Interleukin 2 prevents graft-versus-host disease while preserving the graft-versus-leukemia effect of allogeneic T cells

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ABSTRACT We have recently demonstrated that interleukin 2 (IL-2), when administered in high doses for several days beginning on the day of allogeneic bone marrow transplantation (BMT), markedly diminishes graft-versus-host disease (GVHD) mortality in lethally irradiated mice. An optimal anti-GVHD effect was attained by coadministering T-cell-depleted (TCD) syngeneic marrow. We demonstrate here that the full graft-versus-leukemia effect of allogeneic T lymphocytes is obtained even when GVHD is markedly diminished by the coadministration of IL-2 and TCD syngeneic marrow. This methodology represents an approach to the treatment of leukemia in which the beneficial effects of allogeneic T cells can be exploited while their major deleterious effect, GVHD, is avoided. These results may thus have an impact on the clinical use of BMT for the treatment of hematologic malignancies.

T-cell depletion of allogeneic bone marrow, while successful in preventing severe graft-versus-host disease (GVHD), is associated with an increased incidence of failure of alloengraftment, increased probability of leukemic relapse (1–4), and delayed recovery of T-lymphocyte functions (5). Thus, the development of methods of retaining the beneficial graft-versus-leukemia (GVL) and alloengraftment-promoting effects of allogeneic T cells, while abrogating their ability to cause GVHD, remains a major goal of bone marrow transplantation (BMT) research. Previous studies from this laboratory have demonstrated that T-cell-depleted (TCD) syngeneic bone marrow can delay GVHD mortality when administered to lethally irradiated recipients of allogeneic bone marrow and spleen cells (6). Recent studies demonstrated that such coadministration of TCD syngeneic marrow, nevertheless, did not diminish the GVL effect of T cells in allogeneic marrow inocula (7). This result, and the complete allogeneic lymphohematopoietic repopulation observed in such animals (6, 7), suggested that GVH reactions could be confined to the lymphohematopoietic system without causing clinically apparent GVHD. Unfortunately, we were unable to demonstrate an anti-GVHD effect of TCD syngeneic marrow in animals that enjoyed the GVL effect of allogeneic T cells, because of the limited potency of protection from acute GVHD and because of the inability of TCD syngeneic marrow to protect against chronic GVHD mortality, which occurred at the same time as leukemic mortality. More recently, however, we reported that interleukin 2 (IL-2) has a much more potent ability than TCD syngeneic marrow to abrogate both acute and chronic GVHD mortality, while preserving the ability to achieve complete allogeneic lymphohematopoietic repopulation (8). When a suboptimal dose (10,000 units twice daily for 5 days) of IL-2 was administered, coadministration of TCD syngeneic marrow was required to produce a maximal protective effect. With higher doses of IL-2 (50,000 units twice daily for 5 days), a protective effect was also observed for animals not receiving TCD syngeneic marrow, but the greatest protection from acute GVHD mortality was still observed in animals receiving both components (syngeneic marrow and IL-2). Clinically apparent IL-2 toxicity was completely eliminated and the entire anti-GVHD effect was retained when the duration of administration of the higher dose was diminished to 2.5 or 3 days, beginning just prior to BMT. Early timing of IL-2 administration was critical in producing such protection (8). In the present report, we attempted to determine whether or not IL-2 treatment would, like other clinically available methods of avoiding GVHD, reduce the GVL effect of allogeneic T cells. The EL4 tumor model was particularly suitable for examination of this question since the GVL effect was dependent on the administration of allogeneic T cells, and IL-2 treatment alone did not markedly delay mortality due to EL4. Our results indicate that the entire GVL effect of allogeneic T cells was preserved in IL-2-treated mice, suggesting that this therapeutic combination (IL-2, TCD autologous bone marrow cells (BMCs), and allogeneic BMCs) could potentially resolve the major limitation of clinical BMT for the treatment of leukemia—namely, the loss of GVL effects and failure of alloengraftment, which result when T cells are depleted for the prevention of GVHD.

MATERIALS AND METHODS

Mice. Female C57BL/10SnCr (B10, H-2<sup>b</sup>) and A/J (H-2<sup>d</sup>) mice were obtained from the Frederick Cancer Research Facility of the National Cancer Institute.

Experimental Design. BMT was performed as described (9). Briefly, recipient B10 mice, 12–16 weeks old, were lethally irradiated (1025 R, 137Cs source, 110 R/min; 1 R = 0.258 mC/kg) and reconstituted on the same day with BMCs obtained from sex-matched donors. Animals were housed in sterilized microisolator cages, in which they received autoclaved food and autoclaved acidified drinking water; 5 × 10<sup>6</sup> syngeneic (B10) BMCs, TCD with rabbit anti-mouse brain antiserum and complement as described (7), 10–15 million (except where indicated) untreated A/J BMCs, 6–9 × 10<sup>6</sup> A/J spleen cells, and 500 EL4 cells were administered to the mice as indicated. EL4<sup>F</sup> cells (referred to here as EL4), a subline of the B6 T-cell leukemia/lymphoma EL4 (10), were thawed from frozen vials and maintained in culture for 4–14 days prior to each experiment. BMCs, EL4 cells, and spleen cells were coadministered in a single 1-ml intravenous injection as described (7). Animals from different cages were randomly put into experimental groups prior to injection and were randomly returned to cages after injection, so that each cage contained animals from several different experimental groups. Survival was checked on a daily basis for 100 days.

Abbreviations: IL-2, interleukin 2; BMT, bone marrow transplantation; TCD, T-cell depleted; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia; BMC, bone marrow cell; mAb, monoclonal antibody; LAK, lymphokine-activated killer; MHC, major histocompatibility complex.

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T-Cell Depletion of Spleen Cells. T-cell depletion was performed as described (11) using ascites of αCD4 monoclonal antibody (mAb) GK1.5 (1:1000 dilution) plus αCD8 mAb 2.43 (1:800 dilution), followed by treatment with low toxicity rabbit complement (1:11 dilution).

IL-2 Administration. Recombinant human IL-2 was generously provided by Cetus and was administered intraperitoneally as described (8). IL-2 (5×10⁴ units) was administered 1–3 hr before BMT and approximately every 12 hr thereafter for a total of six doses.

Peripheral Blood Lymphocyte Phenotyping. For phenotyping of surviving animals, peripheral blood lymphocytes were isolated and split into two tubes, one of which was stained with biotinylated mAb 5F1 (anti-K⁺) (12); the other was stained with biotinylated 34-2-12 (anti-D9) (13) followed by fluorescein isothiocyanate–avidin as described (14). One-color flow cytometry was performed as described (14). Percentage staining was determined from one-color fluorescence histograms and comparison with those obtained from normal donor and host-type animals, which were used as positive and negative controls, as described (14).

Statistical Analyses. Survival probability was determined by the censored data technique of Kaplan-Meier, and statistical significance was determined by the method of Wilcoxon and Breslow. All statistical results are expressed as P values, and values of <0.05 are considered to be significant.

Necropsy Evaluation. After death, carcasses were fixed in formaldehyde and were subsequently evaluated for gross evidence of tumor by an observer who was unaware of which treatment the animals had received. Carcasses were classified as showing no gross evidence for tumor, probable evidence for tumor, or definite evidence for tumor (7). The presence of two or more of the following were considered to be definite evidence for tumor: splenomegaly, an enlarged thymus, enlarged kidneys, retroperitoneal masses, mesenteric masses, and liver nodules. Evidence for involvement of only one of these organs constituted probable evidence for tumor. Animals with probable or definite evidence for tumor were considered to have a tumor, since previous studies have shown that such categorization predicts detectable tumors on independent histologic evaluation by an observer who was unaware of which treatment animals had received, with a sensitivity of 93% and a specificity of 89% (7).

RESULTS AND DISCUSSION

The EL4 H-2b leukemia model, which we recently described in mixed allogeneic BMT recipients (7), was used. This tumor is highly virulent, and as few as 100 cells were shown to be sufficient to kill lethally irradiated, syngeneically reconstituted mice within 3.5 weeks (7). Since allogeneic BMCs were previously shown to mediate a GVL effect when administered without spleen cells, a higher dose of EL4 cells was used here to increase the leukemic challenge so that allogeneic BMCs alone would be insufficient to mediate a marked GVL effect. Female B10 (H-2b) mice were lethally irradiated and reconstituted with 5×10⁴ TCD syngeneic BMCs plus 5×10³ EL4 leukemia/lymphoma cells. All such recipients died of tumor by day 19 (Fig. 1). Treatment with IL-2 on days 0–3 led to slight prolongation of survival, with all animals dying by day 23 (Fig. 1; P < 0.02). The addition of 10⁷ A/J BMCs plus 10⁷ A/J spleen cells to inocula containing TCD syngeneic marrow plus EL4 was associated with a highly significant GVL effect in animals also receiving IL-2 (Fig. 1) (P < 0.002). The median survival time was extended to 33 days, and 2 of 10 animals were apparently cured, surviving longer than 100 days. A GVL effect was also observed in animals receiving a similar treatment regimen without TCD syngeneic marrow, but a greater percentage of these animals died in the 2nd week from GVHD, rendering the improvement in survival not statistically significant (P = 0.09).

Similarly, a lesser degree of GVHD protection was provided by IL-2 administered without TCD syngeneic marrow in control animals not receiving EL4 (Table 1). IL-2 without TCD syngeneic marrow provided significant protection from GVHD (P < 0.01), but optimal protection from acute GVHD was observed in animals receiving TCD syngeneic marrow along with the same A/J inoculum plus IL-2 (P < 0.001), consistent with previous results (8). Comparison of the survival of groups receiving A/J BMCs and spleen cells plus

![Fig. 1. Survival of lethally irradiated B10 mice receiving intravenous inocula containing TCD B10 BMCs plus EL4 leukemia cells (--; n = 4); TCD B10 BMCs plus EL4 leukemia cells and i.p. IL-2 (---; n = 4); A/J BMCs, A/J spleen cells, EL4 leukemia cells, and i.p. IL-2 (--; n = 9); TCD B10 BMCs, A/J BMCs, A/J spleen cells, EL4 leukemia cells, and i.p. IL-2 (--; n = 10).](image-url)
IL-2 along with EL4 in Fig. 1 with similarly treated control groups not receiving EL4 (Table 1) indicates that the majority of deaths in such groups in Fig. 1 were due to tumor and not to GVHD. Control animals receiving TCD syngeneic plus A/J BMCs without spleen cells with or without IL-2 demonstrated excellent survival (Table 1, groups 4 and 5); thus, mortality among the groups shown in Table 1 reflected GVHD caused by administration of allogeneic spleen cells. These results therefore demonstrate a significant GVL effect of allogeneic marrow and spleen cells in animals that were simultaneously protected against GVHD by administration of a high dose of IL-2 plus TCD syngeneic marrow. All surviving animals demonstrated complete allogeneic reconstitution of peripheral blood lymphocytes, regardless of whether or not they had received TCD syngeneic marrow and EL4 in addition to IL-2 (data not shown). IL-2 plus TCD syngeneic marrow therefore reduces GVHD mortality while permitting a GVL effect and engraftment of allogeneic cells. Allogeneic BMCs in the numbers used in the above experiment were insufficient to mediate a GVL effect against the 500 EL4 cells that were administered. As shown in Table 2, administration of A/J BMCs without spleen cells did not prolong the survival of recipients of TCD syngeneic marrow, EL4, and IL-2 (Table 2, group 2 vs. group 1; \( P = 0.4 \)). In contrast, animals receiving \( 7 \times 10^8 \) A/J spleen cells in addition to EL4, A/J BMCs, TCD syngeneic marrow, and IL-2 demonstrated significantly improved survival (Table 2, group 3 vs. group 1, \( P < 0.0008 \); group 3 vs. group 2, \( P < 0.0004 \)), with 3 of 9 animals surviving longer than 100 days. Comparison of groups 3 and 5 (Table 2) demonstrates in this experiment that IL-2 was necessary to prevent acute GVHD mortality, since 70% of control recipients of TCD B10 BMCs plus A/J BMCs and spleen cells without IL-2 (group 5) died of acute GVHD by day 9. In contrast, mortality in group 3 occurred significantly later (days 25–50), and the finding of gross tumor in these animals at necropsy (Table 2) was consistent with the conclusion that the deaths in this group were due to tumor rather than to GVHD. In a repeat experiment producing similar results, recipients of A/J BMCs and spleen cells with or without EL4 cells showed an identical mortality pattern (\( P = 1.0 \)), indicating that EL4 cells had no effect on GVHD mortality. Control animals receiving A/J BMCs plus TCD syngeneic marrow with or without IL-2 demonstrated 100% survival (Table 2, groups 7 and 8), indicating that BMCs alone were insufficient to cause GVHD mortality.

Since spleen cells are a good source of precursors of lymphokine-activated killer (LAK) cells, which can kill some tumors \textit{in vivo} (15) and which are activated by treatment with IL-2 (16, 17), it was important to determine whether the GVL effect of allogeneic spleen cells was due to the activity of alloreactive T cells or of LAK cells not specifically recognizing alloantigen of the recipient. Spleocytes depleted of CD4+ and CD8+ T cells are still capable of generating LAK activity associated with lysis of tumors \textit{in vivo} (18, 19). To determine which splenocyte cell populations mediated GVL effects against EL4, T cells were depleted from A/J spleen cell inocula by using mAbs against the CD4 and CD8 T-cell subset markers followed by treatment with rabbit complement. Comparison of groups 5 and 6 in Table 2 demonstrates that, in animals not treated with IL-2, acute GVHD mortality was completely prevented by removal of CD4+ and CD8+ T cells from A/J spleen cell inocula. Chronic GVHD, however, was not completely abrogated (compare group 6 with group 7, which did not receive any spleen cells), suggesting that some T cells remained after treatment of spleen cells. In animals also receiving EL4 cells and IL-2, however, removal of CD4+ and CD8+ T cells was associated with a complete loss of the GVL effect of A/J spleen cells (group 3 vs. group 4; \( P < 0.0006 \)). All animals receiving T-cell-depleted spleen cells died with gross evidence of tumor at necropsy, and no GVL effect was observed compared to recipients of similar

### Table 1. Protection from GVHD in IL-2-treated animals not receiving EL4 cells

<table>
<thead>
<tr>
<th>Group</th>
<th>B10-T BMCs</th>
<th>A/J BMCs</th>
<th>A/J spleen</th>
<th>IL-2</th>
<th>MST, days</th>
<th>At 25 days</th>
<th>At 100 days</th>
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<td>+</td>
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<td>–</td>
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<td>44</td>
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<tr>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>80</td>
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<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>&gt;100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>&gt;100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>5</td>
<td>&gt;100</td>
<td>100</td>
</tr>
</tbody>
</table>

MST, median survival time.

### Table 2. GVL effect of allogeneic splenic T cells

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>B10 BMCs*</th>
<th>A/J BMCs†</th>
<th>A/J spleen‡</th>
<th>EL4</th>
<th>IL-2</th>
<th>MST, days</th>
<th>At 25 days</th>
<th>At 100 days</th>
<th>Evidence of tumor at necropsy</th>
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<td>9/9</td>
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<td>9</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>23</td>
<td>33</td>
<td>11</td>
<td>8/8</td>
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<tr>
<td>3</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>C§</td>
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<td>+</td>
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<td>89</td>
<td>33</td>
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<td>9</td>
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<td>+</td>
<td>αCD4/8/C§</td>
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<td>+</td>
<td>23</td>
<td>22</td>
<td>0</td>
<td>9/9</td>
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<tr>
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<td>10</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>–</td>
<td>–</td>
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<td>30</td>
<td>20</td>
<td>0/71</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>αCD4/8/C</td>
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<td>100</td>
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<td>0/11</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>&gt;100</td>
<td>100</td>
<td>100</td>
<td>–</td>
</tr>
</tbody>
</table>

MST, median survival time. Evidence of tumor is expressed as number of animals with gross evidence of tumor on autopsy per number of animals autopsied.

*\( 5 \times 10^6 \) TCD B10 BMCs.
†\( 1.25 \times 10^6 \) A/J BMCs.
‡\( 7 \times 10^6 \) A/J spleen cells.
§Spleen cells treated with complement only.
αCD4 cells treated with \( \alpha \)CD4 plus \( \alpha \)CD8 mAbs followed by complement.
‖Animals died of GVHD.
Table 3. Effect of syngeneic vs. allogeneic spleen cells on leukemic mortality in IL-2-treated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>B10 n</th>
<th>BMCs*</th>
<th>Splenê</th>
<th>EL4+</th>
<th>IL-2²</th>
<th>% survival at 25 days</th>
<th>MST, median survival time</th>
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<td>22</td>
<td>11</td>
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<td>10</td>
<td>A/J</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>&gt;35</td>
<td>80</td>
<td>0.03³</td>
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<tr>
<td>5</td>
<td>10</td>
<td>A/J</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>10</td>
<td>0</td>
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<tr>
<td>6</td>
<td>5</td>
<td>B10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>&gt;35</td>
<td>100</td>
</tr>
</tbody>
</table>

MST, median survival time. The experiment had been followed for 35 days at the time of writing.

115 x 10⁶ untreated BMCs.
26.5 x 10⁶ untreated spleen cells.
3500 EL4 cells.
450,000 units twice daily for 3 days.
5Compared to group 1; NS, not significant.
6Compared to group 4.

Inocula without spleen cells (compare groups 2 and 4, P = 0.6). Since none of the control recipients of TCD A/J spleen cells without EL4 died during this period (group 6), the deaths in group 4 were clearly due to tumor and not GVHD. In contrast, as noted above, recipients of complement-treated spleen cells (group 3) were protected both from tumor-related mortality and from GVHD-related mortality. Although the deaths that occurred in this group were due to tumor and not GVHD (see above), a highly significant GVL effect was observed (P < 0.004; see above). Thus, the GVL effect observed in the present model was completely dependent on the administration of large numbers of T cells in A/J spleen cell inocula, even though GVHD mortality, which is also mediated by T cells, was abrogated by treatment with IL-2. Additional experiments are necessary to determine whether the observed GVL effect is mediated by CD4⁺, CD8⁺, or both subsets of T cells. Since EL4 does not express class II antigens and CD4⁺ T cells interact with class II and not with class I molecules, it is believed that CD8⁺ T cells mediate the GVL effect against EL4. Since we have been unable to produce acute GVHD by administering large numbers of A/J CD8⁺ T cells without CD4⁺ T cells to lethally irradiated B10 mice (M.S., unpublished data), a possible mechanism for the dissociation of GVHD from GVL in IL-2-treated mice is that CD4⁺ T-cell reactivity is selectively abrogated by IL-2, leaving CD8-mediated GVL effects intact.

Since the EL4 tumor line was established from a C57BL mouse 45 years ago (10), it is highly probable that there are minor histocompatibility differences between EL4 and the B10 recipients used here, and that EL4 is therefore not truly syngeneic to the recipients. However, our previous studies using this model demonstrated that T cells contained in B10 or other H-2b bone marrow inocula do not mediate GVL effects, in contrast to similar numbers of major histocompatibility complex (MHC)-disparate BMCs. These results and the T-cell dependence of the phenomenon (e.g., see Table 2) suggested that alloreactivity against host MHC molecules was necessary for a GVL effect. However, it remained possible that LAK cells derived from CD8⁺ splenic precursors in IL-2-treated mice were capable of killing EL4 in vitro, since LAK cells derived from such precursors are capable of killing EL4 in vitro (19). To evaluate this possibility, we compared the ability of similar numbers of allogeneic (A/J) vs. syngeneic (B10) spleen cell/BMC mixtures to protect against leukemic mortality in IL-2-treated mice. As shown in Table 3, B10 spleen cell/BMC mixtures did not mediate GVL in IL-2-treated mice (group 1 vs. group 2), whereas similar numbers of A/J spleen cells plus BMCs mediated a significant GVL effect (group 1 vs. group 4) in such mice. Highly significant GVHD protection was provided by IL-2 (group 4 vs. group 5). Thus, host-type splenic LAK cells did not protect against EL4 leukemia, consistent with the interpretation that MHC-specific alloreactive T cells, and not LAK cells, are effective in eliminating EL4 cells in vivo.

Since animals receiving A/J spleen cells without IL-2 died of GVHD, it could not be determined from the above studies whether or not the magnitude of the GVL effect of A/J spleen cells was reduced by the coadministration of IL-2. To address this question, we examined the effect of IL-2 administration on the GVL effect of a larger dose of A/J BMCs (3 x 10⁶), which, perhaps because of its low T-cell content (=2%), does not cause GVHD (7). This experiment was performed twice with similar results, which are summarized in Table 4. The inability of A/J BMCs to cause GVHD mortality is confirmed in Table 4 (groups 1 and 2), in which control recipients of A/J BMCs plus TCD B10 BMCs with or without IL-2 demonstrated excellent survival. Comparison of groups 3 and 4 in Table 4 demonstrates that this dose of A/J BMCs, when coadministered with TCD syngeneic marrow, mediated a small but significant GVL effect, similar to previous results (20). The addition of IL-2 to the regimen did not reduce the magnitude of this GVL effect (group 3 vs. group 5 in Table 4). In the same experiments, administration of IL-2 to recipients

Table 4. GVL effect of allogeneic BMCs in IL-2-treated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>B10(T) BMCs*</th>
<th>A/J BMCs†</th>
<th>A/J spleen‡</th>
<th>EL4</th>
<th>IL-2</th>
<th>n</th>
<th>At 25 days</th>
<th>At 100 days§</th>
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*5 x 10⁶ TCD B10 BMCs.
†3 x 10⁶ untreated A/J BMCs.
‡6.5 x 10⁶ A/J spleen cells.
§One of the two experiments included here had been followed for only 84 days at the time of writing, and 100-day survival statistics therefore include some withdrawals at day 84.
§Group 1 vs. group 6.
†Group 2 vs. group 7.
**Group 3 vs. group 4.
††Group 3 vs. group 5.
‡‡Group 6 vs. group 7.
of TCD syngeneic marrow, A/J BMCs, and A/J spleen cells was associated with significant protection from acute GVHD mortality (Table 4, group 6 vs. group 7). Thus, IL-2 can protect against GVHD mortality without reducing the magnitude of even a weak GVL effect. In additional experiments, we have demonstrated that the GVL effect of allogeneic bone marrow cells is mediated by CD4+ and/or CD8+ T cells in this model (7). Thus, similar mechanisms are probably responsible for the GVL effects of bone marrow and spleen cells, and the preservation, in IL-2-treated mice, of the small degree of protection afforded by allogeneic BMCs is strong evidence that the more potent protective effect of allogeneic splenic T cells is also not attenuated by IL-2.

T-cell depletion, which remains the most effective known method of abrogating GVL in human BMT recipients, has been associated with an increased probability of leukemic relapse in several hematologic malignancies (3, 4). Evidence has also been obtained that the widely utilized immunosuppressive agent cyclosporin may also increase leukemic relapse probability (21). In contrast, IL-2 is known to be capable of shrinking solid tumors in humans and animals (15, 22, 23), and recent evidence also suggests that IL-2 may have GVL effects (24, 25). The ability of IL-2 to prolong survival only slightly in recipients of EL4 without allogeneic T cells does not rule out the possibility that IL-2 might mediate stronger protective effects against other hematologic malignancies, since tumors vary in their in vivo sensitivity to IL-2 (15). In contrast to other methods of GVHD prophylaxis, therefore, IL-2 may actually mediate GVL effects rather than attenuate them.

The GVL effect observed in this model appears to depend on alloreactivity of donor T cells recognizing disparate MHC molecules expressed on EL4 cells. Since the majority of patients who might benefit from BMT lack a suitable HLA-identical donor, a method of preventing GVHD in HLA-disparate combinations, such as IL-2 administration, might significantly broaden the availability of BMT as a therapeutic modality. BMT across greater HLA disparities might also lead to increased GVL effects above those that have been observed in HLA-identical combinations, as we have observed in the EL4 model. Such beneficial effects of anti-MHC alloreactivity may have been previously masked by the increase in GVHD observed when clinical BMT has been attempted across such barriers (26).

The importance of the data presented here is not related to the absolute magnitude of the GVL effect of allogeneic T cells against EL4, which is an exceptionally virulent tumor, and which was intentionally used in a relatively high dose for the experiments described here. Instead, the important conclusion that can be drawn from the present results is that IL-2, while protecting against GVHD, preserves the entire GVL effect of allogeneic T lymphocytes; the absolute magnitude of the effect of such cells would be expected to be highly dependent on the tumor model used. EL4 serves as an excellent model for examining the GVL effects of T cells rather than of other cell types with antitumor activity, and the results are therefore particularly relevant to the field of clinical bone marrow transplantation, in which the importance of allogeneic T cells in eliminating residual leukemia has been well documented (1–4). Further studies are necessary to elucidate the mechanism whereby the GVL- and GVHD-producing effects of alloreactive T cells can be so clearly dissociated by administration of IL-2 plus TCD syngeneic marrow.

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