Peripheral Deletion After Bone Marrow Transplantation with Costimulatory Blockade Has Features of Both Activation-Induced Cell Death and Passive Cell Death

Thomas Wekerle, Josef Kurtz, Mohamed H. Sayegh, Hiroshi Ito, Andrew D. Wells, Steven Bensinger, Juanita Shaffer, Laurence A. Turka and Megan Sykes

*J Immunol* 2001; 166:2311-2316; doi: 10.4049/jimmunol.166.4.2311
http://www.jimmunol.org/content/166/4/2311

---

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

---

**References**

This article cites 38 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/166/4/2311.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Peripheral Deletion After Bone Marrow Transplantation with Costimulatory Blockade Has Features of Both Activation-Induced Cell Death and Passive Cell Death

Thomas Wekerle, Josef Kurtz, Mohamed H. Sayegh, Hiroshi Ito, Andrew D. Wells, Steven Bensinger, Juanita Shaffer, Laurence A. Turka, and Megan Sykes

Two major pathways of death of previously activated T cells have been described: activation-induced cell death can be triggered by restimulating activated T cells with high concentrations of Ag, is Fas-dependent, and is not influenced by proteins of the Bcl family, and is blocked by cyclosporin A; in contrast, passive cell death is induced by the withdrawal of growth factors and activation stimuli, is Fas-independent, and is blocked by Bcl family proteins. We examined the role of these two forms of cell death in the peripheral deletion of donor-reactive host T cells after allogeneic bone marrow transplantation and costimulatory blockade with anti-CD154 plus CTLA4Ig in two murine models. The substantial decline in donor-reactive CD4 cells seen in wild-type recipients 1 wk after bone marrow transplantation with costimulatory blockade was largely inhibited in Fas-deficient recipients and in Bcl-xL-transgenic recipients. We observed these effects both in a model involving low-dose total body irradiation and a conventional dose of bone marrow, and in a radiation-free regimen using high-dose bone marrow transplantation. Furthermore, cyclosporin A did not completely block the deletion of donor-reactive CD4+ T cells in recipients of bone marrow transplantation with costimulatory blockade. Thus, the deletion of donor-reactive T cells occurring early after bone marrow transplantation with costimulatory blockade has features of both activation-induced cell death and passive cell death. Furthermore, these in vivo data demonstrate for the first time the significance of in vitro results indicating that proteins of the Bcl family can prevent Fas-mediated apoptosis under certain circumstances. The Journal of Immunology, 2001, 166: 2311–2316.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This study was supported by National Institutes of Health Grant R01 HL-49915 and in part by a sponsored research agreement between Massachusetts General Hospital and BiocTransplant. M.H.S. is a recipient of the National Kidney Foundation Clinician Scientist Award. T.W. was supported by fellowships from the Max Kade Foundation and the Austrian Science Fund (Fonds zur Förderung der wissenschaftlichen Forschung).

2 Current address: Department of Surgery, Vienna General Hospital, University of Vienna, Währingerguertel 18, A-1090 Vienna, Austria.

3 Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Received for publication September 14, 2000. Accepted for publication November 27, 2000.
of the Fas-mediated and Bcl-inhibited cell death pathways. To better characterize the mechanism of peripheral deletion after costimulatory blockade and BMT, we examined this phenomenon using Fas-deficient (lpr) and Bcl-x<sub>L</sub>-transgenic recipients.

**Materials and Methods**

**Animals**

Female C57BL/6 (B6; I-E<sup>+</sup>), B6.lpr (lpr; Fas-deficient; I-E<sup>+</sup>), B10.A (B10.A; I-E<sup>+</sup>), and A.SW mice were purchased from Frederick Cancer Research Center (Frederick, MD) or from The Jackson Laboratory (Bar Harbor, ME). B6.Bcl-x<sub>L</sub>-transgenic mice (with transgenic expression of the long form of the Bcl-x gene (Bcl-x<sub>L</sub>) targeted to the T cell lineage (Ref. 24; B6.Bcl-x<sub>L</sub>; I-E<sup>+</sup>) and B6 nontransgenic littermates (B6; I-E<sup>+</sup>) were bred in the colony of Dr. L.A. Turka. Mice were maintained in a specific pathogen-free microisolator environment, as described (25).

**BMT protocols**

In the standard-dose BMT model, recipient mice were treated with 3 Gy total body irradiation (TBI) and were injected i.v. on the same day (day 0) with ~2 × 10<sup>6</sup> unseparated BM cells (BMC) harvested from fully MHC-mismatched B10.A donors. In the high-dose BMT model, recipients were injected i.v. with ~200 × 10<sup>6</sup> unseparated BMC harvested from B10.A donors. In both BMT models, a hamster anti-mouse CD154 mAb (MR1; 0.5 or 2 mg) was injected i.p. on day 0, and murine CTLA4Ig (0.5 mg) was injected i.p. on day +2. In the high-dose BMT experiment using B6.Bcl-x<sub>L</sub> recipients, rat anti-mouse B7.1 (G110) and rat anti-mouse B7.2 (2D10) mAbs (0.5 mg of each on day +2) were used instead of CTLA4Ig. CyA was administered s.c. at a dose of 20 mg/kg/d for the first 2 wk after BMT where indicated. CTLA4Ig was a gift of Bristol-Myers Squibb (Seattle, WA); the MR1 hybridoma was provided to us by Dr. Randolph I. Noelle; and the anti-B7.1 and anti-B7.2 mAbs were purchased from Bioexpress (West Lebanon, NH).

**Flow cytometric (FCM) analysis of TCR V<sub>β</sub> families**

PBL were stained with anti-V<sub>β</sub>5.1/2-FITC, V<sub>β</sub>11-FITC, and V<sub>β</sub>8.1/2-FITC mAbs vs PE-conjugated anti-CD4 (all purchased from PharMingen, San Diego, CA). Two-color FCM analysis was performed on gated CD<sup>4</sup><sup>+</sup> cells. Background staining (as determined with nonreactive mAb H0PC-FITC) was subtracted from the percentage of cells staining with each anti-V<sub>β</sub> mAb. All <i>p</i> values were calculated using a two-tailed Student’s <i>t</i> test.

**FCM analysis of thymocyte subpopulations**

Thymocytes were stained with anti-CD4-PE vs anti-CD8-CyChrome, and the percentages of CD4 or CD8 single-positive, CD4 CD8 double-positive, and double-negative thymocytes were determined by two-color FCM analysis.

**CFU assay**

CFUs were determined using a complete methylcellulose medium with recombinant cytokines (MethoCult GF M3434; Stem Cell Technologies, Vancouver, British Columbia, Canada) plus 1 x penicillin/streptomycin plus 1 x glutamine. BM from one tibia and one femur was flushed into IMDM with 2% FBS. RBCs were lysed in 2% acetic acid, and nucleated cells were counted. Cells were diluted to 1.33 × 10<sup>5</sup> cells/ml. 1 ml of medium was plated into 35-mm petri dishes. After incubation for 7–8 days at 37°C with 5% CO<sub>2</sub>, the total number of colonies was counted on an inverted microscope.

**Results**

**Peripheral deletion of donor-reactive CD4 cells after BMT with costimulatory blockade in a model involving low-dose TBI**

Wild-type B6, Fas-deficient B6.lpr, Bcl-x<sub>L</sub>-transgenic B6 mice, and nontransgenic littermate controls were treated with 3 Gy TBI, 20 × 10<sup>6</sup> fully MHC-mismatched B10.A BMC, and costimulatory blockade consisting of one injection each of anti-CD154 plus CTLA4Ig. Seven days after BMT, the percentages of CD4 cells bearing certain V<sub>β</sub> subunits on their TCR were determined by two-color FCM analysis. The donor strain B10.A expresses I-E<sup>+</sup>, which is required to present superantigens derived from mammary tumor virus (Mtv)-8 and -9 endogenous retroviruses encoded in the B6 background. Developing thymocytes whose TCR contained V<sub>β</sub>11 or V<sub>β</sub>5.1/2, which bind to these superantigens, are deleted in the thymus of I-E<sup>+</sup>-positive B10.A mice, but not in B6 mice, because they do not express I-E<sup>+</sup> (26–29). V<sub>β</sub>5.1 and V<sub>β</sub>11<sup>+</sup> CD4 cells can also be subject to deletion in the periphery when they recognize superantigen plus donor MHC class II (I-E<sup>+</sup>) under specific circumstances. T cells whose TCR contain V<sub>β</sub>8.1/2 do not bind these superantigens, are therefore not deleted, and thus served as an irrelevant control to assure specificity of the deletion.

In the first experiment, we sought to examine the role of the Fas pathway in early deletion of donor-reactive CD4<sup>+</sup> cells. As reported previously (6), wild-type B6 BMT recipients demonstrated a substantial decline in the percentages of V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> CD4<sup>+</sup> PBL, by 74 and 59%, respectively, compared with B6 controls receiving costimulatory blockade and TBI without BM (<i>p</i> < 0.00005) (Fig. 1A). The percentage of V<sub>β</sub>8<sup>+</sup> CD4<sup>+</sup> PBL was not reduced in any group (data not shown), indicating that the observed deletion was specific for endogenous superantigens presented by the donor. In marked contrast, B6.lpr recipients showed a reduction of only 39 and 22%, respectively, in the percentages of V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> CD4<sup>+</sup> PBL, compared with B6.1pr controls treated with costimulatory blockade alone (<i>p</i> = 0.1 for V<sub>β</sub>11, <i>p</i> = 0.003 for V<sub>β</sub>5). A significant difference was seen between wild-type B6 and Fas-deficient BMT recipients in the extent of deletion (<i>p</i> < 0.001 for V<sub>β</sub>5 and V<sub>β</sub>11).

We next evaluated the effect of constitutive Bcl-x<sub>L</sub> expression on early deletion of donor-reactive CD4<sup>+</sup> cells. Again, a substantial reduction of V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> CD4<sup>+</sup> PBL was seen 1 wk after BMT in B6 hosts (65 and 68% reduction, respectively, compared with controls receiving costimulatory blockade alone) (Fig. 1B). (As expected, nontransgenic littermate controls behaved similarly to wild-type B6 mice; therefore, these mice are presented together.) In contrast, Bcl-x<sub>L</sub>-transgenic BMT recipients showed reductions of only 20 and 1% in V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> CD4<sup>+</sup> PBL, respectively (compared with Bcl-x<sub>L</sub>-transgenic mice treated with costimulatory blockade alone, <i>p</i> > 0.05). The difference in the extent of the deletion between wild-type B6 and Bcl-x<sub>L</sub>-transgenic BMT recipients was highly significant (<i>p</i> < 0.002).

The above results were surprising because they implicated both AICD (Fas-dependent) and PACD (blocked by Bcl-x<sub>L</sub> expression) in the deletion of donor-reactive CD4<sup>+</sup> cells in BMT recipients. CyA has been shown to inhibit AICD (15), but should not block PACD because it reduces IL-2 production. Therefore, we were interested in determining the effect of CyA on deletion after BMT with costimulatory blockade. We treated wild-type B6 mice with 3 Gy TBI, 20 × 10<sup>6</sup> BMC, and costimulatory blockade (<i>n</i> = 12) and compared V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> deletion 1 wk after BMT with that in a group receiving the same protocol plus daily CyA treatment beginning on the day of BMT (<i>n</i> = 13). Recipients of BMT with costimulatory blockade again showed the expected reduction of V<sub>β</sub>5<sup>+</sup> and V<sub>β</sub>11<sup>+</sup> CD4<sup>+</sup> PBL (73 and 67% reduction, respectively, compared with normal B6 controls). In mice receiving CyA in addition to these treatments, there was still substantial deletion, but it was diminished to some degree (55 and 40% reduction, respectively; <i>p</i> = 0.09 for V<sub>β</sub>5, <i>p</i> < 0.01 for V<sub>β</sub>11 compared with the group without CyA) (Table I). Control mice receiving TBI, BMC, and CyA (but no costimulatory blockade, <i>n</i> = 5) did not show significant deletion (<i>p</i> > 0.05, compared with normal B6).

**Radiosensitivity of Bcl-x<sub>L</sub>-transgenic mice**

TBI is an essential component of the BMT model described above. Therefore, it was important to rule out the possibility that Fas deficiency or Bcl-x<sub>L</sub> overexpression indirectly influenced the peripheral deletion of CD4 cells by abrogating a participatory role for TBI. This becomes especially relevant in view of evidence that...
in wild-type mice ($p < 0.05$ comparing the change in percent double-positive cells after irradiation in wild-type vs Bcl-xL-transgenic mice). Thus, although the radiosensitivity of some cell populations differed between wild-type mice and Bcl-xL-transgenic mice, other cell types seemed to react similarly.

**Peripheral deletion in a radiation-free model of high-dose BMT with costimulatory blockade**

Because both lpr and Bcl-xL-transgenic mice react differently to radiation than wild-type mice, we next used a radiation-free model to evaluate mechanisms of peripheral T cell deletion after BMT with costimulatory blockade. We have recently demonstrated that early peripheral deletion of donor-reactive CD4$^+$ T cells also occurs after a protocol involving high-dose BMT with costimulatory blockade without cytoreductive host conditioning (8). Wild-type B6, Fas-deficient B6/lpr, Bcl-xL-transgenic B6 mice, and B6-nontransgenic littermate controls were treated with $200 \times 10^6$ fully MHC-mismatched BMC and anti-CD154 plus CTLA4Ig (or anti-CD154 plus anti-B7.1 and anti-B7.2; Ref. 8). As in the experiments described above, the percentages of V$\beta^{5+}$, V$\beta^{11+}$, and V$\beta^{8+}$ CD4$^+$ PBL were determined 7 days after BMT. Similar to previous results, in wild-type B6 recipients the percentages of V$\beta^{5+}$ and V$\beta^{11+}$ (but not V$\beta^{8+}$) CD4$^+$ PBL declined by 81 and 79%, respectively, after high-dose BMT with costimulatory blockade compared with controls receiving BMT only without costimulatory blockade ($p < 0.05$) (Fig. 2A) (8). In contrast, Fas-deficient B6/lpr recipients showed no significant reduction ($p > 0.05$) in either V$\beta^{5+}$ or V$\beta^{11+}$ CD4$^+$ PBL compared with normal B6/lpr mice ($p < 0.005$ for comparison of B6 and B6/lpr BMT recipients).

In an experiment to examine the role of Bcl-xL in the peripheral deletion of donor-reactive CD4$^+$ cells, B6-nontransgenic littermate controls receiving high-dose BMT with costimulatory blockade demonstrated the expected reduction in V$\beta^{5+}$ and V$\beta^{11+}$ CD4$^+$ PBL (58 and 59% reductions, respectively, compared with naive lpr mice ($n = 3$) or lpr controls ($n = 4$) receiving costimulatory blockade without BM ($p = 0.1$ for V$\beta^{11}$, $p = 0.003$ for V$\beta^{5}$; group A). The difference in the percentages of V$\beta^{11}$ and V$\beta^{5}$ between B6/lpr and B6 BMT recipients was statistically significant ($p < 0.001$ comparing groups B and D). B, B6 and B6-nontransgenic littersmate BMT recipients (group I; $n = 10$) demonstrated a significant reduction in the percentages of V$\beta^{5+}$ and V$\beta^{11+}$ CD4$^+$ PBL 1 wk after BMT compared with B6 controls (group H; $n = 4$) ($p < 0.002$). In contrast, Bcl-xL-transgenic BMT recipients (group G; $n = 6$) showed no significant reduction in the percentages of V$\beta^{5+}$ and V$\beta^{11+}$ CD4$^+$ PBL compared with Bcl-xL-transgenic controls receiving costimulatory blockade alone without BM (group F; $n = 3$) ($p > 0.05$). The difference in the percentages of V$\beta^{11}$ and V$\beta^{5}$ between B6/Bcl-xL and B6 BMT recipients was statistically significant ($p < 0.002$ comparing groups G and I). All experimental mice were irradiated with 3 Gy TBI and received anti-CD154 and CTLA4Ig. BMT recipients were injected with $20 \times 10^6$ B10.A BMC. The percentages of V$\beta^{5+}$, V$\beta^{11+}$, and V$\beta^{8+}$ CD4$^+$ PBL were determined by two-color FCAM analysis 7 days after BMT, with V$\beta^{8+}$ serving as nonspecific control. Co.-bl denotes costimulatory blockade.

**Discussion**

Costimulatory blockade can exert its effects through several mechanisms, with anergy (1), immune deviation (2), suppression (3), and apoptosis (4, 5) having been observed in various models. We have recently demonstrated that BMT under cover of costimulatory blockade (anti-CD154 and CTLA4Ig) leads to the peripheral deletion of donor-reactive host T cells immediately after BMT (6–8). This mechanism of peripheral deletion seems to provide an effective way to specifically eliminate T cells with a certain Ag specificity from an adult animal, and thus has potential for widespread clinical application. Here we describe studies in two BMT models that indicate that this form of peripheral deletion is: 1) largely dependent on the presence of functional Fas in the host; 2) preventable by the constitutive expression of Bcl-xL; and 3) not completely inhibited by CyA.

In theory, either AICD or PACD could be responsible for the deletion after BMT with costimulatory blockade. The injection of a large amount of Ag in the form of fully allogeneic BMC could lead to repeated activation of host T cells and subsequent AICD. In addition, several cell types with veto-like activity contained in the
unseparated BM inoculum might induce Ag-specific apoptosis of host cells. Indeed, in the high-dose BMT studies, a slight reduction of donor-reactive CD4$^+$ cells was observed in control mice receiving only the BM without costimulatory blockade, but this effect was not observed in a Fas-deficient control mouse (Fig. 2A and Ref. 8). Early deletion after BMT with costimulatory blockade was also observed to a similar extent when T cell-depleted BM was used (J.K. and M.S., unpublished data), suggesting that donor T cells are not required for the deletion and that they are not required as mediators of putative veto activity. Therefore, it is possible that the effect of anti-CD154 and CTLA4Ig is primarily to prevent the effect of anti-CD154 and CTLA4Ig is primarily to prevent the rejection of the injected BM (possibly by inducing anergy or suppression), and that the prolonged presence of donor Ag is enough to lead to continuous extrathymic deletion of donor-reactive T cells through AICD or other BM-mediated pathways. Additional support for a partial role of AICD in the deletion after BMT with costimulatory blockade comes from the observation that CyA, which has been shown to prevent AICD, seemed to inhibit deletion to some degree (but by far not completely). A key feature of AICD is its dependence on Fas (10–13). Consistent with this characteristic, the lack of functional Fas in lpr mice prevented ~50% or more of the peripheral deletion observed in wild-type control mice. However, proteins of the Bcl-family are thought to be unable to prevent AICD (4, 11, 13, 19). An additional argument against AICD as the main mechanism of deletion in these BMT models is that we have not found evidence for expansion of donor-reactive T cells (J.K. and M.S., unpublished observation), which preceded AICD at an early time point in several studies (33, 34). However, in another system, superantigen was able to induce deletion of CD28$^+$ T cells without evidence of preceding T cell expansion (35). Thus it seems possible that AICD can occur without preceding T cell expansion in the absence of an intact CD28 pathway, which would be the case in our model by day 2 when CTLA4Ig is administered.

PACD can be induced as a consequence of the absence of growth factors and activation stimuli due to a lack of costimulation signals (9, 17, 36). The CD28 pathway, which can be blocked by CTLA4Ig, is considered to provide the most important costimulatory signal for mature T cells by inducing IL-2 and other essential growth and activation signals (37). A hallmark of PACD is that it can be prevented by the overexpression of survival genes of the Bcl-2 family (4, 11, 18). In our experiments, peripheral deletion of V$\beta$5$^+$ and V$\beta$11$^+$ CD4$^+$ cells was effectively prevented in Bcl-xL-transgenic recipients. Because the Bcl-xL transgene does not augment proliferation of T cells with or without costimulation (but in fact seems to delay cell cycle kinetics somewhat (Ref. 4, and A.D.W. and L.A.T., unpublished observation), it is unlikely that the powerful net effect on deletion observed in Bcl-xL-transgenic mice reflects increased proliferation rather than reduced deletion. Also, the lack of evidence for expansion before the deletion and the inability of CyA to prevent deletion completely is consistent with a major role for PACD. However, PACD is generally considered not to be mediated by Fas.

How can the observations that the extrathymic deletion in these BMT models is partly Fas-dependent but preventable by Bcl-xL expression be reconciled? First, Fas deficiency did not completely inhibit deletion in our studies, suggesting that there is a component of apoptosis that is Fas independent. This component of apoptosis could be due to PACD. However, because Bcl-xL is able to prevent deletion almost entirely, there seems to be a component that is

### Table I. Influence of CyA on early deletion after BMT with costimulatory blockade

<table>
<thead>
<tr>
<th></th>
<th>V88</th>
<th>V85</th>
<th>V811</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBL6</td>
<td>17.24 ± 0.3</td>
<td>3.17 ± 0.4</td>
<td>5.28 ± 0.71</td>
</tr>
<tr>
<td>NBL10.A</td>
<td>16.8 ± 0.4</td>
<td>0.03 ± 0</td>
<td>0.04 ± 0</td>
</tr>
<tr>
<td>CyA + BM</td>
<td>17.32 ± 0.6</td>
<td>2.06 ± 0.7</td>
<td>4.1 ± 0.9</td>
</tr>
<tr>
<td>co-bl. + BM</td>
<td>18.57 ± 0.7</td>
<td>0.86 ± 0.5</td>
<td>1.75 ± 1.1</td>
</tr>
<tr>
<td>co-bl. + BM with CyA</td>
<td>18.54 ± 1</td>
<td>1.44 ± 1</td>
<td>3.19 ± 1.3</td>
</tr>
</tbody>
</table>

* Wild-type B6 mice were treated with 3 Gy TBI, 20 × 10^6 BMC, and costimulatory blockade with (co-bl. + BM with CyA) or without the addition of CyA (co-bl. + BM). a control group received 3 Gy TBI, 20 × 10^6 BMC and CyA (CyA + BM). The percentage of V88, V85, and V811 CD4$^+$ PBL was determined by FCM analysis 1 wk after BMT. Combined results from two similar experiments (except control group) are shown as mean ± SD.

* $p < 0.01$ compared to group receiving co-bl. + BM. Co-bl. denotes costimulatory blockade.

### Table II. Effects of 3 Gy TBI on lymphocytes and hemopoietic cells of Bcl-xL-transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>Thymus</th>
<th>CD4 SP</th>
<th>CD8 SP</th>
<th>DP</th>
<th>DN</th>
<th>Lymph Node</th>
<th>Spleen</th>
<th>BM</th>
<th>CFUs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBL6 0 Gy</td>
<td>153.5 ± 19.1</td>
<td>27.9 ± 0.9</td>
<td>14.5 ± 0.2</td>
<td>93.3 ± 12.7</td>
<td>9.6 ± 0</td>
<td>2.6 ± 0.1</td>
<td>78.5 ± 12.0</td>
<td>18 ± 3.4</td>
<td>473 ± 4.2</td>
</tr>
<tr>
<td>NBL6 3 Gy</td>
<td>23.7 ± 2.4</td>
<td>10.3 ± 0.9</td>
<td>7.4 ± 1.2</td>
<td>0.2 ± 0</td>
<td>3.9 ± 1.6</td>
<td>0.4</td>
<td>12.9 ± 2.3</td>
<td>8.2 ± 1.2</td>
<td>167.3 ± 40.7</td>
</tr>
<tr>
<td>B6.Bcl-xL 0 Gy</td>
<td>116.5 ± 2.1</td>
<td>16.6 ± 4.1</td>
<td>8.2 ± 1.1</td>
<td>72.4 ± 12.4</td>
<td>2.4 ± 2.3</td>
<td>4.38 ± 0.2</td>
<td>40 ± 7.1</td>
<td>19.3 ± 1.4</td>
<td>442 ± 5.7</td>
</tr>
<tr>
<td>B6.Bcl-xL 3 Gy</td>
<td>38 ± 4.4</td>
<td>10.5 ± 1.8</td>
<td>7.4 ± 2.6</td>
<td>13.5 ± 1.5</td>
<td>4 ± 0.7</td>
<td>0.8</td>
<td>23.9 ± 1.0</td>
<td>4.1 ± 1.1</td>
<td>172.7 ± 31</td>
</tr>
</tbody>
</table>

* To determine the sensitivity of lymphocytes and BMC to irradiation, Bcl-xL-transgenic mice and normal B6 mice were treated with 3 Gy TBI. Four days after TBI, the number of viable cells was determined (by trypan-blue exclusion) in thymus, lymph node, spleen, and BM (expressed as mean counts to be multiplied by 1 × 10^6 SD). Total numbers of CFUs were determined after culturing the BM of one femur plus one tibia for 7–8 days (mean ± SD). Numbers in parentheses indicate the percentages of cell counts in relation to nonirradiated mice; numbers in brackets [:] indicate the percentages of thymocyte subpopulations in relation to total thymocyte numbers. The percentages of thymocyte subpopulations were determined by two-color FCM analysis (mean ± SD). Two mice in the nonirradiated groups were compared to three mice in the irradiated groups. Results from one of two similar experiments are shown. SP, single-positive; DP, double-positive; DN, double-negative.

* $p < 0.01$ comparing percent change in cell counts in irradiated wild-type vs irradiated Bcl-xL-transgenic mice.

* $p < 0.05$ comparing the change in the percent thymocyte subpopulation in irradiated wild-type vs irradiated Bcl-xL-transgenic mice.
FIGURE 2. In a radiation-free model of high-dose BMT with costimulatory blockade, early peripheral deletion of donor-reactive CD4 cells is still inhibited in Fas-deficient and in Bcl-xL-transgenic recipients. A. B6 recipients of high-dose BMT with costimulatory blockade (group D; n = 7) showed a substantial reduction of the percentages of Vβ5+ and Vβ11+ (but not Vβ8+, data not shown) CD4+ PBL 1 wk after BMT compared with naive B6 (n = 2) (p < 0.02) or B6 controls receiving BM without costimulatory blockade (group C; n = 2) (p < 0.05). In contrast, B6.lpr recipients of high-dose BMT with costimulatory blockade (group B; n = 5) showed no significant reduction of Vβ5+ and Vβ11+ CD4+ PBL compared with normal B6.lpr (n = 2) (p > 0.05) or a control receiving BM without costimulatory blockade (group A). The difference in the percentages of Vβ11 and Vβ5 between B6.lpr and B6 BMT recipients was statistically significant (p < 0.005 comparing groups D and B). B, B6-nontransgenic littermate controls receiving high-dose BMT with costimulatory blockade (group F; n = 4) showed the expected reduction of Vβ5+ and Vβ11+ CD4+ PBL 1 wk after BMT (p < 0.05 for comparison with naive nontransgenic littermate controls; n = 2). In contrast, Bcl-xL-transgenic recipients of high-dose BMT with costimulatory blockade (group E; n = 3) did not show a substantial reduction of Vβ5 and Vβ11 (p = 0.3 for Vβ11 and p = 0.05 for Vβ5 compared with naive nontransgenic littermate controls). The difference in the percentage of Vβ11+ CD4 cells between B6.Bcl-xL and B6 BM recipients was statistically significant (p < 0.05 comparing groups E and F; p > 0.05 for Vβ5). BMT recipients were treated with 200 × 10^6 B10.A BMC and anti-CD154 plus CTLA4Ig (A), or anti-CD154 plus anti-B7.1 and anti-B7.2 (B), respectively. The percentages of Vβ5+, Vβ11+, and Vβ8+ CD4+ PBL were determined by two-color FCAM analysis 7 days after BMT. Co-bl denotes costimulatory blockade.

Acknowledgments

We thank Drs. Arlene Sharpe and Stephen Alexander for helpful review of the manuscript, and Julia Lundell for expert secretarial assistance.

References


