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_J Immunol_ 2013; 190:1341-1350; Prepublished online 21 December 2012;
doi: 10.4049/jimmunol.1201554
http://www.jimmunol.org/content/190/3/1341

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/12/31/jimmunol.1201554.DC1

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Granzyme B–Mediated Damage of CD8+ T Cells Impairs Graft-versus-Tumor Effect

Guanglin Bian,* Xilai Ding,* Nicholas D. Leigh,* Youzhou Tang,*† Maegan L. Capitano,* Jingxin Qiu,‡ Philip L. McCarthy,‡ Hong Liu,‡ and Xuefang Cao*

Allogeneic hematopoietic cell transplantation is an established treatment for hematologic and other malignancies. Donor-derived immune cells can identify and attack host tumor cells, producing a graft-versus-tumor (GVT) effect that is crucial to the treatment. Using multiple tumor models and diverse donor–host combinations, we have studied the role of granzyme B (GzmB) in GVT effect. We first confirmed previous findings that GzmB deficiency diminished the ability of a high dose of CD8+ T cells to cause lethal graft-versus-host disease. However, when GVT studies were performed using a moderate cell dose that the hosts could tolerate, GzmB−/− CD8+ T cells demonstrated a significantly enhanced GVT effect. GzmB-mediated, activation-induced cell death in wild-type CD8+ T cells was found responsible for their reduced GVT activity. Conversely, GzmB−/− CD8+ T cells exhibited enhanced expansion, skewed toward an effector or effector memory phenotype, and produced higher amounts of IFN-γ and Fas ligand that might contribute to GzmB-independent tumor control. These findings demonstrate for the first time, to our knowledge, that GzmB-mediated damage of CD8+ T cells impairs the desired GVT effect. This study suggests that inhibiting donor-derived GzmB function may represent a promising strategy to improve GVT effect without exacerbating graft-versus-host disease.

*Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263; †Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263; and ‡Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263

Received for publication June 7, 2012. Accepted for publication November 18, 2012.

This work was supported by a Young Investigator Development Award (to X.C.) from the Roswell Park Alliance Foundation and by a Basic Research Fellow Scholar Award (to X.C.) from the American Society of Hematology.

Address correspondence and reprint requests to Dr. Xuefang Cao, Department of Immunology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263. E-mail address: Xuefang.Cao@RoswellPark.org

The online version of this article contains supplemental material.

Abbreviations used in this article: AICD, activation-induced cell death; AML, acute myeloid leukemia; BM, bone marrow; BMT, bone marrow transplantation; FasL, Fas ligand; GVHD, graft-versus-host disease; GVT, graft-versus-tumor; GzmB, granzyme A; GzmB, granzyme B; TCD, T cell depletion; Treg, regulatory T; WT, wild-type.

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Materials and Methods

Animals and tumor cells

129/SvJ (H-2b) and C57BL/6 (H-2b) mice were obtained from The Jackson Laboratory. BALB/c (H-2d) and DBA/2 (H-2d) mice were purchased from the National Cancer Institute. Granzyme A (GzmA)^−/− and GzmB^−/− mice in 129/SvJ strain and GzmB^−/− mice in C57BL/6 strain were developed as described (15–19). A20 lymphoma and WEHI-3 leukemia cells, derived from the BALB/c strain, and P815 mastocytoma cells, derived from the DBA/2 strain, were transduced to express luciferase as described (15). MB0 acute myeloid leukemia (AML) cells were derived from 129/SvJ strain as described (15). All mice were maintained in specific pathogen-free housing, and all experiments were conducted in accordance with the animal care guidelines in the Roswell Park Cancer Institute using protocols approved by the animal studies committee.

Reagents and Abs

Abs including anti-mouse CD3, CD45.1, IFN-γ, CD4, CD8, H-2Kb, CD16/32, CD44, CD62L, Annexin V apoptosis detection kits, and IFN-γ ELISA kits were purchased from eBioscience. Fasl ELISA kits were purchased from R&D Systems. BruU flow kits were purchased from BD Biosciences.

Donor cell preparation

All donor BM cells were isolated from WT mice. TCD was performed with auto-MACS by using anti-CD90.2 microbeads (Miltenyi Biotec). Donor CD8^+ T cells (purity ∼85%) were purified by the spleens by using the Pan T Isolation Kit II (Miltenyi Biotec) combined with biotin-conjugated anti-CD4. Donor CD4^+CD25^+ T cells (purity >90%) were purified by using the Pan T Isolation Kit II combined with biotin-conjugated anti-CD8 and anti-CD25 Abs.

Acute GVHD model

The BALB/c hosts were irradiated with 9 Gy from [137]Cs source. One day later, the hosts were injected i.v. with 2 × 10^6 TCD-BM cells only or combined with 0.3–1.5 × 10^6 CD8^+ T cells or 0.1–0.2 × 10^6 CD4^+CD25^+ T cells isolated from 129/SvJ WT or GzmB^−/− mice. The hosts were weighed every week and monitored for survival.

GVMT model

The donor mice were either 129/SvJ strain or C57BL/6 strain (both H-2b). The BALB/c (H-2d) host mice were irradiated with 9 Gy. One day later, the hosts were inoculated i.v. with 1 × 10^6 A20 cells or 4 × 10^4 WEHI-3 cells. Then, immediately via another i.v. injection, these mice received 2 × 10^6 TCD-BM cells only or combined with 0.3–1.5 × 10^6 CD8^+ T cells or 0.1–0.2 × 10^6 CD4^+CD25^+ T cells isolated from 129/SvJ WT or GzmB^−/− mice. The hosts were weighed every week and monitored for survival.

Histopathological analysis of GVHD target organs

GzmA^−/− mice were inoculated with A20 cells as described above and then transplanted with 2 × 10^6 TCD-BM cells combined with a mixture of 1.5 × 10^6 CD45.1 WT CD8^+ T cells and 1.5 × 10^5 GzmB^−/− CD8^+ T cells. The BALB/c host mice were irradiated and inoculated with A20 cells as described above and then transplanted with 2 × 10^6 TCD-BM cells combined with a mixture of 1.5 × 10^6 CD45.1 WT CD8^+ T cells and 1.5 × 10^5 GzmB^−/− CD8^+ T cells.

Results

GzmB deficiency diminishes the ability of a high dose of CD8^+ T cells to cause lethal GVHD

Previous studies have shown that GzmB deficiency diminishes the ability of allogeneic CD8^+ T cells to cause lethal GVHD (9, 10). Those studies used an MHC-disparate model in which the hosts were transplanted with syngeneic BM combined with MHC-mismatched T cells harvested from GzmB^−/− or WT donors. Although such an MHC-disparate system could examine T cell-mediated damage to the hematopoietic system of the host, it was not strictly representative of clinical transplantation in which donor-derived T cells are always syngeneic to the BM graft. In this study, we developed an allogeneic transplantation model that is more clinically relevant. We harvested both TCD-BM and CD8^+ T cells from 129/SvJ donors (H-2b) and used BALB/c (H-2d) mice as hosts. Thus, the CD8^+ T cells and BM graft are syngeneic to each other, but allogeneic to the host. Using this improved model, we performed transplants with a high dose of CD8^+ T cells to recapitulate the previous studies. Transplants of 1.5 × 10^6 WT CD8^+ T cells caused progressive weight loss and histopathological and lethal GVHD in ∼50% of the hosts (Fig. 1A, Supplemental Fig. 1), whereas transplants of the same dose of GzmB^−/− CD8^+ T cells did not induce substantial lethal GVHD. This result confirms that GzmB is required for a high dose of allogeneic CD8^+ T cells to cause lethal GVHD.

To further determine the role of GzmB in moderate condition of GVHD, we performed dose-finding experiments and found that 0.3–0.6 × 10^6 CD8^+ T cells could be tolerated by the BALB/c hosts without lethality. Therefore, we performed transplants of 2 × 10^6 TCD-BM cells combined with 0.3 × 10^6 CD8^+ T cells isolated from WT or GzmB^−/− mice. Compared to the hosts receiving BM only, host mice receiving this low dose of CD8^+ T cells demonstrated moderate weight loss and histopathological evidence of GVHD (Fig. 1B). However, no significant differences were observed between host mice receiving WT and GzmB^−/− CD8^+ T cells. This result suggests that GzmB does not affect moderate GVHD under the condition of a low dose of CD8^+ T cells.

We have also attempted to determine the role of GzmB in CD4^+ T cell–mediated GVHD in this model. To exclude any potential effect that CD4^+CD25^+ regulatory T (Treg) cells may have on GVHD because they may also use GzmB (15), we transplanted CD4^+CD25^+ T cells. A low dose of 0.2 × 10^6 or 0.1 × 10^6 CD4^+CD25^+ T cells (Fig. 1C, 1D) caused rapid and lethal GVHD to 100% of the hosts. Notably, GzmB deficiency did not affect the ability of CD4^+CD25^+ T cells to cause severe GVHD in this model, which is also consistent with the previous findings (9, 10).
mice, we monitored their survival. All of the tumor-bearing hosts receiving TCD-BM only died of tumor outgrowth. Transplant of WT CD8+ T cells prolonged host survival but failed to rescue the hosts from death caused by tumor growth (Fig. 2B). Remarkably, GzmB−/− CD8+ T cells rescued the majority of the hosts, consistent with the dramatically reduced tumor burden observed in these mice. To rule out a donor strain–specific effect, we prepared TCD-BM and CD8+ T cells from WT and GzmB−/− mice in the C57BL/6 strain (H-2b) and performed equivalent experiments. Indeed, we observed similar results (Fig. 2C). Furthermore, to rule out a tumor model–specific effect, we inoculated the BALB/c mice with syngeneic WEHI-3 leukemia cells and performed the same transplants. Again, we observed similar results (Fig. 2D). These results demonstrate that GzmB deficiency, by an unidentified mechanism, enhances CD8+ T cell–mediated GVT effect.

GzmB−/− CD8+ T cells exhibit enhanced GVT effect in an MHC-matched model

To further confirm this unusual phenotype, we extend this project to study P815 mastocytoma model (H-2b). Lethally irradiated DBA/2 host mice were first inoculated with 1 × 10^5 syngeneic P815 tumor cells and then transplanted with TCD-BM and CD8+ T cells isolated from the 129/SvJ donor mice (H-2b). Compared to the transplant of 2 × 10^6 TCD-BM cells only, 0.6 × 10^6 WT CD8+ T cells combined with TCD-BM cells significantly inhibited tumor growth (Fig. 3A). Transplant of the same number of GzmB−/− CD8+ T cells led to further reduced tumor burden compared with the transplant of WT CD8+ T cells. To further examine the impact of transplant, we monitored host survival. WT CD8+ T cells prolonged host survival but failed to rescue the hosts from death caused by tumor growth (Fig. 3B). GzmB−/− CD8+ T cells rescued ~40% of the hosts, consistent with the reduced tumor burden observed in these mice.

GzmB−/− CD8+ T cells exhibit enhanced GVT effect in an MHC-matched model

To further study GVT effect in a clinically more relevant system, we have developed a MHC-matched transplant model. In this model, lethally irradiated 129/SvJ host mice were first inoculated with 1 × 10^5 syngeneic MB0 AML cells and then transplanted with 2 × 10^6 TCD-BM cells alone or combined with 0.2 × 10^6 CD4+CD25− T cells harvested from 129/SvJ WT or GzmB−/− mice. Kaplan-Meier survival curves are shown (Fig. 2D). Indeed, we observed similar results (Fig. 2C). Furthermore, to rule out a donor strain–specific effect, we prepared TCD-BM and CD8+ T cells from WT and GzmB−/− mice in the C57BL/6 strain (H-2b) and performed equivalent experiments. Indeed, we observed similar results (Fig. 2C). Furthermore, to rule out a tumor model–specific effect, we inoculated the BALB/c mice with syngeneic WEHI-3 leukemia cells and performed the same transplants. Again, we observed similar results (Fig. 2D). These results demonstrate that GzmB deficiency, by an unidentified mechanism, enhances CD8+ T cell–mediated GVT effect.

GzmB−/− CD8+ T cells exhibit enhanced GVT effect in a clinically relevant system

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Together (Figs. 2, 3), these results demonstrate that GzmB deficiency enhances CD8+ T cell–mediated GVT effect in three different strains of hosts with four different tumor models.
GzmB deficiency does not affect GVHD in the tumor-bearing hosts

The surprising GVT results prompted us to fully examine GVHD in the tumor-bearing mice. We collected tissue samples at 34 d after transplantation, a critical period when some of the hosts started to die of tumor growth. We performed histopathological analyses of the skin, liver, and small and large intestines. No evidence of GVHD was observed in the skin. The liver samples did not yield conclusive result because of various amounts of tumor cell infiltrates, making it difficult to distinguish GVHD damage from tumor-induced damage. Nevertheless, we observed consistent evidence of GVHD in both large and small intestines that were free from tumor infiltration. We used an established scoring system to evaluate GVHD severity (22). Although a high dose ($1.5 \times 10^6$) of CD8$^+$ T cells induce severe and lethal GVHD, only mild to moderate condition of GVHD was observed in the hosts receiving the low dose ($0.3 \times 10^6$) of CD8$^+$ T cells (Supplemental Fig. 1). Notably, GzmB deficiency did not affect GVHD under the condition of the low CD8$^+$ T cell dose either in the A20 tumor-bearing mice (Fig. 4A) or in the WEHI-3 tumor-bearing mice (Fig. 4B). These results

FIGURE 2. GzmB$^{-/-}$ CD8$^+$ T cells exhibit enhanced GVT effect in the A20 and WEHI-3 tumor models. BALB/c hosts were inoculated with luciferase-expressing tumor cells and then transplanted with $2 \times 10^6$ TCD-BM cells only or combined with $0.3 \times 10^6$ CD8$^+$ T cells of the indicated genotypes. (A) Tumor burden in the hosts measured by bioluminescence imaging after receiving $1 \times 10^6$ A20 cells and transplantation from 129/SvJ donors. Summary data from two experiments are shown as mean ± SD, with six to eight mice in each group. (B) Kaplan-Meier survival curves of the hosts described in (A). (C) Tumor burden in the hosts after transplantation from C57BL/6 donors. Summary data from two experiments are shown as mean ± SD, with six mice in each group. (D) Tumor burden in the hosts after receiving $4 \times 10^6$ WEHI-3 cells and transplantation from 129/SvJ donors. Representative data from one of two experiments are shown as mean ± SD, with four mice in each group. Two-way ANOVA was performed to determine statistically significant differences between hosts receiving WT versus GzmB$^{-/-}$ CD8$^+$ T cells ($p < 0.05$, ***$p < 0.001$).

FIGURE 3. GzmB$^{-/-}$ CD8$^+$ T cells exhibit enhanced GVT effect in the P815 and MB0 tumor models. DBA/2 mice were inoculated with $1 \times 10^5$ luciferase-expressing P815 cells and then transplanted with $2 \times 10^6$ TCD-BM cells only or combined with $0.6 \times 10^6$ CD8$^+$ T cells isolated from 129/SvJ WT or GzmB$^{-/-}$ mice. (A) Bioluminescence imaging was performed to measure tumor burden. Representative data from one of two experiments are shown as mean ± SD, with five mice in each group. Two-way ANOVA was performed to determine statistically significant differences between hosts receiving WT versus GzmB$^{-/-}$ CD8$^+$ T cells ($p < 0.05$). (B) Kaplan-Meier survival curves of the hosts, summarized from two experiments described in (A). (C) Lethally irradiated 129/SvJ mice were inoculated with $1 \times 10^5$ MB0 AML cells and then transplanted with $2 \times 10^6$ TCD-BM cells only or combined with $0.6 \times 10^6$ CD8$^+$ T cells isolated from C57BL/6 WT or GzmB$^{-/-}$ mice. Kaplan-Meier survival curves of the MB0 tumor-bearing mice are shown.
are consistent with the GVHD results in the tumor-free host mice receiving the same low dose of CD8+ T cells (Fig. 1B).

GzmB−/− CD8+ T cells exhibit increased expansion in the tumor-bearing host

To dissect the mechanism underlying the enhanced GVT effect of GzmB−/− CD8+ T cells, we used flow cytometry to examine the lymphocytes in the tumor-bearing host. In the control group that received TCD-BM only, very few CD8+ T cells were detected in the spleens within the first 3 wk after transplant (Fig. 5A). In contrast, an average of 3 × 10^6 donor CD8+ T cells were detected between day 11 and day 20 in the spleens of the hosts receiving WT CD8+ T cells, suggesting that donor CD8+ T cells underwent significant expansion. Notably, GzmB−/− CD8+ T cells exhibited an expansion that is 2-fold higher than that of WT CD8+ T cells (Fig. 5B). These data indicate that GzmB deficiency in the donor CD8+ T cells somehow increases their expansion, which results in enhanced GVT activity. Next, we examined T cell proliferation with BrdU assays. Significant amount of BrdU incorporation was observed in donor CD8+ T cells. However, no significant difference was observed between WT and GzmB−/− CD8+ T cells (data not shown), indicating that GzmB did not affect donor T cell proliferation.

GzmB-mediated, activation-induced cell death of donor CD8+ T cells

Previous reports showed that GzmB was involved in activation-induced cell death (AICD) of CD4+ and CD8+ T cells (24, 25). Therefore, we hypothesized that GzmB-mediated AICD may account for the different GVT effects between WT and GzmB−/− CD8+ T cells. First, to test whether this cell-intrinsic mechanism played a role in this BMT model, we performed CD45.1-based competition experiments (Fig. 6A). When an equal number of GzmB−/− and WT CD8+ T cells were mixed and transplanted into the same tumor-bearing host, GzmB−/− cells significantly out-competed WT cells (Fig. 6B), suggesting that a GzmB-mediated cell-intrinsic mechanism is responsible for this phenotype. Interestingly, perforin-deficient CD8+ T cells were also found to out-compete WT CD8+ T cells (Supplemental Fig. 3), which is consistent with previous reports that perforin also plays an important role in AICD for CD8+ T cells (26–29). Next, we used Annexin V staining to measure CD8+ T cell death in the tumor-bearing hosts (Fig. 6C). We examined CD8+ T cell death at various time points. Results indicated that GzmB-dependent AICD contributed to up to 40% of donor T cell death in the early phase (days 7–9) after transplant (Fig. 6D). However, in the late phase (days 11–20), significant donor T cell death was still observed but appeared to occur via a GzmB-independent fashion, which may have accounted for the precipitous contraction of GzmB−/− T cells observed between days 14 and 20 after transplant (Fig. 5B). Notably, substantially higher rates of CD8+ T cell death were observed in the lymph nodes than in the spleen. This may be due to the fact that lymph nodes are the primary sites for T cell activation or that the spleen has more phagocytes that quickly engulf dead cells. Even so, a similar trend of reduced cell death was observed for GzmB−/− CD8+ T cells in the spleen. These results reveal that GzmB contributes to AICD of donor CD8+ T cells in the tumor-bearing mice. Therefore, GzmB deficiency results in a survival advantage that leads to the increased expansion of GzmB−/− CD8+ T cells.

GzmB−/− CD8+ T cells skew toward an effector or effector memory phenotype and produce higher levels of IFN-γ and FasL that may inhibit tumor growth

Recent studies have suggested that different phenotypes of T cells may play differential roles in GVHD and GVT effect (30–34). CD8+CD44+ naive T cells were found to induce severe GVHD, whereas CD8+CD44high memory T cells were shown to mediate potent GVT activity without causing severe GVHD (30, 33). To understand why GzmB deficiency enhances GVT effect without causing more severe GVHD, we examined the phenotypic differentiation of donor CD8+ T cells (Fig. 7A). Between days 10 and 14 after transplant, all donor CD8+ T cells were found to be CD44high cells, and the majority (>70%) exhibited a CD44+CD62L− effector or effector memory phenotype, whereas only a minority (<30%) showed a CD44+CD62L+ central memory phenotype. Notably, GzmB deficiency skewed CD8+ T cells toward the effector or effector memory phenotype. Compared to WT

FIGURE 4. GzmB−/− CD8+ T cells do not exacerbate GVHD in the tumor-bearing host. Thirty-four days after tumor inoculation and transplantation described in Fig. 2, tissues sections were prepared and assessed for GVHD as described in the Materials and Methods. (A) GVHD score in the A20 tumor-bearing mice after transplantation from C57BL/6 donors. (B) GVHD score in the WEHI-3 tumor-bearing mice after transplantation from 129/SvJ donors. Summary data from two independent experiments are shown, with four to eight mice assessed in each group. Two-tailed t tests were performed to determine statistically significant differences of GVHD scores between hosts receiving WT versus GzmB−/− CD8+ T cells.
CD8⁺ T cells, GzmB⁻/⁻ CD8⁺ T cells demonstrated an 8–10% increase in the CD44⁺CD62L⁻ population at this early stage. At the late stage of 34 d, ~20–30% of donor CD8⁺ T cells showed a CD44⁻naive phenotype, which were probably differentiated from stem or progenitor cells in the BM graft. Nevertheless, GzmB⁻/⁻ CD8⁺ T cells still exhibited a 15% shift toward the CD44⁺CD62L⁻ phenotype. To explore the GzmB-independent mechanisms that control tumor growth, we performed ELISA to measure soluble IFN-γ and FasL in the tumor-bearing mice. Starting from day 7 after transplant, significantly higher levels of IFN-γ were found in the peripheral blood of the hosts receiving GzmB⁻/⁻ CD8⁺ T cells (Fig. 7B). Intracellular IFN-γ staining did not show a significant difference between WT and GzmB⁻/⁻ CD8⁺ T cells, suggesting that the enhanced IFN-γ levels were due to the increased expansion of GzmB⁻/⁻ CD8⁺ T cells. Also, significantly higher amounts of FasL were found in the hosts receiving GzmB⁻/⁻ CD8⁺ T cells. Interestingly, IFN-γ and FasL showed very different expression profiles, which suggest that IFN-γ and FasL may be produced by different subsets of CD8⁺ T cells and may mediate GVT effect at different stages. Finally, to determine whether IFN-γ and FasL may contribute to tumor control, we used various doses of IFN-γ and FasL recombinant proteins to treat tumor cells in vitro.
Indeed, both IFN-γ and FasL were able to suppress the proliferation of all three tumor models, even though different tumor types showed different sensitivity. Together, these results suggest that IFN-γ and FasL might have accounted for a part of the enhanced GVT activity delivered by CD8+ T cells. This notion is consistent with previous studies showing that IFN-γ and FasL contribute to CD8+ T cell–mediated GVT activity without causing severe GVHD (35–37).

**Discussion**

A great challenge for transplantation immunology is to separate the adverse GVHD effect from the desirable GVT effect. Various cellular targets, including CD4+Foxp3+ Treg cells and NK cells, have been tested to prevent GVHD without sacrificing the GVT effect (38–40). In this study, we have performed a complete analysis of the role of GzmB in allogeneic T cell–mediated GVT effect. Because Treg cells have been shown to use GzmB to promote tumor growth (15), this study did not involve Treg transplant, and we did not observe Foxp3+ Treg conversion after transplantation (data not shown). As we and other groups have shown, allogeneic CD4+CD25+ T cells induce rapid and lethal GVHD at extremely low doses. In contrast, the hosts can tolerate moderate doses of allogeneic CD8+ T cells without developing severe GVHD. Therefore, we have focused on investigating the role of GzmB in CD8+ T cell–mediated GVT effect.
Using an improved and more clinically relevant transplant system, we first confirmed previous findings that GzmB is required for a high dose of CD8+ T cells to cause lethal GVHD (9, 10). To proceed with GVT studies, we used moderate doses of CD8+ T cells that the hosts could tolerate. Surprisingly, transplant of GzmB<sup>−/−</sup> CD8+ T cells led to dramatically reduced tumor burden and significantly improved host survival in both MHC-matched and MHC-mismatched models compared with WT CD8+ T cells.

Mechanistic study reveals that following transplantation, GzmB expression causes some donor CD8+ T cells to undergo AICD, which results in the reduced GVT activity of WT CD8+ T cells. Importantly, this T cell–intrinsic mechanism applies to diverse donor–host combinations and multiple tumor models. These results illustrate a detrimental role for GzmB in allogeneic BMT because it not only mediates GVHD damage, but also diminishes the antitumor effect by causing AICD in donor CD8+ T cells.

**FIGURE 7.** GzmB<sup>−/−</sup> CD8+ T cells skew toward an effector or effector memory phenotype and produce higher amounts of IFN-γ and FasL that may control tumor growth. (A) BALB/c mice were inoculated with A20 cells and received transplants from 129/SvJ donor mice as described in Fig. 2A. Shown in the left panel are representative dot plots of CD44 and CD62L expression on the gated H-2Kb+CD8+ T cells at days 10 and 34 after transplantation. Shown in the right panel are the percentages of donor H-2Kb+CD8+ T cells that are CD44<sup>+</sup>CD62L<sup>−</sup> at day 0 before and the indicated dates. (B) ELISA was performed to measure IFN-γ and soluble FasL in the serum of the tumor-bearing mice. Summary data of two experiments are shown, with four to six mice assessed in each group. Two-tailed t tests were used to determine statistically significant differences between the hosts receiving WT and GzmB<sup>−/−</sup> CD8+ T cells. (C) Total of 800 A20 cells, 400 WEHI-3 cells, and 400 P815 cells were seeded in 1 ml of complete media in triplicate wells. rIFN-γ and FasL were added in the beginning and added again at day 3. Tumor burden was measured by bioluminescence imaging after culturing for 5 d. Representative data from one of three experiments are shown as mean ± SD. Two-tailed t tests were performed to determine statistically significant differences (*p < 0.05, **p < 0.01, ***p < 0.001). ND, Nondetectable.
contrast, GzmB deficiency confers a survival advantage to donor CD8+ T cells. This leads to an increased expansion of CD8+ T cells that use other mechanisms, including IFN-γ and FasL, to control tumor growth.

It is intriguing to observe that the enhanced expansion of GzmB−/− CD8+ T cells leads to stronger GVT activity, but not more severe GVHD. First, these CD8+ T cells are inefficient in causing GzmB and therefore less efficient in causing tissue damage. Secondly, it is important to note the skewing of GzmB−/− CD8+ T cells toward the effector or effector memory phenotype. Several recent studies proposed that different phenotypes of T cells may play differential roles in GVHD and GVT effect (30–34). CD8+ CD44high memory T cells were shown to mediate GVT activity without causing severe GVHD (30, 33). Also of note, a previous study using a virus model also showed that GzmB deficiency or overexpression of a GzmB inhibitor, Sp6, protected CD8+ memory T cells from GzmB-mediated apoptosis and led to an increase of CD8+ memory T cells after virus infection (41). To better understand GzmB-dependent phenotypic change, we have characterized the phenotypic distributions in the input CD8+ T cells of the 129/SvJ and C57BL/6 strains, two donor strains used throughout this study. Interestingly, the composition in the 129/SvJ strain, but not the C57BL/6 strain, appears to be different between WT and GzmB−/− CD8+ T cells. As shown in Supplemental Fig. 4, in the 129/SvJ-derived graft, GzmB−/− CD8+ T cells contain a higher percentage of CD44+CD62L+ central memory cells and a lower percentage of CD44−CD62L− naive cells than WT CD8+ T cells. In contrast, the phenotypic distributions in the C57BL/6-derived grafts are identical between WT and GzmB−/− CD8+ T cells, but these C57BL/6 donor cells produced the same GzmB-dependent GVT and GVHD effects (Figs. 2C, 3C, 4A). Therefore, although the input phenotypic difference in the 129/SvJ-derived graft could partially affect GVT/GVHD responses, it is not the dominating factor. In contrast, GzmB-dependent AICD that occurs after transplantation appears to make the major impact on the observed differential GVT and GVHD effects. Together, these studies suggest that GzmB is not only simply a cytotoxic molecule that T cells use to kill their targets, but also an important regulator that influences the phenotypic and functional differentiation of T cells.

In summary, this work prompts us to re-examine the critical paradigms of GzmB function. First, GzmB has been viewed as a major effector molecule for lymphocytes to eliminate foreign pathogens (6, 7, 42, 43). Secondly, GzmB-mediated AICD may have evolved as a negative feedback to terminate an overreactive immune response (24, 25). In the setting of allogeneic transplantation, the first mechanism can indeed mediate GVHD, whereas the second mechanism, as we discovered in this study, turns out to be detrimental to the desired GVT effect. Fortunately, GzmB deficiency cannot only alleviate severe GVHD, but also preserve the subset of CD8+ T cells that are critical for GVT effect. Therefore, this discovery illuminates an unforeseen pathway that can separate the beneficial GVT effect from GVHD. This unique separation suggests that inhibiting donor-derived GzmB function may represent a promising strategy that can improve the effectiveness of allogeneic BMT without exacerbating GVHD.

Acknowledgments
We thank Aimin Jiang, Scott Abrams, Mary Lynn Hensen, Jeremy Waith, Kelvin Lee, and Elizabeth Repasky for helpful advice and technical assistance.

Disclosures
The authors have no financial conflicts of interest.


