Elimination of Leukemia in the Absence of Lethal Graft-Versus-Host Disease After Allogenic Bone Marrow Transplantation

William R. Drobyski, Maria Gendelman, Sanja Vodanovic-Jankovic and Jack Gorski

*J Immunol* 2003; 170:3046-3053; doi: 10.4049/jimmunol.170.6.3046
http://www.jimmunol.org/content/170/6/3046

**References**
This article cites 29 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/170/6/3046.full#ref-list-1

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

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

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Elimination of Leukemia in the Absence of Lethal Graft-Versus-Host Disease After Allogeneic Bone Marrow Transplantation

William R. Drobyski, Maria Gendelman, Sanja Vodanovic-Jankovic, and Jack Gorski

Donor T cells are able to effect a graft-vs-leukemia (GVL) response but also induce graft-vs-host disease (GVHD) after allogeneic bone marrow transplantation. We used an AKR leukemia murine transplant model, analogous to human acute lymphoblastic leukemia, in which donor T cells expressed a thymidine kinase suicide gene, to test whether separation of GVL and graft-vs-host (GVH) responses was feasible by selectively eliminating alloactivated donor T cells at defined time points posttransplant. Under experimental conditions where untreated mice could not be cured of disease without dying from GVHD, mice transplanted with thymidine kinase-positive T cells and subsequently administered ganciclovir (GCV) could eliminate leukemia without lethal GVHD. Timing of GCV administration, donor T cell dose, and preexisting leukemia burden were observed to be critical variables. Eradication of leukemia without lethal GVHD in GCV-treated mice implied that the kinetics of GVL and GVH responses were asynchronous and could therefore be temporally dissociated by timely GCV administration. That this strategy was feasible in a murine leukemia model in which GVHD and GVL reactivity are tightly linked suggests that this approach may be relevant to the treatment of selected human leukemias where similar constraints exist. This strategy represents an alternative approach to separating GVL and GVH reactivity and challenges the current paradigm that separation of these responses is dependent upon the administration of donor T cells with restricted specificity for leukemia as opposed to host Ags. The Journal of Immunology, 2003, 170: 3046–3053.

The therapeutic efficacy of allogeneic marrow transplantation derives not only from the conditioning regimen which is myeloablative, but also from the allogeneic marrow graft itself which is able to mediate an antileukemic (graft-vs-leukemia (GVL)) effect against residual malignant cell populations (1). In both clinical and experimental bone marrow transplantation (BMT), the GVL effect has typically been coexpressed with graft-vs-host disease (GVHD) (2–5). This is due to the fact that both GVL and graft-vs-host (GVH) reactivity are mediated primarily by donor T cells, although cytokines and other secondary effector cell populations (e.g., NK cells, macrophages, etc.) also appear to play a role (6–8). Thus, cure of leukemia is often at the expense of GVHD (2, 3) that can be fatal or require administration of protracted immunosuppressive therapy for effective control. The fact that some patients appear to have cured their disease before GVHD ceases to be a clinical problem (2–4) suggests that the time course of GVL and GVH responses may not be synchronous and therefore may be exploitable from a therapeutic perspective. However, clinically, the precise temporal regulation of GVL/GVH responses has not been feasible due to the fact that immunosuppressive agents are not able to selectively eliminate alloreactive donor T cells at defined time points in the posttransplant period. Thus, whether timed elimination of alloreactive donor T cells posttransplant is a feasible strategy to preserve GVL reactivity while at the same time mitigating GVH lethality under the same time mitigating GVH is unknown. To test this hypothesis, we used a murine AKR leukemia model, analogous to human acute lymphoblastic leukemia, and used donor T cells that were genetically modified to express the herpes simplex virus thymidine kinase (TK) suicide gene. The incorporation of a suicide gene into donor T cells allowed us to precisely regulate donor T cell survival in vivo at selected time points posttransplant by the administration of the antiviral agent ganciclovir (GCV). Transplantation of TK+ T cells into MHC-mismatched leukemia-bearing animals followed by posttransplant GCV administration was then performed to determine whether this strategy was capable of eliminating leukemia and reducing GVH lethality under experimental conditions where the vast majority of untreated mice could not be cured of leukemia in the absence of fatal GVHD.

Materials and Methods

Mice

C57BL/6 (B6; H-2b, Thy1.2+) and AKR/J (H-2k, Thy1.1+) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). TK-transgenic mice on a pure B6 background in which the TK gene is targeted to the T cell using a CD3 promoter/enhancer construct have been previously described (9). 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

GCV (Cytovene; Roche Laboratories, Nutley, NJ) was dissolved in distilled water and stored in aliquots at −20°C. GCV was thawed before use and administered to animals i.p. in PBS at a dosage of 50 mg/kg/day.
Leukemia

T cell acute lymphoblastic leukemia/lymphoma that develops spontaneously in AKR/J mice (AKR-L) (10, 11) was used as a source of tumor. Stocks of leukemia were obtained by serial passage of leukemia cells in AKR mice. Frozen stocks of AKR-L cells were used in GVL experiments. A dose of 100 cells is lethal in irradiated recipients transplanted with T cell-depleted (TCD) bone marrow (BM) alone.

Bone marrow 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-Thy1.2 mAb (clone 30-H12, rat IgG2b; American Type Culture Collection, Manassas, VA) plus low toxicity rabbit complement (C-SIX Diagnostics, Meqoun, WI). BM cells were washed and resuspended in DMEM before injection. Naïve donor T cells were obtained by passing erythrocyte-depleted spleen cells through nylon wool columns to remove non-T cells. Host mice were conditioned with total body irradiation 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 TCD BM (10^7 cells) with or without added TK⁺ T cells within 24 h.

CD4⁺ and CD8⁺ T cell subset enrichment

In some experiments, to obtain highly enriched populations of CD4⁺ T cells, spleen cells from either normal or transgenic animals 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 FACS to confirm purity before transplantation. Typically >90% purity was obtained for the positively selected T cell subset with <2% contamination of the reciprocal subset.

Experimental model

In GVL studies, recipient animals were irradiated and then given specified numbers of AKR-L cells. The following day, mice were transplanted with TCD BM alone or together with TK⁺ T cells. All leukemia-bearing animals (except one animal in one experiment) were autopsied at the time of death to determine whether leukemia was present. Animals were deemed to have relapsed if there was evidence of splenomegaly, hepatomegaly, or lymphadenopathy at autopsy or if mice had exhibited any degree of limb paralysis antemortem. Mice that died without morphologic evidence of leukemia were classified as having died from GVHD.

Flow cytometric analysis and assessment of chimera

mAb conjugated to either FITC or PE were used to assess chimeraism in marrow transplant recipients. PE-anti-CD8 (clone CT-CD8a, rat IgG2a) was obtained from Caltag (San Francisco, CA). PE-anti-TCR a/b (clone H57-597, hamster IgG), PE-anti-CD4 (clone GK1.5, rat IgG2b), FITC-anti-Thy1.2 (clone 30-H12, rat IgG2b), and FITC-anti-H-2Kb (clone AF6-88.5, mouse IgG2a) were all purchased from BD PharMingen (San Diego, CA). Spleen and thymus cells were obtained from chimeras at defined intervals posttransplant, processed into single-cell suspensions, and stained for two-color analysis. Red cells were removed when necessary by hypotonic lysis. Cells were analyzed on a FACScan flow cytometer (BD Biosciences, Mountain View, CA). At least 10,000 cells were analyzed for each determination whenever possible.

Statistics

Survival curves were constructed using the Kaplan-Meier product limit estimator and compared using the log rank test. A value of p = 0.05 was deemed to be significant in survival experiments.

Results

GVL and GVH reactivity are tightly linked in non-GCV-treated mice

The murine model used was designed to evaluate the GVL effect under conditions where the competing risks of graft rejection and GVHD were also operative (9), similar to human transplantation. Initial studies were performed to determine whether there was a minimal T cell dose that cured all animals of leukemia without fatal GVHD. Sublethally irradiated (850 cGy) AKR mice were administered a lethal dose of AKR-L cells (10^5 cells) and then transplanted with TCD B6 BM plus graded doses of TK⁺ T cells. A dose of 5 × 10^5 B6 T cells that prior studies had shown capable of preventing graft rejection (9) resulted in significantly superior survival compared with leukemia control mice (p = 0.008) but did not effect a long-lasting GVL response as the majority of animals died from leukemia (Fig. 1A). When mice were transplanted with 1.5 × 10^5 T cells, survival was again significantly higher than in control animals (p = 0.017) and leukemia was not detected at autopsy in any mice (Fig. 1B). However, all of these animals died from GVHD with significant weight loss and characteristic features of hunched posture, perianal dermatitis, and ruffled fur. Thus, animals could not be cured of leukemia in the absence of fatal GVHD, indicating that GVL and GVH reactivity were tightly linked (Fig. 1C).

Retention of GVL reactivity in the absence of lethal GVHD is dependent upon the GCV administration schedule

The goal of these studies was to determine whether selective elimination of alloreactive donor T cells by timed GCV administration was capable of prolonging leukemia-free survival under conditions where GVHD was the primary cause of treatment failure. Initial experiments were performed to determine whether the administration of GCV alone had any antileukemic activity in mice transplanted with TCD BM alone. Cohorts of irradiated mice administered 100 AKR-L cells (n = 4 per group) were transplanted with TCD B6 BM alone and then either left untreated or treated with GCV on days 4–14 post-BMT. This schedule encompassed GCV administration schedules used in subsequent studies. The median time to death in untreated mice (17 days; range, 16–27) did not differ from that in GCV-treated animals (20 days; range, 17–23) (p = 0.83). Thus, GCV alone had no direct antitumor activity in this leukemia transplant model.

The timing of GCV administration was initially examined using three different administration schedules (i.e., days 4–8, 7–11, and 10–14). A T cell dose of 1.5 × 10^6 was used in these studies because this dose was the minimal dose necessary to eradicate leukemia (Fig. 1B). Administration of GCV on days 4–8 post-BMT resulted in significantly superior survival compared with leukemia control mice (p = 0.007). However, there was no statistically significant difference in survival between GCV-treated and non-GCV-treated mice (p = 0.70) (Fig. 2A). However, the cause of death in these two groups was different. The majority of GCV-treated mice (71%) died with evidence of leukemia at autopsy, while all untreated animals died from GVHD without morphologic evidence of disease recurrence. Delaying the administration of GCV until days 7–11 resulted in similar outcomes to that observed when GCV was administered on days 4–8. GCV-treated mice had significantly superior survival compared with leukemia control animals (p = 0.019), but survival was no different from that of untreated animals (p = 0.76) (Fig. 2B). In untreated mice, the primary cause of death was GVHD, while the majority of GCV-treated mice died with evidence of leukemia, similar to that observed in animals treated with GCV on days 4–8. Thus, administration of GCV on either days 4–8 or 7–11 substantially compromised the GVL response.

In contrast, administration of GCV on days 10–14 resulted in significantly superior survival compared with both leukemia control animals (p = 0.014) and untreated mice (p = 0.006) with 40% of these mice being long-term survivors (Fig. 2C). The incidence of leukemia recurrence at day 90 was also lower in recipients treated with GCV on days 10–14 vs either days 4–8 (p = 0.06) or 7–11 (p = 0.03 by Fisher’s exact test). To confirm that surviving mice treated with GCV on days 10–14 were free of residual leukemia, spleen cells were obtained from these animals 97–109
planted with TCD B6 BM alone (Leuk Control) (filtered a lethal dose of AKR-L (100 cells) and the following day were transplanted mice. Sublethally irradiated (850 cGy) AKR mice were adminis-

FIGURE 1. ELIMINATION OF LEUKEMIA WITHOUT LETHAL GVHD

3048 ELIMINATION OF LEUKEMIA WITHOUT LETHAL GVHD

data in residual leukemia was present. The percentage of all mice that relapsed with either $5 \times 10^6$ (A; $n = 11$) or $1.5 \times 10^6$ (B; $n = 12$) TK$^+$ T cells. All animals were autopsied at the time of death to determine whether residual leukemia was present. The percentage of all mice that relapsed with leukemia as determined by autopsy is shown. Actual survival is depicted. Data in A and B were derived from two independent experiments each. C. The percentage of mice dying from either leukemic recurrence (■) or GVHD (■) in these experiments.

days posttransplant (two mice each from two independent experiments). Pooled cell suspensions were then administered i.v. to normal unirradiated AKR mice ($1 \times 10^6$/mouse; $n = 4$ per group, total of 8 mice). Whereas 100 AKR-L cells caused death within 36 days in all normal AKR mice ($n = 8$), no animals that received chimeric spleen cells died from leukemia after 80 days of observation. Thus, surviving mice treated with GCV on days 10–14 appeared to have cured their leukemia. These GCV-treated mice ($n = 4$) were also analyzed for splenic and thymic reconstitution as well as extent of donor chimerism. All mice had complete donor T cell engraftment (mean, 99%) in the spleen and thymus (mean, 100%). However, serial weights in GCV-treated animals indicated that these animals had lost ~10% of their pretransplant body weight (mean pre-BMT weight, 25.4 g vs 22.3 g at day 90). Splenic and thymic cellularity (mean, $27 \times 10^6$ and $69 \times 10^6$ cells, respectively) was also reduced compared with that of normal mice, indicating that these animals were not completely free from GVHD.

Therefore, additional studies were performed to determine whether the administration of a second course of GCV could further reduce GVHD in treated recipients. Mice were transplanted as in Fig. 2C with the exception that GCV was administered on days 10–14 and 18–22. Survival in mice administered two courses of GCV (Fig. 2D) was similar to that observed in animals adminis-
tered a single course of GCV (Fig. 2C). A composite analysis of results from Fig. 2, C and D, demonstrated statistically significant higher survival for GCV-treated mice when compared with either leukemia controls ($p = 0.002$) or untreated mice ($p = 0.0006$). Notably, mice administered a second course of GCV had no in-
crease in leukemia recurrence relative to animals treated once indicating that the GVL response was not adversely affected. Al-
though mortality from GVHD was similar in both groups (30%), surviving animals treated with two courses of GCV had an in-
crease in weight with time posttransplantation (mean pre-BMT weight, 24.9 g vs 27.4 g at day 90) in comparison to mice treated with one course. These data were consistent with the interpretation that GVHD was less severe in these mice. Collectively, these studies demonstrated that retention of GVL reactivity in the absence of lethal GVHD was critically dependent upon the GCV administra-
tion schedule and required that GCV treatment be delayed for at least 10 days posttransplantation in this murine model.

Effect of donor T cell dose escalation on GVL/GVH reactivity

Prior studies using TK$^+$ T cells to attenuate the severity of GVHD have suggested that administration of GCV immediately after BMT may provide optimal protection (12, 13). Because we observed that early GCV treatment on days 4–8 or 7–11 compromised the GVL effect in mice transplanted with $1.5 \times 10^6$ TK$^+$ T cells, we examined whether escalating the dose of donor TK$^+$ T cells in the setting of early GCV administration could provide a more effective GVL response, yet still result in mitigation of GVHD severity. Leukemia-bearing AKR mice were transplanted with TCD B6 BM alone or together with $3 \times 10^6$ TK$^+$ T cells. This dose of T cells was sufficient to eradicate leukemia in all untreated animals similar to what was observed at a lower T cell dose ($1.5 \times 10^6$). However, there was no difference in survival between leukemia control animals and non-GCV-treated mice ($p = 0.07$) (Fig. 3), due to the fact that mice transplanted with $3 \times 10^6$ cells all died early post-BMT due to GVHD. Similarly transplanted cohorts of mice were also treated with GCV on days 4–8, a schedule that had resulted in a high probability of disease recur-
rence in mice transplanted with $1.5 \times 10^6$ TK$^+$ T cells (Fig. 2A). This resulted in no statistically significant difference in survival between leukemia controls and mice that received GCV ($p = 0.08$) or between GCV-treated and untreated mice ($p = 0.96$).
Notably, all GCV-treated mice died from GVHD, indicating that escalation of the donor T cell dose effected a stronger GVL response, but also resulted in more severe GVHD. The net result was that survival in mice transplanted with $3 \times 10^6$ T cells and treated with GCV on days 4–8 was no different compared with similarly treated animals transplanted with $1.5 \times 10^6$ B6 T cells (median survival, 27 vs 34 days, respectively; $p = 0.22$ by log rank test).

Role of CD4$^+$ and CD8$^+$ T cell subsets in GVL and GVH reactivity

Both CD4$^+$ and CD8$^+$ T cells have been shown to contribute to the GVH and GVL effects in the AKR leukemia model (14, 15). In studies using unseparated T cells, GCV administration eliminated both of these T cell subsets; however, even under optimal conditions (Fig. 2, C and D), a proportion of mice still relapsed from their disease. We reasoned that selective elimination of either CD4$^+$ or CD8$^+$ T cells might provide a more potent GVL effect due to the persistence of the reciprocal non-TK$^+$ T cell population after GCV administration. To test this hypothesis, leukemia-bearing AKR mice were initially transplanted with TCD B6 BM alone or together with purified CD4$^+$ T cells and CD8$^+$ non-TK$^+$ T cells. Recipients received absolute numbers of CD4$^+$ T cells and CD8$^+$ non-TK$^+$ T cells that were identical with that in mice transplanted with unseparated T cells (see Fig. 4). Untreated animals all died at a median of 24 days posttransplant from severe GVHD and had no statistically significant difference in survival compared with leukemia controls ($p = 0.45$) (Fig. 4A). However, mice that were administered GCV on days 4–8 had significantly different survival compared with untreated controls ($p < 0.001$) (Fig. 4A). Mice transplanted with TCD B6 BM alone or together with CD4$^+$ T cells exhibited similar survival regardless of GCV administration (Fig. 4B and C). However, mice transplanted with TCD B6 BM alone or together with CD8$^+$ non-TK$^+$ T cells exhibited no survival benefit from GCV administration (Fig. 4D).

**FIGURE 2.** Retention of GVL reactivity after GCV administration is schedule dependent. Sublethally irradiated (850 cGy) AKR mice were administered a lethal dose of AKR-L (100 cells) and the following day were transplanted with TCD B6 BM alone (■, $n = 9–13$ per group) or together with $1.5 \times 10^6$ B6 T cells. Cohorts of mice transplanted with TK$^+$ T cells were either left untreated (No GCV; □, $n = 10–17$ per group) or treated with GCV for 5 days on one of four schedules: days 4–8 (A), 7–11 (B), 10–14 (C), or 10–14 and 18–22 (D) post-BMT (●, $n = 10–17$ per group). Actual survival is depicted. Data are pooled from two to three independent experiments at each GCV schedule.
prolonged survival relative to untreated animals \((p = 0.006)\), although survival in GCV-treated mice was not significantly different from that of leukemia controls \((p = 0.09)\). The cause of death in GCV-treated mice was attributable to GVHD, as no mice had morphologic evidence of leukemia at autopsy. This was in contrast to mice transplanted with an equivalent number of unseparated cells and treated on the same GCV schedule where the majority of animals relapsed (Fig. 2A). In subsequent studies, reciprocal experiments were performed in which mice were transplanted with equivalent numbers of CD4\(^+\) non-TK\(^+\) T cells and CD8\(^+\) TK\(^+\) T cells (Fig. 4B). Administration of GCV on days 4–8 resulted in no statistically significant difference in survival between untreated and GCV-treated mice \((p = 0.19)\) as all animals in both groups died from GVHD. These results indicated that a strategy of selective elimination of either CD4\(^+\) or CD8\(^+\) T cells prevented leukemia relapse but exacerbated GVHD. Optimal results were observed when CD4\(^+\)TK\(^+\) T cells were used in conjunction with CD8\(^+\)non-TK\(^+\) T cells.

Preservation of GVL reactivity is dependent upon leukemia cell burden

To quantify the magnitude of the GVL effect in GCV-treated mice, AKR mice were administered either \(10^5\) or \(10^6\) AKR-L cells and then transplanted with TCD B6 BM plus \(1.5 \times 10^6\) TK\(^+\) T cells. This represented a one- and two-log increase in the leukemia cell dose, respectively. Animals were then treated with GCV on days 10–14 which had proven to be the optimal schedule for effecting a GVL response when mice were challenged with a lower dose of leukemia (100 cells) (Fig. 2, C and D). Control mice administered \(10^3\) AKR-L cells all died from leukemia by a median of 15 days post-BMT (Fig. 5A). This was \(\sim 1\) wk earlier than mice given 100 AKR-L cells (Fig. 2, A–D). Mice transplanted with TCD BM plus \(1.5 \times 10^6\) TK\(^+\) T cells had significantly prolonged survival relative to that of leukemia control animals \((p = 0.035)\), although all ultimately died from GVHD within 2 mo of transplant. GCV treatment of similarly transplanted animals resulted in significantly prolonged survival compared with both leukemia control \((p = 0.004)\) and untreated \((p = 0.013)\) mice. Approximately 40% of GCV-treated mice died with evidence of leukemia at autopsy, similar to what was observed when animals were administered 100 AKR-L cells. When mice were given \(10^6\) AKR-L cells before transplantation, control animals all died rapidly from leukemia at a median of 13 days post-BMT (Fig. 5B). Mice treated with GCV \((p = 0.004)\) and untreated animals \((p = 0.004)\) had significantly superior survival compared with leukemia control mice. However,
in contrast to what was observed at lower doses of leukemia, GCV-treated mice had significantly inferior survival relative to untreated animals (p = 0.05). This was attributable to a higher incidence of and more rapid death due to leukemia (92% of mice with leukemia at autopsy). The percentage of untreated mice that died with evidence of leukemia was also substantial (73%) and indicated that there was a significant loss of the GVL effect at this leukemia cell dose. Thus, in these animals, any salutary effects derived from GCV treatment on attenuating the severity of GVHD were outweighed by a corresponding loss of GVL reactivity. These data indicated that the ability of GCV treatment to preserve GVL reactivity and reduce GVHD severity at a fixed donor T cell dose was dependent upon the leukemia cell burden of transplanted animals.

Discussion

Current approaches for separating GVL and GVH reactivity are largely predicated on the ex vivo administration of donor T cells with restricted and/or preferential specificity for leukemia as opposed to host Ags (16–20). In this study, we considered an alternative approach in which donor T cells are allowed to initiate an antihost reaction in vivo for a defined period of time before it is abbreviated by GCV administration. The underlying assumption for these studies was that the kinetics of GVL and GVH responses might be asynchronous and separation of GVL and GVH might be feasible with an approach that allowed for timed elimination of donor T cells. We addressed this question under conditions in which dose escalation of donor T cells resulted in no apparent therapeutic window (Fig. 1, A and B). Thus, the vast majority of mice could not be cured of leukemia without dying from GVHD. Our studies demonstrated that administration of GCV to mice transplanted with TK⁺ T cells could eliminate leukemia without fatal GVHD. However, timing of GCV was critical because mice treated on days 4–8 or 7–11 had no increase in survival relative to untreated animals due to the fact that the decrease in GVHD mortality was offset by a corresponding increase in leukemic recurrence. However, prolongation of survival relative to both leukemia and GVHD control mice was observed when GCV administration was delayed until days 10–14 posttransplantation. This was attributable to a reduction in the relapse rate without a commensurate increase in GVHD mortality and indicated that GVL and GVH reactivity could be temporally dissociated by timely GCV administration.

The other major findings were that the T cell dose, preexisting tumor burden, and subset composition of TK⁺ T cells were variables that affected both retention of GVL reactivity and attenuation of GVHD lethality. Escalation of the donor T cell dose (3 × 10⁶) in transplanted animals to compensate for the loss of GVL reactivity with early GCV administration (days 4–8) improved the antileukemic effect, but also significantly increased the severity of GVHD, resulting in all animals dying from this complication (Fig. 3). Transplantation of selected (CD4⁺ or CD8⁺) TK⁺ T cell subsets was effective in prolonging survival relative to GVHD control animals only when CD4⁺TK⁺ T cells were cotransplanted with non-TK⁺CD8⁺ T cells (Fig. 4A). Notably, this required earlier administration of GCV than when unseparated TK⁺ T cells were used. However, a comparative survival analysis between mice transplanted with unseparated TK⁺ T cells and treated with GCV beginning on days 10–14 (GCV group, Fig. 2, C and D, n = 20) vs animals transplanted with CD4⁺TK⁺ and CD8⁺ non-TK and treated with GCV on days 4–8 (GCV group, Fig. 4A, n = 12) revealed no statistically significant difference in survival (p = 0.25), suggesting that these approaches may be therapeutically equivalent. In contrast, transplantation with CD8⁺TK⁺ and CD4⁺non-TK⁺ T cells resulted in no improvement in survival relative to untreated mice. Elimination of leukemia was also dependent upon the preexisting leukemia burden. When mice were administered AKR-L cell doses of 10³–10⁵, GCV-treated mice had significantly superior survival relative to both leukemia and untreated controls (Figs. 2C and 5A). However, when animals were challenged with 10⁵ AKR-L cells, administration of GCV adversely affected survival due to more rapid death from leukemia recurrence. Collectively, these studies indicated that there was a narrow therapeutic window within which mice could be cured of leukemia without developing lethal GVHD.

The fact that GCV-treated mice could be cured of leukemia without fatal GVHD implied that the time course of the GVL and
GVH responses differed and that donor T cells preferentially targeted leukemia cells as opposed to host tissues within this temporal window. In effect, the leukemia was eradicated before donor T cells could induce sufficient collateral host tissue damage to kill the recipient. The ability to dissociate GVL and GVH responses in this study can be conceptualized in two ways that are based on the relative specificities of donor T cells for leukemia and host alloantigens. One derives from the assumption that there exist two separate populations of donor T cells with relative, if not absolute, specificity for leukemia as opposed to recipient Ags. In this scenario, GVL-reactive T cells have an earlier kinetic response against leukemia cells than do GVH-reactive T cells against host Ags. By the time of optimal GCV administration, GVL-reactive T cells have eliminated the leukemia, but GVH-reactive T cells have not initiated a fatal GVH reaction. However, to date, leukemia-specific T cells have not been identified in either AKR syngeneic hosts (21) or in AKR recipients of allogeneic marrow grafts (22). Moreover, we would predict that the dominant immune response in this MHC-incompatible model would be directed against H-2k alloantigens present on both host and leukemia cells. Therefore, we currently favor an alternative hypothesis based on the premise that no separate GVL and GVH populations exist, but rather donor T cells are able to respond to alloantigens present on both cells. In this scenario, donor T cells target the leukemia preferentially such that mice are cured of leukemia without lethal GVHD, as long as the leukemia burden is not excessive (Fig. 5). An unresolved question that is relevant regardless of which of these two hypotheses is correct is why leukemia cells would be more favorable targets for donor T cells early post-BMT. Studies are currently ongoing in an attempt to address this issue.

An important practical point is that the use of TK-modified donor T cells to preserve the GVL effect and reduce GVHD is a viable strategy regardless of whether or not separate GVL and GVH donor T cell populations exist. In human marrow transplantation, both scenarios may be operative. For example, an optimal GVL effect has typically been dependent upon the presence of GVHD in diseases such as acute lymphoblastic leukemia (23) suggesting the presence of donor T cells with overlapping specificities. Conversely, GVL and GVH responses have been more easily dissociable in diseases such as chronic myelogenous leukemia (24) where leukemia-reactive T cells have been demonstrable (18, 25, 26). One would predict that the major limitation of this approach would occur in diseases where GVL and GVH reactivity are tightly linked, such as in the AKR leukemia model. That a therapeutic window existed under these conditions suggests that this strategy may be relevant for the treatment of human leukemias as well (27, 28). Testing this approach in a murine model in which donor and recipient differ only at minor histocompatibility Ags that are more analogous to the majority of transplants performed in man would provide additional confirmation.

We observed that not all surviving mice that were cured of their leukemia were completely free of GVHD. There are several possible explanations for this observation. The first is that GCV failed to eliminate all alloantigen-specific donor T cells either because the drug concentration was inadequate at tissue sites or because not all host-reactive donor T cells were actively dividing at the time of GCV administration. The fact that mice treated with a second course of GCV (Fig. 2D) had improved weight curves supports the premise that not all GVH-reactive T cells may have been completely eliminated in all instances. Alternatively, alloantigen-specific donor T cells may have been completely eliminated but caused irreversible host tissue damage to target organs such as intestinal tract, skin, liver, and thymus before elimination by GCV. In that regard, thymus from some GCV-treated mice that were cured of their leukemia were depleted of thymocytes relative to normal animals, indicating that thymic damage had occurred in these mice. Damage to the host thymus has been reported to result in the emergence of autoreactive BM-derived donor T cells that can themselves contribute to GVHD (29, 30). Finally, secondary effectors of GVHD pathology (i.e., NK cells, macrophages, and proinflammatory cytokines) that would not be directly responsive to GCV may have become recruited to perpetuate the GVH response (6, 7, 31). The fact that we observed GVHD in some surviving recipients indicates that this genetic strategy for prevention and/or mitigation of GVHD may have finite limitations. This interpretation is supported by limited clinical data derived from studies by Bonini et al. (32) and Tiberghien et al. (33) who used this approach in allogeneic marrow transplant recipients with underlying hematologic malignancies. In both studies, there were patients who failed to have complete resolution of GVHD after administration of GCV.

In summary, this study demonstrates that leukemia can be eliminated in the absence of fatal GVHD using donor T cells that express a TK suicide gene. Separation of GVL/GVH reactivity was due to temporally discordant GVL and GVH responses that could be exploited therapeutically by timed GCV administration. This protective effect was contingent upon a number of variables that included the timing of GCV administration, the number and over-all composition of donor T cells, and the preexisting leukemia burden at the time of transplantation. That this strategy was feasible in a murine leukemia model in which GVHD and GVL reactivity are tightly linked suggests that it may be relevant to the treatment of selected human leukemias where similar constraints exist. This strategy represents an alternative approach to separating GVL and GVH reactivity and challenges the current paradigm that separation of these responses is dependent upon the administration of donor T cells with restricted specificity for leukemia as opposed to host Ags.

References


