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IL-7 Is the Limiting Homeostatic Factor that Constrains Homeostatic Proliferation of CD8$^+$ T Cells after Allogeneic Stem Cell Transplantation and Graft-versus-Host Disease

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Immune reconstitution after allogeneic hematopoietic stem cell transplantation relies primarily on homeostatic proliferation (HP) of mature T lymphocytes, but this process is typically impaired during graft-versus-host disease (GVHD). We previously showed that low IL-7 levels combined with lack of dendritic cell (DC) regeneration constrain CD4$^+$ T cell HP during GVHD. However, it is not clear whether these alterations to the peripheral CD4$^+$ T cell niche also contribute to impair CD8$^+$ T cell regeneration during GVHD. We found that IL-7 therapy was sufficient for restoring CD8$^+$ T cell HP in GVHD hosts while forcing DC regeneration with Flt3-L had only a modest effect on CD8$^+$ T cell HP in IL-7 treated mice. Using bone marrow chimeras, we showed that HP of naïve CD8$^+$ T cells is primarily regulated by MHC class I on radio-resistant stromal cells, yet optimal recovery of CD8$^+$ T cell counts still requires expression of MHC class I on both radio-resistant and radio-sensitive hematopoietic cells. Thus, IL-7 level is the primary limiting factor that constrains naïve CD8$^+$ T cell HP during GVHD, and accessibility of MHC class I on stromal cells explains how IL-7 therapy, as a single agent, can induce robust CD8$^+$ T cell HP in the absence of DCs.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is the best treatment option for numerous patients with high-risk hematologic malignancies. Unfortunately, graft-versus-host disease (GVHD) is the major cause of post-transplant morbidity and mortality that contributes to significantly diminish the survival of transplanted patients [1]. Allo-SCT is typically characterized by a phase of profound lymphopenia that can last several months or years [2]. During this period, patients are at risk of developing infectious complications and relapse. Regeneration of T cells after lymphopenia can occur via thymopoiesis and/or via homeostatic proliferation (HP) of mature lymphocytes contained in the graft [3]. After allo-SCT and GVHD, thymopoiesis is dysfunctional and lymphocyte reconstitution occurs primarily through HP [4]. Although the regeneration of CD8$^+$ T cells is relatively fast after T cell depletion, it is significantly postponed by GVHD [5,6].

The current model put forth to explain the adverse impact of GVHD on lymphocyte reconstitution relates to two primary factors. First, GVHD induces damage to the microenvironment of the thymus and bone marrow (BM), which are critical for T cell production [7,8]. Second, GVHD induces damage of the peripheral niche controlling the survival and the expansion of naive T cells in the periphery [9–13]. In GVHD hosts, dendritic cells (DCs) and systemic IL-7 are diminished and contribute to limit immune reconstitution of CD4$^+$ T cells [13–17]. Although DC depletion could have a profound effect of peripheral CD4$^+$ T cells, it is not clear whether CD8$^\pm$ T cell homeostasis also depends on DCs [18,19]. For instance, in lymphopenic non-GVHD settings, HP of naïve CD8$^+$ T cells is more efficient than HP of naïve CD4$^+$ T cells, where the ubiquitous expression of MHC class I relative to the restricted expression pattern of MHC class II has been invoked to explain these differences. In addition, after T cell depletion CD8$^+$ T cell recovery always precedes CD4$^+$ T cell recovery, raising the prospect that some elements of the peripheral CD4$^+$ and CD8$^+$ T cell niches do not overlap with each other [20–22]. In this work, we evaluated the impact of DC depletion and low IL-7 levels on naïve CD8$^+$ T cell HP after allo-SCT and GVHD.

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METHODS

Mice and Administration of IL-7 or Flt3 Ligand

C57BL/6.SJL (B6.SJL; H-2^b, Ptprc^a Pep3^b, CD45.1$^+$, cat. no. 002014), C57BL/6.129S7-Rag1$^{tmMom/J}$ (Rag$^{-/-}$; H-2^b, CD45.2$^+$, cat. no. 002216), and (C57BL/6 X DBA2/J) F_1 (B6D2F$_1$; H-2$^{b/d}$, CD45.2$^+$, cat. no. 100006), B6.129P2-B2mtm1Unc/J (β2m$^{-/-}$; H-2^b, CD45.2$^+$, cat no. 002087) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). OT-I Rag$^{-/-}$ mice (OT-I Rag$^{-/-}$; H-2^b; CD45.2$^+$) were provided by Dr. Nathalie Labrecque (Centre de recherche de l'hôpital Maisonneuve-Rosemont). Rag$^{-/-}$ X B6.129P2-B2mtm1Unc/J (Rag$^{-/-}$β2m$^{-/-}$; H-2^b, CD45.2$^+$) mice were provided by Dr. Heather Melichar (Maisonneuve-Rosemont Hospital Research Center). All animals were housed at the Maisonneuve-Rosemont Hospital animal facility, and animal studies were performed in accordance with the Maisonneuve-Rosemont Hospital Animal Care Committee. Recombinant human IL-7 (rhIL-7) was supplied by Cytheris Inc. (now Revimmune, France). Vehicle (PBS) or rhIL-7 (5 μg) was administered to mice for 6 days as a daily i.p. injection. rhFlt3-L was purchased from BioXcell (West Lebanon, NH) and administered daily to mice as an i.p. injection of 10 μg for 14 consecutive days.

BM Transplantation and GVHD

We used a "parent into F1" mouse model (B6/B6D2F1) for allo-SCT using C57BL/6 mice as donors and B6D2F1 mice as recipients. On day 0, 10^7 BM cells from Rag$^{-/-}$C57BL/6 donor mice (H-2^b) were injected i.v. into lethally irradiated (10 Gy) B6D2F1 recipients (H-2$^{b/d}$) along with 1×10^6 purified T cells (T cell enrichment kit; StemCell Technologies, Vancouver, Canada) from B6.SJL mice (GVHD-causing T cells) or B6D2F1 mice (syngeneic T cells, no GVHD). Mice were weighed every 3 days and monitored for clinical signs of GVHD. A weight loss of 20% beyond day 14 after transplantation or signs of distress (immobility, arched back, blepharospasm) were considered survival endpoints entailing euthanasia. Five weeks after engraftment mice were used for adoptive transfer of enriched OT-I$^+$ lymphocytes.

Cell Trace Violet Staining and Adoptive Transfer of Lymphocytes

Spleen and lymph nodes from OT-I mice were homogenized and lymphocytes enriched by negative selection (T cell enrichment kit; StemCell Technologies). Enriched CD8$^+$ T cells were suspended at 10^7 cells/mL in PBS and incubated for 15 minutes at room temperature with 1 μL/mL of 5 mM Cell Trace Violet (CTV; Invitrogen, Burlington, Canada). Cells were washed twice in PBS, and recipient mice received 10^6 CTV-labeled T cells by i.v. injection. After 7 days mice were sacrificed and congenic T cells analyzed for CTV content with a Fortessa flow cytometer (BD Bioscience, San Jose, CA). FlowJo software (TreeStar, Ashland, OR) was used for all analysis.

Flow Cytometry

Cells were resuspended at a density of 10^7 cells/mL in FACS buffer and were incubated 30 minutes on ice with diluted monoclonal antibodies and then washed and resuspended in FACS buffer for immediate analysis. The following monoclonal antibodies from BioLegend (San Diego, CA) were used: PE-anti-Vα2 (B20.1), APCCy7-, and APC-anti-CD4 (GK1.5); PeCy7-anti-CD8 (53-2.7); PerCPCy5.5-anti-CD45.1 (A20); PE- and FITC-anti-CD11c (N418); PerCPCy5.5-anti-CD11b (M1/70); FITC-anti-TCRβ (H57-597); APC-anti-CD127 (A7R34); APC-anti-H2-K^d (SF1-1.1); APC-Cy7-anti-I$_A$I$_E$ (M5/114.15.2); and PE-anti-H2-K^b (AF6-88.5). The following monoclonal antibodies from BD Biosciences were used: PE-anti-Stat5 (47/Stat5(pY694)).

pSTAT5 Staining

For in vitro detection of phosphorylated STAT5, cells were stimulated for 30 minutes with different concentrations of rhIL-7. Cells were then fixed in warm (37°C) lyse/fix buffer, washed twice in PBS, and made permeable in iced Perm Buffer III (BD Biosciences). Cells were then stained for pSTAT5 and surface receptors.

Apoptosis Assay

Enriched 10^6 T cells from the spleen and lymph nodes of Rag$^{-/-}$OT-I mice were transferred into chimeric mice by i.v. injection. Seven days later, mice were killed, and OT-I CD8$^+$ T cells from the spleen were labeled with Annexin V-FITC Apoptosis Detection Kit (BD Bioscience), according to the manufacturer's protocol. Acquisition was performed with a Fortessa flow cytometer (BD

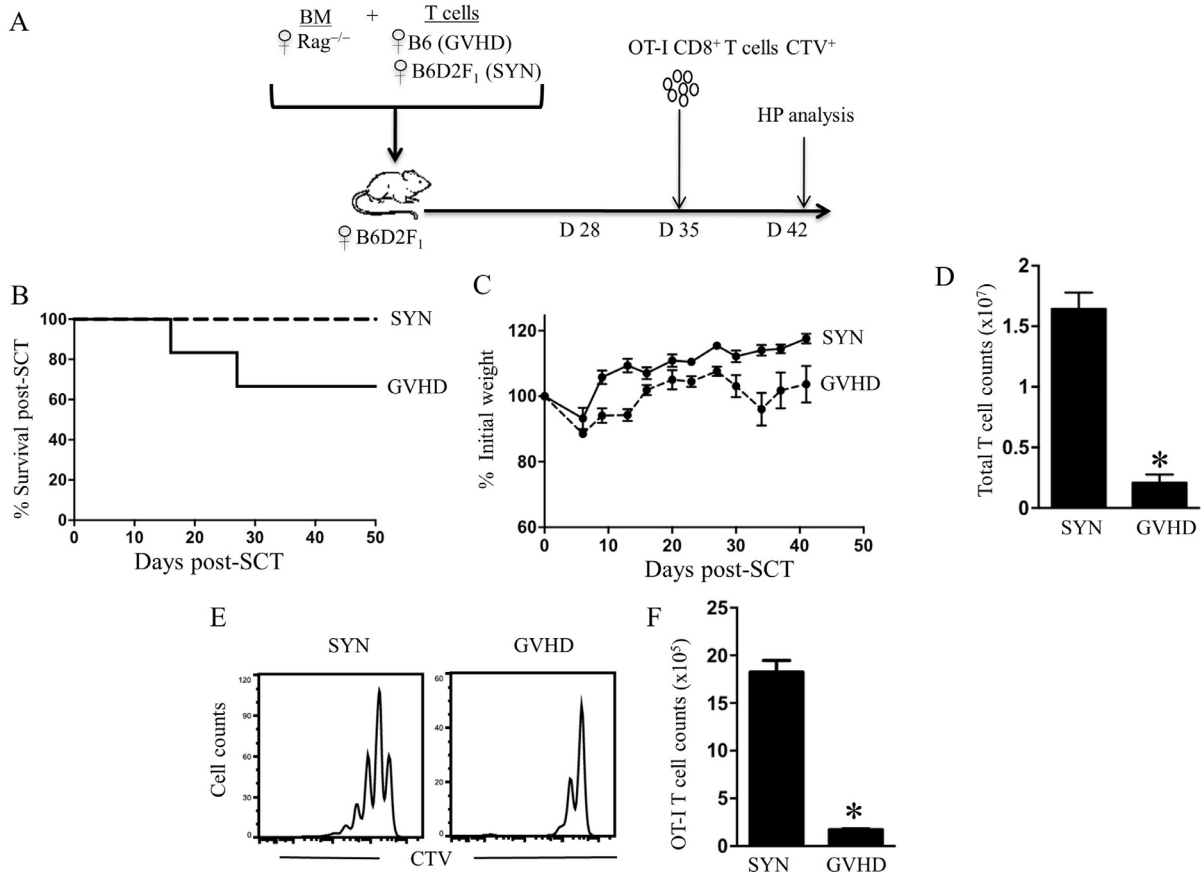

Figure 1. GVHD constrains CD8$^+$ T cell HP after allo-SCT. (A) The experimental design. (B) Survival curve of GVHD and non-GVHD mice. (C) Body weight of mice after allogeneic or syngeneic SCT. (D) Histogram showing total T cell counts in the spleen in non-GVHD and GVHD hosts. (E) Proliferation of OT-I CD8$^+$ T cells 7 days after their transfer in non-GVHD and GVHD hosts. (F) OT-I T cell counts 7 days after their transfer in non-GVHD and GVHD hosts. Data are representative of 3 independent experiments, 3 or more mice per group. Results show mean ± standard error of the mean. *P* values were determined by a Mann-Whitney test ($^*P \leq .05$).

Bioscience), and analyses were performed with FlowJo software (TreeStar). Apoptotic cells were defined as Annexin V$^+$7-AAD$^+$ positive.

Stromal Cell Isolation and Quantitative Real-Time PCR

BM from femurs and tibias were flushed and bones cut into little pieces and digested in dispase (.1%; Gibco, Burlington, Canada) + type 1 collagenase (.25%; Sigma, St. Louis, MO) for 30 minutes at 37°C. Stromal cells were filtered through a 70-μm cell strainer and washed with RPMI buffer (2% FBS, 1% Pen-Strep). RNA was prepared from stromal and BM cells using Trizol (Life Technologies, Burlington, Canada). Expression of IL-7 mRNA was measured with an ABI-Prism 7500 Sequence Detection System (Applied Biosystems, Burlington, Canada). RNA expression for each sample was normalized to the expression of the housekeeping gene *Gapdh* (encoding glyceraldehydes 3-phosphate dehydrogenase). Samples were run in triplicates, and RNA expression was determined using standard curve method.

Statistical Analysis

Prism 5.0 (GraphPad Software, La Jolla, CA) was used for all statistical analyses. The nonparametric Mann-Whitney test was used to compare pairs of data, and the Kruskal-Wallis test was used to compare more than 3 data. $P \leq .05$ was considered significant. Data are presented as mean ± standard error of the mean.

RESULTS

HP of Naïve CD8$^+$ T Cells Is Constrained in GVHD Hosts

IL-7 and TCR stimulation by professional antigen presenting cells have been invoked to be essential for peripheral T cell homeostasis, and accessibility to these resources is compromised during GVHD [13]. We used the parent into F1 (B6→B6D2F1) mouse model to understand the effect of GVHD on the CD8$^+$ T cell peripheral niche. Body weight, survival of transplanted mice, and T cell counts in the spleen were used as indicators of GVHD (Figure 1B-D). To understand the effect of GVHD on CD8$^+$ T cell HP, we transferred CTV-labeled TCR transgenic anti-ovalbumin CD8$^+$ T cells (OT-I) into GVHD or non-GVHD hosts and assessed their proliferation (CTV dilution) 7 days later (Figure 1A). It is important to stress that OT-I CD8$^+$ T cells do not undergo antigen-driven T cell activation in this model because they are specific for the ovalbumin xenoantigen. Furthermore, OT-I CD8$^+$ T cells are not alloreactive for either the B6D2F$_1$ recipient or the donor B6 T cells because they also share a B6 background. Thus, in this GVHD mouse model HP of OT-I CD8$^+$ T cells cannot be confounded with proliferation resulting from TCR-driven T cell activation.

In control mice (no GVHD), B6Rag$^{-/-}$→B6D2F1 + syngeneic B6D2F1 T cells, we found robust HP of OT-I CD8$^+$ T cells. In GVHD mice, however (B6Rag$^{-/-}$→B6D2F1 + allogeneic B6 T cells), HP of OT-I CD8$^+$ T cells was greatly reduced (Figure 1E), and in some mice there was a complete absence of HP. Diminished HP of naïve OT-I CD8$^+$ T cells was associated with much

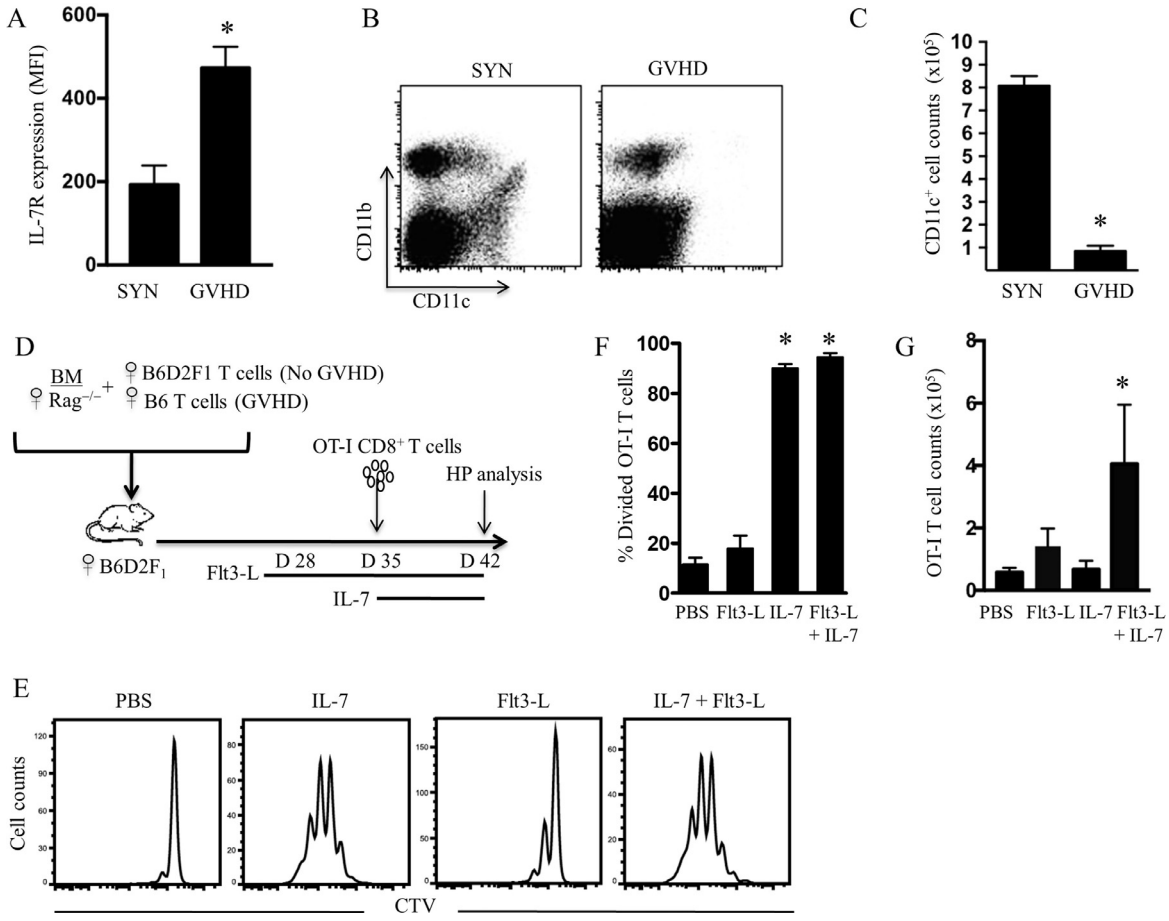

Figure 2. IL-7 administration can restore naïve OT-I CD8$^+$ T cell HP in GVHD hosts. (A) Estimation of systemic IL-7 by transferring OT-I CD8$^+$ T cells into non-GVHD and GVHD hosts and assessing IL-7Rα expression 24 hours later. Histogram showing the mean fluorescence of IL-7Rα on OT-I CD8$^+$ T cells in indicated recipients. (B) Dot plot showing DCs at day +28 after allo-SCT in non-GVHD and GVHD hosts. (C) Absolute counts of splenic CD11c$^+$ cells at day +28 after allo-SCT in non-GVHD and GVHD hosts. (D) Schematic representation of the experimental design in which GVHD mice were treated with Flt3-L (from d28-42) and/or IL-7 (from d35-42) and received OT-I CD8$^+$ T cells. The proliferation of OT-I CD8$^+$ T cells was analyzed at day +42 post-SCT. (E) CTV experiment showing OT-I CD8$^+$ T cell proliferation 7 days after their transfer into GVHD mice treated with IL-7 and/or Flt3-L. (F) Percentage of OT-I CD8$^+$ T cells that have divided 7 days after transfer into GVHD mice treated with IL-7 and/or Flt3-L. (G) Absolute counts of OT-I CD8$^+$ T cells in GVHD mice treated with IL-7 and/or Flt3-L. Data are representative of 2 independent experiments, 3 mice per group. Results show mean ± standard error of the mean. *P* values were determined by a Mann-Whitney test or a Kruskal-Wallis test followed by a post-hoc Dunn's test, (*$P \leq .05$).

lower recovery of OT-I CD8$^+$ T cells in the spleen of GVHD versus non-GVHD hosts (Figure 1F). Thus, as for CD4$^+$ T cells, CD8$^+$ T cell HP is blunted in GVHD hosts.

Limited Access to IL-7 but Not DCs Constrains CD8$^\pm$ T cell HP during GVHD

CD4$^+$ T cell HP is limited in GVHD hosts because of low IL-7 levels and a decrease in the number of MHC class II expressing DCs. We wanted to determine if CD8$^+$ T cell HP in GVHD is constrained by these same factors. We first evaluated whether our GVHD model also exhibited reduced levels of IL-7 and DCs. To validate that IL-7 levels were reduced in our GVHD model, we transferred enriched OT-I CD8$^+$ T cells into GVHD and non-GVHD hosts and studied IL-7Rα expression 24 hours after their transfer. Indeed, IL-7 induces rapid down-modulation of IL-7Rα on T cells such that the level of systemic IL-7 inversely correlates with IL-7Rα on T cells [23, 24]. As predicted, we found much higher expression of IL-7Rα on OT-I CD8$^+$ T cells isolated from GVHD mice compared with OT-I CD8$^+$ T cells isolated from non-GVHD mice, confirming a limited amount of systemic IL-7 in GVHD hosts (Figure 2A). We next evaluated DCs in GVHD hosts and found fewer CD11c$^+$ DCs in GVHD animals (Figure 2B-C). These data confirmed that our GVHD mouse model has limited accessibility to IL-7 and DCs, both of which could constrain CD8$^+$ T cell HP during GVHD.

To understand how lower IL-7 levels and/or reduced numbers of DCs could contribute to the loss of CD8$^+$ T cell HP during GVHD, we treated GVHD mice with IL-7 and/or Flt3-L to increase systemic IL-7 and/or DC numbers, respectively (Figure 2D). The proliferation of OT-I CD8$^+$ T cells was restored in GVHD mice treated with Flt3-L and IL-7 (Figure 2E). Importantly, IL-7 administration alone was sufficient to induce OT-I CD8$^+$ T cell HP to a level similar to that observed in mice treated with both Flt3-L and IL-7 (Figure 2E). In contrast, Flt3-L administration alone had only a very modest effect on OT-I CD8$^+$ T cell HP despite a significant rise in DC counts. We finally evaluated OT-I CD8$^+$ T cell recovery in GVHD mice treated with IL-7 and/or Flt3-L and, despite robust HP of OT-I CD8$^+$ T cells induced by IL-7 administration, the recovery of OT-I CD8$^+$ T cells was lower compared with mice receiving both IL-7 and Flt3-L cytokines (Figure 2F-G). Together, our data support a model wherein low systemic IL-7 levels are the primary limiting factor that constrains CD8 T cell HP, whereas optimal CD8$^+$ T cell recovery requires both IL-7 and Flt3-L cytokines.

MHC I Expression by Radio-Resistant Nonhematopoietic Cells Is Sufficient for CD8$^\pm$ T Cell HP in Lymphopenic Hosts

Although increasing the number of antigen-presenting cells has been shown to be required to improve CD4$^+$ T cell HP during GVHD [13], the modulation of DC numbers by Flt3-L appears to be nonessential for optimal CD8$^+$ T cell HP. We confirmed the absolute requirement of TCR stimulation by MHC I to promote CD8$^\pm$ T cell HP as OT-I CD8$^+$ T cells failed to proliferate and disappeared rapidly on transfer into MHC class I null

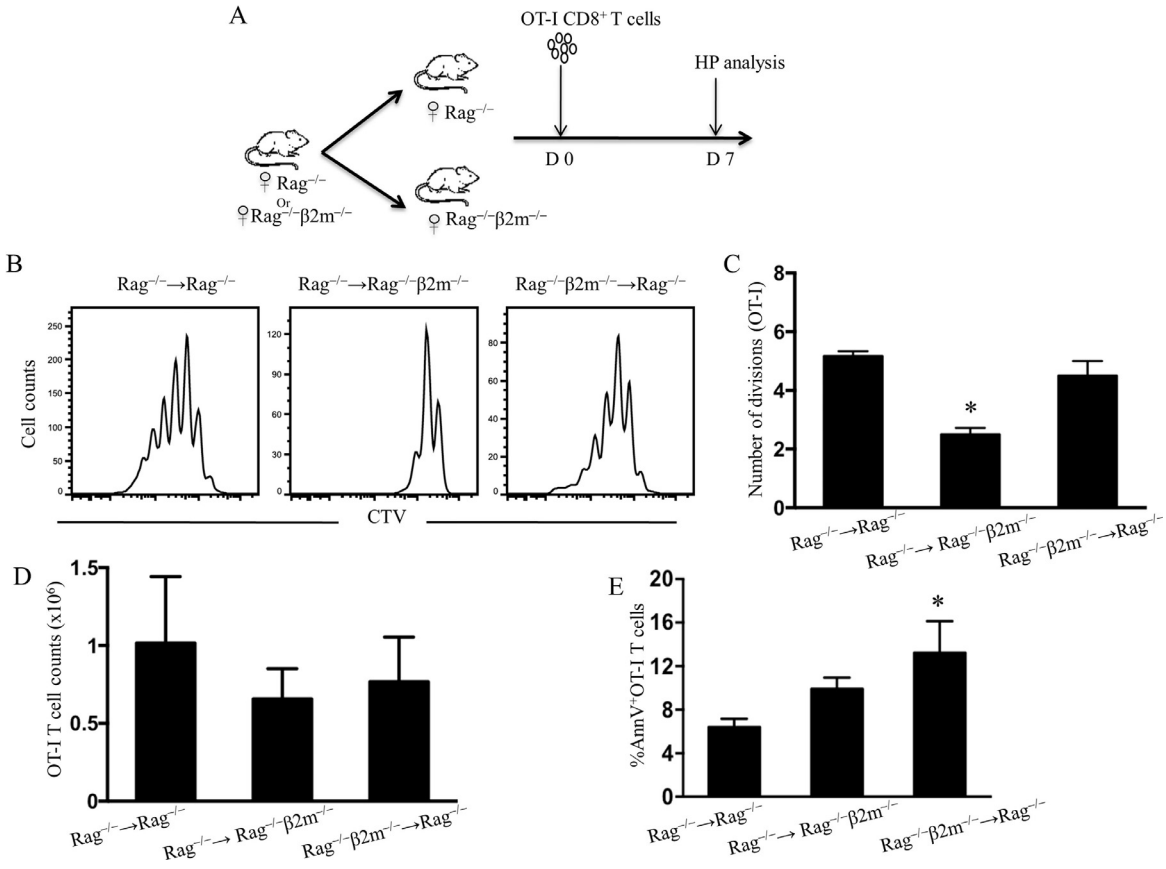

Figure 3. MHC class I expression by nonhematopoietic cells is sufficient for OT-I CD8$^+$ T cell HP mediated by IL-7 therapy. (A) The experimental design. (B) OT-I CD8$^+$ T cell proliferation 7 days after their transfer into BM chimeras described in (A). (C) Number of divisions of OT-I CD8$^+$ T cells 7 days after their transfer in BM chimeras. (D) OT-I CD8$^+$ T cell counts 7 days after their transfer in BM chimeras. (E) Percentage of OT-I CD8$^+$ T cells positive for annexin V 7 days after their transfer in BM chimeras. Data are representative of 2 independent experiments, 3 to 6 mice per group. Results show mean ± standard error of the mean. P values were determined by a Kruskal-Wallis test followed by a post-hoc Dunn's test. (*P ≤ .05).

$Rag^{-/-}\beta2m^{-/-}$ recipients (data not shown). To understand the contribution of radio-sensitive hematopoietic and radio-resistant nonhematopoietic cells at providing MHC class I to promote HP of naïve $CD8^+$ T cells during lymphopenia, we engineered BM chimeras in which the expression of MHC class I was restricted to either radio-resistant stromal cells or radio-sensitive hematopoietic cells using $Rag^{-/-}\beta2m^{-/-}$ or $Rag^{-/-}$ mice as donor or recipient. These chimeric mice lack both $CD4^+$ and $CD8^+$ T cells to limit any potential interference with $CD8^+$ T cell HP. Two months after transplantation, chimeric mice were used for adoptive transfer of enriched OT-I $CD8^+$ T cells to study the requirement of MHC class I expression by hematopoietic and nonhematopoietic cells to promote HP (Figure 3A).

We first evaluated OT-I $CD8^+$ T cell HP in mice expressing MHC class I on hematopoietic cells ($Rag^{-/-}\rightarrow Rag^{-/-}\beta2m^{-/-}$) and found weaker HP compared with mice expressing MHC class I on hematopoietic and nonhematopoietic cells ($Rag^{-/-}\rightarrow Rag^{-/-}$) (Figure 3B-C). We next evaluated OT-I $CD8^+$ T cell HP in mice expressing MHC class I on nonhematopoietic cells ($Rag^{-/-}\beta2m^{-/-}\rightarrow Rag^{-/-}$) and, consistent with our data obtained in GVHD mice, OT-I $CD8^+$ T cell HP was stronger than HP that occurred when MHC class I was restricted to hematopoietic cells, yet the number of cell divisions was lower compared with control mice expressing MHC class I on both hematopoietic and nonhematopoietic cells. Despite stronger HP of OT-I $CD8^+$ T cells in $Rag^{-/-}\beta2m^{-/-}\rightarrow Rag^{-/-}$ chimeras compared with $Rag^{-/-}\rightarrow Rag^{-/-}\beta2m^{-/-}$, the number of OT-I $CD8^+$ T cells recovered in both chimeras was similar but still lower compared with OT-I $CD8^+$ T cell recovered in control $Rag^{-/-}\rightarrow Rag^{-/-}$ mice (Figure 3D). To understand why OT-I cells failed to accumulate normally in $Rag^{-/-}\beta2m^{-/-}\rightarrow Rag^{-/-}$ hosts, we evaluated apoptosis of OT-I $CD8^+$ T cells in different BM chimeras. While OT-I $CD8^+$ T cells in $Rag^{-/-}\rightarrow Rag^{-/-}$ hosts had the lowest levels of annexin V^+7AAD^+, the proportion of annexin V^+7AAD^+ cells was significantly increased on OT-I $CD8^+$ T cells recovered from hosts expressing MHC class I exclusively on stromal cells (Figure 3E). Thus, despite robust OT-I $CD8^+$ T cell HP mediated by stromal-derived MHC class I, increased levels of apoptosis of OT-I $CD8^+$ T cells likely contribute to limit $CD8^+$ T cell expansion.

$CD8^+$ T Cells Respond to Lower Systemic IL-7 Concentrations than $CD4^+$ T Cells

We and others have demonstrated that TCR stimulation controls cytokine responsiveness in $CD4^+$ and $CD8^+$ T cells [25–27]. The ubiquitous MHC class I expression prompted us to determine whether $CD8^+$ T cells could respond to lower IL-7 concentration than $CD4^+$ T cells. We first measured STAT5 phosphorylation in $CD4^+$ and $CD8^+$ T cells exposed to varying

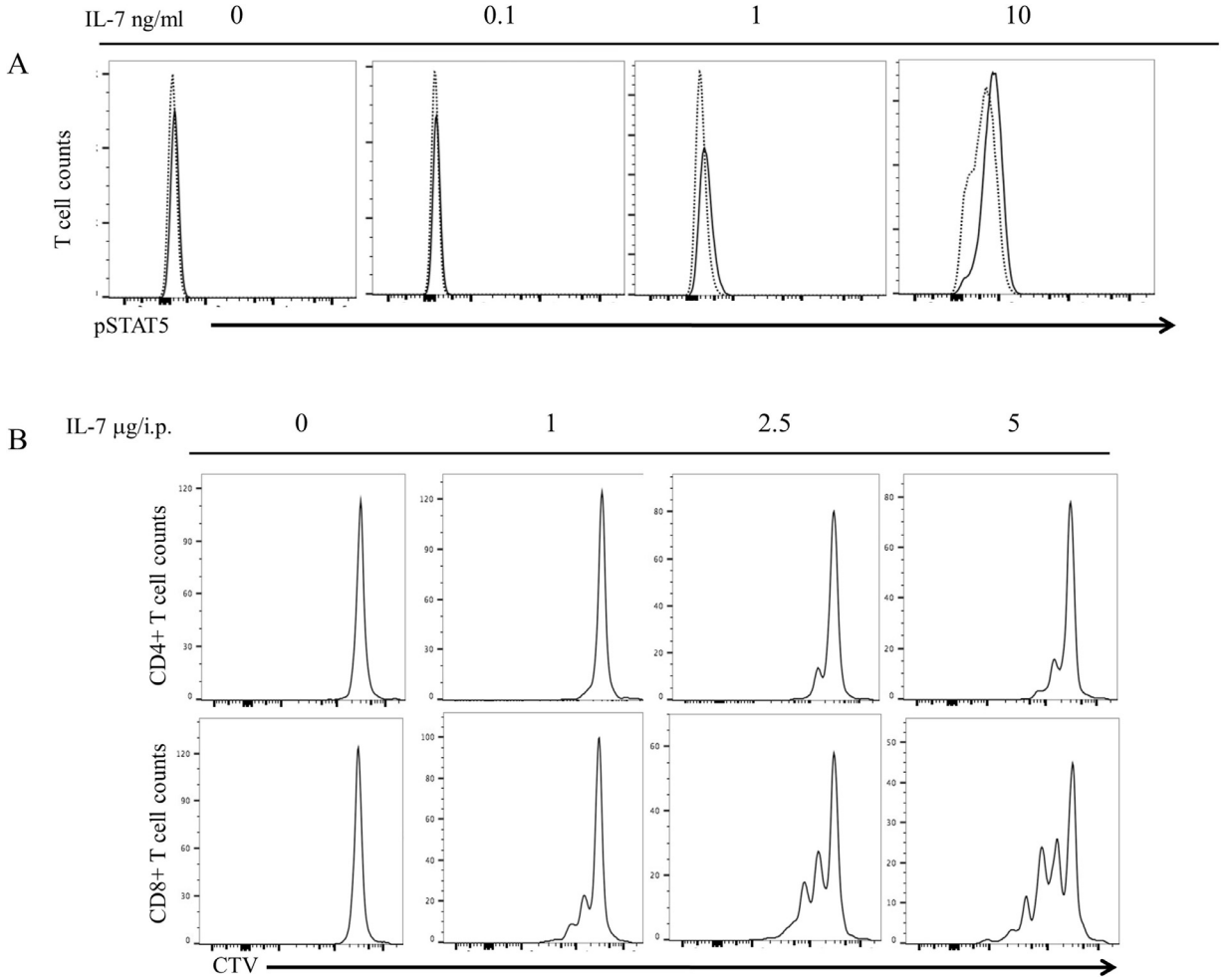

Figure 4. $CD8^+$ T cells respond to lower IL-7 concentration than $CD4^+$ T cells. (A) STAT5 phosphorylation in $CD8^+$ (*solid line*) versus $CD4^+$ (*dashed line*) T cells exposed to varying concentrations of rhIL-7. (B) Representative flow cytometry analysis of proliferation of polyclonal $CD8^+$ vs $CD4^+$ T cells recovered 7 days after their transfer into wild-type mice treated with varying doses of IL-7. Data are representative of 2 independent experiments, 3 mice per group.

concentrations of IL-7. We found that 1 ng/mL of IL-7 could induce STAT5 phosphorylation in $CD8^+$ T cells, whereas higher concentrations of IL-7 were required to induce STAT5 phosphorylation in $CD4^+$ T cells (Figure 4A). We then studied the proliferation of congenic $CD4^+$ and $CD8^+$ T cells in wild-type mice treated with varying doses of IL-7. The administration of 1 μg/day of IL-7 was sufficient to induce $CD8^+$ T cell HP, whereas 2.5 μg/day of IL-7 were required to induce $CD4^+$ T cell HP (Figure 3, Figure 4B). Thus, in addition to the bioavailability of MHC class I, which is considerably higher than the bioavailability of MHC class II, $CD8^+$ T cells respond to lower IL-7 concentrations, which likely contributes to fundamental differences in $CD8^+$ versus $CD4^+$ T cell immune reconstitution in most clinical settings of lymphopenia, notably after allo-SCT and GVHD.

DISCUSSION

Multiple studies have demonstrated that $CD8^+$ T cell regeneration occurs relatively early after lymphopenic insults, whereas $CD4^+$ T cells take longer and normally require thymopoiesis [3,20,21,28–31]. After allo-SCT and GVHD, immune reconstitution of both $CD4^+$ and $CD8^+$ T lymphocytes is dramatically postponed with quantitative and qualitative defects seen in both subsets [32–34]. Although $CD8^+$ T cell counts may normalize during the first year after allo-SCT, the diversity of the TCR repertoire remains poor and also requires thymopoiesis for normalization [35–39]. Thus, in the absence of thymopoiesis, $CD8^+$ and $CD4^+$ T cell immune reconstitution are both impaired, which contributes to the long-lasting immunosuppression seen in GVHD patients.

TCR triggering and IL-7 are both essential for thymopoiesis and T cell maintenance in the periphery [27,40–45]. Although MHC class II expression is largely restricted to antigen-presenting cells, MHC class I expression is ubiquitous and found on most cells. $CD11c^+$ DCs are important for CD4 HP, as for CD8 HP [13,18,19,46]. During GVHD, $CD4^+$ T cell HP is impaired because DC counts and IL-7 levels are diminished, and improvement of $CD4^+$ T cell HP requires not only the administration of IL-7 but also the administration of cytokines to increase DC counts [13]. Using a similar approach, we confirmed that systemic IL-7 levels were low and associated with diminished IL-7 production in the spleen and BM (see Supplementary Figure S1) [13]. Surprisingly, loss of DCs during GVHD did not appear to have a dramatic impact on $CD8^+$ T cell HP because IL-7 therapy alone could induce robust $CD8^+$ T cell HP despite profound DC depletion. Studies have demonstrated that HP of T cells occurs in the T cell zones of secondary lymphoid organs such as the spleen and lymph nodes, where fibroblastic reticular cells express MHC class I and produce large amounts of IL-7 [47–50]. Fibroblastic reticular cells are immunosuppressive and express self-antigens presented to $CD8^+$ T cells, which could be important for $CD8^+$ T cell homeostasis [51]. GVHD insults to fibroblastic reticular cells have been described after MHC mismatch SCT and could limit the accessibility to MHC class I and IL-7 [52]. In this mouse model, lymph nodes were severely atrophic and prevented us from evaluating $CD8^+$ T cell HP at this specific site. Although data from the spleen conclusively demonstrate the absence of $CD8^+$ T cell HP in GVHD hosts, we cannot rule out the possibility of total absence of $CD8^+$ T cell HP in other niches that may have taken place without being investigated. However, given that IL-7 therapy alone dramatically improves $CD8^+$ T cell HP in the spleen, we postulate that accessibility to IL-7, and not MHC class I, is the primary limiting factor that constrains $CD8^+$ T cell HP during GVHD. Whether TCR stimulation is provided by fibroblastic reticular cells themselves or other nonhematopoietic cells remains largely unknown. Additional studies are needed to identify which cell type(s) can present MHC class I to $CD8^+$ T cells to promote HP during IL-7 therapy.

MHC class I accessibility appears to be instrumental to explain differences in the magnitude of $CD8^+$ versus $CD4^+$ T cell HP in lymphopenic hosts. When MHC class I expression is limited to hematopoietic cells, $CD8^+$ T cell HP is diminished and the profile of proliferation is similar to $CD4^+$ T cells in lymphopenic $Rag^{-/-}$ hosts [24,27]. Although nonhematopoietic cells appear to be sufficient for $CD8^+$ T cell HP, normalization of $CD8^+$ T cell counts still requires MHC class I expression by hematopoietic cells in addition to nonhematopoietic cells. TCR stimulation has been shown to control cytokine response in both $CD4^+$ and $CD8^+$ T cells, and greater accessibility to MHC class I likely explains why lower IL-7 concentration can induce $CD8^+$ T cell HP [26,27]. In the hematopoietic compartment, DCs are most likely the prominent cell type providing MHC class I to induce $CD8^+$ T cell HP [18,19,46], but we cannot exclude the contribution of other immune cell types because they also express MHC I. However, there are several DC subsets in mice, and it is not clear which subset if any can sustain $CD8^+$ T cell HP during lymphopenia. DCs can produce and trans-present IL-15 which is critical for the homeostasis of memory $CD8^+$ T cells. Importantly, naïve $CD8^+$ T cells undergo substantial phenotypic changes during HP, and upregulation of IL-15Rβ could allow IL-15 responsiveness by these cells [53–55]. Because IL-7Rα expression downregulation occurs in response to IL-7 and HP [23,24], IL-15 trans-presentation by DCs could play a critical role by increasing $CD8^+$ T cell survival [45,56–59]. In addition, we cannot rule out that quantitative and qualitative differences in TCR stimulation by DCs versus stromal cells could impact $CD8^+$ T cell survival. Finally, despite greater $CD8^+$ T cell HP induced by nonhematopoietic cells, apoptosis is significantly higher, suggesting fundamental differences between MHC class I on hematopoietic and stromal cells to stimulate $CD8^+$ T cell HP and immune reconstitution during lymphopenia.

In conclusion, this study has several potential clinical implications. We showed that systemic IL-7 is the primary limiting factor that constrains $CD8^+$ T cell HP during GVHD, and IL-7 administration can efficiently improve $CD8^+$ T cell HP. Although IL-7 therapy can worsen GVHD [60–62], its effect on alloreactive T cells is modest when administered during GVHD, in part because alloreactive T cells are already activated and express lower IL-7Rα levels compared with non-alloreactive resting lymphocytes (see Supplementary Figure S2A-B) [13,63]. Whether IL-7 administration can diminish infectious complications by expanding antipathogen-specific $CD8^+$ T cells after GVHD remains unknown [64]. It will be particularly interesting to investigate this treatment strategy in appropriate models to determine its clinical applicability. We also found a lack of DC regeneration, and this observation represents a significant concern given their essential role in the priming of naïve T cells [65]. Several studies have confirmed that $CD8^+$ T cell HP is more efficient than $CD4^+$ T cell HP, and we provided conclusive evidence that accessibility to MHC class I versus class II explains these differences. $CD8^+$ T cells respond to lower IL-7 concentrations, and small fluctuations of IL-7 are likely to have more a dramatic effect on $CD8^+$ than $CD4^+$ T cells. As IL-7 production recovers after SCT, this could explain why $CD8^+$ T cell counts tend to rise before $CD4^{\pm}$ T cell counts because they respond to lower IL-7 concentrations and do not require DCs to undergo HP. Thus, strategies to improve T cells counts after allo-SCT must consider the different requirements of naïve $CD4^+$ versus $CD8^+$ T cells for homeostatic resources that appear to be more stringent for $CD4^+$ T cells.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.066.

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