Effect of Recombinant Human Macrophage Colony-Stimulating Factor in Irradiated Murine Recipients of T-Cell–Depleted Allogeneic or Non-Depleted Syngeneic Bone Marrow Transplants

By Bruce R. Blazar, S. Lea Aukerman, and Daniel A. Vallera

Recombinant macrophage colony-stimulating factor (rM-CSF), which reacts exclusively with cells of monocyte lineage, was evaluated in the murine bone marrow (BM) transplant setting for in vivo effects on recipient survival, hematologic recovery, and engraftment. Two types of fully allogeneic donors were selected based on the expression (BALB/c), or lack of expression (DBA/2J), or lack of expression (DBA/1J) antigens. These antigens are established targets for monocyte and/or natural killer (NK) cell-mediated graft rejection. Irradiated C57BL/6 mice were used as recipients for all experiments. Recipients of T-cell–depleted (TCD) BALB/c BM and a 14-day continuous subcutaneous infusion of 16.8 μg/d rM-CSF (n = 30) showed a significant decrease in donor cell engraftment as compared with recipients of donor BM administered pumps delivering saline. These mice administered rM-CSF also displayed significantly reduced levels of circulating leukocytes (predominantly lymphocytes) on day 14 posttransplant (compared with saline controls). Neither engraftment effects nor leukocyte effects were observed when C57BL/6 recipients were administered H-2 nonexpressing C57BL/6 BM cells (n = 30), suggesting that the monocyte/macrophage population is important in long-term alloengraftment in certain donor-recipient strain combinations in which donor H-2 antigens can serve as target antigens for host effector cells, but are not important in strain combinations in which they are not recognized. Circulating tumor necrosis factor α (TNFα) levels measured at two time periods during rM-CSF infusion were not elevated. Thus, the reduction in alloengraftment is not likely to be directly related to TNFα. However, in vivo elimination of NK cells in the BALB/c into C57BL/6 model prevented the impairment of engraftment mediated by rM-CSF. Thus, rM-CSF–mediated inhibition of alloengraftment is contingent on the presence of host NK cells with antiderivative reactivity. Survival was unaffected when rM-CSF was administered in either allogeneic BM transplant model, but was significantly reduced when rM-CSF was administered to C57BL/6 recipients of syngeneic BM transplants. These data are the first analyzing the effects of rM-CSF in murine allogeneic BM transplantation and extend our previous studies using the BALB/c into C57BL/6 model in which in vivo infusions of recombinant granulocyte-macrophage CSF, but not recombinant granulocyte-CSF, lead to decreases in alloengraftment. These data show that rM-CSF–induced stimulation of monocytes may increase BM graft rejection in instances in which NK cells are involved in the rejection process. These data may have future clinical implications for the use of rM-CSF in allogeneic BM transplantation.

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allogeneic donor marrow in which BM progenitor cells express Hh1 antigens that serve as a target for natural killer (NK) cell-mediated and monocyte-facilitated rejection.\textsuperscript{10,11} The present study was constructed to determine if monocytes are important in long-term reconstitution studies in donor-recipient strain combinations in which these antigens are or are not expressed and, if so, to provide insight as to the mechanisms involved in these processes. These data should provide important information relative to the in vivo effects of rM-CSF in human allogeneic BMT recipients being treated with rM-CSF as an anti-infectious adjuvant.

**MATERIALS AND METHODS**

**BMT procedure.** BALB/c (H-2\textsuperscript{b}) donors were purchased from Charles River (Wilmington, MA) and C57BL/6 (H-2\textsuperscript{d}) recipients were purchased from Jackson Laboratory (Bar Harbor, ME). DBA/1 (H-2\textsuperscript{b}) donors were purchased from the University of Connecticut (Farmington, CT). Animals were housed in conventional cages with filter lids and fed a fat-supplemented diet and antibiotic-supplemented water for 2 days pre-BMT and for 30 days post-BMT. Donors (age, 5 to 8 weeks) and recipients (10 to 12 weeks) were female mice. Total body irradiation (TBI) was administered from a Philips RT 250 Orthovoltage Therapy Unit (Philips Medical Systems, Hamburg, Germany) filtered 0.35 mm Cu.\textsuperscript{23,24} The final absorbed dose rate was 0.41 Gy/min at 225 kV and 17 mA to a total dose of 6.5 Gy/mouse for recipients of DBA/1 marrow, 7.0 Gy/mouse for recipients of BALB/c marrow, and 9.0 Gy/mouse for nonmanipulated syngeneic BM. Doses of irradiation chosen for the various strain combinations were based on our previous\textsuperscript{14} and recent studies\textsuperscript{25} in which TBI dose and BM titration experiments were performed in the DBA/1 into C57BL/6 system to establish a new model of mixed chimerism with hematopoietic reconstitution.\textsuperscript{15} To establish this system, a total of 145 mice in three experiments were transplanted with varying doses of TBI (6.0 to 7.5 Gy) and BM cell doses (2.5 \texttimes 10\textsuperscript{6} to 20 \texttimes 10\textsuperscript{6}). Survival, hematopoietic recovery, and aloenograftation were monitored. Irradiated (6.5 Gy TBI) C57BL/6 recipients of 10 \texttimes 10\textsuperscript{6} TCD DBA/1 BM had mixed chimerism, a 90\% 100-day actuarial survival rate, and evidence of hematopoietic recovery by day 14 post-BMT. Our transplant methodology has been described in detail.\textsuperscript{12,13} Briefly, donor marrow was collected into RPMI 1640 medium by flushing it from the shafts of femurs and tibias. The cells were resuspended, and clumps of debris allowed to settle out. Ex vivo T-cell depletion of 18 \texttimes 10\textsuperscript{6} BALB/c marrow cells/recipient or 10 \texttimes 10\textsuperscript{6} DBA/1 marrow cells/recipient was performed using anti-Thy 1.2 monoclonal antibody (MoAb) (hybridoma 30-H12, rat IgG2b; American Tissue Culture Collection, Rockville, MD) plus complement, as previously described.\textsuperscript{21,22} The BM cell doses were chosen on our prior experience.\textsuperscript{14,15} For syngenic transplants, 10\% nondepleted marrow cells were infused into each recipient. After treatment, all aliquots were washed, resuspended, and adjusted for caudal vein injection. For natural killer (NK) cell depletion in vivo, 400 \mu\textsubscript{g} of purified anti-NK1.1 antibody (hybridoma PK1.36, mouse IgG2a)\textsuperscript{23} was injected on day 1, as previously reported.\textsuperscript{17,18}

**Long-term engraftment as measured by flow cytometry.** Chimerism of peripheral blood mononuclear cells (PBMC) was analyzed at 6 to 9 weeks post-BMT. Engraftment was quantitated by analyzing the cell surface binding of anti-H-2\textsuperscript{MoAb} linked to fluorochromes on a FACScan (Becton Dickinson, Mountain View, CA). For quantitation of donor cell engraftment, anti-H-2\textsuperscript{b} (hybridoma 34-5-8, mouse IgG2a; provided by Dr. David Sachs, National Institutes of Health, Bethesda, MD)\textsuperscript{19} or anti-H-2\textsuperscript{d} (hybridoma 66-3-5, mouse IgG2a; provided by Dr. Sachs)\textsuperscript{20} were directly conjugated to fluorescein isothiocyanate (FITC). For host cell quantitation, anti-H-2\textsuperscript{a} (hybridoma EH144, mouse IgG2a; provided by Dr. T. V. Rajan, Albert Einstein University, New York, NY) was directly conjugated to phycoerythrin (PE). All samples were first incubated with 2.4G2 for 15 to 30 minutes at 4°C to block Fc receptor binding.\textsuperscript{21} The PBMC were then incubated with an optimal concentration of fluorochrome-labeled MoAb for 45 minutes at 4°C. Cells were washed three times and resuspended for analysis. An antihuman CD7 (hybridoma 3A1E, mouse IgG2a; provided by Dr. Barton Haynes, Duke University, Durham, NC)\textsuperscript{22} was conjugated to FITC or PE to determine the degree of background binding. Flow cytometry was performed on a FACScan (Becton Dickinson). Ten thousand cells (determined by forward light scatter) were analyzed. Background binding was subtracted and the percentage of donor or host cells were calculated as described.\textsuperscript{23} Zero to 1\% of the control PBMC stained positive with the irrelevant anti-H-2 MoAb linked to fluorescence, while 99\% to 100\% of cells were positive for the relevant anti-H-2-specific MoAb linked to fluorescence.

**rM-CSF.** rM-CSF is a highly purified recombinant human protein produced in Escherichia coli\textsuperscript{24} by Cetus Corporation (Emeryville, CA). The E coli-produced rM-CSF is nonglycosylated. The specific activity is 1 \times 10\textsuperscript{7} U/mg. One unit is defined as being equal to the amount of protein that stimulates the generation of one colony in mouse BM culture.

**In vivo administration of rM-CSF.** For continuous subcutaneous delivery of rM-CSF, a 14-day mini-osmotic pump (ALZA Corporation, Palo Alto, CA) was implanted (under general anesthesia) in control and experimental mice on the day of BM transfer as previously described.\textsuperscript{45} No other schedules were tested. Two hundred-microliter volumes containing 235 \mu\textsubscript{g} rM-CSF were injected into the pump and 16.8 \mu\textsubscript{g} was delivered per day (0.5 \mu\textsubscript{L}/h). Control pumps were injected with phosphate-buffered saline (PBS). Fifteen mice per group were transplanted in each experiment.

**Hematologic evaluation of recipients post-BMT.** Fifty microliters of PB was obtained by retro-orbital venipuncture on days 7, 14, and 28 post-BMT. Leukocyte number and morphology were determined by examination of Wright-Giemsa-stained slides.\textsuperscript{45} Hematocrit (Hct) values were determined by capillary tube red blood cell plasma volume ratios after centrifugation. Due to platelet clumping, accurate platelet counts were not possible.

**Murine TNFa levels.** Sera was collected and pooled on days 6 and 12 post-BMT from irradiated (6.5 Gy TBI) C57BL/6 mice administered TCD BALB/c BM and a 14-day continuous subcutaneous infusion of rM-CSF (16.8 \mu\textsubscript{g}/d) or PBS. TNFa (R & D Systems, Minneapolis, MN) was analyzed in an enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s specifications, as previously described.\textsuperscript{45} Sera from the experimental group was not diluted. A Bio-Rad (Cambridge, MA) data manager program was used. Values for TNFa were calculated from the linear portion of a dose-response curve. The correlation coefficient of this assay (R\textsuperscript{2}) value was 0.9953.

**Assessment of graft-versus-host disease (GVHD).** Although all allogeneic recipients received TCD marrow and syngeneic recipients would not be expected to acquire GVHD under these BMT conditions, nonetheless, we monitored animals for the occurrence of acute, subacute, and early chronic GVHD. All groups were weighed twice weekly and examined for the presence or absence of ruffled fur, diarrhea, or alopecia, as previously described.\textsuperscript{22,25}

**Statistical analyses.** Groupwise comparisons of continuous data were made by Student’s t-test. Survival data were analyzed by lifetable methods using the Mantel-Peto-Cox summary of \chi\textsuperscript{2}.\textsuperscript{26} Hematologic data were analyzed as individual values.
RESULTS

The effect of 14-day continuous subcutaneous infusions of rM-CSF on hematologic recovery post-BMT. Hematologic data for recipients of TCD allogeneic marrow are shown in Table 1. Data from two independent experiments for each donor strain combination (ie, a total of four experiments) were pooled for analysis. By day 14 post-BMT, C57BL/6 recipients of TCD BALB/c BM and rM-CSF had significantly (P < .05) lower total leucocyte numbers than controls due to decreases in absolute numbers of circulating mononuclear cells. The mononuclear cells were predominantly lymphoid in morphology with a paucity of circulating monocytes (≤0.2 x 10^9/μL in any group at all time periods) (data not shown). By day 28 post-BMT, these differences had resolved. Interestingly, C57BL/6 recipients of TCD DBA/1 BM and r-M-CSF were not observed to have decreases in circulating leukocytes 14 days post-BMT.

In contrast to the allogeneic BM transplanted mice, mice receiving syngeneic BM transplants and rM-CSF had transient decreases in the numbers of circulating leukocytes due to decreases in the absolute numbers of mononuclear cells (similar to recipients of BALB/c marrow) but not in Hct values (Table 2).

A modest decrease in the mean values for Hct for C57BL/6 recipients of either TCD BALB/c BM or TCD DBA/1 BM, and rM-CSF was noted on day 14, but not day 28 post-BMT. No significant differences were present in C57BL/6 recipients of syngeneic BM and rM-CSF (Table 2). The effect of 14-day continuous subcutaneous infusions of rM-CSF on survival post-BMT. The cumulative actuarial survival data for the allogeneic and syngeneic experiments are presented in Figs 1 through 3. First, we compared the actuarial survival of 15 C57BL/6 recipients of TCD BALB/c BM and rM-CSF at a dose of 16.8 μg/d for 14 days to 15 C57BL/6 recipients of TCD BALB/c BM at a higher dose (25.2 μg/d) (data not shown). Mice receiving the higher dose of rM-CSF had a significantly (P < .05) lower actuarial survival than mice receiving the lower dose. Therefore, for all subsequent experiments, we used rM-CSF at a dose of 16.8 μg/d for 14 days. Thirty recipients of TCD BALB/c marrow and rM-CSF had an identical 100-day actuarial survival as compared with 30 control recipients of PBS (82%) (Fig 1).

No significant differences in survival were noted in the 30 recipients of DBA/1 BM and rM-CSF as compared with PBS controls (73% v 50%; P = .27) (Fig 2). However, a decrease in survival was measured in 15 mice administered syngeneic BM transplants and rM-CSF as compared with controls (94% v 66%; P < .05) (Fig 3). There was no evidence of GVHD in any of these recipients. Moreover, weight curves were comparable in rM-CSF-treated and PBS-treated groups at all time periods.

The effect of 14-day continuous subcutaneous infusions of rM-CSF on engraftment. To determine the effect of continuous administration of rM-CSF on donor cell engraftment, cells from the mice in the above allogeneic experiments were H-2 phenotyped (Table 3). In recipients of TCD BALB/c BM (n = 25/group), mean donor cell percentages were significantly (P < .05) higher (59.6 ± 36.0) and host cell percentages significantly (P < .05) lower (37.0 ± 61.0) in PBS controls than in the M-CSF–treated mice, consistent with an rM-CSF–induced reduction in aloengraftment in this strain combination.

Aloengraftment differences were not observed in 26 recipients of DBA/1 marrow grafts administered PBS as compared with the 18 recipients of rM-CSF.

In vivo NK cell depletion prevents the rM-CSF–mediated reduction in aloengraftment in C57BL/6 recipients of TCD BALB/c BM. To determine if the adverse effects of rM-CSF infusion on aloengraftment were mediated by

Table 1. Effect of rM-CSF on the Hematologic Recovery of Recipients of Fully Allogeneic TCD Marrow Grafts

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Studied</th>
<th>WBC (x 10^9/μL)</th>
<th>ANC (x 10^9/μL)</th>
<th>AMNC (x 10^9/μL)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c donors (H1 disparate) Day 14 post-BMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>29</td>
<td>2.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>38.0 ± 1.0</td>
</tr>
<tr>
<td>M-CSF</td>
<td>27</td>
<td>1.3 ± 0.2*</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1*</td>
<td>33.2 ± 0.2*</td>
</tr>
</tbody>
</table>

| Day 28 post-BMT | | | | | |
| PBS | 13 | 5.3 ± 0.7 | 1.4 ± 0.2 | 4.0 ± 0.6 | 37.4 ± 0.3 |
| M-CSF | 12 | 5.9 ± 1.7 | 3.2 ± 1.5 | 2.7 ± 0.6 | 37.0 ± 0.2 |

| DBA/1 donors (H1mm) Day 14 post-BMT | | | | | |
| PBS | 28 | 1.8 ± 0.3 | 1.3 ± 0.3 | 0.5 ± 0.1 | 36.5 ± 0.7 |
| M-CSF | 22 | 2.5 ± 0.9 | 2.0 ± 0.8 | 0.4 ± 0.1 | 35.1 ± 0.1* |

| Day 28 post-BMT | | | | | |
| PBS | 28 | 1.9 ± 0.3 | 1.1 ± 0.2 | 0.7 ± 0.1 | 34.9 ± 1.0 |
| M-CSF | 22 | 1.8 ± 0.4 | 1.2 ± 0.3 | 0.7 ± 0.1 | 35.2 ± 1.4 |

Irradiated (6.5 to 7.0 Gy TBI) C57BL/6 received 18 x 10^6 TCD BALB/c donor marrow or 10 x 10^6 TCD DBA/1 marrow. On the day of transplantation, mice were implanted subcutaneously with pumps that delivered 16.8 μg/d rM-CSF or PBS for 14 days. Peripheral blood smears were examined on days 14 and 28 post-BMT. Data are presented as mean ± 1 standard error of the mean. Abbreviations: WBC, white blood cell count; AMNC, absolute mononuclear cell count; ANC, absolute neutrophil count. *P < .05 (as compared with PBS controls by Student’s t-test).
Table 2. Effect of rM-CSF on the Hematologic Recovery of Recipients of Syngeneic Marrow Grafts

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Studied</th>
<th>WBC (x 10^3/µL)</th>
<th>ANC (x 10^5/µL)</th>
<th>AMNC (x 10^3/µL)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 donors</td>
<td></td>
<td></td>
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<tr>
<td>Day 14 post-BMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>14</td>
<td>1.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>39.3 ± 0.1</td>
</tr>
<tr>
<td>M-CSF</td>
<td>12</td>
<td>0.8 ± 0.1*</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1*</td>
<td>38.6 ± 1.2</td>
</tr>
<tr>
<td>Day 28 post-BMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>14</td>
<td>2.6 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>42.7 ± 0.7</td>
</tr>
<tr>
<td>M-CSF</td>
<td>11</td>
<td>3.4 ± 0.6</td>
<td>0.8 ± 0.1</td>
<td>2.6 ± 0.5</td>
<td>41.8 ± 0.7</td>
</tr>
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</table>

Irradiated (9.0 Gy TBI) C57BL/6 received 10^6 nondepleted syngeneic marrow cells. On the day of transplantation, mice were implanted subcutaneously with pumps that delivered 16.8 µg/d rM-CSF or PBS for 14 days. Peripheral blood smears were examined on days 14 and 28 post-BMT. Data are presented as mean ± 1 standard error of the mean.

Abbreviations: WBC, white blood cell count; AMNC, absolute mononuclear cell count; ANC, absolute neutrophil count.

*P < .05 (as compared with PES controls by Student’s t-test).

monocyte products which stimulated NK cell rejection of BM, we compared the alloengraftment status of murine recipients of PBS or rM-CSF that did or did not receive anti-NK1.1 antibody to deplete NK cells. Fifteen mice per group were transplanted and all surviving recipients (n = 10 to 15 per group) were H-2 phenotyped 47 days post-BMT. Murine recipients of anti-NK1.1 (day –1), when compared with mice administered PBS, had a significantly (P < .001) increased mean percentage of donor cells (83 ± 4 vs 31 ± 12) and corresponding lower mean host cell percentage (12 ± 4 vs 62 ± 12). Recipients administered anti-NK1.1 and then a 14-day infusion of rM-CSF (16.8 µg/d) beginning on day 0 of BMT had similar alloengraftment (mean donor cell percentage, 78 ± 5; mean host cell percentage, 16 ± 5) compared with the anti-NK cell-treated control group, indicating that prior NK cell depletion prevented the detrimental effect of rM-CSF on alloengraftment of TCD BALB/c BM in irradiated C57BL/6 recipients. To determine if rM-CSF infusions induced high circulating levels of TNFα with the potential of stimulating NK cell-mediated rejection of TCD BALB/c BM, we quantitated TNFα levels in five mice per group that received rM-CSF or PBS. No circulating levels of TNFα (sensitivity, ≤4 pg/mL) was detectable on days 6 or 12 post-BMT in either group.

**DISCUSSION**

The effects of 14-day continuous subcutaneous administration of rM-CSF was investigated in recipients of TCD major histocompatibility disparate or nondepleted syngeneic donor grafts to determine the potential direct or indirect role of monocytes in engraftment. We have been particularly interested in the function of monocytes post-BMT because (1) in short-term 5 to 7 day [Iabeled BMT, monocyte elimination with or carageenan resulted in higher splenic-derived BM proliferation indices. This suggested a direct or indirect inhibitory role of monocytes on engraftment in certain donor-recipient strain combinations (eg, Hhl homozygous expressing donors transplanted into F1 recipients) but not in others (eg, Hhl donors). (2) Long-term alloengraftment studies in our laboratory have indicated that the infusion of rGM-CSF, which stimulates monocytes and granulocytes and their

![Fig 1. Survival of irradiated C57BL/6 mice receiving continuous subcutaneous infusions of rM-CSF (16.8 µg/d) immediately after transplantation of TCD BALB/c BM cells. Animals were monitored for survival, engraftment (Table 3), and hematologic recovery (Table 1). Survival data were plotted in an actuarial manner.](image1)

![Fig 2. Survival of irradiated C57BL/6 mice receiving continuous subcutaneous infusions of rM-CSF (16.8 µg/d) immediately after transplantation of TCD DBA/1 BM cells. Animals were monitored for survival, engraftment (Table 3), and hematologic recovery (Table 1). Survival data were plotted in an actuarial manner.](image2)
transplantation with syngeneic C57BL/6 BM cells. Animals were
monitored for survival and hematologic recovery (Table 2). Survival
data were plotted in an actuarial manner. The

Fig 3. Survival of irradiated C57BL/6 mice receiving continuous
subcutaneous infusions of rM-CSF (16.8 pg/d) immediately after
transplantation with syngeneic C57BL/6 BM cells. Animals were
monitored for survival and hematologic recovery (Table 2). Survival
data were plotted in an actuarial manner. The

precursors, but not rG-CSF, which stimulates only granulo-
cytes and their precursors but not monocytes, was associ-
ated with a higher incidence of mixed chimerism and/or
autologous recovery. Because rGM-CSF administration
also lead to an increased 100-day actuarial survival, it is
possible that rGM-CSF infusion stimulated residual radiore-
sistant host cells to rescue mice, which, without treatment,
would have died post-BMT. Studies with rM-CSF would
provide additional information on these issues.

Irradiated C57BL/6 recipients of TCD BALB/c, but not
DBA/1 marrow grafts, did have a significantly reduced level
of donor cell chimerism 6 to 9 weeks post-BMT compared
with controls. These findings were similar to those in
irradiated C57BL/6 recipients of TCD BALB/c marrow
grafts receiving rGM-CSF administered by continuous
subcutaneous infusion. Because survival in recipients of
BALB/c marrow grafts and rM-CSF was not superior to
PBS-treated controls, these data support the hypothesis
that rGM-CSF administration stimulated homozgyous ex-
pressing Hhl", H-2" host monocytes and/or resulted in the
release of monocyte products to stimulated host NK cells
that are involved in the rejection of homozgyous Hhl", H-2" donor marrow grafts.

Monocyte-facilitated allograft rejection may be occurring
as a result of increasing the number and function of
monocytes that present donor alloantigens to host T cells.
Alternatively, an increase in the number of monocytes and
their products (such as interferons) may stimulate the
total number of NK cells and/or may induce non-lytic NK
precursor cells to become lytic. The contribution of NK cells
to the rejection of certain (eg, BALB/c, Hhl"), but not other
(eg, DBA/1, Hhl") donor marrow cells in C57BL/6
recipient has been established using both short-term17 and
long-term18,19 engraftment assays. We did, in fact, show that
NK cells are a component of the rM-CSF-induced predilec-
tion toward host (rather than donor) cell repopulation by
selectively depleting NK cells immediately before rM-CSF
infusions. TNFa, one of the array of soluble products of
monocyte activation, is a known stimulator of natural
cytotoxic (including NK cell) function. TNFa also is an
in vitro35 and in vivo36 inhibitor of hematopoiesis. TNFa was
not detectable in the circulation of mice receiving rM-CSF
infusion. However, this does not entirely exclude its involve-
ment because membrane-associated TNFa by itself may
confer cytolytic function, thus not detectable as a circu-
lating protein. Finally, because rM-CSF reduced alloengraft-
ment in a donor-recipient strain specific fashion, a direct
inhibitory effect of rM-CSF on donor progenitor cells as
a cause for the inhibition of alloengraftment is unlikely.

A significant, but transient, relative lymphopenia was
observed 14 days after transplantation in recipients of
rM-CSF and TCD BALB/c or nondepleted syngeneic
control grafts. These data are consistent with the known
effects of rM-CSF on circulating lymphocytes. Lymphope-
nia was not statistically significant in the recipients of TCD
DBA/1 BM grafts and rM-CSF, perhaps because the
control mice had approximately half of the number of
circulating lymphocytes in the PBS-treated control groups
as compared with the other two groups.

Monocytosis was not observed at either day 7, 14, or 28
post-BMT. Other investigations of rM-CSF in nontrans-
planted rodent species show an early monocytopenia,
which occurs within minutes of rM-CSF administration,
and a short-lived monocytosis, which peaks 1 to 2 days after
a single dose of rM-CSF. It should also be noted, however,
that rM-CSF-treated nontransplanted rodents have normal
numbers of circulating monocytes and monocyte precursors
at the time of rM-CSF administration, in marked distinc-
tion to our rM-CSF-treated transplanted mice in which the
highest monocyte number observed in the controls is only
0.2 x 109/μL during the rM-CSF infusion period.

Neutrophil levels did not appear to be positively or
negatively affected by rM-CSF administration. Because
M-CSF induces the production of TNF, IL-1, and
G-CSF, each of which can induce a neutrophil response, a
relative neutrophilia would have been the predicted neutro-
phil response to rM-CSF administration to transplanted

Table 2. Chimerism of Irradiated Mice Receiving TCD Fully Allogenic
Marrow Grafts and M-CSF Post-BMT

<table>
<thead>
<tr>
<th>Group</th>
<th>No. BMT</th>
<th>No. H-2 Typied</th>
<th>% Donor</th>
<th>% Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c donor marrow (Hhl disparate)</td>
<td>30</td>
<td>25</td>
<td>59.6 ± 5.0</td>
<td>37.0 ± 7.5</td>
</tr>
<tr>
<td>M-CSF</td>
<td>30</td>
<td>25</td>
<td>36.0 ± 8.6*</td>
<td>61.0 ± 8.4*</td>
</tr>
<tr>
<td>DBA/1 donor marrow (Hhl&quot;)</td>
<td>30</td>
<td>26</td>
<td>33.6 ± 7.4</td>
<td>60.7 ± 7.5</td>
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<tr>
<td>M-CSF</td>
<td>30</td>
<td>18</td>
<td>41.4 ± 0.1</td>
<td>52.9 ± 8.8</td>
</tr>
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C57BL/6 mice were irradiated with 7.0 Gy TBI and transplanted with
18 x 106 TCD BALB/c or 10 x 106 TCD DBA/1 marrow. Fourteen-day
miniosmotic pumps were implanted on the day of BMT. Pumps
contained PBS or M-CSF (16.8 μg/d) for 14 days. Mice from Table 1
were H-2 phenotyped beginning 6 weeks post-BMT. Data for each
donor strain are pooled from experiments. Data are presented as
the mean ± 1 standard error of the mean.

*P < .05 (as compared with PBS controls by Student's t-test).
mice. In fact, previous reports have indicated that rM-CSF administration increases the number of circulating neutrophils in humans with chronic neutropenia or undergoing allogeneic BMT and in normal rodents. The neutrophilia typically resolves within 1 to 3 days after a single intravenous injection of rM-CSF in rodents. It is possible that a neutrophilia was indeed present in the recipients but was not detected when analyzed 1 day postinjection (day 14).

In our studies, recipients of allogeneic BM and rM-CSF also had a mild transient relative anemia. Ulrich et al noted a rM-CSF dose-dependent (approximately 90 to 900 µg/kg) induction of monocytes, neutrophilia, and lymphopenia in rats. rM-CSF toxicity studies in rats administered in doses ranging from 100 to 1,000 µg/d for 14 days was associated with a reversible thrombocytopenia as the only hematopoietic toxicity. In nonhuman primate toxicology studies, in addition to the dose-related reversible thrombocytopenia, slight reversible decreases in red blood cell count, hemoglobin, and Hct were not detected when analyzed 1 day postinjection (day 14).

We observed that rM-CSF (16.8 µg/d for 14 days) is a tolerable dose in C57BL/6 recipients of TCD BALB/c or DBA/1 BM grafts. We had anticipated a similar or superior survival in recipients of DBA/1 or syngeneic marrow grafts because (1) augmentation of in vivo neutrophil levels has been reported due to a direct or indirect effect of rM-CSF on myeloid cells or their precursors; (2) augmentation of monocyte function as a consequence of rM-CSF (or GM-CSF in our earlier studies) stimulation could provide anti-infection advantages to rM-CSF-treated mice, as has been reported in vitro and in recent human studies in vivo. We could not easily anticipate the survival results of the recipients of BALB/c grafts because the potential positive effects of rM-CSF, as listed above, could be offset by the theoretical negative effects of increasing host antidonor monocyte-facilitated graft resistance processes culminating in poorer hematopoietic recovery.

The reasons for the significantly decreased survival noted in recipients of syngeneic BM and rM-CSF are not clear. Differences in the syngeneic as compared with allogeneic transplants include: (1) lack of allogeneicity of the donor graft; (2) use of nonmanipulated (versus TCD) BM as a source of donor inoculum; (3) lower BM cell dose (10^6 v 10 to 18 x 10^6); (4) higher TBI dose (9.0 ± 6.5 to 7.0 Gy). The first two differences are difficult to reconcile as causative factors for the decreased survival noted in syngeneic BMT. We favor either or both of the last two differences as potentially contributory to the decreased survival. It is possible that low numbers of BM cells in the syngeneic system are more sensitive to monocyte and/or NK inhibitory products. Alternatively, it is possible that the higher dose of TBI predisposes the recipients to toxicity from activated macrophages (eg, alveolar cells in lungs of lethally irradiated recipients) or monocyte and/or NK products.

Taken together, these data suggest that the rM-CSF has variable effects on recipient lymphohematopoiesis and engraftment dependent on the particular donor-recipient strain MHC combination tested. These data may have implications for human clinical trials of rM-CSF in the context of TCD unrelated donor transplants, although caution should be taken in the extrapolation of these data to human BMT recipients receiving rM-CSF at later periods of time post-BMT for antifungal therapy.

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Effect of recombinant human macrophage colony-stimulating factor in irradiated murine recipients of T-cell-depleted allogeneic or non-depleted syngeneic bone marrow transplants

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