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Host Conditioning Is a Primary Determinant in Modulating the Effect of IL-7 on Murine Graft-versus-Host Disease

Maria Gendelman,* Toby Hecht,§ Brent Logan,† Sanja Vodanovic-Jankovic,* Richard Komorowski,‡ and William R. Drobyski 2*

Interleukin-7 has been shown to enhance T cell reconstitution after allogeneic bone marrow transplantation, in part, by expansion of mature donor T cells, but whether IL-7 also exacerbates graft-vs-host disease (GVHD) remains unresolved. To address this issue, we examined the effect of IL-7 on GVHD induction using a well-defined murine GVHD model (B6→B6AF1/J). Administration of IL-7 to nonirradiated B6AF1/J recipients of B6 T cells resulted in expansion of splenic donor CD4⁺ and CD8⁺ T cells and increased GVHD mortality. In contrast, administration of IL-7 on the same schedule failed to increase GVHD mortality in either sublethally or lethally irradiated animals that received graded doses of T cells designed to induce varying degrees of GVHD severity. Moreover, IL-7 failed to increase the number of alloreactive T cells when examined in a murine model (B6→BALB.B) that allowed for direct quantitation of graft-vs-host-reactive T cells. The combination of irradiation and transplantation of alloreactive donor T cells resulted in significantly increased levels of endogenous splenic IL-7 mRNA when compared with nonirradiated transplanted animals, providing a potential explanation for why exogenous IL-7 did not increase GVHD severity in these mice. We conclude that host conditioning modulates the ability of exogenous IL-7 to exacerbate GVHD and that this occurs through induction of endogenous IL-7 production. The Journal of Immunology, 2004, 172: 3328–3336.

Impaired T cell reconstitution is one of the major complications after allogeneic bone marrow transplantation (BMT), and is largely responsible for the increased risk of opportunistic infections that exists in this patient population (1, 2). T cell reconstitution posttransplantation has been shown to be dependent upon the generation of new donor T cells in the recipient thymus as well as the expansion of mature T cells in the periphery (3). Unfortunately, both pathways of T cell development are often adversely affected by the conditioning regimen itself or complications that arise in the post-BMT period. The generation of new donor T cells is impaired in the setting of an age-related decline in thymic function that is operative in the majority of transplant patients (4). Moreover, toxicity from the conditioning regimen causes direct damage to the thymus and inhibits the production of growth factors necessary for thymocyte development (5, 6). Finally, graft-vs-host (GVH) disease (GVHD) can directly target the thymus and further compromise thymic function (7–9). In the majority of patients, the T cell repertoire and effective T cell immunity are the consequence of mature T cells transferred from the marrow graft (10). Because these cells are not tolerant of host alloantigens, they are capable of inducing GVHD. Moreover, survival of both host-reactive and nonhost-reactive donor T cells can be compromised due to an increased susceptibility to undergo apoptotic cell death in the setting of a GVH reaction (11–13). The consequence of these events is that T cell immunity against endogenous and exogenous infectious agents is often compromised.

The clinical consequences of impaired T cell reconstitution have been the impetus for preclinical approaches designed to augment T cell immunity posttransplantation as a way to improve overall host immunity. One promising approach has focused on the use of IL-7 as a means to accelerate T cell reconstitution. IL-7 is a cytokine that is produced by both thymic and marrow stromal cells as well as dendritic cells (14–18), and has been shown to be critical for maintenance of T cell homeostasis (19, 20). IL-7 has also been shown to have a number of other effects on T cells, including the ability to provide a costimulatory signal for T cell activation (21), inhibit programmed cell death (22), and enhance Th1-type T cell responses (23, 24). A number of murine studies have demonstrated that administration of IL-7 can enhance lymphocyte recovery in murine recipients undergoing BMT (25–27). This has been attributable to augmentation of thymopoiesis as well as expansion of mature peripheral T cells (28). Although expansion of mature donor T cells may be beneficial with respect to augmenting immunity early after BMT, these same cells have the potential to exacerbate GVHD because they are not tolerant of recipient alloantigens. The ability of specific cytokines to exacerbate or inhibit GVHD, however, is dependent upon a number of variables such as timing of administration (29, 30) and the intensity of the conditioning regimen (30, 31). The purpose of this study was to examine how these variables affected the ability of IL-7 to modulate the severity of GVHD in murine allogeneic marrow transplant recipients.

Materials and Methods

Mice

C57BL/6 (B6) (H-2b), BALB.B (H-2b), and (C57BL/6 × A/J)F1 (B6AF1/J, H-2b) mice were bred in the Animal Resource Center at the Medical College of Wisconsin or purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were housed in the American Association for...
Laboratory Animal Care-accredited Animal Resource Center of the Medical College of Wisconsin. Mice received regular mouse chow and acidified tap water ad libitum.

**Reagents**

Human rIL-7 was obtained from the National Cancer Institute (Frederick, MD). IL-7 was reconstituted in PBS and administered at a dose of 5 μg/day to murine transplant recipients.

**CD4+ and CD8+ T cell subset enrichment**

To obtain highly enriched populations of CD4+ T cells, B6 spleen cells were passed through nylon wool columns, and then CD4+ T cells were positively selected using the MACS magnetic cell separation system (Miltenyi Biotec, Auburn, CA). A similar procedure was done to isolate highly enriched CD8+ T cells. Cells were analyzed by flow cytometry to confirm purity before transplantation. Typically, >90% purity was obtained for the positively selected T cell subset with <2% contamination of the reciprocal subset.

**BM transplantation**

BM was flushed from donor femurs and tibias with DMEM and passed through sterile mesh filters to obtain single cell suspensions. BM was T cell depleted in vitro with anti-Thy-1.2 mAb plus low toxicity rabbit complement (C-SIX Diagnostics, Mequon, WI). The hybridoma for 30-H12 (anti-Thy-1.2, rat IgG2b) Ab was purchased from the American Type Culture Collection (Manassas, VA). BM cells were washed and resuspended in DMEM before injection. Naive donor T cells were obtained by passing erythrocyte-depleted spleen cells through nylon wool columns to remove non-T cells. Red cells were removed by hypotonic lysis using distilled water. Host mice were conditioned with total body irradiation (TBI) administered as a single exposure at a dose rate of 67 cGy using a Shepherd Mark I cesium irradiator (J.L. Shepherd and Associates, San Fernando, CA). Irradiated recipients received a single i.v. injection of T cell-depleted (TCD) BM (10^7 cells) with or without added T cells. Nonirradiated recipients received T cells alone without BM.

**Experimental design**

GVHD was first assessed in a parent→F1, model to examine GVH reactivity in the absence of a conditioning regimen. In this model, nonirradiated B6AF1/J mice were transplanted with 25 × 10^6 purified B6 T cells. In subsequent studies, the effect of a conditioning regimen on GVHD mortality was assessed using the same model in which B6AF1/J recipients were sublethally (500 cGy) or lethally (1000 cGy) irradiated and transplanted with TCD B6 BM alone or admixed with graded doses of B6 T cells.

**Flow cytometric analysis and assessment of chimerism**

mAbs conjugated to either FITC or PE were used to assess chimerism in marrow transplant recipients. PE anti-CD5 (clone CT-CD5a, rat IgG2a) was obtained from Caltag (San Francisco, CA). PE anti-TCR αβ (clone H57-597, hamster IgG), PE anti-CD4 (clone GK1.5, rat IgG2b), CyChrome anti-CD3, and FITC anti-CD8-β2 (clone AF3-12.1, mouse IgG1) were all purchased from BD Pharamingen (San Diego, CA). Spleen cells were obtained from chimeras at different time points after transplantation, processed into single cell suspensions, and stained for two- or three-color analysis. Cells were analyzed on a FACScan flow cytometer (BD Biosciences, Mountain View, CA). The absolute number of splenic donor or host T cell subpopulations was determined by analyzing cells within a gate that included the entire spleen cell population after exclusion of debris and residual red cells. At least 10,000 cells were analyzed for each determination whenever possible.

**Histological analysis**

Representative samples of liver and colon were obtained from transplanted recipients and fixed in 10% neutral-buffered formalin. Samples were then embedded in paraffin, cut into 5-μm-thick sections, and stained with H&E. Semi-quantitative scoring systems were used to account for histological changes consistent with GVHD. The degree of portal triad lymphocytic infiltration was assessed in the liver, while the severity of crypt cell apoptosis and overall crypt destruction was evaluated in the colon. Histological tissue damage compatible with GVHD in the liver was graded as 0 for normal, 1 for mild, 2 for moderate, and 3 for severe, while tissue injury in the colon was graded as 0 for normal, 1 for mild, and 2 for moderate. Scores were added to provide a composite score for each animal (maximal score 5/mouse). All slides were coded and read in a blinded fashion.

**Quantitation of GVH-reactive T cells**

Soluble H-2Kb Ig dimer (BD Pharamingen) was incubated with the immunodominant peptides from H-2Db (PEVYRNK) or OVA (SIINFEKL) for 48 h at 4°C, according to the manufacturer’s instructions. Peripheral blood or spleen cells were blocked with 1 μg of purified anti-mouse IgG Ab and 0.5 μg of Fc block for 20 min. Cells were then incubated with 4 μg of peptide-loaded H-2Kb: Ig fusion protein at 4°C for 1 h and then stained with PE anti-mouse IgG and FITC anti-CD8 Abs for 30 min at 4°C. At least 25,000 events were analyzed for each determination whenever possible. The percentage of H60-specific CD8+ T cells was determined by subtracting the percentage of CD8+ OVA+ T cells (background) from the percentage of CD8+ H60+ T cells for each individual animal.

**Quantification of IL-7 gene expression by real-time PCR**

Total RNA was isolated from spleen cells after extraction from TR1zol reagent (Invitrogen, Carlsbad, CA). Five hundred nanograms of RNA were reverse transcribed into cDNA using a Superscript II First-Strand RT-PCR system (Invitrogen), according to the manufacturer’s instructions. Primers and probes for 18S ribosomal RNA and IL-7 were purchased from Applied Biosystems (Foster City, CA). cDNA was suspended in 2× master mix along with 18S or IL-7 primer/probes, according to the manufacturer’s specifications. Real-time PCR was performed on a DNA Engine Opticon-2 (MJ Research, Watertown, MA). Reaction conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The amount of IL-7 transcript, normalized to amount of 18S ribosomal RNA as an internal standard, was calculated by the comparative C_t method (ABI User Bulletin 2; Applied Biosystems).

**Statistics**

Group comparisons of the absolute number of splenic donor and host T cells were performed using the Mann-Whitney U test. The relative increase in IL-7 mRNA levels between groups was compared using the unpaired Student’s t test. Survival curves were constructed using the Kaplan-Meier product limit estimator and compared using the log rank rest. A value of p ≤ 0.05 was deemed to be significant in all experiments.

**Results**

Administration of IL-7 results in increased donor and host T cell expansion after BMT in nonirradiated mice

The effect of IL-7 administration on donor and host T cell expansion was first examined in the absence of a conditioning regimen by performing transplant experiments using a parent→F1, model in which there is no competing host-vs-graft response. B6AF1/J recipients were transplanted with 25 × 10^6 B6 T cells and administered either PBS or IL-7 (5 μg/day) on days 0−9, unless terminated before completion of the regimen. Cohorts of mice were then sacrificed either 6 or 11−13 days after transplant to assess the kinetics of T cell reconstitution in the spleens of transplant recipients. Although host T cells were the predominant T cell population in both groups of animals 6 days post-BMT, IL-7 treatment resulted in a significant increase in the absolute number of host αβ T cells compared with what was observed in PBS-treated mice (72 × 10^6 vs 51 × 10^6, p = 0.03) (Table I). Administration of IL-7 also increased the absolute numbers of donor CD4+ T cells (14 × 10^6 vs 10 × 10^6, p = 0.02), but there were no statistically significant differences in donor CD8− or αβ T cells. By days 11−13 posttransplantation, donor T cells were the primary T cell population in both cohorts of animals. IL-7–treated mice had a greater absolute number of splenic donor αβ T cells when compared with animals treated with PBS (55 × 10^6 vs 32 × 10^6, p = 0.03) (Table I). This difference was observed for both CD4+ (18 × 10^6 vs 11 × 10^6, p = 0.002) and CD8− (33 × 10^6 vs 19 × 10^6, p = 0.005) T cell populations. The absolute number of host αβ and CD4+ T cells was also significantly greater in IL-7–treated animals, although there was no difference in total host CD8+ T cells. Due to the fact that IL-7–augmented expansion of both donor and host T cells, there was no statistically significant difference in the percentage of donor T cell engraftment in the spleens of PBS- vs IL-7–treated mice (55 vs 59%, respectively; p = 0.23). The ratio of...
Table 1. Administration of IL-7 results in increased donor and host T cell expansion in nonirradiated recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Donor (×10^6)</th>
<th>Host (×10^6)</th>
<th>Donor (×10^6)</th>
<th>Host (×10^6)</th>
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</thead>
<tbody>
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<td>CD4⁺/CD8⁺ αβ</td>
<td>CD4⁺/CD8⁺ αβ</td>
<td>CD4⁺/CD8⁺ αβ</td>
</tr>
<tr>
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<td>10 ± 1</td>
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<td></td>
<td>22 ± 2</td>
<td>72 ± 6</td>
<td>15 ± 3</td>
<td>35 ± 2</td>
</tr>
</tbody>
</table>

* Nonirradiated B6AF1/J mice were transplanted with 25 × 10^6 B6 T cells and then treated with either PBS (n = 8–10/group) or IL-7 (n = 9–13/group) on days 0–9 posttransplantation, unless sacrificed earlier. Mice from each group were sacrificed either 6 or 11–13 days posttransplantation. Spleen cells from individual chimeras were obtained, and the percentage of donor and host T cells was quantitated. Data were derived from two separate experiments per time point and are presented as mean ± 1 SE. Values were rounded to the nearest integer. p ≤ 0.03; †p ≤ 0.008.

Administration of IL-7 exacerbates GVH lethality in the absence of a conditioning regime

Because administration of IL-7 resulted in expansion of both donor and host T cells relative to PBS-treated control mice, we performed experiments to determine whether administration of IL-7 to transplanted animals affected the severity of GVHD. Nonirradiated B6AF1/J recipients were transplanted with 25 × 10^6 B6 T cells and then treated with either PBS or IL-7 on days 0–9. Mice treated with PBS had 20% survival 60 days after BMT. In contrast, survival in IL-7-treated animals was significantly worse, with all mice dying by 33 days posttransplantation (Fig. 1A, p < 0.03). Weight loss was comparable in both groups of mice (Fig. 1B), with the exception that a percentage of PBS-treated mice survived for the duration of the experiment. To further characterize the severity of GVHD in both cohorts of mice, additional experiments were performed in which mice were sacrificed 13 days posttransplantation for histological analysis of GVHD target organs. Mice treated with IL-7 had significantly more GVHD pathology in both the liver and colon than PBS-treated mice (mean score 3.5 ± 0.4 vs 2.3 ± 0.4, p = 0.03) (Fig. 1C). Because IL-7 has been shown to enhance thymopoiesis after allogeneic BMT (25), we also examined the thymi of mice that were administered either PBS or IL-7. There was no difference in thymus size between PBS-treated (mean 21 × 10^6 cells, n = 10) and IL-7-treated mice (mean 14 × 10^6 cells, n = 13) when analyzed 11–13 days post-BMT (p = 0.23). The percentage of CD4⁺CD8⁺ thymocytes, however, was significantly lower in IL-7-treated vs PBS-treated animals (29 vs 48%, p = 0.008) consistent with more severe GVHD. Thus, administration of IL-7 exacerbated GVHD lethality and did not enhance thymopoiesis.

Administration of IL-7 does not exacerbate GVHD mortality when administered to lethally or sublethally irradiated recipients

Irradiation is known to exacerbate GVHD through the production of inflammatory cytokines that directly or indirectly contribute to the pathophysiology of GVHD (32–34). Consequently, we questioned whether differences in survival between mice transplanted with or without IL-7 administration would exist under conditions in which GVHD severity was not solely dependent upon donor T cells. To examine this question, we performed studies in which mice were preconditioned with varying doses of TBI. Several donor T cell doses were also examined because an earlier study (35) had indicated that the ability of IL-7 to exacerbate GVHD was dependent, in part, upon T cell dose. In initial studies, B6AF1/J mice were lethally irradiated (1000 cGy) and then transplanted with TCD B6 BM alone or together with either 0.75 or 1.5 × 10^6 B6 T cells. A dose of 1.5 × 10^6 T cells resulted in death in all lethally irradiated recipients not administered IL-7, while 0.75 × 10^6 cells caused fatal GVHD in ~50% of transplanted animals (our unpublished observations). Similarly transplanted mice were then treated with either PBS or IL-7 on days 0–9 post-BMT using the same schedule that was used in nonirradiated recipients. There was no significant difference in survival between PBS- and IL-7-treated mice at donor T cell doses of either 1.5 × 10^6 (Fig. 2A, p =...
0.14) or 0.75 \times 10^6 (Fig. 2B, p = 0.59) cells. Because a significant percentage of mice transplanted with 0.75 \times 10^6 T cells did not die from GVHD, we also assessed weight loss and parameters of immune reconstitution as more sensitive indicators of GVHD. Serial weight curves demonstrated no significant difference between groups when a subthreshold number of donor T cells were transplanted into recipients (Fig. 2C). Both spleen (0.5 \times 10^6 vs 1.4 \times 10^6, p = 0.14) and thymus size (0.8 \times 10^6 vs 14 \times 10^6, p = 0.42) were not significantly different between PBS- and IL-7-treated mice (n = 5/group), respectively, when examined 60 days post-BMT. Thus, IL-7 did not exacerbate lethality under conditions in which GVHD was uniformly lethal or under conditions in which GVHD mortality was attenuated due to transplantation of a sub-threshold number of donor T cells.

Studies have shown that T cell immune reconstitution is enhanced in allogeneic marrow transplant recipients when IL-7 is administered several weeks posttransplant (27). We reasoned that the administration schedule might be a variable that affected the ability of IL-7 to exacerbate GVHD. We therefore repeated these experiments and administered IL-7 for a similar 10-day course, but beginning later on days 14–23. Lethally irradiated B6AF1/J mice were transplanted with TCD B6 BM and 0.75 \times 10^6 B6 T cells. Animals treated with IL-7 had no statistically significant difference in survival when compared with mice administered PBS (Fig. 3, p = 0.85), indicating that this delayed administration schedule also had no adverse effect on GVHD mortality.

Because IL-7 failed to exacerbate GVHD in the presence of a lethal conditioning regimen, we performed additional studies to

FIGURE 2. Administration of IL-7 does not exacerbate GVHD lethality when administered early posttransplant to lethally irradiated recipients. A, Irradiated (1000 cGy) B6AF1/J mice were transplanted with 10 \times 10^6 TCD B6 BM (○, n = 8) alone or together with 1.5 \times 10^6 B6 T cells. Mice transplanted with T cells were then treated with PBS (■, n = 10) or IL-7 (5 μg/day) (■, n = 10) on days 0–9. Actual survival is depicted. Data are cumulative results from two separate experiments at each T cell dose. Mean serial weight measurements from mice transplanted in B are shown in C.

FIGURE 3. Delayed administration of IL-7 does not exacerbate GVHD mortality in lethally irradiated recipients. Irradiated (1000 cGy) B6AF1/J mice were transplanted with 10 \times 10^6 TCD B6 BM (○, n = 9) alone or together with 0.75 \times 10^6 B6 T cells. Mice transplanted with T cells were then treated with PBS (□, n = 17) or IL-7 (5 μg/day) (■, n = 17) on days 14–23. Actual survival is depicted. Data are cumulative results from three separate experiments.
FIGURE 4. Administration of IL-7 does not exacerbate GVHD lethality when administered early posttransplant to sublethally irradiated recipients. Irradiated (500 cGy) B6AF1/J mice were transplanted with 10 × 10^6 TCD B6 BM (●, n = 8) alone or together with 5 × 10^6 B6 T cells. Mice transplanted with T cells were then treated with PBS (□, n = 10) or IL-7 (5 µg/day) (■, n = 10) on days 0–9. Actual survival is depicted. Data are derived from two separate experiments.

assess the effect of IL-7 administration when the conditioning regimen was less intense. In these experiments, mice were sublethally irradiated (500 cGy) and then transplanted with TCD B6 BM alone or together with either 1.5 × 10^6 or 5 × 10^6 T cells. Because GVHD was not lethal at a dose of 1.5 × 10^6 T cells in either PBS- or IL-7-treated mice, serial weight curves were examined as a more sensitive indicator of GVHD. No difference in weight loss was observed in IL-7- vs PBS-treated mice (data not shown). When mice were transplanted with 5 × 10^6 T cells that resulted in more GVHD mortality in untreated animals, no statistically significant difference in survival was observed between either of the two groups (Fig. 4). Collectively, these studies demonstrated that IL-7 did not exacerbate GVHD lethality when transplants were performed at several different TBI and T cell doses designed to induce varying degrees of GVHD severity.

IL-7 administration does not result in the expansion of GVH-reactive T cells in irradiated hosts

In nonirradiated mice, we observed that exacerbation of GVHD mortality by IL-7 was associated with a significant increase in the absolute number of donor T cells. We therefore conducted a similar analysis in irradiated recipients to determine whether IL-7 enhanced expansion of donor T cells. Lethally irradiated B6AF1/J mice were transplanted with TCD B6 BM plus a limiting number (0.75 × 10^6) of B6 T cells, and then administered either PBS or IL-7 on days 0–9 post-MT, unless terminated before completion of the regimen. Animals were sacrificed on either day 6 or 11 post-BMT, and the absolute numbers of donor splenic T cells were determined. There was a statistically significant, but minimal increase in the absolute number of donor CD4^+ T cells on day 6 post-BMT. Otherwise, there were no other statistically significant differences in the absolute number of donor or host CD4^+ or CD8^+ T cells in the spleens of PBS- vs IL-7-treated animals at either 6 or 11–13 days posttransplantation (Table II).

Because this approach does not specifically quantitate GVH-reactive T cells, but rather all donor T cells, we performed additional BMT experiments using a B6→BALB.B murine model in which donor and recipient animals differ at multiple defined minor histocompatibility Ags. One of these minor Ags, termed H60, has been shown to be an immunodominant Ag that is preferentially recognized by donor CD8^+ T cells during a GVH reaction in this strain combination (36). Lethally irradiated BALB.B mice were transplanted with TCD B6 BM alone or together with 5 × 10^6 B6 T cells. Within the first 10 days of a GVH reaction, B6 H60-specific CD8^+ T cells are found in increased numbers in the spleens of BALB.B recipients (38). Cohorts of mice were treated with either PBS or IL-7 on days 0–9 post-MT. Animals (n = 7/group) were bled on days 12–13 and then sacrificed 13 days posttransplantation to determine the percentage of H60-reactive CD8^+ T cells. There was no difference in the percentages of CD8^+ T cells that recognized H60 in either the peripheral blood or spleens of PBS- vs IL-7-treated animals (Fig. 5). Moreover, there was no statistically significant difference in spleen cellularity or total number of CD8^+ T cells (Table III). Thus, the absolute numbers of splenic H60-specific CD8^+ T cells were not significantly different between groups, indicating that IL-7 did not effect an expansion of GVH-reactive donor T cells.

Host conditioning plus transplantation of alloreactive donor T cells results in increased endogenous IL-7 mRNA production in recipient mice

We reasoned that production of endogenous IL-7, or lack thereof, by recipient animals after allogeneic BMT might be a factor that affected the ability of exogenous IL-7 to exacerbate GVHD mortality. To address this question, the level of IL-7 mRNA in the spleens of mice on day 7 posttransplantation was quantitated by real-time PCR. Groups of animals that were evaluated consisted of lethally irradiated (1000 cGy) B6AF1/J mice (no transplant), lethally irradiated B6AF1/J mice transplanted with TCD B6 BM alone, lethally irradiated B6AF1/J mice transplanted with TCD B6 BM plus 1.5 × 10^6 B6 T cells, nonirradiated B6AF1/J mice transplanted with 25 × 10^6 B6 T cells, and normal nontransplanted

Table II. Administration of IL-7 has no effect on donor T cell reconstitution in irradiated recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 6 (Donor ×10^6)</th>
<th>Day 11 (Donor ×10^6)</th>
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<tr>
<td></td>
<td>CD4^+</td>
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</tr>
<tr>
<td>IL-7</td>
<td>1 ± 0</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

* Lethally irradiated B6AF1/J mice were transplanted with 10 × 10^6 TCD B6 BM plus 0.75 × 10^6 B6 T cells and then treated with either PBS (n = 6/group) or IL-7 (n = 5/group) on days 0–9 posttransplantation, unless sacrificed earlier. Cohorts of mice were sacrificed on day 6 or 11 posttransplantation. Spleen cells from individual chimeras were obtained, and the absolute number of donor and host T cells was quantitated. Data are presented as mean ± 1 SE. Values were rounded to the nearest integer. p = NS (all groups), except for day 6, * donor CD4, p = 0.02 (nonrounded values PBS, 0.8 ± 0.1 vs IL-7, 1.2 ± 0.1).
B6AF1/J mice. To control for the adequacy of template, 18S ribosomal RNA was also amplified from the same cDNA samples in individual mice, as detailed in Materials and Methods. There was no statistically significant difference in IL-7 levels between normal nontransplanted B6AF1/J mice and nonirradiated mice transplanted with B6 T cells (p > 0.66), indicating that transplantation of alloreactive donor T cells alone did not augment endogenous IL-7 production (Fig. 6). In contrast, IL-7 levels in all irradiated groups of mice were significantly greater than levels in normal B6AF1/J animals, demonstrating that host conditioning was a potent stimulus for induction of IL-7. In GVHD models, we observed that exogenous IL-7 administration exacerbated GVHD mortality (Figs. 2–4). Notably, in the absence of exogenous IL-7, the combination of TBI plus transplantation of alloreactive donor T cells resulted in a 72-fold higher level of IL-7 mRNA when compared with that observed in nonirradiated animals transplanted with donor T cells alone. Thus, irradiated mice undergoing GVHD had significantly more endogenous IL-7 production than nonirradiated mice with GVHD.

Discussion
Effective T cell reconstitution after allogeneic marrow transplantation is attributable to mature T cells present in the marrow graft and the generation of new T cells from BM-derived precursors that undergo maturation and appropriate selection in the thymus. This

Table III. **IL-7 does not increase the relative or absolute number of GVH-reactive T cells***

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood</th>
<th>Spleen</th>
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<tbody>
<tr>
<td></td>
<td>% CD8&lt;sup&gt;+&lt;/sup&gt; H60&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Cellularity (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
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<tr>
<td>PBS</td>
<td>9 ± 2</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>IL-7</td>
<td>9 ± 2</td>
<td>73 ± 7</td>
</tr>
</tbody>
</table>

* Lethally irradiated (800 cGy) BALB.B mice (n = 7) were transplanted with 10 × 10<sup>6</sup> TCD B6 BM alone or together with 5 × 10<sup>6</sup> B6 T cells. Mice transplanted with T cells were then treated with either PBS or IL-7 (5 μg/day) on days 0–9 posttransplantation. Mice in each cohort were bled on day 12–13 before being sacrificed on day 13 post-BMT to sample both peripheral blood and spleen cells. Flow cytometric analysis of CD8<sup>+</sup> T cells that recognized the H60 immunodominant peptide is shown from representative individual mice in each of the three cohorts.
In that regard, we have previously shown that inhibition of T cell apoptosis alone can exacerbate GVHD lethality in nonirradiated recipients (37). In these studies, mice transplanted with donor T cells that were resistant to passive apoptotic cell death through overexpression of Bcl-xL had more severe GVHD. The implication of these data was that any intervention that increased T cell survival in nonirradiated mice would exacerbate GVHD lethality. The results of the current study support this premise. Moreover, the present results provide further support for the interpretation that, under conditions in which GVHD severity is determined primarily by donor T cells as opposed to contributions from the conditioning regimen, factors that prolong donor T cell survival adversely affect GVHD. Thus, our data would predict that use of IL-7 in the setting of delayed donor lymphocyte infusions (38–40) or in instances in which IL-7 might be used to enhance the cytotoxic effector function of adoptively transferred donor T cells posttransplantation (41, 42) might be associated with an increase in the severity of GVHD.

Because the severity of GVHD is dependent upon a number of factors, including the conditioning regimen as well as the donor T cell dose (32–34), we examined the effect of IL-7 on GVHD severity in the presence of a TBI-based conditioning regimen as well as at several different donor T cell doses. In mice preconditioned with TBI, we were unable to distinguish any difference in survival between groups treated with and without IL-7, regardless of the T cell dose, intensity of the conditioning regimen, or IL-7 administration schedule. It should be pointed out that these results were observed under conditions in which GVHD was fatal in all recipients as well as conditions in which GVHD was not uniformly lethal. Furthermore, unlike what was observed in nonirradiated mice treated with IL-7, administration of IL-7 did not result in an increase in the absolute numbers of donor CD4+ and CD8+ T cells in the spleen early posttransplantation, even when assessed under conditions in which GVH-reactive donor T cells could be specifically identified and quantitated (Table III). These data indicated that the ability of IL-7 to effect donor T cell expansion and exacerbate GVHD was abrogated in mice that received pretransplant conditioning with TBI. We hypothesized that a potential explanation for these discordant results was that the conditioning regimen might augment the production of endogenous IL-7 and thereby attenuate the effect of exogenous IL-7 administration. In support of this premise, Levy et al. (43) have shown that irradiated mice transplanted with either syngeneic or allogeneic BM with or without added T cells had detectable IL-7 mRNA in the spleen, although they did not examine whether a GVH reaction in the absence of host conditioning was sufficient to induce IL-7 transcription. Moreover, IL-7 levels have been demonstrated to be increased under conditions of T cell depletion (44, 45), as occurs after intensive conditioning regimens. We observed that there were no significant differences in IL-7 mRNA levels between normal mice and nonirradiated animals transplanted with donor T cells (Fig. 6). In contrast, irradiated mice, irradiated animals transplanted with TCD BM, and irradiated mice transplanted with TCD BM and allogeneic donor T cells all had significantly higher levels of splenic IL-7 mRNA. Notably, the combination of TBI and transplantation of allogeneic donor T cells resulted in a 72-fold higher IL-7 mRNA level than observed in nonirradiated mice transplanted with donor T cells alone. In light of these data, we believe the most likely interpretation for the discordant effects of IL-7 on GVHD mortality in these two groups of mice is that donor T cells in nonirradiated B6→B6AFl/J chimeras were capable of a more robust response to exogenous IL-7 due to the limited endogenous production of the cytokine. Thus, IL-7 was able to expand the number of donor T cells in these animals, which led to worsening of GVHD. Conversely, T cells
obtained from the spleens of irradiated GVHD animals were already exposed to significant levels of endogenous IL-7, and therefore the administration of exogenous IL-7 resulted in no further donor T cell expansion in vivo. This would explain why IL-7 administration did not exacerbate GVHD in these animals. The specific cells responsible for IL-7 secretion are not resolved by these studies, although dendritic cells located in the spleen are a prime candidate (17, 18, 46).

Prior studies that have examined the role of IL-7 on GVHD induction have been conducted exclusively in lethally irradiated recipients and have yielded discordant results. Sinha et al. (35) showed that IL-7 could exacerbate GVHD when a subthreshold dose of donor T cells was transplanted into lethally irradiated recipients. These investigators examined the effect of IL-7 at three donor T cell doses, all of which were insufficient to cause lethal GVHD in all recipients. IL-7 administered over a 28-day period exacerbated GVHD lethality at the highest T cell dose examined (7.5 x 10^6), but did not worsen GVHD mortality at two lower doses. IL-7 did increase the degree of weight loss at all T cell doses examined. Histologic evidence of GVHD was also significantly worse in IL-7-treated animals at two of three T cell doses tested (i.e., 1 x 10^6 and 10 x 10^6), but not 5 x 10^6 as well as in syngeneic recipients, suggesting that IL-7 may have some toxicity in the absence of alloreactive donor T cells. These data suggested that IL-7 administration could exacerbate GVHD under conditions in which the dose of donor T cells was insufficient to effect uniform lethality in transplanted recipients. These studies stand in juxtaposition to those of Alpdogan et al. (27), who reported that IL-7 administration did not exacerbate GVHD under conditions in which GVHD was uniformly lethal in untreated animals when examined in three different murine models. There were, however, differences between these two studies that may have affected the observed outcomes. First of all, the dose of IL-7 was significantly higher in the report by Sinha et al. (35) (i.e., 5 μg/day) than that used by Alpdogan et al. (27) (1 μg/day). Second, the duration of therapy was longer in the former study (28 vs 14 days). Finally, the more severe GVHD elicited in the latter models may have obscured effects of IL-7 on GVHD induction. In the current study, we used the higher dose of IL-7 (5 μg/day), as used by Sinha et al. (35), and examined GVHD induction under a variety of TBI and donor T cell doses designed to effect varying degrees of GVHD severity. Nonetheless, we were unable to uncover any scenario under which IL-7 increased GVHD mortality in irradiated animals. It should be noted that the duration of IL-7 administration was shorter in our study, and therefore we cannot exclude the possibility that this variable is responsible for the observed differences between the two studies.

In summary, these studies demonstrated that IL-7 has potent effects on the survival and expansion of T cells that are sufficient to significantly increase GVHD mortality in the absence of a conditioning regimen. In contrast, under inflammatory conditions resulting from TBI conditioning, the dose and administration schedule used in these experiments did not result in an exacerbation of GVHD mortality. These discordant results appear to be attributable, in part, to the increased endogenous production of IL-7 induced by the combination of TBI and transplantation of alloreactive donor T cells that serves to attenuate any detrimental effects on GVHD resulting from exogenous IL-7 administration. These data also establish that another manner in which the conditioning regimen can contribute to the pathophysiology of GVHD is by the induction of cytokines that can directly promote T cell survival and proliferation.

References
EFFECT OF IL-7 ON MURINE GVHD


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