CD62L\(^-\) memory T cells enhance T-cell regeneration after allogeneic stem cell transplantation by eliminating host resistance in mice

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A major challenge in allogeneic hematopoietic cell transplantation is how to transfer T-cell immunity without causing graft-versus-host disease (GVHD). Effector memory T cells (CD62L\(^-\)) are a cell subset that can potentially address this challenge because they do not induce GVHD. Here, we investigated how CD62L\(^-\) T cells contributed to phenotypic and functional T-cell reconstitution after transplantation. On transfer into allogeneic recipients, CD62L\(^-\) T cells were activated and expressed multiple cytokines and cytotoxic molecules. CD62L\(^-\) T cells were able to deplete host radioresistant T cells and facilitate hematopoietic engraftment, resulting in enhanced de novo T-cell regeneration. Enhanced functional immune reconstitution was demonstrated in CD62L\(^-\) T-cell recipients using a tumor and an influenza virus challenge model. Even though CD62L\(^-\) T cells are able to respond to alloantigens and deplete host radioresistant immune cells in GVHD recipients, alloreactive CD62L\(^-\) T cells lost the reactivity over time and were eventually tolerant to alloantigens as a result of prolonged antigen exposure, suggesting a mechanism by which CD62L\(^-\) T cells were able to eliminate host resistance without causing GVHD. These data further highlight the unique characteristics of CD62L\(^-\) T cells and their potential applications in clinical hematopoietic cell transplantation. (Blood. 2012;119(26):6344-6353)

Introduction

Hematopoietic cell transplantation begins with a preparatory regimen that destroys the host immune system (especially T cells), thereby allowing the engraftment of donor stem cells.\(^1\) The reconstitution of T cells after hematopoietic cell transplantation depends on the mature T cells in the graft and on the de novo regeneration of T cells from hematopoietic stem cells.\(^2\) Donor-type mature T cells provide immediate immunity against infectious agents and tumor cells to the host.\(^2\) However, donor T cells also cause life-threatening graft-versus-host disease (GVHD).\(^3\) Moreover, GVHD and the immunosuppressive treatments used to prevent or control GVHD result in severely impaired thymopoiesis and T-cell deficiency in the graft recipient.\(^4\) De novo T-cell regeneration from hematopoietic stem cells is a very slow process, usually taking months and even years.\(^5-8\) Under current treatment protocols, the overall T-cell recovery can be very slow after allogeneic hematopoietic cell transplantation, making hematopoietic cell recipients extremely susceptible to a variety of opportunistic infections for a significant period of time.\(^5-9\) As a result, infections have remained a major cause of morbidity and mortality after hematopoietic cell transplantation.\(^9\)

Because of the slow de novo regeneration of stem- and progenitor-derived T cells, a population of T cells that does not cause GVHD would be extremely helpful to protect the recipients from infections in the first few months after transplantation before new T cells can be generated from hematopoietic stem or progenitor cells. We and others have recently observed that allogeneic effecter memory T cells (T\(_{EM}\), CD62L\(^-\))\(^10\) do not cause GVHD and contribute directly to posttransplantation T-cell recovery.\(^11-16\) We further demonstrated that CD62L\(^-\) T cells contribute to after transplantation T-cell reconstitution not only through peripheral expansion but also through thymopoiesis.\(^13\) These important observations suggest that CD62L\(^-\) T cells are capable of protecting hematopoietic cell recipients from infections early after transplantation by providing immediate recall immunity and later by promoting more diverse T-cell regeneration through thymopoiesis. Because depletion of host radioresistant T cells is associated with the enhancement of immune reconstitution,\(^11\) it is likely that CD62L\(^-\) T cells enhance stem/progenitor cell mediated de novo T cell regeneration through facilitating hematopoietic cell engraftment.

Here, we further investigated whether and how CD62L\(^-\) T cells enhanced functional immune reconstitution after allogeneic stem cell transplantation. CD62L\(^-\) T cells were able to prolong the survival of T cell–depleted (TCD) bone marrow (BM) recipients after challenge with a tumor cell line or with live influenza viruses. CD62L\(^-\) T cells facilitated hematopoietic progenitor engraftment, leading to enhanced immune reconstitution after hematopoietic stem cell transplantation. On transfer into irradiated BALB/c recipients, donor CD62L\(^-\) C57BL/6 T cells were activated, secreted multiple inflammatory cytokines, and expressed many cytotoxic molecules such as perforin and granzyme B. We also investigated why the activation of CD62L\(^-\) T cells by alloantigens only led to host cell depletion but not GVHD.

Methods

Mice

BALB/c (H2\(^d\), CD45.2, Thy1.2, Mls-2\(^a\), Mls-3\(^a\)), C57BL/6, CD45.2, Thy1.2 (H2\(^k\), Mls-2\(^b\), Mls-3\(^b\), termed B6 CD45.2 mice), BALB/c severe


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immunodeficiency (SCID), NOD.Cg-Prkd<sup>−/−</sup> (NSG) mice were purchased from The Jackson Laboratory. Rag2<sup>−/−</sup>γC<sup>−/−</sup> C57BL/6 mice<sup>17</sup> were purchased from Taconic Farms. The breeders of C57BL/Ka, CD45.1, Thyl.1 mice (H2<sup>b</sup>, Mls<sup>−/−</sup>, Mls<sup>−/−</sup>, termed B6 CD45.1 mice) were provided by Dr Jos Domen (Duke University, Durham, NC). All animals were female and were used when they were 8 to 12 weeks old except that some T-cell donors were male and up to 14 months old. Animal were housed in sterile microisolator cages in a specific pathogen-free facility throughout the study. This study was approved by the Duke University Institution Animal Care and Use Committee.

**BCL1 cell lines**

BCL1 (a kind gift from Drs Defu Zeng and Samuel Strober, Stanford University, Stanford, CA) is a spontaneous B-cell leukemia/lymphoma cell line of BALB/c origin.<sup>19</sup> Purified BCL1 cells were obtained from spleen cells from BCL1-bearing mice by positive selection using anti-BCL1 idiotype antibody<sup>19</sup> (rat IgG2a, clone 6A5; a generous gift from Dr Ellen S. Vietta, University of Texas, Dallas) followed by using goat anti–rat immunoglobulin G magnetic beads (Miltenyi Biotec).

**T-cell separation**

CD62L<sup>−</sup> T cells were isolated based on a published protocol from our laboratory, with modification.<sup>20</sup> The only difference was that a mouse pan-T-cell selection kit (Miltenyi Biotec) was used to negatively purify splenic T cells.

**Hematopoietic cell transplantation**

Recipient BALB/c mice were lethally irradiated (8.5 Gy for regular BALB/c mice; 5 Gy for BALB/c SCID mice; 3.25 Gy for NSG mice; 8 Gy for Rag2<sup>−/−</sup>γC<sup>−/−</sup> C57BL/6 mice). Within 4 hours after irradiation, all recipients were transplanted via tail-vein injection. For the rejection model, recipients were transplanted with 5 × 10<sup>5</sup> TCD BM cells. For the immune reconstitution model, recipients were transplanted with 1 × 10<sup>5</sup> TCD BM cells. For the immune reconstitution model, recipients were transplanted with 1 × 10<sup>5</sup> TCD BM cells from 5000 hematopoietic stem cells (c-Kit<sup>+</sup>Thy1,1<sup>int</sup>Lin<sup>−/low</sup>Sca-1<sup>+</sup> or KT1<sup>+</sup>CD62L<sup>+</sup> cells<sup>21-23</sup>) with or without 1 × 10<sup>5</sup> T cells. Except where indicated, the experiments were performed in the C57BL/6→BALB/c system.

**Peripheral blood cell counts**

Blood cells were counted in an automatic hematologic analyzer (HEMAVET HV950FS; Drew Scientific).

**Intracellular staining**

Spleen cells were first stained with surface markers. Cells were then fixed, permeabilized, and intracellularly stained according to the manufacturer’s instructions (eBioscience). Except where indicated, all antibodies were purchased from eBioscience. Isotype control was used to define the negative populations.

**Tumor model**

Lethally irradiated BALB/c or Rag2<sup>−/−</sup>γC<sup>−/−</sup> mice were transplanted with 1 × 10<sup>5</sup> TCD BM cells from B6 CD45.2 mice or total BM cells from Rag2<sup>−/−</sup>γC<sup>−/−</sup> C57BL/6 mice with or without CD62L<sup>−</sup> T cells (1 × 10<sup>6</sup>) from B6 CD45.1 mice via tail-vein injection. BCL1 cells (5 × 10<sup>5</sup>) were injected intravenously to recipients at different times after transplantation. Mortality was recorded daily. Biopsies were taken from spleen, lung, and liver for histologic analyses for evidence of leukemia or lymphoma.

**Flu model**

Lethally irradiated BALB/c mice were transplanted with TCD BM cells (1 × 10<sup>5</sup>) with or without CD62L<sup>−</sup> T cells (1 × 10<sup>6</sup>) from C57BL/6 mice via tail-vein injection. At day 7 after transplantation, all recipients were administered with 1.4 × 10<sup>5</sup> PFU influenza virus (A/PR/8/34 murine influenza; Charles River Laboratories) in 20 μL of PBS by intranasal inoculation. Mock-challenged controls (normal BALB/c mice) were administered 20 μL of PBS in the same way. Weight loss, change in body temperature, and survival were monitored daily. Mice with more than 25% weight loss were humanely killed.

**Statistical analysis**

Data were presented as mean ± SD or mean ± SD. Comparison of continuous data between groups was performed by either unpaired t test (2 groups) or ANOVA (>2 groups). Survival data were analyzed by log rank test. Statistical analyses were performed using StatView Version 5 (SAS Institute), Excel 2010 (Microsoft), or Prism software Version 5 (GraphPad Software). P values < .05 were considered significant.

**Other methods**

Other methods used in this study have been described in detail previously.<sup>24</sup>

**Results**

**CD62L<sup>−</sup> T cells enable the ability of TCD BM recipients to prevent tumor growth**

Our previously published study<sup>11</sup> has demonstrated that CD62L<sup>−</sup> T cells enhance phenotypic T-cell recovery. To determine whether CD62L<sup>−</sup> T cells enhance functional immune recovery, lethally irradiated BALB/c recipients were first transplanted with 1 × 10<sup>5</sup> TCD BM cells with or without 1 × 10<sup>6</sup> CD62L<sup>−</sup> T cells from B6 CD45.2 mice. At day 7 after transplantation, the recipient mice were challenged with 5 × 10<sup>5</sup> BCL1 tumor cells. BCL1 is a host-type leukemia/lymphoma cell line.<sup>18</sup> In an allogeneic transplantation setting, T cells play a critical role in preventing BCL1 growth.<sup>19</sup>

Development of tumor(s) and survival were monitored, and the results are summarized in Figure 1A-B. Although 6 of 8 TCD BM only recipients developed tumors and died within 30 days after transplantation, none of CD62L<sup>−</sup> T-cell recipients developed tumors, and all recipients survived for more than 100 days after transplantation (P < .001). Consistent with our previously published results,<sup>11</sup> none of CD62L<sup>−</sup> T-cell recipient mice developed GVHD (data not shown).

To determine whether the enhancement of antitumor immunity in CD62L<sup>−</sup> T-cell recipients is the direct effect of transplanted CD62L<sup>−</sup> T cells or newly generated immune cells from stem or progenitor cells, we repeated the above-mentioned experiment using bone marrow from T, B, and natural killer (NK) cell–deficient Rag2<sup>−/−</sup>γC<sup>−/−</sup> B6 mice. Similar to the data shown in Figure 1A and B, none of CD62L<sup>−</sup> T-cell recipients who also received TCD BM from wild-type donors (B6 CD45.2) developed tumors, and all recipients survived for more than 100 days after transplantation. By contrast, all CD62L<sup>−</sup> T-cell recipients transplanted together with Rag2<sup>−/−</sup>γC<sup>−/−</sup> bone marrow developed tumors, and 14 of 20 mice died by 100 days (Figure 1C; P < .0001). Similar results were also obtained when Rag1<sup>−/−</sup> mice (T and B but not NK cell deficient) were used as bone marrow donor (Figure 1C). It is of note that B6 CD45.2 TCD BM only group survived longer than both Rag1<sup>−/−</sup> and Rag2<sup>−/−</sup>γC<sup>−/−</sup> BM only groups (Figure 1C; P < .05), probably because tumor cells were inoculated on day 7 when new T and B cells had already been generated from B6 CD45.2 but not from Rag1<sup>−/−</sup> or Rag2<sup>−/−</sup>γC<sup>−/−</sup> marrow.

**CD62L<sup>−</sup> T cells enhance the ability of TCD BM recipients to counteract the effects of viral infection in vivo**

The ability of CD62L<sup>−</sup> T cells to enhance functional immune recovery in allogeneic hematopoietic cell recipients was further
tested using an influenza virus challenge model. Similar to the tumor challenge model (Figure 1A–B), CD62L+ T-cell recipients were challenged with 1.4 × 10^5 PFU influenza virus at day 7 after transplantation. Weight change, body temperature, and survival were monitored. The results were summarized in Figure 2A. On challenge with influenza virus, TCD BM only recipients rapidly developed symptoms of influenza; namely, rapid weight loss and decrease in body temperature, followed by death of all animals within 17 days after challenge. The onset of disease was significantly delayed in allogeneic CD62L+ T-cell recipients compared with the TCD BM only recipients. Even though all allogeneic CD62L+ T-cell recipients developed flu disease, they survived significantly longer than TCD BM control animals (median survival time, 15 vs 30.5 days; P < .0001).

To understand how CD62L+ T cells enhance the ability of TCD BM recipients to fight against influenza virus infection, we performed similar experiments as described in Figure 1C. Although CD62L+ T-cell recipients transplanted with B6 CD45.2 TCD BM survived much longer than TCD BM only recipients (Figure 2B; P < .0001), CD62L+ T cells did not help Rag2−/−γC−/− bone marrow recipients survive longer (Figure 2B; P = NS). To further define the specific role of NK cells, we also transplanted CD62L+ T-cell recipients together with Rag1−/− bone marrow. In contrast to antitumor responses (Figure 1C), CD62L+ T-cell recipients transplanted with Rag1−/− bone marrow survived longer than Rag1−/− bone marrow alone recipients (Figure 2B; P < .01). Even though it was not statistically significant, CD62L+ T-cell recipients transplanted with B6 CD45.2 bone marrow trended to survive longer than those transplanted together with Rag1−/− bone marrow (median survival time, 22 vs 18 days; P = .06; Figure 2B).

**CD62L+ T cells enhance hematopoietic cell engraftment after TCD BM transplantation**

It was reported previously that CD62L+ T cells were able to kill host radiosensitive T cells and host-type tumor cells, suggesting that CD62L+ T cells might facilitate hematopoietic stem cell engraftment. To determine whether CD62L+ T cells could truly facilitate hematopoietic stem cell engraftment, we used a rejection model in which lethally irradiated BALB/c mice were transplanted with a minimum number (5 × 10^5) of B6 CD45.2 TCD BM cells. As illustrated in Figure 3A, only 40% of TCD BM only recipients survived more than 100 days after transplantation because of engraftment failure. Both total white blood cell and platelet counts were significantly lower in TCD BM only recipients compared with CD62L+ T-cell recipients at day 30 (Figure 3B; P < .05). All TCD BM only recipients had a mixed chimera phenotype when measured at day 30 (Figure 3C). By contrast, the addition of 1 × 10^6 CD62L+ T cells to the TCD BM graft rescued 90% of the recipients (P < .01, compared with TCD BM only group), and all recipients became full donor chimeras by day 30 (P < .05). Similar results on chimeraism were obtained at day 70 and day 100 (data not shown), indicating that the engraftment was durable.

It has been demonstrated in mice and humans that naive T cells have strong effects on facilitating hematopoietic cell engraftment. Even though the contamination of CD62L+ T cells was very small (always < 0.5%), there was a possibility that the enhancement of hematopoietic cell engraftment was mediated by the contaminating CD62L+ T cells. To exclude this possibility, we included an additional group in which the same number (4 × 10^6) of CD62L+ T cells contained in the 1 × 10^6 CD62L− T-cell graft was added to the TCD BM graft. As demonstrated in Figure 3A through C, addition of 4 × 10^6 CD62L+ T cells neither significantly prolonged the survival of TCD BM recipients, accelerated hematopoietic recovery, nor converted mixed chimeras into full donor chimeras.

**CD62L+ T cells promote purified hematopoietic stem cell–derived T-cell regeneration**

Because hematopoietic stem cell dose correlates with the speed of immune reconstitution after stem cell transplantation, facilitation of engraftment (Figure 3) by CD62L+ T cells may lead to enhanced immune reconstitution. Indeed, our published data suggest that allogeneic CD62L+ T cells are able to promote stem- or progenitor-derived T-cell generation because TCD BM was used in that study. To directly test whether CD62L+ T cells were able to support the growth of stem cell–derived T cells, we performed an experiment using purified stem cells. In this experiment, we transplanted 1 × 10^6 CD62L− T cells from unprimed B6 CD45.2 mice and 5 × 10^5 KTLs cells from B6 CD45.1 mice into lethally irradiated BALB/c recipients. CD4+ and CD8+ T-cell counts were
monitored in peripheral blood by flow cytometry over time. As illustrated in Figure 4A, the promoting effect of CD62L⁺ T cells on stem cell–mediated T-cell regeneration was maintained in both CD4⁺ and CD8⁺ T cells through day 42, directly demonstrating that allogeneic CD62L⁺ T cells were able to enhance T-cell generation from hematopoietic stem cells.

**Figure 2.** CD62L⁺ T cells enhance the ability of TCD BM recipients to counteract the effects of viral infection in vivo. Bone marrow cells (1 x 10⁷) from different sources and CD62L⁺ T cells (1 x 10⁶) from unprimed B6 CD45.1 mice were transplanted into lethally irradiated BALB/c recipients. The recipient mice were challenged with influenza virus at day 7 after transplantation. Body weight and body temperature were monitored daily in all recipients. Each group contained 10 (A) or 15 (B) mice. A group of normal BALB/c mice (n = 5) was included as mock-challenged control for each experiment. (A) TCD BM was from B6 CD45.2 mice. CD62L⁺ versus TCD BM only (P < .0001); TCD BM only versus mock-challenged (P < .001). (B) Bone marrow used included TCD BM cells from B6 CD45.2 mice or whole marrow from Rag1−/− B6 or Rag2−/−γC−/− B6 mice. For survival, BM only versus their corresponding CD62L⁺ groups except for Rag2−/−γC−/− (P < .01); TCD BM only versus Rag1−/− BM only or Rag2−/−γC−/− BM only (P < .01); P = NS in all other comparisons.

**Figure 3.** CD62L⁺ T cells facilitate hematopoietic cell engraftment after allogeneic TCD BM transplantation. TCD BM cells from B6 CD45.2 (5 x 10⁵) and CD62L⁺ T cells (1 x 10⁶) from unprimed B6 CD45.1 mice were transplanted into lethally irradiated BALB/c recipients. Mortality was recorded daily. Each group contained 5 to 10 animals (TCD BM only, 10; CD62L⁺, 10; and contaminating, 5). The data are the representative of 2 independent experiments with similar data. (A) Survival. CD62L⁺ versus TCD BM only or contaminating (P < .01); TCD BM only versus contaminating (P = .86). (B) Hematologic recovery. The recovery of white blood cells (WBCs) and platelets was analyzed in peripheral blood at day 30 after transplantation. All values represent means ± SD (*P < .05; CD62L⁺ vs TCD BM only or contaminating in both WBC and platelets). The mean values of WBCs and platelets in peripheral blood of normal BALB/c mouse (age, 8-12 weeks; female) were 6.1 ± 1.5 K/μL and 716.1 ± 142.4 K/μL, respectively. (C) Chimerism analysis. Chimerism in different cell subsets was analyzed in peripheral blood by flow cytometry on day 30 after transplantation. All values represent means ± SD (*P < .05; CD62L⁺ versus TCD BM only or contaminating in all cell subsets except CD62L⁺ vs contaminating in CD19⁺ cells).
CD62L<sup>−</sup> T cells lose the promoting effects on stem cell–mediated T-cell regeneration in recipients with limited or no host resistance

Our results were consistent with a model in which CD62L<sup>−</sup> T cells promoted T-cell regeneration from stem cells via depleting host radioresistant T cells that could interfere with T-cell regeneration. Specifically, the finding that host radioresistant T cells were depleted in CD62L<sup>−</sup> T-cell recipients but not in the TCD BM control mice suggested that CD62L<sup>−</sup> T cells might eliminate radioresistant host cells. To test directly whether CD62L<sup>−</sup> T cells promote new T-cell generation by overcoming T cell–mediated host resistance, we repeated the immune reconstitution experiments using BALB/c SCID mice (lacking both T and B cells but not NK cells) as recipients. We transplanted 1 × 10<sup>6</sup> CD62L<sup>−</sup> T cells from unprimed B6 CD45.1 mice and 1 × 10<sup>7</sup> B6 CD45.2 TCD BM cells into lethally irradiated SCID BALB/c recipients. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts were monitored in peripheral blood by flow cytometry over time. The results presented in Figure 4B and C demonstrated that the promoting effect of CD62L<sup>−</sup> T cells was dramatically decreased when SCID mice were used as recipients compared with BALB/c mice.

It was observed that there was a trend toward higher T-cell counts in SCID recipients of CD62L<sup>−</sup> T cells after day 28 even though the differences were not statistically significant (Figure 4B). These potential differences could have reflected the resistance mediated by radioresistant NK cells. To investigate whether that was the case, we repeated the experiment using NSG mice (lacking T, B and NK cells and no known host resistance) as recipients.
Using NSG mice as recipients, the promoting effect of CD62L<sup>H11002</sup> T cells could not be observed any more (Figure 4B-C). These data further indicated that allogeneic CD62L<sup>H11002</sup> T cells enhanced immune reconstitution through depletion of host radioresistant immune cells and enhancing hematopoietic stem or progenitor cell engraftment.

CD62L<sup>H11546</sup> T cells are activated, secrete multiple cytokines, and express cytotoxic molecules on transfer into recipients

Even though CD62L<sup>H11002</sup> T cells do not induce GVHD,<sup>11</sup> the ability of these cells to deplete host-radioresistant T cells<sup>11</sup> and kill host-type tumor cells<sup>25,26</sup> suggests that CD62L<sup>H11002</sup> T cells from unprimed donors may be activated on transfer into allogeneic recipients. To confirm this, we transplanted 1 × 10<sup>6</sup> CD62L<sup>H11546</sup> T cells from unprimed B6 CD45.1 mice and 1 × 10<sup>7</sup> B6 CD45.2 TCD BM cells into lethally irradiated BALB/c recipients. At day 6, splenocytes were harvested, and the expression of activation molecules, cytokines, and cytotoxic molecules by CD62L<sup>H11546</sup> T cells was analyzed directly by flow cytometry without further in vitro stimulation. On transfer into irradiated BALB/c recipients, both CD4<sup>H125</sup> (Figure 5A) and CD8<sup>H172</sup> (data not shown) CD62L<sup>H11546</sup> T cells expressed many activation markers (eg, CD25, CD30, CD69, and KLRG1) and secreted IFN-γ at similar or even higher levels than not only normal B6 CD45.1 T cells but also transferred bulk and CD62L<sup>H11002</sup> T cells. Moreover, CD8<sup>H172</sup> CD62L<sup>H11546</sup> T cells produced similar or higher amounts of multiple cytotoxic molecules, including perforin, granzyme B, CD107α, FasL, TRAIL, and TNF-α compared with both bulk and CD62L<sup>H11002</sup> T cells (Figure 5B). The total numbers of CD62L<sup>H11546</sup> T cells expressing most of the activated markers except perforin and FasL were also similar or higher than those of bulk and CD62L<sup>H11002</sup> T cells (Figure 5Aiv,Biv). These results demonstrated that CD62L<sup>H11546</sup> T cells could be rapidly and fully activated on transfer into allogeneic recipients, explaining why CD62L<sup>H11546</sup> T cells from unprimed donors were able to kill host radioresistant T cells<sup>11</sup> and to enhance hematopoietic cell engraftment.

CD62L<sup>H11546</sup> T cells lose alloreactivity over time on transfer into allogeneic but not syngeneic recipients

The data presented here have convincingly demonstrated that, on transfer into allogeneic recipients, CD62L<sup>H11546</sup> T cells from unprimed donors can be activated (Figure 5) and are able to kill host-radioresistant T cells (Figure 3) and host-type tumor cells (Figure 1C). How can we reconcile these data with the previously
published data demonstrating that these cells do not induce GVHD. To explain this dichotomy, we hypothesized that donor CD62L\(^+\) T cells could react to alloantigens initially but the allosequences could not be sustained on transfer into GVHD recipients based on our previously published in vitro observations. To test this hypothesis in vivo, we injected BCL1 cells \((5 \times 10^5)\) into lethally irradiated BALB/c recipients of Rag2\(^{-/-}\) CD62L\(-/-\) BM only vs CD62L\(-/-\) T cells (same as B without BCL1 cell challenge) more than 100 days after transplantation. Percentages of V\(\beta\) T cells were determined by flow cytometry. Each group contained 10 to 12 mice. The data were pooled from 2 independent experiments with similar data (*\(P < .05\), normal C57BL/6 vs CD62L\(-/-\) [allogeneic]).

Figure 6. CD62L\(^+\) T cells lose alloreactivity over time on transfer into allogeneic but not syngeneic recipients. Bone marrow cells \((1 \times 10^6)\) from Rag2\(^{-/-}\)\(\gamma C^{-/-}\) B6 mice and CD62L\(-/-\) T cells \((1 \times 10^6)\) from unprimed B6 CD45.2 mice were transplanted into lethally irradiated BALB/c (A and Bii, allogeneic) or Rag2\(^{-/-}\)\(\gamma C^{-/-}\) B6 (Biii, syngeneic) recipients. BALB/c origin BCL1 cells \((5 \times 10^5)\) were injected into bone marrow recipients at different times after transplantation (Ai, day 7; Aii, day 14; Aiii, day 21; B, day 28). The recipient mice were monitored for development of leukemia/lymphoma and survival. For V\(\beta\) family experiments (C), peripheral blood was obtained from allogeneic or syngeneic recipients of CD62L\(-/-\) T cells in allogeneic and syngeneic recipients (Figure 6Bii-iii), indicating that CD62L\(+\) responsible for the loss of alloreactivity of CD62L\(+\) T cells in allogeneic and syngeneic recipients.

Prolonged exposure to antigens has been reported to induce clonal deletion or clonal anergy of antigen-specific T cells in GVHD recipients, we compared the ability of CD62L\(+\) T cells to kill host-type tumor cells in allogeneic and syngeneic recipients 28 days after transplantation. To test only the ability of donor CD62L\(+\) T cells to kill tumor cells, Rag2\(^{-/-}\)\(\gamma C^{-/-}\) B6 mice were used as syngeneic recipients and BM donors (Figure 6Bi). Although CD62L\(+\) T cells completely lost the ability to kill BCL1 cells in allogeneic recipients (Figure 6Bi, \(P = .67\); Rag2\(^{-/-}\)\(\gamma C^{-/-}\) BM only vs CD62L\(+\)), they retained the reactivity in syngeneic recipients (Figure 6Biii, \(P < .01\); Rag2\(^{-/-}\)\(\gamma C^{-/-}\) BM only vs CD62L\(+\)). These data demonstrated that the gradual loss of CD62L\(+\) T cells’ ability to respond to alloantigens on transfer into allogeneic recipients was a result of prolonged exposure to alloantigens.

To further determine whether prolonged exposure of CD62L\(+\) T cells to alloantigens leads to clonal anergy or deletion of antigen-specific T cells, we measured the percentages of V\(\beta\)3\(^+\) and V\(\beta\)5.1/5.2\(^+\) cells in peripheral blood from allogeneic recipients of CD62L\(-/-\) T cells more than 100 days after transplantation (no BCL1 challenge). V\(\beta\)3\(^+\) and V\(\beta\)5.1/5.2\(^+\) cells recognize Mls-2 and Mls-3 superantigens that are present in the recipient BALB/c mice but are absent in the donor C57BL/6 mice. As shown in Figure 6C, CD62L\(+\) T-cell allogeneic recipients had similar percentages of V\(\beta\)3\(^+\) in both CD4\(^+\) and CD8\(^+\) T-cell subsets compared with normal C57BL/6 mice and CD62L\(-/-\) T-cell syngeneic recipients. Even though the percentages of V\(\beta\)5.1/5.2\(^+\) among both CD4\(^+\) and CD8\(^+\) T cells in CD62L\(-/-\) T-cell allogeneic recipients were lower than those in normal C57BL/6 mice \((P < .05)\), the differences between CD62L\(-/-\) T-cell allogeneic and syngeneic recipients were not significant. As a negative control, the percentages of V\(\beta\)8.1/
8.2% among both CD4+ and CD8+ T cells were similar in normal C57BL/6 mice, CD62L+ T-cell allogeneic and syngeneic recipients.

Discussion

Previously published data from several different groups have independently demonstrated that CD62L+ effector TEM from unprimed donors do not induce GVHD.11,13,14,16 Our current work further demonstrates that these T cells are able to facilitate hematopoietic engraftment and T-cell reconstitution. CD62L− T cells contribute to posttransplantation T-cell reconstitution not only directly and also by promoting de novo T-cell regeneration from hematopoietic stem cells. The ability of CD62L+ T cells to enhance stem cell–mediated T-cell reconstitution is of great clinical significance because now CD62L+ T cells can not only provide immediate recall immunity11 to the host but also dramatically boost regeneration of new T cells through thymopoesis. Newly generated T cells would presumably be more potent and have a broader T-cell repertoire capable of protecting the recipients from a wider range of opportunistic infections.2 Indeed, we demonstrate in 2 different models that CD62L+ T cells enhance functional immune reconstitution against tumor (Figure 1) and viral (Figure 2) antigens. CD62L+ T cells have direct antitumor effects as demonstrated in Figures 1C and 6Ai. In addition, enhanced reconstitution of stem cell–derived adaptive immunity contributes significantly to antitumor effects after transplantation of CD62L+ T cells because antitumor activity is dramatically decreased when Rag1−/− BM is used (Figure 1C). In contrast, CD62L− T cells from unprimed donors have very limited direct anti-influenza immunity because CD62L− T cells completely lose their anti-influenza activity when Rag2−/−γC−/− BM is used (Figure 2B). Enhancement of NK cell reconstitution is mainly responsible for enhanced anti-influenza activity after CD62L− T-cell transplantation because anti-influenza immunity is preserved even when Rag1−/− BM is used (Figure 2B). Because CD62L+ T-cell recipients transplanted with B6 CD45.2 bone marrow seem to survive longer than those transplanted together with Rag1−/− bone marrow (Figure 2B), stem cell–derived adaptive immunity also may contribute to antiviral immunity. Even though CD62L+ T cells from unprimed donors have limited direct antiviral activity, it is predicted that the anti-influenza immunity could be enhanced if the donor is immunized against influenza antigens before transplantation.10,12

Previous studies have demonstrated that CD62L+ T cells are able to deplete host-radiosensitive T cells11 and host-type tumor cells25,26 without causing GVHD, suggesting that allogeneic CD62L+ T cells may facilitate hematopoietic engraftment. We further characterized the effect of allogeneic CD62L+ T cells on hematopoietic stem or progenitor engraftment using a rejection model, in which a minimum numbers of TCD bone marrow cells were infused. Data from this experiment (Figure 3) demonstrate that CD62L+ T cells rescue TCD bone marrow recipients from engraftment failure and significantly prolong survival. The ability of CD62L+ T cells to facilitate hematopoietic cell engraftment is further supported by faster hematologic recovery (Figure 3B) and the conversion from mixed to full chimerism (Figure 3C) after addition of CD62L+ T cells to the graft. These data are in line with recent data published by Dutt et al.26

Because higher numbers of hematopoietic stem24 and progenitor2,23 cells correlate with faster T-cell reconstitution after transplantation, facilitation of engraftment may lead to accelerated T-cell reconstitution. Indeed, we did observe in the previous study that CD62L− T cells enhance T-cell generation from TCD bone marrow cells.11 However, it is possible that T cells are derived from residual T cell in the TCD bone marrow graft in that study. To further confirm CD62L− T cells indeed promote T-cell regeneration from stem cells, we repeated the experiments using purified stem cells. The data from this experiment (Figure 4A) conclusively demonstrate that the T cells promoted by CD62L− T cells are derived from hematopoietic stem cells. This hypothesis also is supported by the results obtained from the recipients with limited or no host resistance. When BALB/c SCID mice (lacking T and B cells) are used as recipients, the promoting effect of CD62L− T cells is dramatically decreased (Figure 4B-C). Because classic SCID mice contain NK cells34 and NK cells are able to reject stem cell graft,35 host resistance remains in these mice. To further exclude the role of host radiosensitive NK cells, we repeated the experiment using NSG mice, in which all T, B, and NK cells are absent. Data from this experiment (Figure 4B-C) demonstrate that the promoting effect of CD62L− T cells is completely lost when host immune barrier is absent. The observation that CD62L− T cells have decreased ability to prevent tumor growth when Rag2−/−γC−/− marrow is used (Figures 1C and 6A) provide functional evidence confirming that CD62L+ T cells are able to enhance stem cell–mediated immune recovery. All these data strongly support that facilitation of hematopoietic engraftment by CD62L+ T cells lead to enhanced stem cell–mediated T-cell regeneration. It has been reported that CD4+ T cells can stimulate hematopoiesis directly.36 Multiple hematopoietic cytokines that can support hematopoiesis are also produced during allevotheses.37 However, inability of CD62L− T cells to enhance stem cell–mediated T-cell regeneration in allogeneic SCID recipients (Figure 4B-C) argues against these 2 mechanisms.

How do CD62L− T cells deplete host-radiosensitive T cells11 and facilitate hematopoietic cell engraftment (Figure 3)? Previously published data20,22 suggest that CD62L− T cells may be activated on transfer into allogeneic recipients. The data from the current work (Figure 5) clearly demonstrate that CD62L− T cells can be activated by alloantigens in vivo. Multiple activation markers including CD25, CD30, CD69, and KLRG1 can be detected in CD62L− T cells. The activated CD62L− T cells are able to produce IFN-γ, a critical cytokine in T-helper 1/cytotoxic T1 immune response.38 Multiple cytotoxic pathways including perforin, granzyme B, FasL, TRAIL, and TNF-α are activated in CD62L− T cells and likely responsible for the depletion of host-radiosensitive T cells. These data are consistent with the data published by Zheng et al.,20,25 indicating that cytolytic function is essential for effector memory T cell–mediated GVL effect. Data from the in vivo tumor model (Figures 1C and 6A) demonstrate that CD62L− T cells from unprimed donors have direct effects on host-type tumor cells.

A critical question is why CD62L− T cells are able to kill host radiosensitive T cells11 and tumor cells20,25 without inducing GVHD. Previously published in vitro24 and in vivo16,39 observations suggest that CD62L− T cells can react to alloantigens initially but the alloresponses cannot be sustained on transfer into GVHD recipients. Aborted alloresponses may result in decreased overall responses14,25 that fall below the threshold required for GVHD induction20,41 and eventually lead to tolerance against host antigens. To test whether this is the case in vivo, we injected host-type tumor cells (BCL1) into lethally irradiated BALB/c recipients of Rag2−/−γC−/− BM and CD62L− T cells from B6 CD45.2 mice at different times (days 7, 14, 21, and 28) after transplantation. Because Rag2−/−γC−/− mice do not contain and cannot generate T, B, and NK cells,17 the alloreactivity of CD62L− T cells could...
be clearly assessed through their ability to kill host-type tumor cells. The data from this experiment (Figure 6A) clearly demonstrate that CD62L R T cells are able to respond to alloantigens initially but lose alloreactivity over time on transfer into allogeneic recipients. Because previous studies have demonstrated that chronic antigenic stimulation can diminish antigen-specific T-cell responses in vivo,42,43 we speculate the reason why CD62L R T cells lose alloreactivity is because CD62L R T cells become tolerant to host alloantigens because of persistent antigen exposure. Indeed, this is the case because only CD62L R T cells transplanted into allogeneic but not syngeneic recipients lose the ability to kill host-type tumor cells (Figure 6B). Allospecific tolerance is mainly a result of clonal anergy because Vβ3 R and Vβ5.1/5.2 R cells, which recognize Mls-2 and Mls-3 superantigens expressed on host BALB/c cells, are not deleted (Figure 6C; Chen et al11) and do not respond to restimulation against the host H2 as well as Mls antigens.11

As summarized in Figure 7, besides providing immediate recall immunity to the host,11,13 allogeneic CD62L R T cells from unprimed donors can deplete host-radiosensitive T cells and facilitate hematopoietic cell engraftment on transfer into allogeneic recipients. Facilitation of hematopoietic engraftment results in enhanced de novo T-cell regeneration from hematopoietic stem cells. Even though CD62L R T cells are able to respond to alloantigens and deplete host-radiosensitive immune cells, CD62L R T cells become tolerant to host antigens as a result of prolonged antigen exposure in GVHD recipients, explaining why CD62L R T cells are able to eliminate host resistance without causing GVHD. It is important to point out that only the alloreactive portion of CD62L R T cell-mediated “abortive” responses. The nonalloreactive portion of CD62L R T cells can survive and function long term.11 The data presented in this article further highlight the unique characteristics of CD62L R T cells and their potential applications in clinical hematopoietic cell transplantation.

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Authorship

Contribution: J.Z., E.R., and B.J.C. designed research, performed research, analyzed data, and wrote the paper; and B.E.B., W.M., D.D., J.S., and X.C. designed research, performed research, and analyzed data.

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CD62L− memory T cells enhance T-cell regeneration after allogeneic stem cell transplantation by eliminating host resistance in mice

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