Allogeneic bone marrow cells that facilitate complete chimerism and eliminate tumor cells express both CD8 and T-cell antigen receptor–αβ

Fengshuo Lan, Defu Zeng, Philip Huie, John P. Higgins, and Samuel Strober

Nonmyeloablative host conditioning regimens have been used in clinical allogeneic bone marrow and hematopoietic progenitor transplantation to effectively treat lymphohematopoietic tumors and reduce early toxicity. However, severe graft-versus-host disease (GVHD) remains a major problem. The goal of the current study was to determine whether specific subsets of cells in allogeneic bone marrow transplants can effectively treat the BCL1 B-cell lymphoma in nonmyeloablated BALB/c mouse hosts given a single dose of sublethal (450 cGy) total body irradiation, without inducing severe GVHD. The experimental results show that high doses of whole bone marrow cells from major histocompatibility complex (MHC)-mismatched donors eliminate both normal and malignant host-type lymphohematopoietic cells without causing injury to nonlymphohematopoietic host tissues. The CD8 T-cell antigen receptor–αβ (TCRαβ1) T cells within the marrow transplants mediated the killing of the tumor cells via both perforin- and FasL-dependent pathways. Cells present in marrow transplants from either CD8+ or TCRα−/− donors failed to eliminate malignant and normal host lymphohematopoietic cells. Addition of small numbers of blood mononuclear cells to the marrow inoculum caused lethal GVHD. Thus, the resident allogeneic bone marrow CD8+ TCRαβ+ T cells had the unique capacity to eliminate the host lymphohematopoietic cells without nonlymphohematopoietic tissue injury. (Blood. 2001;97: 3458-3465)

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Introduction

Recent advances in clinical allogeneic bone marrow and hematopoietic progenitor transplantation have reduced the toxicity of host-conditioning regimens such that chimerism has been achieved in nonmyeloablated hosts.1-4 Residual tumor cell elimination has been associated with the spontaneous transition from mixed to complete chimerism or with the subsequent infusion of donor blood lymphocytes. A major side effect despite the reduced toxicity regimens is the high incidence of severe graft-versus-host disease (GVHD) even with HLA-matched sibling combinations.1-4 However, mouse models of allogeneic bone marrow transplantation (BMT) in nonmyeloablated hosts conditioned with combinations of sublethal total body irradiation (TBI), thymic irradiation, anti–T-cell monoclonal antibodies (mAbs), and/or chemical cytoreduction show little or no GVHD in major histocompatibility complex (MHC)-mismatched combinations.5-8 The lack of GVHD in the latter models of mixed chimerism is likely explained by the lack of contamination of the BMTs with donor peripheral T cells especially peripheral blood T cells, because only small numbers of the latter cells (∼1 × 105) added to the mouse marrow inoculum induce acute lethal GVHD.9,10 Peripheral blood hematopoietic progenitor transplants mobilized by human bone marrow and granulocyte colony-stimulating factor are highly contaminated with donor blood cells.11-14

Although MHC-mismatched BMT with or without added peripheral T cells can be used to treat lymphomas and leukemias in myeloablated mice,15-18 it is not clear whether this treatment is effective in nonmyeloablated mice with high tumor burdens conditioned with TBI. It is possible that peripheral T cells that induce GVHD are required to eliminate tumor cells in nonmyeloablated hosts. Purified populations of CD8 T cells from the lymph nodes and spleen effectively kill lymphohematopoietic tumor cells in myeloablated hosts given TBI after addition to T-cell-depleted (TCD) or whole BMTs, but severe GVHD develops as the dose of purified CD8+ T cells increases.16,18 A narrow dose range of purified CD8+ T cells from the bone marrow or spleen has been reported to effectively treat the BCL1 lymphoma/leukemia in myeloablated hosts given purified allogeneic hematopoietic stem cell transplants without subsequent development of the clinical signs of GVHD.19 However, it is not clear whether this dose range of purified CD8+ T cells is effective in treating tumors in nonmyeloablated hosts and whether higher doses may cause GVHD. Recently, a CD8+ non-T cell has been identified in the mouse bone marrow that is able to facilitate the engraftment of hematopoietic stem cells in myeloablated allogeneic hosts without the development of GVHD.20,21 The ability of these CD8+ “facilitator cells” to kill or eliminate tumor cells in vivo has not been determined in either myeloablated or nonmyeloablated hosts.

The object of the current study was to determine whether allogeneic BMT can effectively treat the BCL1 tumor in nonmyeloablated hosts given a single dose of TBI, and to determine the nature of cells in the transplant that can mediate tumor elimination. We used high doses of MHC-mismatched whole bone marrow cells to achieve engraftment in nonmyeloablated hosts because increasing the number of infused hematopoietic stem cells or progenitor cells can overcome engraftment barriers in myeloablated allogeneic hosts.21 Previous studies showed that inclusions of CD8+...
T cells and CD8 non-T cells facilitate the engraftment of these progenitors in these myeloblasted hosts.21 The results of the current study show that infusion of the high doses of marrow cells not only engrafted and eliminated residual normal host lymphohematopoietic cells in sublethally irradiated hosts, but also eliminated tumor cells that had been injected 2 weeks earlier. The development of complete chimerism and elimination of tumor cells was mediated by CD8 T cells in the bone marrow, and the contribution of CD8 non-T cells was minimal. Although the donor marrow CD8 T cells eliminated residual normal host normal and malignant lymphohematopoietic cells, microscopic examination of nonlymphohematopoietic tissues including the skin and large intestine showed no evidence of GVHD or obvious tissue injury.

Materials and methods

Animals

Wild-type C57BL/6 (H-2b) male mice, 6 to 8 weeks old, and male BALB/c (H-2d) mice, 8 to 10 weeks old, were purchased from the Department of Comparative Medicine, Stanford University. CD8+ (C57BL/6-CD8a(+/+) ), perf0rmin+ (C57BL/6Pgp105(+/+)), T-cell antigen receptor α (TCRα)-+/-(C57BL/6-TCRα-(+/+)), and FasL−/− (B6Smn.C3H+Fas(+/+)) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). CD4+ (C57BL/6-CD4+/+) and N7) mice were purchased from Taconic Farms (Germantown, NY). All gene-deficient mice were male, 8 to 10 weeks old.

BCL1 tumor cell passage and injection

BCL1 is a B-cell leukemia/lymphoma derived from BALB/c mice with an IgM surface Ig phenotype.23 This cell line was maintained by serial passage in BALB/c mice as described previously.23 BCL1 cells (1 × 106) from the spleen of BALB/c mice were injected intravenously into normal BALB/c recipients, to allow the lymphoma to grow in the recipients for 13 days before the conditioning treatment.

Sublethal TBI

Recipients injected with BCL1 cells were given a single dose of TBI, 450 cGy. The irradiation was performed with a Philips x-ray unit (200 kV, 10 mA; Philips Electronic Instruments, Rahway, NJ) at a rate of 84 cGy/min with a 0.5-mm Cu filter.

Cell preparation

Femoral and tibial bones taken from donor C57BL/6 mice were rinsed and the residual muscle on the bones were removed. Bone marrow cells were prepared by flushing the bones with phosphate-buffered saline (PBS) (Gibco BRL, Grand Island, NY) with 2% fetal bovine serum, and the cell suspension was filtered through nylon mesh to remove aggregates, washed once before injection. To prepare TCD bone marrow cells, whole C57BL/6 bone marrow cells were incubated with biotinylated anti-Thy1.2 mAb (5a-8; CalTag, Burlingame, CA), and then with streptavidin-conjugated magnetic beads (Miltenyi Biotec, Auburn, CA). Cells were passed through a MACS medi-column (Miltenyi Biotec) twice; the flow through cells contained less than 0.1% TCRαβ+ cells as judged by subsequent immunofluorescent staining. The cells retained in the column were harvested and stained with allophyco-cyanin (APC)-anti-TCRβ, phycoerythrin (PE)-anti-CD4, and fluorescein isothiocyanate (FITC)-anti-CD8 fluorochrome-conjugated mAbs and sorted for CD8+ TCRβ and CD4+ TCRαβ+ T cells as described in detail previously.10 Peripheral blood mononuclear cells (PBMC) were obtained by applying heparinized blood from the tail to Ficoll-Hypaque gradients as described previously.10

Bone marrow cell infusion

Single bone marrow cell suspensions were injected via the lateral tail vein of recipients 24 hours after irradiation. Cell suspensions for each injection were adjusted to 0.5 mL to minimize variation of infused cell volumes and numbers.

Flow cytometric analysis and sorting

Blood samples for chimerism and BCL1 analysis from transplanted recipients were hemolyzed with ammonium chloride potassium carbonate to remove red cells. The white cell pellets were washed twice with PBS containing 1% fetal bovine serum and 0.05% sodium azide (staining buffer), and incubated on ice for 15 minutes with saturation concentrations of mAbs after incubation with anti-CD16/32 (2.4G2; Pharmingen, San Diego, CA) to block FcR-γII/III receptors as described previously.10 The following conjugated mAbs were used for immunofluorescent staining; biotin-anti-Thy1.2 (5a-8, CalTag), APC-anti-TCRαβ (H57-597, Pharmingen), PE-anti-CD4 (RM4-5, Pharmingen), FITC-anti-CD8 (CT-CD8a, CalTag), APC-anti-B220 (RA3-6B2, CalTag), FITC-anti-BCL1 antibody (gift from Dr. R. Levy, Stanford University), and PE-anti-H-2Kb (CTKb, CalTag). After incubation, cells were washed twice and followed by Texas red-streptavidin (CalTag) staining on ice. After 15 minutes, cells were washed twice, and propidium iodide was added to exclude dead cells. All the samples were analyzed using Version One and Flow software (Becton Dickinson, Mountain View, CA). Sorting of stained cells was performed using a Vantage flow cytometer (Becton Dickinson) as described in detail previously.10

Histopathology of skin and intestine

Histopathologic specimens from the skin, liver, kidney, heart, lung, and small and large intestines of hosts were obtained 40 or 100 days after transplantation and fixed in formalin before embedding in paraffin blocks. Tissue sections were stained with hematoxylin and eosin and examined for histologic changes by light microscopy.

Statistics

Kaplan-Meir survival curves were made using Statview 4.5 software (SAS Institute, Cary, NC), and statistical differences were analyzed using the log-rank (Mantel-Cox) test.

Results

Survival of mice given BCL1 tumor cells and marrow transplants

To determine whether the BCL1 B-cell lymphoma of BALB/c origin could be effectively treated by MHC-mismatched (C57BL/6H-2b) BMT in sublethally irradiated (450 cGy) hosts, control BALB/c (H-2d) mice were given irradiation alone or a single intravenous injection of 50 × 106 C57BL/6 marrow cells after irradiation. In both cases, all hosts appeared healthy and survived for at least 200 days (Figure 1A). Injection of 5 × 106 C57BL/6 PBMC instead of the marrow cells into the irradiated BALB/c hosts resulted in uniform death by 14 days with clinical signs of GVHD including diarrhea, hunched back, and swollen face (Figure 1A). The number of T cells contained within the PBMC was similar to that in the marrow inoculum.10 Addition of the PBMC to the marrow cells resulted in uniform death of the BALB/c hosts by day 16 (Figure 1A). Hosts given a single intravenous injection of BCL1 tumor cells (1 × 106) and TBI 13 days later all died by day 75 after the tumor cell injection (Figure 1B). Control mice given the tumor cell injection without irradiation all died by day 60, and there was no significant effect of the irradiation on survival as judged by the log-rank test (P > .5).

Survival of recipients of the tumor cells was markedly improved by the intravenous injection of 50 × 106 C57BL/6 marrow cells 24 hours after sublethal irradiation, and all survived at least
200 days. When 12.5, 25, or 50 \times 10^6 marrow cells without PBMC were compared, survival was about 25%, 90%, and 100%, respectively, during a 200-day observation period (Figure 1C). Survival of mice given even the lowest dose of marrow cells was significantly increased compared to mice given tumor cells and irradiation without marrow cells (P < .01).

To determine whether the improved survival of irradiated tumor-bearing hosts given MHC-mismatched marrow cells without PBMC was dependent on donor T cells within the marrow transplant, hosts given 50 \times 10^6 marrow cells without PBMC from wild-type C57BL/6 donors were compared to those given the same number of marrow cells from C57BL/6 TCR\textsuperscript{a2} donor mice with deficient T-cell development. Whereas all hosts given wild-type marrow cells survived for 140 days, hosts given the cells from TCR\textsuperscript{a2} donors all died by day 72 (P < .01) (Figure 1D). Because improved host survival depended on the presence of T cells within the marrow transplants, further studies were performed to determine whether donor CD4\textsuperscript{+} or CD8\textsuperscript{+} T cells were responsible for the improved outcome. Accordingly, irradiated tumor-bearing hosts were given 50 \times 10^6 marrow cells from C57BL/6 CD8\textsuperscript{-/} or CD4\textsuperscript{-/} donors. Whereas uniform survival was observed for at least 140 days in hosts given the CD4\textsuperscript{-/} donor cells, all hosts given CD8\textsuperscript{-/} donor cells died by day 70 (P < .01) (Figure 1D). The results indicate that CD8\textsuperscript{+} T cells within the MHC-mismatched BMTs mediate the improved survival of the hosts.

Adoptive transfer of donor marrow CD8\textsuperscript{+} but not CD4\textsuperscript{+} T cells improves survival of tumor-bearing hosts

In several experiments, BALB/c hosts given BCL\textsubscript{1} tumor cells and sublethal irradiation were injected with either 50 \times 10^6 TCD bone marrow cells from C57BL/6 wild-type donors or the latter cells in combination with sorted CD4\textsuperscript{+} or CD8\textsuperscript{+} T cells from the C57BL/6 bone marrow. Figure 2A shows that irradiated tumor-bearing hosts given the TCD marrow cells alone all died by day 74, and survival was not significantly improved compared to hosts given no marrow cells (P > .5; Figure 1B). Addition of either 0.1 or 0.3 \times 10^6 sorted CD4\textsuperscript{+} T cells from the bone marrow failed to significantly improve survival compared to TCD marrow alone (P > 0.2; (Figure 2A). In contrast, addition of 0.1 \times 10^6 sorted CD8\textsuperscript{+} T cells from the bone marrow significantly improved survival compared to TCD marrow alone (P < .01; Figure 2B), and addition of 0.3 \times 10^6 sorted CD8\textsuperscript{+} T cells resulted in uniform survival of hosts for at least 200 days.

Role of perforin and FasL in improved survival after BMT

Because CD8\textsuperscript{+} T cells from the marrow of C57BL/6 donors mediated the improved survival of tumor-bearing BALB/c hosts, further experiments were performed to determine the role of perforin and FasL. Two proteins expressed by CD8\textsuperscript{+} T cells that have been shown previously to contribute to CD8\textsuperscript{+} T-cell killing of tumor cells.\textsuperscript{25,26} Accordingly, irradiated tumor-bearing BALB/c hosts were given BMTs from wild-type, FasL\textsuperscript{−/−}, or perforin\textsuperscript{−/−} C57BL/6 donors. Figure 3A shows that at least 90% of hosts given either 25 or 50 \times 10^6 wild-type marrow cells survived for 200 days. In contrast, hosts given 25 \times 10^6 marrow cells from perforin\textsuperscript{−/−} donors all died by day 65, and only 40% given 50 \times 10^6 perforin\textsuperscript{−/−} marrow cells survived 200 days (Figure 3A). Thus, survival with wild-type cells was significantly improved compared to perforin\textsuperscript{−/−} cells at high and low cell doses (P < .01 and .01, respectively). Similar results were observed when wild-type was compared to FasL\textsuperscript{−/−} marrow cells (Figure 3B). Whereas uniform
expressing the BCL1 idiotype were not detected on day 20 (Figure 4A shows a representative pattern from a host given BCL1 tumor cells and a BMT). Figure 4B shows a survival pattern for a host given BMT s from wild-type (WT) or perforin (Perforin) donors. (B) Survival of hosts given BMT s from WT or FasL (FasL) donors. There were 5 or 10 hosts in each group.

Elimination of BCL1 tumor cells did not occur in mixed chimeras

Irradiated BCL1 tumor-bearing hosts used in the studies described above were examined for the presence of donor-type cells and BCL1 cells in the peripheral blood 20 days after BMT. PBMC from the hosts were stained with fluorochrome-conjugated anti-H-2Kd (donor-type) mAb and anti–BCL1-idiotype mAb (Figure 4A). Figure 4A shows a representative pattern from a host given BCL1 tumor cells and irradiation without BMT. Approximately 99% of cells did not stain for the donor-type (H-2Kd) marker, and about 10% expressed the BCL1 idiotype (enclosed in lower right box) 20 days after irradiation. In contrast, an irradiated tumor-bearing host given a wild-type C57BL/6 BMT (25 × 10^6 cells) had more than 99% donor-type and less than 1% host-type cells in the blood, and cells expressing the BCL1 idiotype were not detected on day 20 (Figure 4B). Table 1 shows that all hosts given either 12.5, 25, or 50 × 10^6 wild-type C57BL/6 marrow cells that were assayed for chimerism had less than 1% host-type (H-2Kd negative) cells at day 20. When the dose of marrow cells was reduced to 6.0 × 10^6 then irradiated, non-tumor–bearing hosts failed to achieve detectable chimerism, and at least 99% of cells were host type (Table 1). All tested hosts given 50 × 10^6 marrow cells from CD4+/- marrow donors had less than 1% host-type cells (Table 1), and these had no detectable BCL1 tumor cells (Figure 4C). Five of 8 hosts given 50 × 10^6 CD8+/- marrow cells had mixed chimerism with 2% to 98% host-type cells in the blood, and 3 of 8 had complete chimerism with less than 1% (Table 1). An example of mixed chimerism in a CD8+/- marrow recipient with 88% host-type, 10% donor-type, and 2.5% BCL1-idiotype–positive cells is shown in Figure 4D. All the hosts given 50 × 10^6 TCRα+/- marrow cells were mixed chimeras with 4% to 10% host-type cells in the blood at day

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>C57BL/6 bone marrow donor</th>
<th>% Host-type cells</th>
<th>Fraction of hosts with complete chimerism (&lt; 1% host-type cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 × 10^6 Wild-type*</td>
<td>&gt; 99 × 5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>12.5 × 10^6 Wild-type</td>
<td>&lt; 1 × 9</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>25 × 10^6 Wild-type</td>
<td>&lt; 1 × 10</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>50 × 10^6 Wild-type</td>
<td>&lt; 1 × 5</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>5 × 10^6 CD4+/-</td>
<td>&lt; 1 × 5</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>50 × 10^6 CD8+/-</td>
<td>98, 89, 11, 2, 1 × 3</td>
<td>3/6</td>
<td></td>
</tr>
<tr>
<td>50 × 10^6 TCRα+/-</td>
<td>10, 6, 6, 6, 5, 4, 4, 4, 4</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>25 × 10^6 Perforin+/-</td>
<td>98, 94, 4, 1 × 4</td>
<td>4/7</td>
<td></td>
</tr>
<tr>
<td>50 × 10^6 Perforin+/-</td>
<td>6, 4, 4, 2, 1 × 5</td>
<td>5/9</td>
<td></td>
</tr>
<tr>
<td>25 × 10^6 FasL+/-</td>
<td>91, 91, 2, 1 × 3</td>
<td>3/6</td>
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</tr>
<tr>
<td>50 × 10^6 FasL+/-</td>
<td>3, 1, 7</td>
<td>7/8</td>
<td></td>
</tr>
</tbody>
</table>

*Recipients without BCL1, tumor cells.
Lack of injury to nonlymphohematopoietic tissues after BMT

To determine whether irradiated tumor-bearing BALB/c hosts given an injection of $25 \times 10^6$ wild-type C57BL/6 bone marrow cells developed microscopic evidence of tissue injury to nonlymphohematopoietic tissues (liver, pancreas, thyroid, small and large intestines, and skin), 6 hosts were euthanized 100 days after BMT, and histologic analysis of the latter tissues was performed. The large intestines and skin are the main targets for GVHD. Histologic sections were examined for lesions characteristic of the latter disease. At the time of tissue harvest, the blood and spleen were examined also for the presence of residual host-type cells and of cells expressing the BCL1 idotype. As in the day 20 analysis, all tested hosts given $25 \times 10^6$ wild-type marrow cells had no detectable host-type or BCL1 idotype-expressing cells in the blood or spleen in the day 100 analysis as judged by flow cytometry (data not shown).

Characteristic lesions of GVHD in the large intestine and skin obtained from positive control BALB/c hosts are shown in Figure 5, panels A and B, respectively. The 6 positive controls were given lethal TBI (800 cGy) and $1.5 \times 10^5$ TCD bone marrow and $0.1 \times 10^6$ spleen cells from wild-type C57BL/6 donors. Tissues were harvested 40 days after the injection of the C57BL/6 cells because these hosts had severe clinical signs of GVHD and were likely to die shortly thereafter. Figure 5A shows that the intestinal crypts were depleted of plump mucin-containing glandular cells, and a lymphocyte infiltrate was observed surrounding the crypts (asterisk), and extending into the crypt epithelial cells (arrowhead).

Table 2. Influence of sorted bone marrow T cells on residual host-type blood cells in BALB/c recipients 20 d after bone marrow transplantation

<table>
<thead>
<tr>
<th>Bone marrow cells</th>
<th>Bone marrow T cells</th>
<th>% Host-type cells</th>
<th>Fraction of hosts with complete chimerism (&lt; 1% host-type cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole BM (25 x 10^6)</td>
<td>None</td>
<td>$&lt; 1 \times 10^4$</td>
<td>10/10</td>
</tr>
<tr>
<td>Whole BM (50 x 10^6)</td>
<td>None</td>
<td>$&lt; 1 \times 5.6/5$</td>
<td>10/10</td>
</tr>
<tr>
<td>TCD BM (25 x 10^6)</td>
<td>None</td>
<td>99, 98, 94, 33, 24, 12, 11, 7.5</td>
<td>0/9</td>
</tr>
<tr>
<td>TCD BM (50 x 10^6)</td>
<td>None</td>
<td>10, 9, 4, 3, 3, 2, 1</td>
<td>0/7</td>
</tr>
<tr>
<td>TCD BM (25 x 10^6)</td>
<td>CD8$^+$ (0.1 x 10^6)</td>
<td>$&lt; 1 \times 10^4$</td>
<td>10/10</td>
</tr>
<tr>
<td>TCD BM (50 x 10^6)</td>
<td>CD8$^+$ (0.1 x 10^6)</td>
<td>$&lt; 1 \times 9$</td>
<td>9/9</td>
</tr>
<tr>
<td>TCD BM (50 x 10^6)</td>
<td>CD8$^+$ (0.3 x 10^6)</td>
<td>$&lt; 1 \times 9$</td>
<td>9/9</td>
</tr>
<tr>
<td>TCD BM (50 x 10^6)</td>
<td>CD4$^+$ (0.1 x 10^6)</td>
<td>33, 31, 30, 6, 2</td>
<td>0/5</td>
</tr>
<tr>
<td>TCD BM (50 x 10^6)</td>
<td>CD4$^+$ (0.3 x 10^6)</td>
<td>82, 64, 50, 15, 14</td>
<td>0/5</td>
</tr>
</tbody>
</table>

TCD indicates T-cell depleted; BM, bone marrow.

Sorted marrow CD8$^+$ T cells facilitate complete chimerism and tumor elimination

Whereas 25 or $50 \times 10^6$ wild-type C57BL/6 marrow cells eliminated detectable host-type cells at day 20 in all BALB/c recipients tested, TCD marrow cells at the same doses failed to eliminate residual host-type cells in all recipients (Table 2). Some of the latter hosts had few if any donor-type cells in the blood (Figure 4E). Addition of 0.1 or $0.3 \times 10^6$ sorted CD8$^+$ T cells from the wild-type marrow to the TCD marrow reduced residual host-type cells to less than 1% in all recipients (Table 2 and Figure 4F). However, it is clear that elimination as judged by flow cytometry at day 20 did not predict long-term survival, because 60% of recipients given TCD marrow and $0.1 \times 10^6$ sorted CD8$^+$ T cells died within 125 days after transplantation (Figure 2B). Addition of $0.3 \times 10^6$ sorted CD8$^+$ T cells was required to give uniform long-term survival (Figure 2B). In contrast, addition of 0.1 or $0.3 \times 10^6$ sorted CD4$^+$ T cells failed to induce complete chimerism in any recipient (Table 2), and failed to improve survival of recipients compared to TCD marrow alone (Figure 2A).
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The addition of 5 \times 10^6 donor blood mononuclear cells to 50 \times 10^6 bone marrow cells transplanted to the nonmyeloablated hosts in the current study resulted in acute lethal GVHD whether or not BCL\textsubscript{1} cells were injected. Thus, blood, but not marrow T cells, is able to induce lethal disease in myeloablated and nonmyeloablated hosts.\textsuperscript{10}

The low potency of marrow T cells is related to their unusual balance of T-cell subsets including a high proportion of natural killer (NK1.1\textsuperscript{+}) T cells that suppress GVHD.\textsuperscript{10}

Irradiated tumor-bearing BALB/c hosts given 25 \times 10^6 wild-type C57BL/6 marrow cells were examined at 100 days after the marrow infusion for microscopic evidence of GVHD and tissue injury in the thyroid, pancreas, liver, small and large intestines, and skin. None of the hosts studied at this time point showed microscopic changes of GVHD or of injury in any of these tissues. In addition, tumor cells bearing the BCL\textsubscript{1} idiotype were not detected in the blood or spleen in any of the hosts at the same point. Despite the lack of microscopic evidence of GVHD, residual host lymphohematopoietic cells were eliminated in the blood or spleen as judged by flow cytometry at day 20 and day 100. The mechanisms by which donor marrow cells selectively eliminate one type of host cell but do not injure another is the subject of continued study. This is a unique feature of donor marrow cells, because donor blood or spleen cells clearly cause injury to nonlymphohematopoietic tissues such as the skin and colon.\textsuperscript{10,26,27}

Previous studies using the nonmyeloablative regimen, total lymphoid irradiation, combined with cyclophosphamide, showed that transplantation of large numbers of C57BL/6 marrow cells could effectively treat the BCL\textsubscript{1} tumor in BALB/c \times C57BL/6 F\textsubscript{1} hosts without GVHD.\textsuperscript{29} A subsequent study using the same model system indicated that marrow CD8 cells were required for tumor eradication, because antitumor activity was lost after ex vivo depletion of CD8 but not CD4 cells.\textsuperscript{30}

Although BALB/c hosts in the current study survived at least 100 days without evidence of BCL\textsubscript{1} idiotype-positive tumor cells in the blood or spleen, it is possible that small numbers of “dormant” tumor cells were present in the hosts below the limits of detection of flow cytometry. Previous studies of the BCL\textsubscript{1} tumor have shown that “dormant” tumor cells can be present in healthy transplant hosts even months after tumor inoculation.\textsuperscript{31,32} The latter cells can be detected by injecting spleen cells from the hosts into normal BALB/c mice, and observing tumor outgrowth over a period of at least 3 months.\textsuperscript{31,32} Assays for “dormant” tumor cells were not performed in the current study, but we found a correlation between the detection of at least 2% idiotype-positive tumor cells by flow cytometry in the blood of healthy hosts described in our previous study\textsuperscript{18} and the outgrowth of tumor in adoptive hosts (data not shown).

The BMT protocol described herein may provide a model for MHC-haplotype–matched or MHC-mismatched nonmyeloablated human transplant recipients such that tumor elimination can be achieved without severe GVHD as long as resident bone marrow T cells but not blood T cells are contained in the transplant. This may require techniques for separating the 2 sources of T cells based on surface markers or other characteristics. Unique phenotypic characteristics of human bone marrow T cells from cadaver vertebral bodies that differ from blood T cells have been reported previously.\textsuperscript{33}

To determine the subsets of donor cells within the BMTs in the current study that were responsible for elimination of normal and malignant host-type lymphohematopoietic cells, marrow cells from wild-type C57BL/6 donors were compared to those from C57BL/6 TCR\textalpha\textsuperscript{+}, CD4\textsuperscript{+}, and CD8\textsuperscript{+} donors. The hosts given TCR\textalpha\textsuperscript{+} marrow cells all died by day 72, and neither normal nor malignant

\textbf{Discussion}

The object of the current study was to determine whether BMT can effectively treat the BCL\textsubscript{1} B-cell lymphoma, in nonmyeloablative (450 cGy TBI) hosts. Tumor cells were transferred to the hosts 13 days before irradiation to allow for substantial growth to provide a model for the treatment of human B-cell lymphoma with any microscopic injury of the nonlymphohematopoietic tissues examined in the surviving hosts.

Amarie, 

None of the 6 irradiated tumor-bearing BALB/c hosts given 25 \times 10^6 C57BL/6 marrow cells had clinical signs of GVHD disease during a 100-day observation period, and none had microscopic lesions characteristic of GVHD or any other abnormalities of the large intestine or skin when tissues were harvested at 100 days. Figure 5E shows a representative section of the large intestine from the latter hosts. The crypts were lined with plump mucin-containing glandular cells, and there was no crypt atrophy, apoptosis, or infiltration. Figure 5F shows a representative section of the skin from these experimental hosts. The epidermis was 2 to 3 cells thick, dermal appendages were frequent, and there was no dermal infiltrate or epidermal blistering. Histologic sections from the thyroid, liver, pancreas, and small intestines had no evidence of tissue injury or necrosis (data not shown). Thus, the injection of 25 \times 10^6 wild-type C57BL/6 bone marrow cells was not associated with any microscopic injury of the nonlymphohematopoietic tissues examined in the surviving hosts.
host-type cells were eliminated by day 20. Thus, donor marrow T cells played a necessary role in the elimination of host-type cells. The “facilitator cells” of allogeneic stem cell engraftment that have been reported to be CD8+ non-T cells,23,24 appear to be unable to mediate elimination of normal and malignant host-type cells in this nonmyeloablative model despite their capacity to facilitate engraftment. A recent study showed the latter “facilitator cells” express a unique heterodimer containing the TCRβ chain covalently linked to a newly described 33-kd protein, and do not express the TCRα chain.34 Thus, “facilitator cells” expected to be present in the marrow of TCRα−/− donors do not appear to function in the current model. Further evidence that CD8+ T cells in the donor marrow mediate host lymphohematopoietic cell elimination after sublethal irradiation was shown by the long-term survival of tumor-bearing hosts given TCD (Thy-1+) marrow transplants in combination with sorted CD8+ TCRβ0+ T cells from the bone marrow. Hosts given the TCD marrow transplants alone all died by day 75. The depleted transplants are likely to be depleted of T cells as well as “facilitator” CD8− non-T cells, because the latter cells express the Thy-1 marker also.21

The uniform death of tumor-bearing hosts given CD8−/− BMTs and uniform survival of hosts given CD4−/− BMTs was consistent with the critical role of CD8+ T cells in this model. As expected, genetic deficiencies in the expression of Fas and perforin by C57BL/6 marrow donors resulted in impaired elimination of normal and malignant host-type cells, because these 2 molecules have been shown previously to play a critical role in the cytolytic activity of CD8+ T cells via 2 different pathways of target cell destruction.23,24

The dominant role of marrow CD8+ T cells in the current study using nonmyeloablated hosts is consistent with a recent report showing that marrow CD8+ T cells effectively eliminate BCL1 tumor cells in lethally irradiated hosts using the perforin and FasL pathways without GVHD.19 However, neither the contribution of “facilitator cells,” nor the feasibility of elimination of high levels of residual normal and malignant lymphohematopoietic host-type cells present in sublethally irradiated hosts, nor the presence of microscopic injury of nonlymphohematopoietic tissues was studied.19 In particular, host-type normal B cells were eliminated by the lethal irradiation conditioning regimen.19 Previous murine studies have shown that donor CD8+ T cells from tissue sources outside the marrow (lymph node and spleen) are capable of eliminating lymphohematopoietic tumors and facilitating stem cell engraftment in myeloablative hosts, but lethal GVHD was observed as the dose of purified CD8+ T cells was increased to achieve uniform tumor elimination.16,18 Although donor blood lymphocyte infusions depleted of CD8+ T cells in humans have been shown to induce remissions after tumor relapse in BMT recipients, the nature of the donor cell that effectively kills the host-type tumor is not clear.35 CD4+ T cells in these infusions may be able to help donor CD8+ T cells in the host to eliminate tumor cells. In addition, CD4+ T cells may arrest the growth of some types of leukemia.36

In conclusion, the current study shows that CD8+ T cells in the donor bone marrow have a unique ability to eliminate normal and malignant host lymphohematopoietic cells without causing GVHD or injury to nonlymphohematopoietic tissues in nonmyeloablated hosts.

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References


Allogeneic bone marrow cells that facilitate complete chimerism and eliminate tumor cells express both CD8 and T-cell antigen receptor Tαβ

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