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 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 rmu 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 post-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.

*1988 by Grune & Stratton, Inc.*
rmu GM-CSF. rmu GM-CSF was purified by high-performance liquid chromatography from the supernatant from yeast that was transformed with a plasmid capable of directing the expression of a murine cDNA for rmu GM-CSF. The final 21-kd glycoprotein product was free of detectable endotoxin (<10 pg/10 µg rmu GM-CSF). The specific activity of rmu GM-CSF was approximately 10⁸ U/mg of protein in colony-forming assays (1 unit = 1 colony-forming unit—granulocyte/macrophage CFU-GM/10⁵ cells plated). 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 x 10⁶ 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 of GVHD, 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 with 13,000 ng/mL rmu GM-CSF (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 x 10⁶ non-T cell-depleted BALB/c donor marrow cells were mixed with 2.5, 5, or 15 x 10⁶ BALB/c splenocytes. 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. 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.

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 methyl 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 x 10⁶ 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 cytolysis was performed as previously described. 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 to 12 x 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 splenocytes (in RPMI 1640/5% FBS) onto a discontinuous Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient as described by Santoni et al with modifications. 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 x 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 alkyltrimethyl ammonium bromide, Sigma Chemical Co., St Louis). The percent cytotoxicity was calculated as follows:

\[
\text{percent specific lysis} = \frac{\text{cpm experimental release} - \text{cpm spontaneous release}}{\text{cpm maximal release} - \text{cpm spontaneous release}} \times 100
\]

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.

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 proliferation in vitro. BALB/c or C57BL/6 bone marrow cells were occurred at 50 to 100 pg/mL. The proliferative response of engraftment, rmu GM-CSF was incubated with T cell-on engrafiment. To determine the effect of GM-CSF on CSF-responsive cell line FDC-P1 was also measured. The GM-CSF.

ng/mL, neither the mean percentage of donor cells nor the host cells 2 months post-BMT. At I .3, 1 3, 1 30, and 1,300 was determined by measuring the percent donor and percent host cells 2 months post-BMT. At 1.3, 13, 130, and 1,300 ng/mL, neither the mean percentage of donor cells nor the percentage of animals that showed engraftment were altered in comparison to 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 transplants 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

![Fig 1](image)

Fig 1. The effect of rmu GM-CSF on bone marrow--induced proliferation in vitro. BALB/c or C57BL/6 bone marrow cells were mixed with rmu GM-CSF, incubated for 24 hours, and then assayed for tritiated thymidine incorporation. Dose-response curves are from two experiments. As shown, bone marrow cells demonstrated a peak proliferative response at 0.1 to 1 μg/mL rmu GM-CSF.
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

![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.](image-url)
infused into lethally irradiated and causing BLAZAR ET

source of T cells) were added to nondepleted bone marrow GM-CSF exposure. To test this possibility, splenocytes (a cyte and macrophage immune functions,28 the immunocompetence of the donor graft is conceivably altered by rmu GM-CSF incubation to establish a baseline of evidence of GVHD. Because rmu GM-CSF augments mono-

cytosis of circulating leukocytes, reticulocyte percentage, or Hct values were observed in recipients of rmu GM-CSF–treated, unwashed marrow as compared with recipients of untreated marrow. No significant differences in leukocyte or erythroid recovery were noted in controls v experimental mice when either the cumulative data, data from individual experiments, or pooled data from experiments in which rmu GM-CSF had a pronounced effect on engraftment were further analyzed.

Figure 3. The effect of rmu GM-CSF treatment of donor marrow on the development of GVHD. BALB/c marrow cells and GVHD-causing splenocytes were 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.

incubated with rmu GM-CSF were noted to have clinical evidence of GVHD. Because rmu GM-CSF augments monocyte and macrophage immune functions,28 the immunocompetence of the donor graft is conceivably altered by rmu GM-CSF exposure. To test this possibility, splenocytes (a source of T cells) were added to nondepleted bone marrow before rmu GM-CSF incubation to establish a baseline of GVHD in controls (Fig 3). Recipients of control grafts had clinical evidence of GVHD and a 100-day survival rate of 55%. Recipients of rmu GM-CSF–treated grafts had clinical evidence of GVHD and a 100-day survival rate of 48%. Differences in actuarial survival rates in the two groups were not statistically significant.

Hematologic recovery. Hematologic data are summarized in Table 3. No significant differences in the rate of total leukocyte recovery or final levels of leukocytes, morphology of circulating leukocytes, reticulocyte percentage, or Hct differences in actuarial survival rates in the two groups were not statistically significant.

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

<table>
<thead>
<tr>
<th></th>
<th>Day 7 Post-BMT</th>
<th>Day 14 Post-BMT</th>
<th>Day 28 Post-BMT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated*</td>
<td>Control</td>
</tr>
<tr>
<td>Leukocytes (x 10⁹/L)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>63.8 ± 2.2</td>
<td>78.4 ± 14.3</td>
<td>70.4 ± 6.7</td>
</tr>
<tr>
<td>Bands (%)</td>
<td>0.3 ± 0.10</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0 ± 0</td>
<td>0.1 ± 0.1</td>
<td>2.7 ± 1.4</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>35.3 ± 2.2</td>
<td>33.6 ± 2.1</td>
<td>34.4 ± 2.5</td>
</tr>
<tr>
<td>ANC (x 10⁹/L)</td>
<td>0.5 ± 0</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>ALC (x 10⁹/L)</td>
<td>0.2 ± 0</td>
<td>0.3 ± 0</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Hct</td>
<td>32.5 ± 1.7</td>
<td>33.6 ± 1.6</td>
<td>31.5 ± 1.3</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>5.3 ± 0.4</td>
<td>6.3 ± 0.4</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>Engraftment</td>
<td>Donor (%)</td>
<td>39 ± 5</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>Host (%)</td>
<td>60 ± 5</td>
<td>44 ± 5</td>
<td>38 ± 0.5†</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.

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...
Twelve days later the mice were killed, and CFU-S formation was assessed.

**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. (x ± 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>
</tr>
<tr>
<td></td>
<td>6 x 10⁴</td>
<td>16.3 ± 5.5</td>
<td>3</td>
</tr>
<tr>
<td>Low degree of engraftment in controls*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2 †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>6 x 10⁴</td>
<td>8.8 ± 4.7</td>
<td>6</td>
</tr>
<tr>
<td>rmu GM-CSF ‡</td>
<td>6 x 10⁴</td>
<td>9.3 ± 2.3</td>
<td>8</td>
</tr>
<tr>
<td>High degree of engraftment in controls*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4 †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>6 x 10⁴</td>
<td>11.1 ± 4.7</td>
<td>8</td>
</tr>
<tr>
<td>rmu GM-CSF</td>
<td>6 x 10⁴</td>
<td>10.2 ± 3.4</td>
<td>9</td>
</tr>
</tbody>
</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.

Factors such as GM-CSF, 29 multi-CSF, 30-33 and granulocyte-CSF. 34 Thus, removal of T cells may have a negative effect on posttransplant recovery. 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.

Thus far, investigators have tested the hematopoietic effects of recombinant proteins in vivo by almost exclusively using normal 29,35-38 or neutropenic animals 37,39 or in the context of syngeneic BMT. 15-17,40,41 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 42; (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 variations between experiments in engraftment rates among controls reflect a similar variation in clinical marrow transplantation and are possibly a consequence of environmental factors.

Because marrow cell populations responded 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. The variable degree of engraft-
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, eosinophil, 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. In our opinion, a limited peri-BMT (eg, day 0 of BMT) exposure of donor marrow to rmu GM-CSF ex vivo or in vivo can facilitate 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 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 followed by infusion of the unwashed inoculum was noted to promote engraftment post-BMT without increasing the incidence of GVHD and without adversely affecting the progenitor cell capacity in the recipient. The mechanism remains unclear, although CFU-S, NK cells, and GVHD-causing T cells do not seem directly involved. Hematologic recovery was slightly but not significantly altered by ex vivo rmu GM-CSF treatment. Perhaps the graft-promoting effect may be improved through future studies combining rmu GM-CSF with other growth factors in vivo. Recombinant murine GM-CSF engraftment promotion may in part obviate the need for substantial increases in host cytotherapeutic therapy, which is currently approaching the limits of tolerability.

ACKNOWLEDGMENT

The authors acknowledge L. Pettit and C. Fox for technical assistance, Dr. Philip Morrissey for scientific discussions and M.J. Hildreth for excellent editorial assistance.

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