Role of Immunoregulatory Donor T Cells in Suppression of Graft-Versus-Host Disease Following Donor Leukocyte Infusion Therapy

Bryon D. Johnson, Emily E. Becker, James L. LaBelle and Robert L. Truitt

*J Immunol* 1999; 163:6479-6487; 
http://www.jimmunol.org/content/163/12/6479

---

**References**

This article *cites 38 articles*, 21 of which you can access for free at:
http://www.jimmunol.org/content/163/12/6479.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
Role of Immunoregulatory Donor T Cells in Suppression of Graft-Versus-Host Disease Following Donor Leukocyte Infusion Therapy

Bryon D. Johnson, Emily E. Becker, James L. LaBelle, and Robert L. Truitt

In murine models of allogeneic bone marrow transplantation (BMT), MHC-mismatched recipients given a delayed infusion of donor leukocytes (DLI) at 21 days posttransplant develop significant GVHD whereas MHC-matched recipients do not. The current study was initially designed to test the hypothesis that small numbers of T cells in the MHC-mismatched donor bone marrow (BM) graft exacerbated graft-vs-host disease (GVHD) when DLI was administered at 21 days after BMT. Ex vivo depletion of Thy1+ cells from the donor BM had no impact on the severity of GVHD after DLI. However, depletion of donor T cells in vivo with a Thy1 allele-specific mAb given after BMT resulted in significantly more severe GVHD after DLI. Similar results were obtained in a MHC-matched model of allogeneic BMT, indicating that this was a general phenomenon and not model dependent. These results indicated that a population of donor-derived Thy1+ cells suppressed graft-vs-host reactivity after DLI. Results of experiments with thymectomized recipients demonstrated that an intact thymus was required for generation of the immunoregulatory donor cells. Experiments using TCR β-chain knockout mice as BM donors indicated that the immunosuppressive Thy1+ cells coexpressed αβ TCR heterodimers. Similar experiments with CD4 and CD8 knockout donor BM suggested that the immunoregulatory Thy1+ αβ TCR+ cells consisted of two subpopulations: a CD4+CD8− subpopulation and a CD4−CD8+ subpopulation. Together, these results show that thymus-derived, Thy1+ αβ TCR+ donor cells generated early after allogeneic BMT suppress the graft-vs-host reactivity of T cells given as DLI. These cells may mediate dominant peripheral tolerance after allogeneic BMT. The Journal of Immunology, 1999, 163: 6479 – 6487.

Cellular immunotherapy with infusions of donor lymphocytes, referred to as delayed (or donor) leukocyte infusion (DLI) therapy, has been used to induce stable remissions in patients with posttransplant leukemia relapse (1–3). Despite the large numbers of donor T cells given during DLI therapy, graft-vs-host disease (GVHD) has typically been less severe than that observed when similar numbers of donor T cells were infused at the time of bone marrow transplant (BMT). Reasons for the decreased severity of GVHD following DLI therapy are unknown.

Experimental data from our laboratory indicated that the generation of alloreactive T cells was inhibited following DLI in murine MHC-matched bone marrow (BM) chimeras (4). Mechanisms responsible for decreased GVHD following DLI might include 1) avoidance of the “cytokine storm” induced by pretransplant conditioning (5–7); 2) presence of fewer host-type APC due to the switch in hematopoiesis from host to donor (8); and/or 3) induction of immunosuppressive regulatory cells in the newly developing immune system (9–11).

While examining mechanisms for decreased graft-vs-host (GVH) reactivity following DLI, we serendipitously observed that administration of a Thy1 allele-specific mAb against donor BM-derived T cells increased the severity of GVHD in full MHC-mismatched murine recipients given DLI. Similar results were obtained in a MHC-matched model. The results led us to test the hypothesis that immunoregulatory Thy1+ cells of donor origin developed early posttransplant and suppressed the GVH reactivity of T cells given as DLI. Using thymectomized hosts as well as marrow from donors lacking αβ TCR+, CD4+, or CD8+ T cells, we determined that the immunoregulatory cell population was 1) αβ TCR+CD4−/−CD8−; 2) derived from the donor BM; and 3) educated in the repopulating host thymus. The origin and regulatory properties of this population suggest that dominant peripheral tolerance contributes to the suppression of GVH reactivity after DLI therapy.

Materials and Methods

Mice

C57BL/6 (H-2b; Thy1.2+), B6.Pl-Thy1.1+ (H-2b; Thy1.1+), B10.BR (H-2d; Thy1.2+), AKR (H-2d; Thy1.1+), C57BL/6-Cd4tm1McAk (CD4 knockout (ko)), C57BL/6-Cd8tm1Mak (CD8 ko), and C57BL/6-Tcrbtm1Mom (TCR β-chain ko) mice (4–6 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.BR-Thy1.1 congenic mice were obtained from Bruce Blazar (University of Minnesota, Minneapolis, MN) and bred at the Medical College of Wisconsin (Milwaukee, WI). All mice were housed in the Medical College of Wisconsin’s American Association for the Accreditation of Laboratory Animal Care approved Animal Resource Center.

Bone marrow transplantation and DLI therapy

Normal or surgically thymectomized (Tx) AKR recipient mice were preconditioned with a lethal dose of 1100 cGy of total body irradiation 2–24

Received for publication July 26, 1999. Accepted for publication October 6, 1999.
h before BMT. BM cells were obtained from healthy C57BL/6, C57BL/6 transgenic (CD4 ko, CD8 ko, or βTCR ko), or B10.BR donor mice by flushing the femurs and tibias with DMEM (Life Technologies, Grand Island, NY). The irradiated AKR recipients were injected i.v. with either 10^8 non-T cell-depleted or 10^7 T cell-depleted (TCD) donor BM cells. For T cell depletion, the BM cells were treated with anti-Thy1.2 mAb (clone 30-H12; obtained from the American Type Culture Collection, Manassas, VA) and Low-Tox-M complement (Accurate Chemical and Scientific, Westbury, CT). Some chimeras were treated with i.p. injections of anti-Thy1.2 mAb 7 days before BMT. Anti-Thy1.2 mAb was used in the form of bio-reactor-derived culture supernatant or partially purified from bio-reactor culture supernatants by ammonium sulfate precipitation. For DLI, 3 × 10^7 B10.BR, congenic B10.BR-Thy1.1, C57BL/6, or congenic B6.PL-Thy1 mice spleen cells were injected i.v. at day 21 or 28 after BMT.

Flow cytometric analysis

Spleen cells and purified T cells were stained with various combinations of anti-Thy1.2 mAb (clone 30-H12; FITC-conjugated), anti-2×Ko mAb (clone A6-88.5; FITC-conjugated), anti-Thy1.1 mAb (clone OX-7; FITC-conjugated), anti-CD8 mAb (clone CT-CD8a; PE-conjugated; Caltag, Burlingame, CA), and anti-CD4 mAb (clone GK1.5; PE-conjugated; Pharmingen, San Diego, CA), and anti-CD4 (clone AF6-88.5; FITC-conjugated), anti-Thy1.1 mAb (clone OX-7; FITC-conjugated) with anti-CD8-conjugated microbeads. Enrichment of H-2b/Thy1.1 cells was confirmed by two-color flow cytometric analysis. To assess alloreactive Th cell frequencies, the Thy1.1-enriched responder cells were cocultured at 10^6 different concentrations in 24-replicate U-bottom microwells with 3 × 10^9 irradiated (3000 cGy) AKR dendritic cells and 10^5 irradiated (700 cGy) AKR B cells (200 μl/well total volume). Dendritic cells and B cells were enriched by MACS cell separation using CD11c-conjugated or B220-conjugated microbeads, respectively. After 4 days of culture in a 37°C, 10% CO_2 incubator, 150 μl of culture supernatant was removed from each well and transferred to a new microwell plate containing 2500 IL-2-dependent SAC 9.12 (12) indicator cells and 1 μCi of [3H]thymidine. The plates were cultured for an additional 18–24 h, and thymidine uptake was assessed. Spontaneous thymidine uptake was determined from the supernatants of 24 wells that had contained stimulator cells only.

To assess CTL frequencies, various numbers of Thy1.1-enriched responder cells were cocultured in U-bottom microwells (24 wells per responder concentration) with 3 × 10^9 irradiated (3000 cGy) AKR dendritic cells and 2 × 10^10 irradiated (700 cGy) AKR B cells. Con A-stimulated rat spleen cell-conditioned medium was added to the wells at a concentration of 50% (v/v) (200 μl/well total volume). The plates were cultured for 8 days. On day 8, 50 μl of culture supernatant was removed from each well and replaced with 50 μl of [51Cr]-labeled AKR Con A-stimulated lymphoblast targets (5000/well). [51Cr] release was assessed after a 3.5-h incubation. Spontaneous and maximum [51Cr] release values were determined from 24 wells containing target cells plus stimulators only or detergent (2.5% TX-100 detergent; Flow Laboratories, McLean, VA), respectively.

Individual wells in the Th cell and CTL assays were scored as positive when experimental values (cpm [3H]thymidine uptake or percent specific [51Cr] release) exceeded the spontaneous control values by at least 3 SDs. The frequencies of alloreactive Th cells and CTL was calculated by χ^2 minimization as described by Taswell (13).

Statistics

Kaplan-Meier survival curves were compared using log-rank statistical analysis. The Student t test was used to analyze the percentages and numbers of donor T cells in the spleens of BMT chimeras given DLI.

FIGURE 1. GVHD following DLI at 21 days after BMT is significantly more severe in MHC-mismatched chimeras than in MHC-matched chimeras. Irradiated AKR recipients were transplanted with 10^7 non-T-depleted B10.BR or C57BL/6 BM cells. The recipients were randomized to receive no further treatment (BM only) or DLI with 3 × 10^7 B10.BR or C57BL/6 spleen cells at 21 days after BMT. The mice were followed for survival (A) and body weight changes (B). Survival curves are the combined data of two experiments, and the body weight curves are from one representative experiment.

Results

Elimination of donor BM-derived Thy1.2^+ cells posttransplant with anti-Thy1.2 mAb increased the severity of GVHD induced by DLI

MHC-matched BM chimeras (B10.BR-into-AKR) given DLI at 21 days after marrow transplantation survived without GVHD (Fig. 1A). In contrast, MHC-mismatched (C57BL/6-into-AKR) chimeras developed severe GVHD after DLI, which resulted in 67% GVH-associated mortality (Fig. 1A). GVHD in the MHC-mismatched chimeras was reflected by a sharp loss in body weight within the first week after donor cell infusion (Fig. 1B), followed by continued body weight loss during the remainder of the experiment. These results confirmed earlier work from our laboratory (4, 14, 15).

To explain the disparate results between the MHC-matched and mismatched BM chimeras, we initially hypothesized that small
numbers of donor T cells in the MHC-mismatched BM grafts (~1–3%) augmented the alloreactivity of donor T cells contained in the DLI inoculum, thereby increasing the severity of DLI-induced GVHD. We used two different experimental approaches to test this hypothesis. First, we depleted the donor BM of T cells ex vivo with anti-Thy1 mAb and complement before BMT. If mature donor T cells in the marrow graft were promoting GVH reactivity of T cells given as DLI, we speculated that ex vivo depletion of the donor T cells would result in less severe GVHD after DLI. AKR recipients were transplanted with untreated or TCD C57BL/6 BM and then randomized to receive no further treatment (BM controls) or to be given DLI at 21 days after BMT (Fig. 2). Control mice given MHC-mismatched BM alone (no DLI) survived without any clinical evidence of GVHD and showed only a transient 10% body weight loss between 3 and 5 wk posttransplant. All BM-only control mice treated with anti-Thy1.2 survived 90 days and did not show any significant body weight loss, indicating that the mAb treatment alone was not toxic. Taken together, these results contradicted our original hypothesis and suggested instead that a population of donor BM-derived Thy1.2<sup>+</sup> cells suppressed, rather than increased, the severity of GVHD after DLI.

Time after BMT is an important variable for avoiding GVHD after DLI therapy (4). We previously reported that GVHD persisted in the circulation of treated animals for several weeks (data not shown). Therefore, to avoid potential problems with residual circulating anti-Thy1.2 mAb, spleen cells from Thy1.1<sup>+</sup> congenic H-2<sup>b</sup> donors (B6.PL-Thy1<sup>a</sup>) were given as DLI. Surprisingly, as shown in Fig. 3A, chimeras pretreated with anti-Thy1.2 mAb in vivo showed accelerated and increased GVHD-associated mortality after DLI as compared with non-Ab-treated chimeras. Mice in both experimental groups had a sharp loss in body weight following DLI (Fig. 3B) and developed clinically evident signs of GVHD including diarrhea and ruffled fur; however, clinical symptoms in the anti-Thy1.2-treated chimeras were more severe. Control mice given MHC-mismatched BM alone (no DLI) survived without any clinical evidence of GVHD and showed only a transient 10% body weight loss between 3 and 5 wk posttransplant. All BM-only control mice treated with anti-Thy1.2 survived >90 days and did not show any significant body weight loss, indicating that the mAb treatment alone was not toxic. Taken together, these results contradicted our original hypothesis and suggested instead that a population of donor BM-derived Thy1.2<sup>+</sup> cells suppressed, rather than increased, the severity of GVHD after DLI.

As a second approach, we treated BM chimeras before DLI with a Thy1.2-specific mAb to deplete donor T cells in vivo. This would determine whether donor T cells generated de novo from BM precursors contributed to GVH reactivity after DLI. In the initial experiments, we administered anti-Thy1.2 mAb on days 10 and 12 after BMT followed by DLI at 21 days after BMT. The mAb
avoided in MHC-mismatched BM chimeras if DLI was delayed from 21 days until 28 days posttransplant (15). Because of the surprising results described above, experiments were done to test whether depletion of donor marrow-derived Thy1.2 \(^+\) T cells would result in increased GVHD when DLI was given at 28 days posttransplant. AKR recipients were transplanted with untreated or TCD BM. Anti-Thy1.2 mAb was given to chimeras on days 10, 12, 24, and 26 after BMT and was followed with DLI at 28 days after BMT. The additional Ab treatments given on days 24 and 26 were to ensure that Thy1.2 \(^+\) cells were depleted at the time of DLI. T depletion was confirmed by flow cytometric analysis (data not shown). As shown in Fig. 4A, 97% (30/31) of chimeras treated with anti-Thy1.2 mAb died within 2 wk after DLI. Death was paralleled by a significant decrease in body weight (Fig. 4B). Ex vivo T depletion of the BM inoculum had no impact on the timing of mortality. Untreated BM chimeras had a significantly higher survival rate than the anti-Thy1.2-treated chimeras, and body weight loss was decreased. Differences in GVH-associated mortality between the BM chimeras transplanted with nondepleted or TCD BM were not significant (Fig. 4A), confirming the results of Fig. 2. These data supported the hypothesis that a donor marrow-derived population of Thy1.\(^+\) “immunoregulatory” cells suppressed GVHD following DLI in MHC-mismatched BM chimeras. The suppressive effect of the cells was more pronounced at 28 days than at 21 days posttransplant, but, in both instances, their removal resulted in intensified GVHD after DLI.

\(\text{Thy}1^+\) regulatory cells also suppressed GVHD in MHC-matched chimeras given DLI

The presence of Thy1.\(^+\) regulatory cells was examined in an established MHC-matched BMT model (B10.BR-into-AKR) to determine whether the observations made in the MHC-mismatched combination were model specific. AKR recipients, transplanted with non-T-depleted B10.BR BM cells, were treated with anti-Thy1.2 mAb on days 10, 12, 17, and 19 after BMT. For DLI, \(3 \times 10^7\) B10.BR-Thy1.1 spleen cells were administered at day 21 after BMT. Control non-Ab-treated BM chimeras given DLI did not develop lethal GVHD and maintained their body weights after DLI (Fig. 5). In contrast, chimeras treated with anti-Thy1.2 mAb developed GVHD after DLI, resulting in 75% mortality (Fig. 5A). GVH reactivity in Ab-treated chimeras was accompanied by a significant loss of body weight (Fig. 5B). These results indicated that suppression of DLI-mediated GVH reactivity by donor-derived Thy1.\(^+\) immunoregulatory cells was a general phenomenon and not model dependent.
Depletion of Thy1.2<sup>+</sup> immunoregulatory cells resulted in elevated numbers of host-reactive CTL and Th cells in the spleens of DLI chimeras

BM chimeras pretreated with anti-Thy1.2 mAb developed significantly more severe GVHD after DLI, as indicated by increased mortality and body weight loss. To determine whether the frequencies of host-reactive CTL and Th cells increased as a result of depletion of the immunoregulatory Thy1<sup>+</sup> cells, spleen cells were collected from MHC-mismatched BM chimeras 7 days after DLI. Flow cytometric and LDA assays were performed. Thy1.1<sup>+</sup> T cells in the DLI inoculum could be distinguished from residual host T cells by differential MHC class I expression (H-2K<sup>b</sup>). As shown in Table I, the anti-Thy1.2-treated chimeras had significantly increased percentages and absolute numbers of splenic T cells derived from the DLI inoculum as compared with non-mAb-treated controls. Infused CD8<sup>+</sup> T cells accounted for the majority of this increase (data not shown). Results of LDA assays performed on splenocyte preparations from anti-Thy1.2-treated chimeras 7 days after DLI indicated that significantly greater numbers of both anti-host-reactive CTL and Th cells were present as compared with the untreated BM chimeras (Table II). Anti-Thy1.2-treated chimeras had 15-fold and 3-fold increases in anti-host-reactive CTL and Th cells, respectively. Together, the data in Tables I and II provided further evidence that donor marrow-derived Thy1<sup>+</sup> cells down-regulated anti-host (GVHD) alloreactivity after DLI.

TABLE I. Elimination of donor BM-derived Thy1<sup>+</sup> cells before DLI resulted in higher percentages and greater numbers of infused donor T cells in the spleens of DLI-treated chimeras

<table>
<thead>
<tr>
<th>Anti-Thy1.2&lt;sup&gt;+&lt;/sup&gt;</th>
<th>% Infused Donor T Cells/Spleen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of Infused T Cells/ Spleen (&lt;i&gt;×10&lt;sup&gt;6&lt;/sup&gt;&lt;/i&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>6.1 ± 1.8</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>Yes</td>
<td>16.0 ± 3.3*</td>
<td>8.5 ± 2.2*</td>
</tr>
</tbody>
</table>

<sup>a</sup> AKR hosts, preconditioned for BMT with 1100 cGy, were transplanted with 10<sup>7</sup> T-depleted C57BL/6 BM cells. On days 24 and 26 posttransplant, designated chimeras were treated with 500 μg anti-Thy1.2 mAb i.p. On day 28 posttransplant, all chimeras were given DLI with 3 × 10<sup>7</sup> B6.PL spleen cells.

<sup>b</sup> The chimeras were sacrificed 7 days post-DLI and individual spleens were tested by flow cytometric analysis to determine the percentage and absolute number of infused donor T cells. The data is from the combined results of two experiments and the values shown are the averages per spleen ± SD.

* p < 0.05.

FIGURE 6. An intact host thymus was required for de novo generation of the donor BM-derived immunoregulatory cells. Irradiated normal or Tx AKR recipients were transplanted with 10<sup>7</sup> T-depleted C57BL/6 BM cells. The hosts were randomized to receive no treatment or injected i.p. with 500 μg of anti-Thy1.2 mAb on days 24 and 26 posttransplant. All chimeras were given DLI with 3 × 10<sup>7</sup> B6.PL-Thy1<sup>a</sup> spleen cells at 28 days after BMT. Survival curves (A) represent the combined data from two experiments; body weight curves (B) are from one representative experiment.

Donor Thy1<sup>+</sup> immunoregulatory cells that suppressed GVHD after DLI are generated de novo in the host thymus

Because pretransplant depletion of T cells had no effect on DLI-induced GVHD, but in vivo depletion of Thy1<sup>+</sup> cells increased GVHD after DLI, we hypothesized that the BM-derived immunoregulatory cells were generated de novo in the repopulating host thymus. Emigration of Thy1.2<sup>+</sup> T cells from the donor-reconstituted host thymus begins ~14 days after BMT (B. Johnson, unpublished data). To prove that the immunoregulatory donor cells were derived from the repopulated host thymus after DLI, AKR recipients were thymectomized ~2 wk before BMT. Tx or normal euthymic AKR recipients were transplanted with T-depleted C57BL/6 BM and then given DLI at day 28 posttransplant. Separate groups of Tx and normal recipients were treated with anti-Thy1.2 mAb on days 24 and 26 after BMT and then given DLI on day 28. All nonthymectomized chimeras treated with anti-Thy1.2 mAb died from severe GVHD within 16 days after DLI (Fig. 6A). The Tx chimeras with or without anti-Thy1.2 mAb treatment developed severe GVHD after DLI. The kinetics of body weight loss and mortality paralleled that of the anti-Thy1.2-treated nonthymectomized group (Fig. 6). These results demonstrated that an intact thymus was required for generation of the donor-derived Thy1<sup>+</sup> immunoregulatory cells. The nonthymectomized chimeras given DLI had an overall survival rate of 78% and maintained their average body weight near pretransplant levels (Fig. 6).
Immunoregulatory donor Thy1\(^+\) cells are αβ TCR\(^+\)CD4\(^+\)CD8\(^-\) and αβ TCR\(^+\)CD4\(^-\)CD8\(^+\)

Experiments were designed to further characterize the donor-derived host-educated Thy1\(^+\) immunoregulatory cells. The first set of experiments addressed whether the immunoregulatory cells co-expressed αβ heterodimers of the TCR by using mice with a ko mutation for the β-chain of the TCR as BM donors. These mice do not produce T cells expressing αβ TCR heterodimers (16). AKR recipients were transplanted with TCD BM from normal C57BL/6 mice or TCD BM from C57BL/6 βTCR ko mice. If the immunoregulatory cells were αβ TCR\(^+\), the severity of DLI-induced GVHD in chimeras transplanted with βTCR ko BM should be comparable to that of chimeras treated with anti-Thy1.2 mAb. The transplanted chimeras were randomized to be treated or not treated with anti-Thy1.2 mAb on days 24 and 26 posttransplant. DLI was administered on day 28 posttransplant. Groups of chimeras given BM alone served as GVH-negative controls. Non-Ab-treated chimeras transplanted with normal BM developed only mild GVHD after DLI and had a 70% survival rate (Fig. 7). In contrast, chimeras transplanted with βTCR ko BM developed severe GVHD, and the kinetics of mortality and body weight loss (data not shown) was similar to the groups treated with anti-Thy1.2 mAb. As described previously, C57BL/6 into-AKR chimeras pretreated with anti-Thy1.2 mAb rapidly developed severe and lethal GVHD following DLI. These results indicated that the donor Thy1\(^+\) immunoregulatory cells were αβ TCR\(^+\).

In a second set of experiments, we addressed whether the Thy1\(^+\)αβ TCR\(^+\) immunoregulatory donor cells belonged to either the CD4 or CD8 T cell subset. For these experiments, mice with ko mutations for CD4 or CD8 were used as BM donors. AKR recipients were transplanted with T-depleted BM from either normal, CD4 ko, or CD8 ko C57BL/6 mice. The transplanted recipients were again randomized to be treated or not treated with anti-Thy1.2 mAb on days 24 and 26 posttransplant, followed by DLI on day 28 after BMT. A group of recipients given normal T-depleted BM only was included as a GVH-negative control. Mice in the experimental groups treated with anti-Thy1.2 mAb (filled symbols in Fig. 8) developed severe and lethal GVHD after DLI regardless of the BM source. Recipients of normal BM and DLI (but no mAb) lost body weight after DLI (data not shown), but there were no deaths (Fig. 8), indicating that GVH reactivity was relatively mild when the immunoregulatory cells were not depleted. Chimeras transplanted with CD8 ko BM also developed only mild GVHD after DLI as indicated by low mortality (18%) and modest weight loss. Survival of these chimeras was not significantly different from that of the GVH-negative controls (p > 0.05). These results indicate that the Thy1\(^+\)αβ TCR\(^+\) immunoregulatory cells do not belong to the CD8 T subset.

Chimeras transplanted with CD4 ko BM developed severe GVHD after DLI as indicated by acute body weight loss (data not shown) and 88% mortality (Fig. 8). However, although survival of these chimeras was significantly worse than that of AKR hosts given normal BM and DLI (p < 0.00008), the kinetics of death was delayed as compared with the anti-Thy1.2-treated chimeras, and median survival time was longer by ~20 days. Because BMT with CD4 ko BM did not completely eliminate the suppression of DLI-induced GVHD, we speculate that there may be two subpopulations of Thy1\(^+\)αβ TCR\(^+\) immunoregulatory cells: one of which is CD4\(^+\) and the other CD4\(^-\).

Discussion

Results shown in Fig. 1 of this paper confirm our previous report (15) that DLI given 21 days after BMT induced lethal GVHD in MHC-mismatched recipients but did not induce GVHD in MHC-matched recipients. Increased immunogenetic disparity in the MHC-mismatched model (i.e., greater alloreactivity) may contribute to the disparate results in these two models. We hypothesized that because of the higher frequency of alloreactive T cells in the MHC-mismatched BMT model, the small numbers of mature T cells in non-T-depleted donor BM intensified GVH reactivity after DLI in MHC-mismatched BM chimeras. This hypothesis was based on our observation that although the number of T cells in the MHC-mismatched marrow grafts were below the threshold needed to induce acute GVHD, transient body weight loss (5–15%) often
occurred 3–6 wk after BMT (for examples, see Figs. 1B and 3B). In contrast, significant body weight loss did not occur in MHC-matched recipients given BM only (Fig. 1B). The early and transient body weight loss in MHC-mismatched recipients was eliminated if the donor BM was depleted of mature T cells, indicating that T cells were involved. Two different strategies were used to address our hypothesis: 1) ex vivo depletion of donor T cells from the BM graft and 2) in vivo depletion of BM-derived donor T cells with anti-Thy 1 mAb. Ex vivo depletion of T cells from the BM graft had no effect on the severity of GVHD after DLI (Fig. 2). To our surprise, when recipient mice were treated in vivo with anti-Thy 1.2 mAb after BM but before DLI, GVH-associated mortality and body weight loss were significantly more severe when DLI therapy was given (Figs. 3 and 4). This phenomenon was not unique to the MHC-mismatched donor-host combination. Similar results were observed in an MHC-matched BMT model (Fig. 5). These serendipitous results led us to hypothesize that a population of Thy1+ donor cells developed early after BMT and suppressed GVH reactivity of the infused donor cells. Subsequent studies revealed that the immunoregulatory Thy1+ cells coexpressed αβTCR heterodimers and that an intact host thymus was required for their development (Figs. 6 and 7). Results from experiments using CD4 and CD8 ko mice as BM donors established that αβTCR CD4+CD8– and αβTCR CD4–CD8+ regulatory cells played key roles in suppressing GVH reactivity after DLI (Fig. 8).

Immunoregulatory CD4+ cells have been shown to be involved in BMT tolerance (17), solid organ allograft tolerance (18), and skin graft tolerance (19, 20). There are numerous reports describing the role of CD4+CD8– immunoregulatory cells in various models of autoimmune disease (reviewed in Ref. 21). Typically, these cells have been found to express the CD45RB molecule at low levels, which is a characteristic of memory T cells. Unlike typical memory T cells, these cells fail to proliferate in response to polyclonal activation and have tolerogenic properties (22, 23). They become detectable in peripheral lymphoid tissues shortly after birth, and an intact thymus is required for their development (24–27). Elimination of the CD4+ regulatory cells in both normal and autoimmune-susceptible animals has resulted in the development and/or acceleration of various autoimmune diseases, suggesting that these cells actively suppress autoreactive T cells that escape thymic selection.

To our knowledge, T cells with a CD4+ phenotype have not been previously described as playing an important role in suppression of GVHD after allogeneic BMT. Tutschka et al. (9–11) published a series of papers in the early 1980s describing the involvement of suppressor cells in immune tolerance after BMT. They reported that histoincompatible rat BM chimeras that survived acute GVHD demonstrated tolerance to host alloantigens and possessed T lymphocytes that suppressed donor-anti-host mixed lymphocyte reactions in vitro (10). Furthermore, chimeric splenocytes containing the suppressor T cells could adoptively transfer suppression of GVHD to secondary hosts. Long-term BM chimeras (250 days after BMT) possessing the suppressor cells did not develop GVHD when given DLI with small numbers of donor-type cells. However, if the chimeras were treated with immunosuppressive agents such as cyclophosphamide or radiation before DLI, tolerance was disturbed and the mice developed GVHD. Perhaps the immunoregulatory Thy1+αβTCR+CD4+−/− cells identified by us are similar to the immunosuppressive T cells described in those earlier studies.

Hess and colleagues (28, 29) have demonstrated that elimination of a peripheral regulatory mechanism is required for the development of cyclosporin A (CSA)-induced autologous GVHD. The primary autoregulatory activity appears to reside within the CD4+ T subset. Recently, Wu and Goldschneider (30) demonstrated that the immunoregulatory CD4+CD8– cells are recent thymic emigrants that are exported to the peripheral lymphoid organs within a few days after cessation of CSA treatment. Their results suggest that the regulatory T cells actively suppress autoreactive T cells.
that have escaped intrathymic negative selection during CSA treatment. Similarly, development of the immunosuppressive regulatory cells described in our studies required an intact thymus (Fig. 6). Modigliani et al. (20, 31) have shown that the thymic epithelium is involved in generating CD4+ regulatory T cells that maintain peripheral tolerance to Ags not present in the thymus. This form of tolerance has been referred to as active or dominant peripheral tolerance (21, 32). Perhaps the αβ TCR CD4+ CD8− regulatory cells identified from our work emerge from the reconstituting thymus after BMT to help maintain dominant peripheral tolerance over anti-host-reactive T cells that have escaped thymic selection. Donor leukocytes infused into chimeric hosts after BMT may be seen by the donor-derived, host thymus-selected immunoregulatory T cells as “autoactive” since they respond to “host-self” but express “donor-self” histocompatibility Ags.

Although administration of CD4 knock-out donor BM significantly reduced the suppression of DLI-induced GVHD (Fig. 8), the suppressive effect was not completely eliminated. These results suggest that αβ TCR CD4+ CD8− cells also are involved in the suppression of GVHD after DLI. Cells with this phenotype have been previously found to exhibit suppressor activity, inhibit GVHD, and facilitate tolerance in mice (33–35). Cells expressing the NK1 Ag with an αβ TCR CD4+ CD8− phenotype have been shown by others to exhibit immunoregulatory properties (36, 37). The NK1+ cells described by Sykes et al. (36) were able to suppress GVHD. Experiments are in progress to examine whether NK1+ αβ TCR+ cells contribute to the suppression of GVHD in our DLI models.

It is noteworthy that when the immunoregulatory Thy1+ αβ TCR+ cells were eliminated before DLI, the kinetics of GVHD-related mortality in MHC-mismatched chimeras was similar to that seen when irradiated hosts were given the donor splenocytes at the time of BMT (15). This suggests that, at 3–4 wk after BMT in mice, active suppression mediated by the immunoregulatory cells described in this report may be the principal mechanism responsible for suppressing GVHD after DLI. We speculate that these immunoregulatory cells are particularly important because they are being actively produced by the newly donor-reconstituted host thymus to mediate dominant peripheral tolerance.

In summary, we have identified an active regulatory/suppressor mechanism in mice which plays an important role in suppressing GVH reactivity after DLI. The regulatory activity appears to be mediated by at least two populations of donor BM-derived, host thymus-selected cells: Thy1+ αβ TCR+ CD4+ CD8− cells and Thy1+ αβ TCR+ CD4+ CD8− cells. These immunoregulatory cells may be important for maintaining dominant peripheral tolerance. If regulatory cells similar to those described in this report can be identified in humans, their immunosuppressive properties could be exploited to reduce the incidence and severity of GVHD following DLI. The possible role of thymic-derived regulatory T cells raises questions about age-related intensification of GVHD after BMT (38, 39) and about the more severe level of GVHD that occurs after DLI in humans (1, 3).

References


10. Tutschka, P. J., A. D. Hess, W. E. Beschorner, and G. W. Santos. 1981. Suppressor cells in transplantation tolerance. III. Evidence that regulatory cells similar to those described in this report can be identified in humans, their immunosuppressive properties could be exploited to reduce the incidence and severity of GVHD following DLI. This possible role of thymic-derived regulatory T cells raises questions about age-related intensification of GVHD after BMT (38, 39) and about the more severe level of GVHD that occurs after DLI in humans (1, 3).

References


10. Tutschka, P. J., A. D. Hess, W. E. Beschorner, and G. W. Santos. 1981. Suppressor cells in transplantation tolerance. III. Evidence that regulatory cells similar to those described in this report can be identified in humans, their immunosuppressive properties could be exploited to reduce the incidence and severity of GVHD following DLI. This possible role of thymic-derived regulatory T cells raises questions about age-related intensification of GVHD after BMT (38, 39) and about the more severe level of GVHD that occurs after DLI in humans (1, 3).

References


