IN VIVO ADMINISTRATION OF INTERLEUKIN 2 PLUS
T CELL-DEPLETED SYNGENEIC MARROW PREVENTS
GRAFT-VERSUS-HOST DISEASE MORTALITY
AND PERMITS ALLOENGRAFTMENT

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Avoiding graft-vs.-host disease (GVHD)[1] while retaining the engraftment-promoting and antileukemic effects of T cells in allogeneic marrow remains a major challenge in the field of bone marrow transplantation (BMT). While T cell depletion reduces the incidence of GVHD, it is associated with an increased probability of engraftment failure (1-6) and a greater risk of leukemic relapse (4, 5, 7). Previous work from this laboratory has demonstrated that the GVHD-related mortality of lethally irradiated, bone marrow–reconstituted mice can be delayed by the coadministration of T cell–depleted (TCD) syngeneic marrow (8). Although this result was encouraging, we have found the magnitude of the protection from acute GVHD mortality to be limited, and no protection from chronic GVHD mortality has been apparent (8). We therefore sought a method of augmenting this protective effect of TCD syngeneic marrow. We have previously demonstrated that TCD syngeneic marrow is responsible for most of the natural suppressor (NS) activity arising in spleens of lethally irradiated mice reconstituted with a mixture of allogeneic plus syngeneic marrow, and have hypothesized that such cells might be responsible for the anti-GVHD effect of TCD syngeneic marrow (9). Since cell lines with in vitro NS activity and in vivo anti-GVHD effects have been successfully cultured in IL-2 (10, 11; Sykes M., K. A. Hoyles, M. L. Romick, and D. H. Sachs, manuscript in preparation), we wished to address the possibility that the administration of IL-2 in vivo to lethally irradiated, bone marrow–transplanted mice might increase the anti-GVHD effect of TCD syngeneic bone marrow. Our results indicate that IL-2 provides significant protection against GVHD mortality from allogeneic lymphocytes while permitting complete repopulation by allogeneic bone marrow cells (BMC). When suboptimal amounts of IL-2 were given, maximal protection was achieved when TCD syngeneic marrow was also administered. Survivors protected in this manner similarly demonstrated complete allogeneic reconstitution.

[1] *Abbreviations used in this paper:* BMC, bone marrow cells; BMT, bone marrow transplantation; FCM, flow cytometry; GVHD, graft-vs.-host disease; LAK, lymphokine-activated killer; NS, natural suppressor; TCD, T cell depleted.

Materials and Methods

Animals. Male and female C57BL/10SnJ (B10, H-2^b, K^{b}I^{b}S^{b}D^b), B10.D2/nSn (B10.D2, H-2^d, K^{d}I^{d}S^{d}D^d), and A/J (H-2^a, K^{k}I^{k}S^{d}D^d) mice were obtained from The Jackson Laboratory, Bar Harbor, ME, or from the Frederick Cancer Research Facility of the National Cancer Institute.

BMT. BMT was performed as previously described (12). Briefly, recipient B10 mice, aged 12–16 wk, were lethally irradiated (1,025 rad, ^{137}Cs source, 110 rad/min) and reconstituted within 8–12 h with BMC obtained from the tibiae and femora of sex-matched donors aged 6–14 wk. Animals were housed in sterilized microisolator cages, in which they received autoclaved food and autoclaved acidified drinking water. Syngeneic (B10) bone marrow was TCD using rabbit anti-mouse brain serum, as previously described (12). 1–1.5 × 10^7 untreated fully MHC-mismatched allogeneic (B10.D2 or A/J) BMC were administered with or without allogeneic spleen cells, varying in dose from 6.5 × 10^6 (A/J spleen cells) to 3.5 × 10^7 (B10.D2 spleen cells). All BMC and spleen cells were coadministered in a single 1-ml intravenous injection. Irradiation controls received no BMC or spleen cells and died 7–12 d after irradiation.

To avoid any cage-related effects on experimental results, animals were randomized both before assigning the experimental groups, and after BMT, so that animals from different experimental groups were randomly mixed in each cage. Survival was checked on a daily basis for 100 d.

IL-2 Administration. The indicated doses of human rIL-2, kindly provided by Cetus Corp. (Emeryville, CA), were injected intraperitoneally in 0.2 ml of HBSS. Unless otherwise indicated, the first dose of IL-2 was administered 1–3 h before BMT, and approximately every 12 h thereafter, for a total of 10 doses. As a control for IL-2 toxicity, additional irradiated animals received IL-2 plus TCD syngeneic marrow with or without allogeneic marrow, and without allogeneic spleen cells.

mAbs. FITC-conjugated mAb 5F1 (anti-K^b) (13) and biotinylated mAb 34-2-12 (anti-D^d) (14) were prepared by standard methods using antibodies purified from ascites using protein A-Sepharose 4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden).

Phenotyping of Chimeras. Phenotyping was performed 9–15 wk after BMT. Animals were bled and PBMC were isolated as described (12). PBMC from each animal were then split into two tubes, and staining with mAbs was performed as described (15). Staining with both donor-specific and host-specific mAbs was performed on each chimera and control animal. One-color flow cytometry (FCM) was performed as described (16). In all experiments, percent 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. The percentage of cells considered positive after staining with a mAb was determined using a cutoff for positivity chosen as the fluorescence level at the beginning of the positive peak of the positive control strain. The relative percent staining of a chimera with a mAb was calculated using the formula: 100 × (chimera percent positive) – (negative control percent positive)/(positive control percent positive) – (negative control percent positive). Since the mAbs used were allele specific for class I H-2 antigens, nearly 100% of cells from positive control animals, and 0% of cells from negative control animals, stained with each mAb in every experiment.

Statistical Analysis. Survival probability was determined using the censored data technique of Kaplan-Meier, and statistical significance was determined using the method of Wilcoxon and Breslow. Since this method of analysis attributes increased weight to the early portion of a survival curve, a two-tailed stratified log rank survival test was substituted when the question of protection from late mortality was specifically being addressed. All statistical results are expressed as p values, and values <0.05 are considered to be significant.

Results

Effects of TCD Syngeneic Marrow and IL-2 on Mortality from Moderate GVHD. The results in Fig. 1 show the effects of TCD syngeneic marrow and IL-2 on mortality from a moderately severe GVHD, which caused early mortality in one third of con-

trol animals. The survival of lethally irradiated B10 control mice given 1.5×10^7 A/J BMC plus 8×10^7 A/J spleen cells is shown by the solid line in Fig. 1, *B–D*. All animals presumably succumbed to GVHD, since control animals not receiving A/J spleen cells demonstrated excellent survival (Fig. 1 *A*). The results in Fig. 1 *B* demonstrate that, while TCD syngeneic marrow prevented early GVHD mortality, all animals eventually succumbed to chronic GVHD, and the overall survival curve was not significantly different from that of the controls. However, similar to previous reports (17), GVHD mortality occurred in two phases, including an acute phase in which deaths occurred between days 7 and 15, and a more chronic phase, usually beginning after day 30. We therefore performed separate statistical analyses on the two phases of the survival curves. Analysis of the early portion of the curves (i.e., the first 25 d) revealed a statistically significant ($p < 0.02$) protective effect of TCD syngeneic marrow against mortality. When the results of this experiment were combined with three others involving similarly mild early GVHD mortality, a significant protective effect of TCD syngeneic marrow was again demonstrated (data not shown).

The effect of IL-2 administration on GVHD mortality is shown in Fig. 1 *C*. In these animals, IL-2 was found to protect against both early and late GVHD mortality (Fig. 1 *C*; $p < 0.008$).

Fig. 1 *D* shows the effect of combined treatment with TCD syngeneic marrow plus IL-2, 50,000 U twice daily from days 0 to 4, on GVHD mortality. This combined regimen significantly reduced both early and late GVHD mortality, so that 63% of animals survived >100 d, compared with only 7% survival among animals receiving neither IL-2 nor TCD syngeneic marrow ($p < 0.0006$). Similar protection from late GVHD mortality by this treatment regimen has been reproducibly observed in another strain combination, B10.D2 into B10 ($p < 0.003$ for the combined results of three experiments; $n = 27$ in each group). Although the difference in survival between the groups receiving IL-2 with or without TCD syngeneic marrow did not achieve statistical significance, long-term survival was slightly greater in recipients of TCD syngeneic marrow (63 vs. 46%), possibly reflecting the improved protection from acute GVHD seen in the group receiving TCD marrow (see below). In two additional experiments comparing chronic GVHD mortality in animals receiving IL-2 with or without TCD syngeneic marrow, the late mortality curves of both groups were also similar (see below).

Effects of TCD Syngeneic Marrow and IL-2 on Mortality from Severe GVHD. We next examined the effects of IL-2 on acute mortality due to a more potent GVHD. In most experiments, administration of $>8 \times 10^7$ A/J spleen cells was sufficient to kill control recipients before day 15, as is shown by the solid line in Fig. 2, *B–D*, for animals receiving 1.1×10^7 A/J BMC plus 9×10^6 A/J spleen cells. Since animals receiving BMC without spleen cells demonstrated excellent survival (Fig. 2 *A*), mortality was most likely due to GVHD. As shown in Fig. 2 *B*, administration of TCD syngeneic marrow without IL-2 had no effect on the rapid mortality from this A/J lymphocyte inoculum ($p > 0.05$), and similar results have been observed in most experiments in which the majority of control animals died in the acute phase of GVHD. The effects of IL-2 on such mortality in mice receiving A/J cells with or without TCD syngeneic marrow were examined. The results in Fig. 2 *C* show that IL-2 (10,000 U twice daily for 5 d) provided no protection against acute GVHD mortality when given without TCD syngeneic marrow ($p > 0.05$). In contrast, ad-
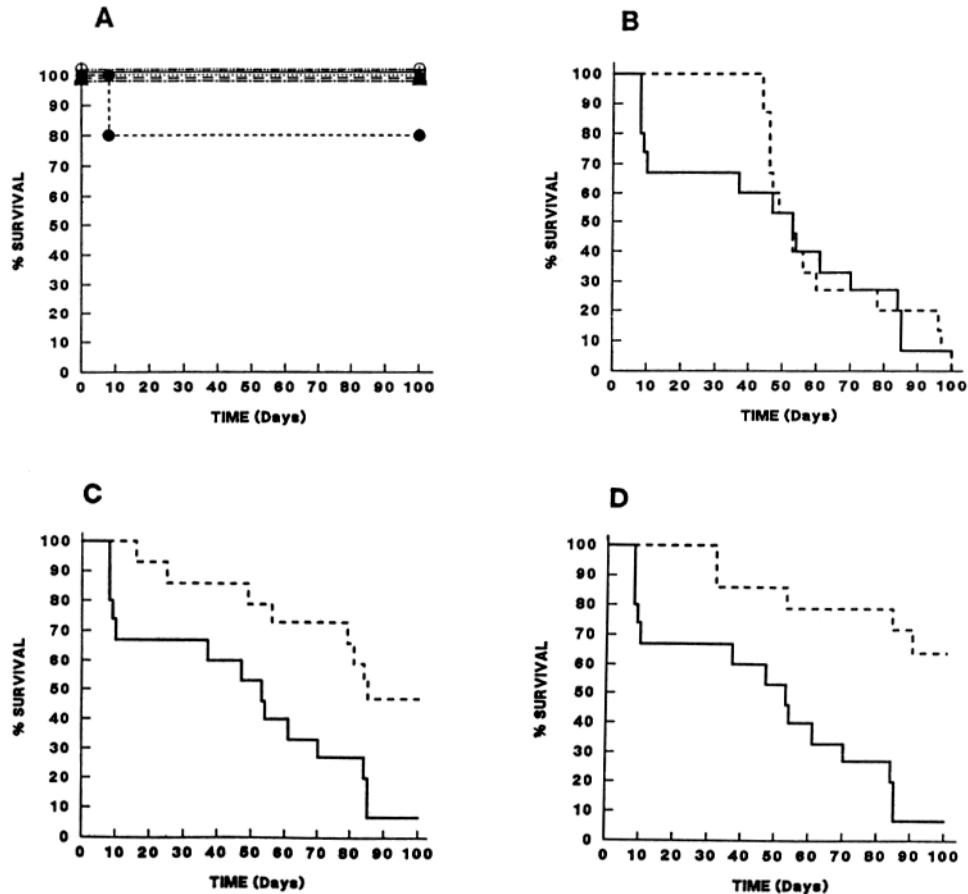
FIGURE 1.   Effect of IL-2 and TCD syngeneic marrow on GVHD mortality from A/J lymphocytes. Lethally irradiated B10 mice received 8×10^6 A/J spleen cells plus 1.5×10^7 A/J BMC, with or without 5×10^6 TCD B10 BMC, and IL-2, 50,000 U, i.p., twice daily for 5 d. (*A*) Survival of control animals not receiving A/J spleen cells; (*) TCD B10 alone (n = 5); (●) TCD B10 BMC plus IL-2 (n = 5); (□) TCD B10 BMC plus A/J BMC (n = 5); (△) TCD B10 BMC plus A/J BMC plus IL-2 (n = 5); (○) A/J BMC (n = 2); (▲) A/J BMC plus IL-2 (n = 3). (*B*, *C*, and *D*) (———) The survival of animals receiving only A/J BMC plus spleen cells (n = 15). (*B*) (- - -) Survival curve of animals receiving A/J BMC plus spleen cells plus TCD B10 BMC (n = 15). (*C*) (- - -) Survival curve of animals receiving A/J BMC plus spleen cells plus IL-2 (n = 15). (*D*) (- - -) Survival curve of animals receiving A/J BMC plus spleen cells, plus TCD B10 BMC plus IL-2 (n = 14).

ministration of TCD syngeneic marrow plus IL-2 was associated with significant protection from GVHD mortality (Fig. 2 *D*; p < 0.02). Thus, coadministration of TCD syngeneic marrow and IL-2 was necessary to protect against mortality from the potent GVHD observed in this experiment. In an additional experiment in which IL-2 alone did not provide optimal protection, a similar effect of TCD syngeneic marrow was observed (data not shown). In other experiments, IL-2 alone was capable of producing marked protection against acute GVHD mortality (e.g., Fig. 3). Nevertheless, maximal early survival was achieved in recipients of TCD syngeneic marrow along with IL-2 in five of five experiments (e.g., Figs. 2 and 3).
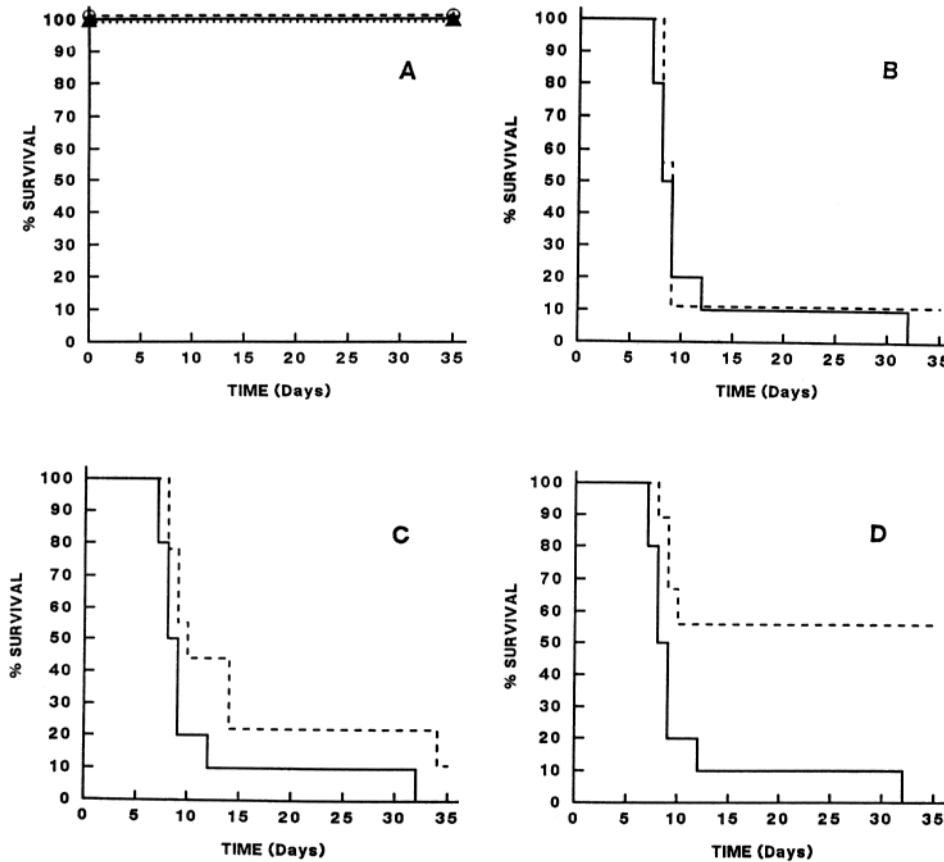
FIGURE 2. Effect of IL-2 and TCD syngeneic marrow on rapid, acute GVHD mortality produced by A/J lymphocytes. Lethally irradiated B10 mice received 9 × 10^6 A/J spleen cells plus 1.1 × 10^7 A/J BMC, with or without 5 × 10^6 TCD B10 BMC and IL-2, 10,000 U twice daily for 5 d. (A) Survival of control animals not receiving A/J spleen cells; (*) TCD B10 BMC plus IL-2 (n = 3); (O) TCD B10 BMC plus A/J BMC (n = 3); (▲) TCD B10 BMC plus A/J BMC plus IL-2 (n = 5). (B, C, and D) (————) The survival of animals receiving A/J BMC and spleen cells alone (n = 10). (B) (- - -) Survival curve of animals receiving A/J BMC plus spleen cells plus TCD B10 BMC (n = 9). (C) (- - -) Survival curve for animals receiving A/J BMC plus spleen cells plus IL-2 (n = 9). (D) (- - -) Survival curve for animals receiving A/J BMC plus spleen cells plus TCD B10 BMC and IL-2 (n = 9).

Relationship of IL-2 Dose to Prevention of GVHD Mortality.   We examined the dose-response relationship of IL-2 and protection from GVHD mortality. In four of five experiments the degree of protection from acute GVHD mortality was directly proportional to the dose of IL-2 administered. These results are summarized in Table I; the difference in mortality in recipients of the 10,000- vs. the 50,000-U dose was significant, but only reflected differences in acute GVHD mortality (see 25-d survival, Table I). Only a small difference in long-term survival was apparent between the two groups, suggesting an increase in chronic GVHD mortality in recipients of the higher, compared with the lower, IL-2 dose (100-d survival, Table I). The magnitude of the acute protective effect in a fifth experiment was inversely proportional
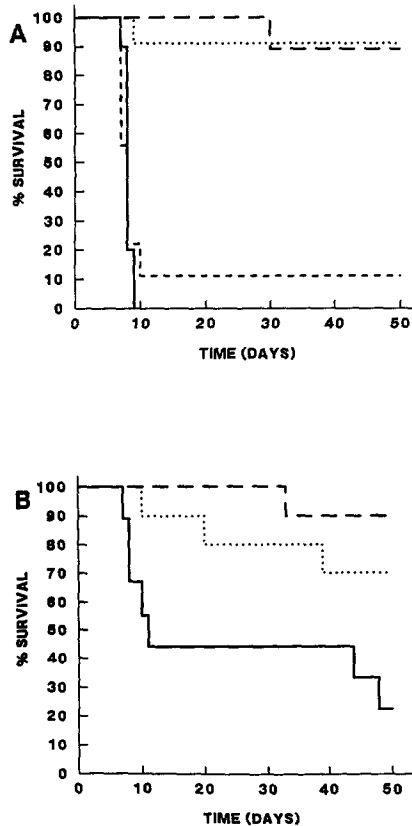
FIGURE 3.   IL-2 alone prevents acute GVHD mortality, but maximal early survival is achieved in recipients of TCD syngeneic marrow plus IL-2. (*A* and *B*) Two independent experiments showing survival in lethally irradiated mice reconstituted with similar inocula containing A/J BMC plus A/J spleen cells, along with: no additional treatment (———); TCD syngeneic marrow coadministered in the reconstituting inoculum on day 0 (- - -); IL-2, 50,000 U twice daily on day 0–4 (*A*) or day 0–2 (*B*) (· · · ·); IL-2, 50,000 U twice daily on day 0–4 (*A*) or day 0–2 (*B*) plus TCD syngeneic marrow coadministered in the reconstituting inoculum on day 0 (—— ——) Each group contained 8–10 animals. Control animals not receiving A/J spleen cells demonstrated excellent survival in both experiments.

to the dose (10,000, 25,000, or 50,000 U twice daily for 5 d) of IL-2 administered. We considered this result to be due to aberrant IL-2 toxicity (discussed below), and therefore elected not to include this experiment in the summary presented in Table I. Control animals receiving TCD syngeneic marrow and/or A/J marrow with or without IL-2 demonstrated uniformly excellent survival (data not shown).

TABLE I

Dose-Response Relationship of IL-2 and GVHD Mortality

(B10(-T) BM + A/J BM + A/J Spleen → B10)

| IL-2 dose* | No. of survivors | | MST‡ | P§ |
	Day 25	Day 100		
U	%	%	*d*	
0 (*n* = 38)	6 (16)	5 (13)	9	<0.002
10,000 (*n* = 38)	21 (55)	14 (37)	47	<0.03
50,000 (*n* = 35)	32 (91)	16 (46)	89	

* The indicated dose was administered twice daily for 5 d, beginning immediately before BMT.

‡ MST, median survival time determined from Kaplan-Meier plots.

§ *P* value comparing group above and below the indicated value. For group receiving 50,000 U IL-2, *P* < 0.0001, compared with group not receiving IL-2. All *P* values were determined using the method of Wilcoxon and Breslow.

Effect of Timing of IL-2 Administration on GVHD Prevention.   Several investigators have reported that in vivo administration of IL-2 is associated with acceleration of GVHD mortality (17–19). One possible explanation for this discrepancy with our own results was that IL-2 was administered by those workers for a prolonged period, or was begun with a delay of 7 or 8 d after BMT (17, 18), whereas we began IL-2 administration on the day of BMT and completed the treatment after 5 d. To assess this possibility, we compared survival in lethally irradiated B10 recipients of TCD B10 marrow plus A/J BMC and spleen cells without IL-2, or with 10,000 U IL-2 administered twice daily for 5 d beginning either on the day of irradiation and BMT, or 7 d later. The results, shown in Fig. 4, indicate that IL-2 was protective only when administration was begun on the day of BMT ($p < 0.01$). Administration of IL-2 beginning on day 7 was associated with a significant acceleration of GVHD mortality ($p < 0.0005$), consistent with previous reports (17, 18). Similar results were obtained in a repeat experiment using the higher dose of IL-2 (50,000 U).

Although the first dose of IL-2 was administered immediately before BMT in all experiments reported here, such timing was not critical, since additional experiments demonstrated that delaying administration until 1 h after BMT did not reduce the anti-GVHD effect of IL-2 (data not shown). Administration of a single high dose of IL-2 (immediately preceeding BMT) did not protect against acute GVHD mortality (Fig. 5).
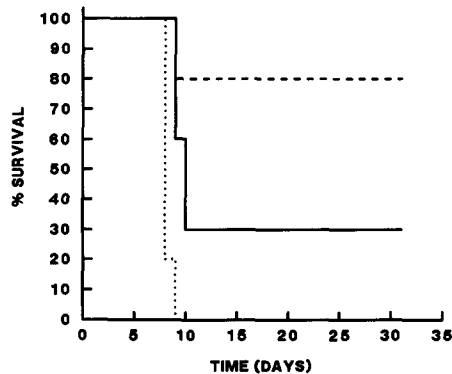

Figure 4.   Effect of timing of IL-2 administration on GVHD mortality. Lethally irradiated B10 mice received 5 × 10⁶ TCD B10 BMC, 10⁷ A/J BMC, and 9 × 10⁶ A/J spleen cells. (————) Survival of control animals not receiving IL-2 (n = 10); (– – –) survival of animals receiving 10,000 U IL-2 twice daily for 5 d beginning on the day of BMT (n = 10); (· · · · ·) survival of animals receiving 10,000 U of IL-2 twice daily for 5 d beginning 7 d after BMT (n = 10). Control animals (not shown; n = 5) receiving A/J marrow plus TCD syngeneic marrow demonstrated 100% survival.
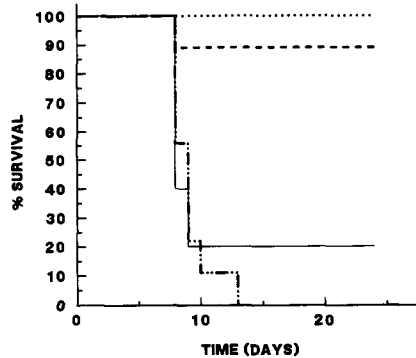

Figure 5.   Assessment of the number of IL-2 doses required for protection against GVHD mortality. Lethally irradiated B10 mice received 5 × 10⁶ TCD B10 BMC, 10⁷ A/J BMC, and 9 × 10⁶ A/J spleen cells. (————) Survival of control animals receiving no IL-2 (n = 10); (– · · –) survival of animals receiving a single dose of 50,000 U IL-2 immediately before BMT (n = 9); (· · · · ·) survival of animals receiving 50,000 U IL-2 twice daily for 2.5 d (i.e., five doses), beginning immediately before BMT (n = 8); (– – –) survival of animals receiving 50,000 U IL-2 twice daily for 5 d (i.e., 10 doses) beginning immediately before BMT (n = 9). Control animals (not shown) receiving TCD syngeneic marrow alone (n = 5) or with IL-2, 50,000 U twice daily for 5 d (n = 5), demonstrated 100% survival.

Since animals in some experiments began to appear ill on the fourth day of high-dose (50,000 U) IL-2 administration, showing lethargy, hunching, and ruffled fur, it seemed possible that this dose of IL-2 might be producing cumulative toxicity. We therefore examined the effect of a shortened 2.5-d (five dose) course of high-dose IL-2 on GVHD mortality. As shown in Fig. 5, this shortened course was at least as protective against early GVHD mortality as was a full 5-d course, and the animals showed no clinical evidence for adverse effects. Similar results were obtained in a repeat experiment. Also, no differences in later mortality have been seen after a 100-d follow-up.

Effect of IL-2 on Engraftment. To examine the effects of IL-2 on alloengraftment, the PBL of long-term BMT survivors were phenotyped using mAbs and FCM. No differences were observed in the level of allogeneic reconstitution between animals receiving or not receiving IL-2 (10,000–50,000 U twice daily for 5 d for one or two courses) along with allogeneic (A/J or B10.D2) spleen cells, BMC, and TCD syngeneic marrow. Examples of FCM profiles from such animals are shown in Fig. 6. Most animals in all groups, regardless of whether or not spleen cells were administered, demonstrated complete allogeneic lymphopoietic repopulation, similar to the results shown in Fig. 6. In some animals receiving IL-2 plus allogeneic BMC and TCD syngeneic BMC without allogeneic spleen cells, however, a small, negative peak representing ∼1–10% of cells was evident on staining with antibody recognizing donor H-2 antigens. This negative peak corresponded to a positive peak on staining with antibody recognizing host H-2 antigens, and tended to disappear with time; such host-type cells were detected in six of seven recipients tested before day 70, and in only 5 of 27 such animals tested after day 95. 1 of 13 simultaneous control
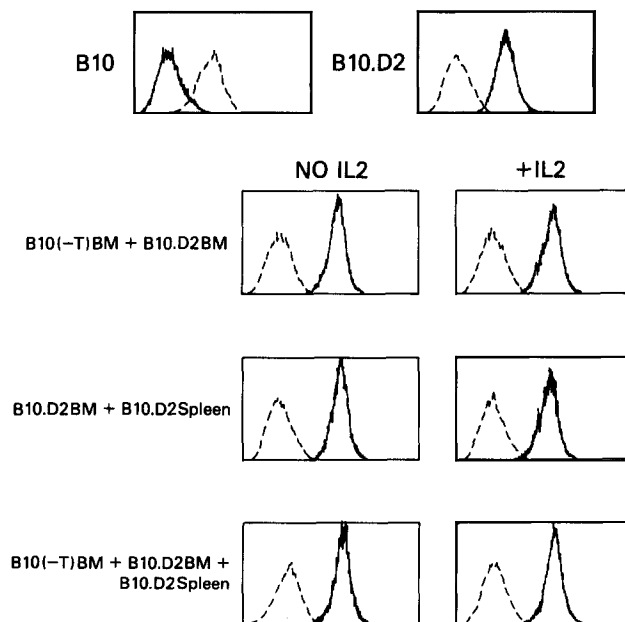

FIGURE 6. Examples of the phenotype of lymphopoietic cells repopulating lethally irradiated B10 mice treated or not treated with IL-2, 50,000 U twice daily for 5 d beginning on the day of BMT. B10 mice were lethally irradiated and reconstituted with either TCD B10 BMC plus B10.D2 BMC, or with TCD B10 BMC, B10.D2 BMC, and B10.D2 spleen cells, or with B10.D2 BMC and spleen cells alone, as indicated. PBL were obtained 15 wk after BMT, stained with mAbs, and analyzed using FCM, as indicated in Materials and Methods. Staining with K^b-specific mAb 5F1 (— — —); staining with D^d-specific mAb 34-2-12 (———).

recipients of TCD syngeneic marrow and allogeneic marrow without IL-2 demonstrated such a peak. Of 21 animals receiving allogeneic spleen cells in addition to TCD syngeneic marrow, allogeneic marrow, and IL-2, none showed any evidence for repopulation by host-type cells at any time.

Discussion

In this report we demonstrate that IL-2 administered in vivo at the time of BMT has a potent effect in preventing mortality due to both acute and chronic GVHD. Furthermore, the combination of IL-2 and TCD syngeneic marrow provides optimal protection against acute GVHD mortality. Neither IL-2 alone nor IL-2 plus TCD syngeneic marrow prevented complete lymphopoietic reconstitution by coadministered allogeneic BMC plus spleen cells. This novel method of achieving complete allogeneic lymphopoietic repopulation while reducing GVHD mortality presents a possible solution to the opposing problems of GVHD versus failure of alloengraftment associated with T cell depletion for the prevention of GVHD (1-6). The possibility that administration of IL-2 might still permit, and even add to (20), the antileukemic effects of allogeneic T cells (4, 5, 7, 21) is currently being explored in a murine leukemia model, which we have recently described (22).

Previous reports from this laboratory demonstrated that TCD syngeneic marrow can delay mortality from acute GVHD (8). In the experiments reported here, we have detected a protective effect of TCD syngeneic marrow against acute GVHD only when the GVHD was mild in severity (e.g., Fig. 1). Furthermore, consistent with the previous report (8), TCD syngeneic marrow alone did not prevent late mortality from chronic GVHD. Thus, TCD syngeneic marrow alone has a limited ability to prevent acute GVHD mortality and no detectable effect on chronic GVHD. In contrast, the addition of high doses of IL-2 leads to increased protection from acute GVHD mortality (e.g., Figs. 2, 4, and 5), as well as significant protection from chronic GVHD mortality (e.g., Fig. 1). Protection from chronic GVHD mortality is apparent regardless of whether or not TCD syngeneic marrow is coadministered.

In the absence of TCD syngeneic marrow, IL-2 also has significant protective activity against acute GVHD mortality, but, in every instance, such protection was less than that observed when TCD syngeneic marrow was coadministered (e.g., Figs. 2 and 3). The ability of TCD syngeneic marrow to increase the protective effect in recipients of IL-2 was most apparent in experiments in which IL-2 alone provided suboptimal protection (e.g., Fig. 2). In some experiments, the degree of protection afforded by IL-2 alone was so potent that there was little room for improvement by the addition of TCD syngeneic marrow (e.g., Fig. 3). The reasons for the variability in the degree of protection afforded by similar doses of IL-2 alone are as yet unclear. If this model were to be applied to larger animals or man, it is possible that IL-2 toxicity would prevent the administration of optimal doses, in which case, the improved protection provided by the addition of TCD autologous marrow might be desirable.

Our data suggest that activation or generation by IL-2 of a cell population contained in TCD syngeneic marrow, such as lymphokine-activated killer (LAK) cells (23-25), NS cells (23, 26), or veto cells (23, 27), might play a role in protecting from GVHD mortality. Both NK and LAK cells have been shown to be activated by treatment with IL-2 in vivo (28-31). Such cell populations in syngeneic marrow might

suppress a GVH response better than similar populations in allogeneic marrow because of their ability to preferentially kill allogeneic and not syngeneic lymphoblasts (32), or to preferentially suppress responses directed against antigens shared by the suppressive population (9, 23, 27). The weaker effect of IL-2 in the absence of TCD syngeneic marrow could reflect the activation of residual cell populations in the irradiated host, or of cell populations in allogeneic BMC and spleens with the ability to suppress GVH reactions in an antigen-nonspecific manner.

Our results appear to conflict with those of other workers, who have found that GVHD is potentiated, rather than abrogated, by in vivo administration of IL-2 (17–19). This discrepancy could be due to several differences in the systems studied, including the fact that these workers did not coadminister TCD syngeneic marrow, administered lower doses of IL-2 (17–19), and used a delayed or prolonged time course of IL-2 administration (17, 18). Consistent with this possibility, we observed protection when IL-2 was administered for 5 d starting on day 0, but acceleration of mortality when administration of the same dose was begun after a 7-d delay. Thus, IL-2 may potentiate GVHD once T cells have already become sensitized, but may prevent or abort the sensitization of previously unprimed T cells. Indeed, IL-2 may be a mediator of the anti-GVHD effect of CD4$^+$ allogeneic T cells observed by Sprent et al. (17) in a class I difference–only GVHD.

Additional possible mechanisms for the anti-GVHD effect of IL-2 include more rapid hematopoietic recovery induced by IL-2 (33), which may be secondary to the production of hematopoietic growth factors by activated NK cells (34). IL-2 may improve resistance to infection through the antiviral, antifungal, antiprotozoan, or antibacterial activities of activated NK cells (35, 36), or by promoting the development of other immunocompetent cell populations; such activities might be highly protective, since infection has been found to play a pivotal role in the induction of, and morbidity from, GVHD (37, 38). The ability of IL-2 to prevent GVHD mortality long after completion of such therapy suggests that protection is either due to the induction of a protective cell population by IL-2, or to the permanent deletion of GVH-reactive clones from the original allogeneic splenocyte inoculum. Experiments are currently underway to distinguish between these possibilities.

In one of five dose-response titrations in the A/J into B10 strain combination, greater protection was provided by decreasing, rather than increasing, doses of IL-2. Although we cannot explain this variability between experiments, these results, the apparent illness of some animals after the third day of high-dose IL-2 treatment, and the observation that IL-2 therapy beginning on day 7 leads to accelerated mortality, suggested that a shorter course of high-dose IL-2 might optimize survival by limiting IL-2 toxicity or by diminishing the exacerbation of GVHD associated with IL-2 administration at later times after BMT. The results of such an approach (Fig. 5) support this notion, and such a protocol may prove to be optimal for avoiding both GVHD and IL-2 toxicity.

We have obtained preliminary evidence for a transient, early increase in NK activity in the spleens of some, but not all, lethally irradiated mice receiving IL-2 and TCD syngeneic marrow (data not shown). Since NK cells have been found to play a role in mediating IL-2 toxicity (39), resisting alloengraftment (40–42), and effecting GVHD-related injury (43), it will be important to dissect the role, if any, of NK cells in the phenomena described here. Despite the increase in NK activity observed

in some animals, complete allogeneic repopulation was achieved in all long-term survivors reconstituted with TCD syngeneic marrow, allogeneic marrow, and allogeneic spleen cells.

In summary, we have presented a new approach to the avoidance of mortality from acute and chronic GVHD that does not prevent alloengraftment. Attempts to understand the mechanism of this effect, and to apply it in a leukemic model (22), will help to determine its potential for clinical application.

Summary

Previous work from this laboratory has demonstrated that T cell–depleted (TCD) syngeneic marrow can delay, but not prevent, the mortality from acute graft-vs.-host disease (GVHD) caused by MHC-mismatched lymphoid cells administered to lethally irradiated mice. We demonstrate here that a protective effect against GVHD is also observed after in vivo treatment with IL-2. Administration of 10,000–50,000 U of IL-2 twice daily for the first 5 d after bone marrow transplantation markedly reduced the mortality from both acute and chronic GVHD induced across complete MHC barriers in lethally irradiated mice, and frequently led to long-term survival. Complete allogeneic reconstitution was demonstrated in all long-term survivors of this treatment regimen. While either IL-2 or TCD syngeneic marrow administered alone was protective in some experiments, the maximal protective effect was observed after administration of both IL-2 and TCD syngeneic marrow, especially when the effects of IL-2 were suboptimal. The timing of IL-2 administration was critical to this beneficial effect, since a delay of 7 d in commencing IL-2 treatment was associated with accelerated GVHD mortality. This new approach to the prevention of GVHD permits the administration of allogeneic T cells, and may therefore avoid the increased incidence of graft failure and loss of antileukemic effects associated with the T cell depletion of allogeneic marrow, which is otherwise required for the prevention of GVHD.

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