 ± 2.5</td>
<td>37.3 ± 2.3</td>
<td>53.6 ± 2.6</td>
<td>49.1 ± 2.6</td>
</tr>
<tr>
<td>ANC (× 10⁹/μL)</td>
<td>0.5 ± 0</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>ALC (× 10⁹/μL)</td>
<td>0.2 ± 0</td>
<td>0.3 ± 0</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Hct</td>
<td>32.5 ± 1.7</td>
<td>33.6 ± 1.6</td>
<td>31.5 ± 1.3</td>
<td>30.0 ± 1.5</td>
<td>26.6 ± 2.1</td>
<td>27.7 ± 2.0</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>5.3 ± 0.4</td>
<td>6.3 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>2.1 ± 0.4</td>
</tr>
</tbody>
</table>

Abbreviations: ANC, absolute neutrophil count; ALC, absolute lymphocyte count.
*Treated with rmu GM-CSF, 13,000 ng/mL, and incubated for 60 minutes at 37°C; unwashed marrow is infused.
†P < .05.
Twelve days later the mice were killed, and CFU-S formation was assessed.

GM-CSF FACILITATED ENGRAFTMENT PROMOTION were established model of murine T-depleted BMT across major histocompatibility barriers. Because one of the more critical issues in allogeneic BMT is posttransplant donor cell repopulation, we have examined the effect of rmu GM-CSF in an established model of murine T-depleted BMT across major histocompatibility barriers. Our results are the first data on the use of rmu GM-CSF in an allogeneic BMT system in which both donor cell engraftment and hematologic recovery are carefully analyzed.

Factors such as GM-CSF, multi-CSF, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and multi-CSF. Thus, removal of T cells may have a negative effect on posttransplant recovery. Because one of the critical issues in allogeneic BMT is posttransplant donor cell repopulation, we have examined the effect of rmu GM-CSF in an established model of murine T-depleted BMT across major histocompatibility barriers. Our results are the first data on the use of rmu GM-CSF in an allogeneic BMT system in which both donor cell engraftment and hematologic recovery are carefully analyzed.

Thus far, investigators have tested the hematopoietic effects of recombinant proteins in vivo by almost exclusively using normal or neutropenic animals or in the context of syngeneic BMT. The primary goal of these studies was to determine the effects of recombinant products on the number and morphology of the circulating cell population and bone marrow-derived elements. Relatively large quantities of the aforementioned growth factors administered in vivo (usually in repeated bolus injections or by continuous infusion) increased the numbers of leukocytes and/or bone marrow cellularity. The goal of the present study was to evaluate the effect of ex vivo treatment of marrow with rmu GM-CSF on the subsequent potential of that marrow to engraft and to influence hematologic recovery in the host in an allogeneic environment.

Characteristics of our murine model that are potentially relevant to BMT clinicians involved in T cell depletion are as follows: (a) our 25% to 75% engraftment rate is within the range reported for human BMT recipients of T cell-depleted, histoincompatible marrow grafts; (b) in the absence of T cell depletion of donor marrow, complete donor cell engraftment uniformly occurs; (c) similar to human recipients of T cell-depleted marrow, murine recipients that reject T cell-depleted donor grafts may experience either autologous recovery of host marrow or may develop lethal marrow aplasia; and (d) the observed variation in clinical marrow transplantation and are possibly a consequence of environmental factors.

Because marrow cell populations respond to rmu GM-CSF over a wide range of concentrations, rmu GM-CSF titration in the allogeneic model was performed over five orders of magnitude beginning at concentrations below 1% of the amount required for receptor saturation and extending to concentrations in excess of receptor saturation. We evaluated a total of 156 mice that received T cell-depleted control or rmu GM-CSF–incubated (at a concentration of 13,000 ng/mL) donor marrow. Recipients were subsequently typed to determine engraftment status. In experiments 1 and 2 in which the controls had low engraftment rates, a more pronounced and statistically significant effect of rmu GM-CSF treatment was measured. Recombinant murine GM-CSF significantly increased the mean percentage of donor cells, decreased the mean percentage of host cells, and increased the overall degree of engraftment. This did not occur in experiments 3, 4, and 5 in which the controls had a high degree of engraftment.

Table 4. Secondary Day 12 CFU-S Formation in Recipients of Nontreated or rmu GM-CSF–Treated Donor Grafts

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells Infused</th>
<th>Colony No. (± 1 SD)</th>
<th>No. Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls†</td>
<td>0</td>
<td>0.3 ± 0.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3 x 10⁴</td>
<td>8.7 ± 1.2</td>
<td>3</td>
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<tr>
<td></td>
<td>6 x 10⁴</td>
<td>16.3 ± 5.5</td>
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<tr>
<td>Low degree of engraftment in controls*</td>
<td></td>
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<td></td>
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<tr>
<td>Experiment 2 †</td>
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<tr>
<td>Nontreated</td>
<td>6 x 10⁴</td>
<td>8.8 ± 4.7</td>
<td>6</td>
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<tr>
<td>rmu GM-CSF †</td>
<td>6 x 10⁴</td>
<td>9.3 ± 2.3</td>
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<tr>
<td>High degree of engraftment in controls*</td>
<td></td>
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<tr>
<td>Experiment 4 †</td>
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<tr>
<td>rmu GM-CSF</td>
<td>6 x 10⁴</td>
<td>10.2 ± 3.4</td>
<td>9</td>
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</table>

*Low engraftment, ≥30% incidence of engraftment in the controls; high engraftment, ≥45% incidence of engraftment in the controls.
†Donors consisted of nontransplanted controls or engrafted mice that had or had not received rmu GM-CSF–incubated bone marrow. Engrafted mice were killed 100 days post-BMT to obtain bone marrow. A quantity of 0, 3 x 10⁴, or 6 x 10⁴ marrow cells was infused into irradiated BALB/c mice. Twelve days later the mice were killed, and CFU-S formation was assessed.
‡rmu GM-CSF: 13,000 ng/mL, incubated for 60 minutes at 37°C; unwashed marrow was infused.

Fig 4. The effect of rmu GM-CSF on NK-enriched splenocytes. Enriched splenocytes were mixed with rmu GM-CSF, incubated for four to six hours with ⁶⁷⁷Cr-labeled YAC-1 target cells at various E:T ratios and measured for cell-mediated cytolyis. Cytolytic curves are from a representative experiment. The presence of rmu GM-CSF during the effector phase of NK activity did not alter the efficacy of cytolyis.
ment in controls and variable efficacy of rmu GM-CSF could be due to numerous factors (eg, environmental factors, progenitor cell numbers, immunocompetence of the donor graft and/or the host, variable efficacy of ex vivo T cell depletion) influencing the engraftment status of recipients in this BMT model. Moreover, this treatment strategy was only partially effective in improving engraftment in mice that had a low propensity for engraftment, thus indicating that rmu GM-CSF probably influenced only some of the variables that affect engraftment.

The high survival rates in this system were not affected by rmu GM-CSF treatment. The reproducibility of this finding in humans is particularly important because rejection of histoincompatible T cell-depleted marrow frequently results in fatal aplasia. The relevance of our model to human transplantation, especially in situations where higher doses of TBI are given, cannot, however, be predicted.

Because GM-CSF affects progenitor cell proliferation and differentiation, we initially speculated that recipients of rmu GM-CSF-incubated donor grafts may have experienced improved hematologic recovery independent of engraftment promotion. The hematologic effects in our allogeneic model, however, were not pronounced. Total circulating leukocyte numbers and absolute neutrophil numbers were only slightly higher in recipients of rmu GM-CSF–treated grafts on days 7 and 14. No change was noted in the proportion of cells of neutrophil, eosinophils, monocyte, or lymphocyte origin. Reticulocyte numbers and hemoglobin values were not altered despite the known burst-promoting effect of GM-CSF. Moreover, no differences in the hematologic data from controls and experimental mice were noted even when analysis was restricted to experiments in which rmu GM-CSF facilitated engraftment. Correlation coefficients (data not shown) of engraftment parameters relative to hematologic parameters were low, which indicated that the engraftment-promoting effects are distinguishable from the hematologic effects.

Because rmu GM-CSF is active in progenitor cell differentiation, we were concerned that rmu GM-CSF treatment of donor grafts may lead to eventual terminal differentiation of progenitor cells and potential, gradually evolving marrow aplasia. As one indicator of marrow stem cell capacity, we quantitated day 12 CFU-S formation in marrow obtained 100 days post-BMT from controls or recipients of rmu GM-CSF–treated marrow grafts. No effect of rmu GM-CSF on day 12 CFU-S formation was evident, even under conditions in which rmu GM-CSF had pronounced engraftment-promoting properties.

Notably, the improvement in engraftment as a consequence of rmu GM-CSF incubation of donor grafts was apparent only at a concentration of rmu GM-CSF that was two to three orders of magnitude greater than that required for murine bone marrow receptor saturation. Thus, we speculated that engraftment promotion may be the result of the intravenous infusion of a single bolus of rmu GM-CSF along with the donor marrow. In an experiment to test this possibility, we injected (a) rmu GM-CSF–incubated marrow that had been washed free of rmu GM-CSF before injection, (b) rmu GM-CSF–incubated, unwashed bone marrow, or (c) in vivo infusion of a single dose of rmu GM-CSF in an amount equivalent to the dose infused in the rmu GM-CSF–incubated, unwashed marrow inoculum. All three treatments were equally effective in promoting engraftment.

To test the possibility that rmu GM-CSF facilitated a donor anti-host reaction (assessed by the severity of the GVHD process), donor bone marrow and splenocytes (as a source of T cells) were pretreated with rmu GM-CSF before BMT. In this setting of GVHD-induced mortality, incubation of donor cells with growth factor slightly decreased the median survival time and the actuarial survival rates as compared with controls. Differences between treated and untreated groups were not, however, statistically significant.

Because in vivo injection of a single dose of rmu GM-CSF improved engraftment, we investigated the possibility that rmu GM-CSF inhibited a well-described process of host NK cell–mediated allograft rejection. At rmu GM-CSF doses that augmented engraftment in our system, no inhibition of NK cell effector function could be demonstrated in vitro. We believe that rmu GM-CSF probably does not directly inhibit the effector phase of NK-mediated allograft rejection, but this does not rule out an indirect mechanism.

In summary, brief ex vivo incubation of donor T cell-depleted, histoincompatible bone marrow grafts with rmu GM-CSF improved engraftment. The high survival rates in this system were not affected by rmu GM-CSF treatment. The reproducibility of this finding in humans is particularly important because rejection of histoincompatible T cell-depleted marrow frequently results in fatal aplasia. The relevance of our model to human transplantation, especially in situations where higher doses of TBI are given, cannot, however, be predicted.

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