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Loss of B7-H1 Expression by Recipient Parenchymal Cells Leads to Expansion of Infiltrating Donor CD8\(^{+}\) T Cells and Persistence of Graft-Versus-Host Disease

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Previous experimental studies have shown that acute graft-versus-host disease (GVHD) is associated with two waves of donor CD8\(^{+}\) T cell expansion. In the current studies, we used in vivo bioluminescent imaging, in vivo BrdU labeling, and three different experimental GVHD systems to show that B7-H1 expression by recipient parenchymal cells controls the second wave of alloreactive donor CD8\(^{+}\) T cell expansion and the associated second phase of GVHD. Loss of B7-H1 expression by parenchymal cells during the course of GVHD was associated with persistent proliferation of donor CD8\(^{+}\) T cells in GVHD target tissues and continued tissue injury, whereas persistent expression of B7-H1 by parenchymal cells led to reduced proliferation of donor CD8\(^{+}\) T cells in GVHD target tissues and resolution of GVHD. These studies demonstrate that parenchymal cell expression of B7-H1 is required for tolerizing infiltrating T cells and preventing the persistence of GVHD. Our results suggest that therapies designed to preserve or restore expression of B7-H1 expression by parenchymal tissues in the recipient could prevent or ameliorate GVHD in humans. The Journal of Immunology, 2012, 188: 724–734.

We recently showed that donor CD8\(^{+}\) T cells transplanted into allogeneic recipients conditioned with 800 cGy total body irradiation (TBI) go through two waves of expansion, each associated with a distinct phase of graft-versus-host disease (GVHD). The first-wave expansion and the first phase of GVHD usually begins at 5–10 d after transplantation and is not lethal. The second wave of expansion and the second phase of GVHD usually begins at 30 d after transplantation and is lethal (1). Zhang et al. (2, 3) have shown that memory CD8\(^{+}\) T cells isolated from recipients at 30 d after transplantation induce severe GVHD in secondary recipients, indicating that memory CD8\(^{+}\) T cells mediate the persistence of GVHD. In contrast, we have shown that donor CD8\(^{+}\) T cells in recipients conditioned with a CD3-specific Ab before transplantation go through only one wave of expansion, which is not associated with clinical evidence of GVHD (1). Previous studies have not defined the mechanisms that mediate or prevent the second wave of donor memory CD8\(^{+}\) T cell expansion and the second phase of GVHD.

B7-H1 (also called PD-L1) is constitutively expressed by hematopoietic cells, including resting T cells, B cells, dendritic cells, and macrophages (4–7). B7-H1 protein is not normally expressed by parenchymal cells but can be induced by proinflammatory cytokines such as IFN-γ and TNF-α (8–11). Activated T cells upregulate expression of PD-1, and its interaction with B7-H1 induces T cell anergy, exhaustion, and apoptosis (5, 12). We and others (13, 14) reported that blockade of B7-H1 and PD-1 interaction augmented acute GVHD. B7-H1 expression by recipient hematopoietic cells and parenchymal cells induced alloreactive CD8\(^{+}\) T cell exhaustion and reduced graft-versus-leukemia effects in TBI-conditioned recipients during the first 2 wk after transplantation (15) and in recipients given delayed donor lymphocyte infusion (16, 17). GVHD recipients usually have lymphopenia due to thymus damage and reduction of de novo-developed T cells as well as deficient proliferation of donor T cells in the peripheral lymphoid tissues (18). However, it is still unknown how GVHD can persist in lymphopenic recipients and how parenchymal cell expression of B7-H1 regulates the GVHD development in the lymphopenic recipients.

In the current studies, we evaluated the role of B7-H1 expression by recipient hematopoietic cells and parenchymal cells in regulating the second wave of donor CD8\(^{+}\) T cell expansion and the second phase of GVHD using three experimental GVHD systems, including TBI-conditioned wild-type (WT) recipients, anti-CD3-
conditioned recipients with or without B7-H1 deficiency, and unconditioned Rag-2–/– recipients with or without parenchymal cell expression of B7-H1. We used in vivo bioluminescent imaging (BLI) and in vivo BrdU labeling to evaluate the proliferation of injected donor CD8+ T cells in recipient lymphoid and GVHD target tissues. Our results demonstrate that loss of B7-H1 expression by recipient parenchymal cells during the course of GVHD permits the second wave of donor CD8+ T cell expansion infiltrating GVHD target tissues, leading to persistence of GVHD and resulting in death.

Materials and Methods

Mice

C57BL/6 (H-2b), BALB/c (H-2d), and Rag-2–/–BALB/c (H-2d) mice were purchased from National Cancer Institute Laboratory (Frederick, MD) or Taconic Farms (Germantown, NY). Luciferase-transgenic (luc+) C57BL/6, congenic C57BL/6 (CD45.1), and B7-H1–/– BALB/c mice were generated as previously described (19, 20). B7-H1–/– Rag-2–/– mice were generated by backcrossing B7-H1–/– BALB/c to Rag-2–/– BALB/c mice. Mice were age-matched for each experiment, and all were 8–12 wk of age. All animals were maintained in a pathogen-free room at City of Hope Animal Research Facilities (Duarte, CA). Animal use protocols were approved by the institutional review committee.

Ab production, conditioning of recipients, transplantation, assessment of GVHD, and in vivo BLI

Production of anti-CD3 mAb (145-2C11) and anti–B7-H1 mAb (10B5) has been previously described (1, 21). Recipients were conditioned with 800 cGy TBI, from a [137Cs] source 8 h before transplantation. Alternatively, recipients were conditioned with one i.v. injection of anti-CD3 (5 µg/g) and three daily i.p. injections of vorinostat (40 mg/g) and busulfan (8.75 µg/g). Bone marrow (BM) and sorted CD8+ T cells from C57BL/6 donors were injected i.v. into BALB/c recipients. Recipients were monitored for donor CD8+ T cell expansion, clinical GVHD, and survival. The assay forGVHD and scoring of clinical GVHD and the procedure for monitoring T cell expansion by in vivo BLI has been described previously (22).

Isolation of mononuclear cells from GVHD target tissues and flow cytometric analysis

Procedures for isolating mononuclear cells (MNC) from GVHD target tissues have been described previously (23, 24). Abs to mouse CD3ε (145-2C11), TCRα (H57-597), CD4 (RM4-5), CD8α (53-6.7), B220 (RA3-6B2), CD11b/Mac-1 (M170), Gr-1 (RB6-8C5), H-2K (AF6-85.5), H-2D (34-2-12), PD-1 (CD279), J43, B7-H1 (CD274, 1-111A), and IL-7ε (CD127) were purchased from BD Pharmingen (San Jose, CA), eBioscience (San Diego, CA), or R&D Systems (Minneapolis, MN). The four-laser MoFlo Immunocytometry System (DK-2600; DakoCytometry, Glostrup, Denmark) or a CyAn immunocytometer (DakoCytomation) was used for flow cytometry, and data were analyzed with FlowJo software (Tree Star, Ashland, OR) as previously described (25–27).

In vivo BrdU labeling

BrdU 2 mg in PBS was injected i.p. daily for 3 d followed by tissue collection and analysis of BrdU incorporation using the BrdU Flow kit protocol (BD Pharmingen).

Proliferation assays

Ex vivo CD4+ T cell proliferation was measured as previously described (22, 25). Briefly, sorted CD4+ T cells (2 × 10^5) were incubated with irradiated (3 Gy) dendritic cells (1 × 10^5) in complete RPMI 1640 media containing 10% FBS, penicillin/streptomycin, L-glutamine, and 2-ME at the bottom of a 96 U-well plate for 5 d. [3H]Thymidine deoxyribose (Tdr; 1 µCi/well) was added to the culture 18 h before harvest.

Histopathology

Tissue specimens were fixed in 10% formalin and embedded in paraffin. Sections were stained with HE and histologic evidence of GVHD was assessed according to a scoring system described previously without knowledge of the assignment to experimental groups (22, 23, 28). Pictures were taken with a Pixera (600CL) cooled charge-coupled device camera (Pixera, Los Gatos, CA).
Loss of B7-H1 expression was not caused by an intrinsic defect in hepatocytes but was associated with increased IL-6 production by infiltrating donor cells

Because IFN-γ can induce parenchymal cell expression of B7-H1 (8–10), and we observed that induction of host parenchymal tissue expression of B7-H1 required donor T cell production of IFN-γ (23), recipients with or without anti-CD3 pretreatment were given a single injection of IFN-γ (150 μg) on day 30 after transplantation. Forty-eight hours later, hepatocytes from recipients in both groups expressed high levels of B7-H1 (Fig. 1E). These results indicate that downregulated expression of B7-H1 by hepatocytes at 30 d after transplantation does not result from an intrinsic cellular defect and must be caused by extrinsic factors that can overcome the effect of IFN-γ.

To identify the extrinsic factor(s), we compared the IFN-γ production by MNC infiltrating the liver and expression of IFN-γR by hepatocytes in recipients with or without anti-CD3 pretreatment. No significant differences were found in the two groups.
IFN-γ with chronic tissue inflammation (31, 32), and IL-6 can inhibit the second wave of donor CD8+ T cell expansion and exacerbated GVHD. T cells and ameliorate the second phase of GVHD. Overexpression of B7-H1 can inhibit the expansion of infiltrating donor T cells and express luciferase protein (30). Therefore, we first tested whether hydrodynamic injection of B7-H1 cDNA could induce hepatocyte expression of B7-H1. B7-H1 cDNA or control plasmid was given via hydrodynamic injection into B7-H1−/− BALB/c mice. Twenty-four hours after the injection, hepatocytes showed markedly upregulated expression of B7-H1 mRNA and cell-surface protein (Supplemental Fig. 1).

We then tested whether hydrodynamic injection of B7-H1 cDNA could ameliorate ongoing GVHD. Accordingly, BALB/c recipients were conditioned with 800 cGy TBI without anti-CD3 preconditioning. The number of donor CD8+ T cells injected on day 0 was decreased from $20 \times 10^6$ to $5 \times 10^6$ to reduce the severity of GVHD and improve the ability of the recipients to tolerate the hydrodynamic injection procedures. Hydrodynamic injections of B7-H1 cDNA or control plasmid were given 20 d after transplantation, when the recipients had recovered from the first phase of clinical GVHD. B7-H1 expression was upregulated 24 h after injection of B7-H1 cDNA but not after injection of control cDNA (Fig. 2A). Compared to recipients treated with vector control, recipients treated with B7-H1 cDNA showed significantly reduced second-phase clinical GVHD ($p < 0.01$; Fig. 2B). Recipients treated with B7-H1 cDNA also showed improved survival as compared with the control (92 versus 69%; $p < 0.05$; Fig. 2C). Fifty days after transplantation, donor-type CD8+ T cells were harvested from the liver and stimulated ex vivo with immobilized anti-CD3. The proliferation of donor CD8+ T cells from the liver of recipients treated with B7-H1−cDNA was 50% lower than seen with CD8+ T cells from the control recipients ($p < 0.01$; Fig. 2D).

Less lymphocyte infiltration was consistently observed in the liver of recipients treated with B7-H1 compared with controls (Fig. 2E). These results support the notion that GVHD target tissue expression of B7-H1 can inhibit the expansion of infiltrating donor T cells and ameliorate the second phase of GVHD.

**Absence of B7-H1 expression allowed the second wave of donor CD8+ T cell expansion and exacerbated GVHD**

In previous studies, we showed that donor CD8+ T cells did not cause GVHD in recipients prepared with a regimen of anti-CD3 with a low dose of vorinostat (26). Under this GVHD-preventative anti-CD3–based conditioning, donor CD8+ T cells showed a single-wave expansion (Supplemental Fig. 2). We compared WT and B7-H1−/− recipients conditioned with this GVHD preventative regimen to test whether expression of B7-H1 in the recipient controls donor CD8+ T cell expansion and prevents GVHD. In WT recipients, donor CD8+ T cells showed a slow and weak single wave of expansion (Fig. 3A, 3B). In B7-H1−/− recipients, donor CD8+ T expansion was rapid and strong, reaching an initial peak at $\sim 13$ d after transplantation and declining slightly thereafter. Donor CD8+ T cells then showed a second wave of expansion beginning at $\sim 30$ d after transplantation. By day 40 after trans-
plantation, the BLI intensity of donor CD8+ T cells in B7-H1−/− recipients was >10-fold higher than in WT recipients (p < 0.01).

The differences in CD8+ T cell expansion kinetics correlated with manifestations of GVHD. WT recipients showed little evidence of GVHD, and all survived for >80 d (Fig. 3C, 3D). B7-H1−/− recipients showed two phases of clinical GVHD that correlated with the two waves of donor CD8+ T cell expansion. The first phase of GVHD peaked at ~13 d after transplantation, as indicated by moderate diarrhea, ruffled fur, and weight loss. The second phase of GVHD began at 35 d after transplantation, as indicated by severe diarrhea, hair loss, and weight loss, and most of the recipients died between days 40 and 60 after transplantation.

At 50 d after transplantation, we compared the yield of CD4+ CD8+ double-positive thymocytes and the percentage and yield of donor-type CD8+ T cells in the spleen, liver, and skin of WT and B7-H1−/− recipients. The numbers of double-positive thymocytes and splenic MNC were ~40-fold higher in WT recipients than in B7-H1−/− recipients (p < 0.01, Fig. 3E, 3F). Whereas the yield of donor-type CD8+ T cells in the spleen was ~7-fold higher in WT recipients than in B7-H1−/− recipients, the number of donor-type CD8+ T cells in the liver and skin was ~2-5-fold lower in WT
recipients than in B7-H1−/− recipients (p < 0.01, Fig. 3G). Histopathological scores were consistently lower in WT recipients than in B7-H1−/− recipients (p < 0.01; Fig. 3H, Supplemental Fig. 3). These results demonstrate that host tissue expression of B7-H1 controls donor CD8+ T expansion and prevents GVHD in the recipients conditioned with a GVHD-preventative anti-CD3-based regimen. The strong in vivo BLI signal and the high yield of donor CD8+ T cells in GVHD target tissues but not in the spleen of B7-H1−/− recipients at 50 d after transplantation suggest that the absence of B7-H1 permits the proliferation of donor CD8+ T cells in GVHD target tissues.

Parenchymal tissue cell expression of B7-H1 prevented the second wave of donor CD8+ T cell expansion and the second phase of GVHD in Rag-2−/− recipients

We tested whether host parenchymal tissue cell expression of B7-H1 was required for prevention of the second wave of donor CD8+ T expansion and the second phase of GVHD, using unconditioned...
Rag-2−/− recipients to avoid any potential influence of TBI or anti-CD3 conditioning. First, we established chimeras that had expression of B7-H1 only in parenchymal tissues by transferring B7-H1−/−Rag-2−/− BM into Rag-2−/− mice. We then evaluated the effect of B7-H1 expression in parenchymal tissues by injecting blocking anti-B7-H1 mAb or control rat IgG every other day for 30 d beginning on day 14 after transplantation. Recipients treated with control IgG showed only mild signs of GVHD, and >90% survived for >80 d, but the recipients treated with anti-B7-H1 mAb showed progressive clinical GVHD, and almost all died by 40 to 60 d after transplantation (p < 0.01; Fig. 4A, 4B). These results suggest that expression of B7-H1 by parenchymal cells in the recipient is required to control late-stage GVHD mediated by donor CD8+ T cells.

We also established chimeras that expressed B7-H1 in hematopoietic cells but not in parenchymal tissues by transferring Rag-2−/− BM cells into B7-H1−/−Rag-2−/− recipients. Control chimeras that expressed B7-H1 both in hematopoietic cells and parenchymal tissues were established by transferring Rag-2−/− BM into Rag-2−/− recipients. In control chimeras, donor CD8+ T cells went through a weak single wave of expansion accompanied by minimal evidence of GVHD. More than 90% of these recipients survived for at least 80 d (Fig. 4C–F). In chimeras with B7-H1 expression limited to hematopoietic cells, donor CD8+ T cells showed a rapid and progressive expansion accompanied by development of severe GVHD, and most of the recipients died within 40 to 60 d. These results demonstrate that expression of B7-H1 by parenchymal tissues in the recipient is required to control the second wave of donor CD8+ T cell expansion and prevent the persistence of GVHD, whereas expression of B7-H1 by recipient hematopoietic cells is not sufficient for these effects. The second wave of donor CD8+ T cell expansion occurred within GVHD target tissues. Further experiments were carried out to characterize differences in the behavior of donor CD8+ T cells according to the presence of GVHD in recipients conditioned with 800 cGy TBI or the absence of GVHD in recipients conditioned with an anti-CD3–based regimen. The injected (CD45.2+) and the de novo developed (CD45.1+) donor-type T cells were distinguished by CD45.2 (Fig. 5). At 35 d after transplantation, recipients with GVHD had marked reductions in percentage and yield of CD4+CD8+ thymocytes and splenic donor-type T cells, especially de novo developed T cells, as compared with recipients without GVHD (p < 0.01; Figs. 5, 6), indicating lymphopenia is associated with GVHD. In contrast, the percentage and yield of donor-type T cells, especially the injected donor CD8+ T cells, in the GVHD target tissues liver and skin was markedly higher in recipients with GVHD than in those without GVHD (p < 0.01; Figs. 5, 6). These results suggested that donor CD8+ T cells proliferate within GVHD target tissues in lymphopenic recipients during the second wave of expansion and the second phase of GVHD.

To test this hypothesis, recipients with and without GVHD were i.p. injected with BrdU daily for 3 d beginning at 35 d after transplantation, and the percentages of BrdU+ proliferating cells among the injected donor-type CD8+ T cells were measured by flow cytometry. In recipients with GVHD, the injected donor CD8+ T cells showed little proliferation in the spleen and lymph nodes and much stronger proliferation in the GVHD target tissues such as liver, gut, and skin (p < 0.01; Fig. 7, Supplemental Fig. 4). In recipients without GVHD, the injected donor CD8+ T cells showed much less proliferation in both the spleen and GVHD target tissues (Fig. 7A, Supplemental Fig. 4). The rapid proliferation of donor CD8+ T cells infiltrating the liver of recipients with GVHD was associated with downregulated expression of B7-H1 expression by hepatocytes. The slower proliferation of donor CD8+ T cells infiltrating the liver of recipients without GVHD was associated with persistent expression of B7-H1 by hepatocytes (Fig. 7B). These results indicate that proliferation of donor CD8+...
T cells in GVHD target tissues correlates inversely with the level of B7-H1 expression.

Because it has been reported that tissue expression of B7-H1 induced donor CD8+ T cells into an exhausted state wherein the T cells upregulate PD-1 and downregulate of IL-7Rα (16), we also compared the expression of PD-1 and IL-7Rα by donor CD8+ T cells from the liver of recipients with or without GVHD on day 35 after transplantation. In both groups, the donor CD8+ T cells infiltrating the liver significantly upregulated expression of PD-1 and downregulated expression of IL-7Rα as compared with donor CD8+ T cells before transplantation (p, 0.01; Fig. 7C). The injected donor CD8+ T cells from recipients with GVHD expressed ~2-fold lower levels of PD-1 but 2-fold higher levels of IL-7Rα compared with those from recipients without GVHD (p < 0.01; Fig. 7C). After ex vivo stimulation with immobilized CD3 Ab, the injected CD8+ T cells isolated from the liver of recipients with GVHD proliferated 10-fold more vigorously than those from the liver of recipients without GVHD (p < 0.01; Fig. 7D). These results indicate that loss of B7-H1 expression by hepatocytes in recipients with GVHD is associated with activation and expansion of infiltrating donor T cells and that persistent expression of B7-H1 by hepatocytes in recipients without GVHD is associated with donor CD8+ T cell tolerance.

Discussion
In this report, we have demonstrated that expression of B7-H1 by parenchymal cells in the recipient prevents the proliferation and expansion of donor CD8+ T cells infiltrating GVHD target tissues and aborts the persistence of GVHD. Loss of B7-H1 expression during the initial phase of GVHD leads to a second wave of alloreactive donor CD8+ T cell expansion in GVHD target tissues and the persistence of GVHD. Anti-CD3 treatment before transplantation maintained parenchymal cell expression of B7-H1, preventing the second wave of donor CD8+ T cell expansion and preventing the persistence of GVHD.

First, the second wave of donor CD8+ T cell expansion was consistent with proliferation of memory donor T cells. Previous reports by Zhang et al. and us (1, 2) showed that injected donor CD8+ T cells in TBI-conditioned recipients first expanded and then contracted ~2 wk after transplantation. The residual CD8+
T cells showed a memory-like phenotype of CD44<sup>hi</sup>CD122<sup>hi</sup>CD25<sup>lo</sup>, and adoptive transfer of these cells into secondary recipients induced severe GVHD (2). In the current studies, we showed that the donor CD8<sup>+</sup> T cells had two waves of expansion in TBI-conditioned recipients. The first wave took place mainly in lymphoid tissues and then contracted. The second wave took place mainly in GVHD target tissues and was associated with lethal GVHD. This observation provides direct evidence that tissue-infiltrating memory CD8<sup>+</sup> T cells mediate the second wave of expansion and persistence of GVHD in TBI-conditioned recipients.

Second, the second wave of donor CD8<sup>+</sup> T cell expansion took place in GVHD target tissues in recipients with lymphopenia in the secondary lymphoid organs. We observed that only a small percentage and low numbers of donor T cells were present in the spleen and lymph nodes during the second wave of donor CD8<sup>+</sup> T cell expansion in TBI-conditioned recipients. Most of these cells were derived from the injected donor CD8<sup>+</sup> T cells, and few had...
developed de novo from the thymus. In contrast, donor T cells and the injected donor CD8⁺ T cells had expanded in GVHD target tissues. In vivo BrdU-labeling assay showed that the injected donor CD8⁺ T cells proliferated much more vigorously in GVHD target tissues such as liver, gut, and skin as compared with the spleen and lymph nodes. This observation is consistent with a recent report that memory CD8⁺ T cells were able to proliferate in nonlymphoid tissues (34). These observations may help to explain why animal recipients and patients can have persistent clinical GVHD despite severe lymphopenia.

Third, downregulated expression of B7-H1 in parenchymal tissues was associated with chronic tissue inflammation and increased production of IL-6. We previously showed that IFN-γ produced by donor T cells induces expression of B7-H1 by parenchymal tissues in the recipients with GVHD (23). In the current study, we observed that hepatocytes expressed similar levels of IFN-γR and that infiltrating MNC produced similar levels of IFN-γ in recipients with or without downregulated expression of B7-H1. We also observed that downregulated expression of B7-H1 by hepatocytes in recipients with GVHD could be reversed by in vivo administration of IFN-γ. These observations indicate that the downregulation of B7-H1 expression of hepatocytes of GVHD recipients does not result from an intrinsic defect or from a direct reduction of IFN-γR/IFN-γ signaling, because enhanced IFN-γR/IFN-γ signaling could overcome the extrinsic factor and re-store B7-H1 expression. The MNC infiltrating the liver of the recipients with hepatocyte downregulation of B7-H1 expression produced five times more IL-6 than those from recipients that maintained hepatocyte expression of B7-H1. IL-6 is usually produced by cells and tissues involved in chronic inflammation (31), and IL-6/IL-6R signaling can upregulate suppressor of cytokine signaling 1 that downregulates IFN-γR/IFN-γ signaling (33). Thus, we speculate that a high level of IL-6 signaling accounts for the loss of B7-H1 expression by hepatocytes in recipients with GVHD.

Finally, the second-wave expansion of injected donor CD8⁺ T cells in GVHD target tissues had a critical role in the pathogenesis of GVHD. We observed that infiltrating donor CD8⁺ T cells in the liver tissues of recipients without GVHD appeared to regulate expression of IL-7Rα and that the injected donor CD8+ T cells in GVHD target tissues had a critical role in the pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. In vivo BrdU-labeling assay showed that the injected donor CD8+ T cells mediate graft-versus-leukemia activity without clinical signs of graft-versus-host disease in recipients conditioned with anti-CD3 monoclonal antibody. J. Immunol. 178: 838–850.


tolerance without GVHD in autoimmune NOD mice conditioned with anti-CD3 mAb. Blood 105: 2180–2188.


Supplemental Figures Legends and Figures

Sup-Fig. 1: Hydrodynamic injection of B7-H1 plasmid induced B7-H1 re-expression in B7-H1−/− mice. B7-H1−/− mice were given hydrodynamic injection of B7-H1cDNA or control vector, in which 20 μg cDNA was dissolved in 1 ml PBS and injected i.v. in 10 seconds. 24 hours after injection, hepatocytes from mice treated with B7-H1 plasmid or vector control were isolated and stained for anti-B7-H1 or IgG isotype control. Hepatocytes were also measured with real-time PCR for B7-H1 mRNA levels. One representative FACS pattern and mean expression levels of B7-H1 mRNA ± SE are shown. N=3.

Sup-Fig. 2: Donor CD8+ T cells induced GVHD in TBI-conditioned recipients but not in anti-CD3-conditioned recipients. WT BALB/c recipients were conditioned with 800 cGy TBI or anti-CD3-based regimen and then given CD8+ T cells (20x10⁶) from luc+ C57BL/6 donors and BM cells (50 x 10⁶) from WT C57BL/6 donors. Recipients were monitored for clinical GVHD 1-2 times a week. Donor CD8+ T cell expansion was visualized with in vivo BLI for up to 40 days after transplantation. (A) A representative series of in vivo BLI pictures depicting donor CD8+ T cell expansion is shown for 1 of 8 recipients in each group. (B) Kinetic curves of whole body emission of photons per second (mean ± SE, N=8). (C) Clinical GVHD scores (mean ± SE, N=8). (D) Survival curves (N=8).

Sup-Fig. 3: Donor CD8+ T cells induced severe tissue damage in B7-H1−/− but not in WT recipients conditioned with anti-CD3. WT or B7-H1−/− BALB/c mice were conditioned with vorinostat, busulfan and anti-CD3 as described in Fig. 3. Seven days after conditioning, the mice were given sorted luc+CD8+ T cells (20x10⁶) from transgenic C57BL/6 donors and BM cells
(50×10^6) from WT C57BL/6 donors. 40 days after transplantation, colon, liver, lung and skin tissues were harvested for histopathologic evaluation. Representative tissue sections from 1 of 6 recipients are shown.

Sup-Fig. 4: The injected donor CD8^+ T cells proliferated in gut and skin tissues of recipients with GVHD but not in recipients without GVHD. As described in Fig. 5, WT BALB/c recipients were conditioned with 800 cGy TBI or with vorinostat, busulfan and anti-CD3, and then given CD8^+ T cells (20×10^6) from WT (CD45.2) C57BL/6 donors and TCD-BM cells (50×10^6) from congenic (CD45.1) C57BL/6 donors. 35 days after transplantation, the recipients were given daily i.p. injections of BrdU (50 μg/g bodyweight) for 3 days. MNCs from LN, skin, and gut were stained for H-2K^b, CD45.2, CD8 and BrdU on day 38. Gated H-2K^b^CD45.2^+CD8^+ donor T cells were shown as BrdU versus CD8 staining. A representative FACS pattern from each group and the mean percentages of BrdU^+ cells are shown (N=4).
Supplemental Figures:

Sup-Fig. 1
Sup-Fig. 2
Sup-Fig. 3
Sup-Fig. 4