Absence of β7 integrin results in less graft-versus-host disease because of decreased homing of alloreactive T cells to intestine

Elisha Waldman, Sydney X. Lu, Vanessa M. Hubbard, Adam A. Kochman, Jeffrey M. Eng, Theis H. Terwey, Stephanie J. Muriglan, Theo D. Kim, Glenn Heller, George F. Murphy, Chen Liu, Onder Alpdogan, and Marcel R. M. van den Brink

The α4β7 integrin plays a central role in the homing of T cells to the gut. We hypothesized that absence of the β7 subunit would result in a reduction of intestinal graft-versus-host disease (GVHD) and an improvement in overall GVHD morbidity and mortality in recipients of hematopoietic stem cell transplantation (HSCT). Analysis of alloreactive β7−/− T cells showed intact activation, proliferation, cytokine production, and cytotoxicity. However, recipients of β7−/− donor T cells in murine HSCT models experienced less GVHD morbidity and mortality than recipients of wild-type (WT) T cells, associated with a decrease in donor T-cell infiltration of the liver and intestine and with an overall significant decrease in hepatic and intestinal GVHD. In graft-versus-tumor (GVT) experiments, we demonstrated intact or even enhanced GVT activity of β7−/− donor T cells. In conclusion, β7−/− donor T cells caused less GVHD morbidity and mortality than WT donor T cells because of selectively decreased T-cell infiltration of the liver and intestines. Our data suggest that strategies to target the β7 integrin have the clinical potential to alleviate or prevent GVHD while sparing or potentiating GVT activity. (Blood. 2006;107:1703-1711)

© 2006 by The American Society of Hematology

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for a variety of malignancies and nonmalignant conditions. In addition to the antitumor effect of the conditioning regimen, the graft-versus-tumor (GVT) activity of donor T cells is increasingly recognized as an important component of the overall antitumor effect of allogeneic HSCT. However, it has been well established that alloreactive T cells also play a critical role in the development of acute graft-versus-host disease (GVHD), which remains one of the main complications of allogeneic HSCT. Acute intestinal GVHD is a significant cause of posttransplantation morbidity and mortality. Damage to the gastrointestinal tract increases the circulation of inflammatory stimuli (such as endotoxin), which can amplify acute GVHD in the intestines and other target organs. Thus, reducing or preventing GVHD, especially of the gut, while preserving the beneficial GVT effect of donor T cells would significantly improve overall survival in patients who undergo allogeneic HSCT.

Although virtually any host tissue is a potential source of alloantigens, GVHD develops only in skin, liver, the gastrointestinal tract, and possibly lung and thymus. The reason for this target organ specificity remains unclear but may be attributed to organ-specific differences in susceptibility to damage from the conditioning regimen, inflammatory cytokine response, activation of antigen-presenting cells, and activation and infiltration of donor T cells. A fundamental hypothesis regarding the pathobiology of GVHD suggests that the dysregulation of leukocyte trafficking is important for the disease process, and recent studies have demonstrated the important role of T-cell homing and its regulation by integrins, chemokine receptors, and ligands in the T-cell immune response and T-cell–mediated diseases, including acute GVHD.

T-cell trafficking through the circulation, secondary lymphoid organs, and specific tissues is a multifaceted process requiring precise communication between lymphocytes, endothelial cells, and the extracellular matrix; chemokines, selectins, integrins, and their receptors play crucial roles in these complex interactions. The expression of specific adhesion molecules and chemokine receptors on T cells, in combination with a spatial and temporal expression pattern of the ligands for these receptors, is largely responsible for the tissue tropism of T-cell migration. The migration patterns of T cells are further influenced by inflammatory stimuli, including cytokines and chemokines associated with inflammation.

Circulating T cells, on activation, may undergo altered surface expression of adhesion molecules and receptors, leading to specific tissue tropism. The αβ integrin, or LPAM-1 (lymphocyte Peyer patch adhesion molecule), is expressed on T cells and acts as an intestinal homing receptor. The integrin exists as a 154-kDa α chain and a 130-kDa β chain joined as a transmembrane heterodimer with a short intracytoplasmic tail. The α4 subunit also associates...
with β1 chains, forming an integrin that binds to VCAM-1 and to fibronectin, resulting in cell adhesion and entry to inflamed tissues.14 The β1 integrin subunit also associates with the α6 subunit, forming an integrin thought to play a role in lymphocyte homing to and retention in the lamina propria of the gut epithelium.5,16 The α6β4 integrin may also play a role in thymocyte adhesion to thymic epithelial cells.17,18

The α6β7 integrin interacts specifically with MAdCAM-1 (mucosal addressin cell adherence molecule-1) on high endothelial venules in the Peyer patches and intestinal lamina propria,19,20 and it interacts less specifically with VCAM-1 and fibronectin.21 Circulating lymphocytes in spleen and mesenteric lymph nodes (MLNs) have low levels of α6β7 expression, which may be up-regulated on activation.22,23 Up-regulation is highly dependent on passage through the Peyer patches, with the subsequent increase in α6β7 expression leading to lymphocyte infiltration of gut endothelium.3,24

It has been well established that the α6β7 integrin plays a specific role in intestinal homing of lymphocytes; previous studies have shown that interference with MAdCAM-1 or with the α4 integrin subunit can prevent or ameliorate the development of various inflammatory conditions of the gut.5,25,26 Administration of monoclonal antibodies directed against the α6β7 integrin or against the α6 integrin subunit has been shown to resolve colitis in a well-established monkey model of inflammatory bowel disease (IBD).27 Recently, natalizumab, a monoclonal antibody directed against the α6 integrin subunit, was demonstrated to ameliorate IBD in human subjects.28-30 The β7 integrin subunit has been targeted in several experimental disease models, also with promising results; monoclonal antibodies directed against the β7 subunit have been shown to ameliorate colitis and to attenuate intestinal allograft rejection in murine models.15,31 Relatively few studies, however, have addressed the role of integrins in T-cell homing in patients with GVHD.

We previously demonstrated in murine models that α6β7- donor T cells cause less GVHD morbidity and mortality than α6β7+ unselected donor T cells, specifically because of reduced homing of donor T cells to host intestinal mucosa.32 However, we also found that even when using α6β7- selected donor T cells, α6β7 expression will be up-regulated on activation, resulting in the delay, but not the prevention, of intestinal infiltration of T cells.32 In a significant departure from these findings, we sought to investigate whether permanent loss of the β7 subunit of the α6β7 integrin would provide similar or better benefits.

The phenotype of β7−/− mice has been well described: mice are healthy and normal in all respects except for impaired development of gut-associated lymphoid tissue (GALT).20 Although lymphocyte numbers in the Peyer patches and intestine are reduced, lymphocytes are found in all other organs in normal quantities.20,33 In addition, lymphocyte development is not affected in β7−/− mice, suggesting that impaired GALT development is secondary to a homing defect and not to defective lymphocyte maturation.20

In this study we used β7−/− donor T cells in murine HSCT models to study the role of the β7 integrin in the trafficking of donor alloreactive T cells in HSCT recipients with GVHD.

Materials and methods

Cell line and antibodies

P815 (H-2b), from ATCC (Manassas, VA), is a mastocytoma cell line of DBA/2 mouse origin. Cell culture medium contained RPMI, 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine. P815-TGL (H-2b) was generated by transducing P815 with a retroviral vector containing a fusion reporter gene coding for HSV1-TK (T), enhanced green fluorescent protein (G), and firefly luciferase (L).34

Antimurine CD16/CD32 FcR block (2.4G2) and fluorescence-labeled antimurine antibodies against CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD62L (MEL-14), Ly-9.1 (30C7), αβ (DATK32), CD44 (IM7), NK1.1-PE (PK136), T-cell receptor β (TCR-β; H57-597), Thy 1.1, H-2k, and H-2d were obtained from Pharmingen (San Diego, CA).

Mice and HSCT

Female B10.Br (H-2b), C57BL/6 (B6, H-2d), B6D2F1 (H-2b/d), B6.Thy1.1 (H-2d), and B6.β7−/− (H-2b) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice used were between 8 and 12 weeks old. HSCT protocols were approved by the Memorial Sloan-Kettering Cancer Center (MSKCC) Institutional Animal Care and Use Committee and have been described previously.35,36 Briefly, BM cells removed from femurs and tibias were T cell-depleted (TCD) with anti-Thy-1.2 and low-TOX-M rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada). Purified splenic T cells were obtained by nylon wool column passage, followed by staining with anti–CD3-FITC antibodies for T-cell purity. Allografts consisted of 5 × 106 TCD wild-type (WT) BM cells with or without 1 × 106 WT or β7−/− T cells. Cells were resuspended in DMEM and injected into lethally irradiated recipients on day 0 after 1300 cGy total body irradiation (cesium Cs 137 [137Cs] source) as a split dose 3 hours apart. Mice were housed in the MSKCC pathogen-free facility in sterilized micro-isolator cages and were given normal chow and autoclaved hyperchlorinated drinking water (pH 3.0).

Tumor induction, assessment of GVHD, and determination of cause of death

P815 cells (1 × 105) were infused on day 0 of HSCT after irradiation. Survival was monitored daily, and mice were individually scored weekly for 5 clinical parameters (weight, posture, activity level, fur ruffling, and skin lesions) on a scale from 0 to 2. A clinical GVHD score was generated by summation of the 5 criteria scores, as described by Cooke et al;37 mice scoring 5 or greater were killed. All animals, regardless of macroscopic tumor at autopsy, underwent histopathologic examination of liver and spleen, performed by a veterinary pathologist (Dr. Krista La Perle, Cornell University Medical College, New York, NY) for evidence of tumor and to determine cause of death (GVHD vs tumor).

Histopathologic analysis

Small and large bowel, liver, and skin were assessed by experts in a blinded fashion. Formalin-preserved organs were embedded in paraffin, sectioned, stained with hematoxylin/eosin, and scored with a semiquantitative scoring system, as previously described.38 Bowl and liver were scored for 19 to 22 different parameters associated with GVHD, as previously described;40 skin was evaluated for number of apoptotic cells per millimeter of epidermis, as previously described.41

Lymphocyte isolation from liver and gut

Mice were killed, and small intestine was dissected from the gastric-duodenal junction to the ileocecal junction. Intestines were flushed with 10% HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid), 10% FBS, cut into 1-cm–long pieces, and incubated for 1 hour at 37°C with continuous shaking. Intestinal pieces were then vortexed for 15 seconds, and the supernatant was strained and centrifuged at 325 g for 5 minutes. Pellets were resuspended in 40% Percoll (Sigma Aldrich, St Louis, MO), overlaid on 70% Percoll, and centrifuged at 1300 g for 30 minutes. Lymphocytes were recovered from the interface. Livers were homogenized and passed through a 70-μm cell strainer. Pellets were resuspended in 40% Percoll, and lymphocytes were isolated as described.
Organ harvest for T-cell infiltrate analysis

Female B10.BR recipients underwent HSCT, as described. Allografts consisted of 5 × 10^6 T-cell–depleted WT BM cells with mixtures of 2 × 10^6 WT (B6.Thy1.1) and 2 × 10^6 β^7^-/− (Thy1.2) T cells infused into lethally irradiated recipients on day 0. Mice were harvested for GVHD target organs on day 8; lymphocytes were isolated for fluorescence-activated cell sorter (FACS) analysis to determine relative percentages of WT and β^7^-/− T cells.

Flow cytometry analysis

Lymphocytes were washed in FACS buffer (phosphate-buffered saline [PBS], 0.5% bovine serum albumin [BSA], 0.1% sodium azide). Cells (10^6) were incubated for 20 minutes at 4°C with anti-CD16/CD32 FcR block and subsequently with fluorochrome-labeled primary antibodies at saturating concentrations for 20 minutes at 4°C. Appropriate isotype controls were used. Cells were resuspended in FACS buffer, and flow cytometry analysis was performed on a FACScalibur (Becton Dickinson, San Jose, CA) with CellQuest software. Data analysis was performed with FlowJo software (Treestar, San Carlos, CA).

Carboxyfluorescein diacetate succinidyl ester labeling

Cells were labeled with CFSE, as described previously.42 Briefly, splenocytes were incubated with CFSE at a concentration of 2.5 μM in PBS at 37°C for 15 minutes, washed with PBS, and infused into lethally irradiated (750 cGy) B10.BR allogeneic recipients. Splenocytes from recipients were harvested 72 hours later and analyzed by FACS, as described.

Cytotoxicity assay

Target cells were labeled with 100 μCi (3.7 MBq) of ^51Cr at 3 × 10^6 cells/ml for 1 hour at 37°C and plated at 2.5 × 10^5 cells/well in 96-well U bottom plates (Costar, Cambridge, MA). Splenocytes from mice 14 days after HSCT with allografts consisting of WT bone marrow and either WT or β^7^-/− T cells were analyzed by FACS for donor markers and CD3/CD8 purity and were added at various effector-target ratios in a final volume of 200 μL in triplicate and incubated for 4 hours at 37°C. Subsequently, 35 μL supernatant was removed from each well and was counted in a gamma counter (Top计-Packard, Meriden, CT) to determine experimental release. Spontaneous release was obtained from wells receiving target cells with medium, and total release was obtained from wells receiving 5% Triton X-100. Percentage cytotoxicity was calculated as follows: percentage cytotoxicity = 100 × (experimental release – spontaneous release)/ (total release – spontaneous release).

Enzyme-linked immunosorbent assay

Blood was obtained by cardiac puncture from GVHD and control animals and was centrifuged at 13 400 g for 1.5 minutes. Sera were collected and stored at −80°C. Concentration of IFN-γ was determined by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

Bioluminescent imaging

Animals that received P815-TGL were given intraperitoneal (150 mg/kg) d-Luciferin (Xenogen, Alameda, CA). Ten minutes after injection, mice were anesthetized with isofluorane and placed supine in the Xenogen IVIS D-Luciferin (Xenogen, Alameda, CA). Ten minutes after injection, mice were anesthetized with isofluorane and placed supine in the Xenogen IVIS bioluminescence imaging system, and recordings were made for 5 minutes. Pseudocolor images showing whole body distribution of bioluminescent signal were superimposed on conventional grayscale photographs.

Statistical analysis

Histopathologic scores and cell counts were compared between groups using the nonparametric unpaired Mann-Whitney U test; the Mantel-Cox log-rank test was used for survival data. The log rank statistic was applied for comparison of survival data between groups. Area under the curve (AUC) was used to summarize the GVHD trajectory of each mouse. Pairwise difference in AUC between groups, using all possible pairwise contrasts, was used to test whether a differential GVHD change occurred between treatment groups. Not all mice were observed for the full length of the study. The primary reason for censoring was death. To account for informative dropouts, AUCs were calculated up to the minimum follow-up time for each pairwise difference in the double sum above. Permutation distribution was used to compute the achieved significance level.

Results

Recipients of β^7^-/− donor T cells experienced significantly less GVHD morbidity and mortality than recipients of WT donor T cells

We performed GVHD experiments in a well-described major histocompatibility complex (MHC)–mismatched murine allogeneic HSCT model: B6→B10.BR. Lethally irradiated recipients were infused with 5 × 10^6 WT TCD-BM cells, and GVHD was induced by the addition of 1 × 10^6 WT or β^7^-/− donor splenic T cells to the allograft. Recipients of allografts with only WT TCD-BM and without GVHD were used as controls.

At a dose of 1 × 10^6 cells, B10.BR recipients of β^7^-/− donor T cells experienced a significant delay and decrease in GVHD mortality compared with recipients of WT T cells (Figure 1A). In addition, clinical GVHD scores in the mice that received β^7^-/− T cells compared with those receiving WT T cells showed significantly worse GVHD morbidity in the WT group (Figure 1B). From these results we conclude that at lower doses, β^7^-/− donor T cells have significantly less potential to induce GVHD than WT T cells. However, with escalating T-cell doses, the survival benefit for
recipients of $\beta_7^{-/-}$ donor T cells becomes insignificant (Figure 1C) at $3 \times 10^6$ T cells, though at a dose of $2 \times 10^6$, a small survival benefit (did not reach statistical significance) favoring the $\beta_7^{-/-}$ T cell recipients could still be observed.

**Alloreactive $\beta_7^{-/-}$ T cells have intact activation, proliferation, and cytotoxicity**

The diminished GVHD activity of alloreactive $\beta_7^{-/-}$ T cells could be attributed to an intrinsic defect in activation, proliferation, or cytotoxicity. To determine the capacity of $\beta_7^{-/-}$ T cells to undergo alloreactive proliferation in vivo, CFSE-labeled T cells were transferred into a sublethally irradiated allogeneic host (B6$\rightarrow$B10.BR), and proliferation kinetics were compared with those of CFSE-labeled WT T cells. We found no differences in the percentages of dividing cells and the numbers of divisions between the 2 selected populations of $\beta_7^{-/-}$ and WT T cells (Figure 2A). Additionally, activation of $\beta_7^{-/-}$ T cells, as determined by CD44 and CD25 up-regulation and CD62L down-regulation on fast proliferative T cells, was comparable to WT T-cell activation (Figure 2B), indicating that alloreactive $\beta_7^{-/-}$ CD4 and CD8 T cells have intact expression of these activation markers and proliferation kinetics.

We also performed further analyses of the phenotypes and percentages of effector, memory, and regulatory T-cell subsets in spleens on day 11 after HSCT. In the spleens, we observed similar percentages of CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells (Figure 2C), indicating that differences in the proliferation of suppressor populations do not account for differences in overall morbidity and mortality. Further phenotypic analysis of donor-derived T cells revealed equal percentages of effector (CD44hi CD62Llo) and central memory (CD44hi CD62Llo) CD4 T cells and effector (CD44hi and CD62Llo) CD8 T cells in the WT and $\beta_7^{-/-}$ T cell groups (Figure 2D-F).

Finally, we assessed the cytolytic capacity of $\beta_7^{-/-}$ T cells against host tumor targets after in vivo stimulation with host antigens (Figure 2G). $\beta_7^{-/-}$ T cells displayed no significant difference in cytolytic activity compared with alloreactive WT T cells. These results indicate that $\beta_7$ deficiency does not impair the activation, proliferation, or cytotoxic activity of alloreactive T cells.

**WT donor T cells infiltrate recipient intestinal mucosa with greater avidity than $\beta_7^{-/-}$ donor T cells**

Our previous study with $\alpha_3\beta_7^+$ and $\alpha_2\beta_7^-$ selected donor T cells demonstrated that recipients of $\alpha_3\beta_7^+$ donor T cells have higher numbers of infiltrating T cells in their intestinal mucosa than do recipients of $\alpha_2\beta_7^-$ donor T cells. Therefore, we hypothesized that impaired intestinal infiltration of $\beta_7^{-/-}$ donor T cells could explain our improved GVHD morbidity and mortality. To test this hypothesis, we determined the percentages of WT versus $\beta_7^{-/-}$ donor T cells in the spleens, mesenteric lymph nodes, and intestinal mucosa of B10.BR recipients on day 8 after transplantation of equal numbers of WT (Thy1.1) and $\beta_7^{-/-}$ (Thy 1.2) donor T cells (Figure 3A). We found that the intestinal mucosa in recipient B10.BR mice contained significantly higher percentages of WT donor T cells compared with $\beta_7^{-/-}$ donor T cells. In contrast, we found significantly higher percentages of $\beta_7^{-/-}$ donor T cells in recipient spleens and mesenteric lymph nodes. This finding was consistent with results of complete blood counts on days 7 and 14 in lethally irradiated B10.BR mice that underwent transplantation of B10.BR TCD-BM ($5 \times 10^6$) and splenic T cells ($1 \times 10^6$) from B6D2F1 HSCT recipients.
WT or $\beta_7^{-/-}$ mice (Figure 3B). Mice receiving $\beta_7^{-/-}$ donor T cells consistently showed a higher number of circulating lymphocytes, with the difference reaching statistical significance by day 14. This could be attributed to a defect in the ability of $\beta_7^{-/-}$ donor T cells to traffic to and infiltrate the gut, resulting in more $\beta_7^{-/-}$ lymphocytes remaining in the general circulation and secondary lymphoid organs.

**Figure 3. Recipients of $\beta_7^{-/-}$ T cells have significantly lower numbers of T cells in their intestinal mucosa but significantly higher numbers of circulating T cells.** (A) Lethally irradiated (1300 cGy) B10.BR mice underwent transplantation such that each mouse received WT TCD-BM ($5 \times 10^7$) and WT (Thy1.1) T cells in combination with $\beta_7^{-/-}$ (Thy1.2) T cells ($2 \times 10^6$ of each type). T cells were analyzed before transfer into recipient mice to ascertain that equivalent percentages of CD4$^+$CD8$^+$ cells were being given (not shown). Mice were killed at day 8, and T cells were isolated and analyzed, as described in "Materials and methods." Donor origin of the isolated T cells was determined by multicolor flow cytometry. Statistical analysis is as follows: for mesenteric lymph nodes and spleen, $P < 0.001$; for gut, $P = 0.004$ (n = 5); experiment repeated 3 times. (B) Lethally irradiated (1300 cGy) B10.BR recipients underwent transplantation with $5 \times 10^7$ WT TCD-BM and either $1 \times 10^6$ WT or $\beta_7^{-/-}$ splenic T cells. Cardiac puncture to obtain blood for complete blood counts was performed on day 7 and day 14. * $P < 0.05$ (n = 8).

$\beta_7^{-/-}$ T cells experience significantly less hepatic and intestinal GVHD

To investigate whether recipients of $\beta_7^{-/-}$ T cells had less severe target organ GVHD damage, we analyzed GVHD-associated organ damage in terminal ileum, colon, and liver. We performed semiquantitative histopathologic analysis in a blinded fashion on tissue samples from the target organs.

We found significantly less GVHD in small intestine, large intestine, and liver in recipients of $\beta_7^{-/-}$ T cells when organs were examined at day 7 after transplantation (Figure 4A). When organs were examined at day 14 after transplantation (Figure 4B), no significant difference was found, though a trend was still evident in small and large intestine toward less damage in recipients of $\beta_7^{-/-}$ T cells. Ear and tongue specimens were analyzed to evaluate skin GVHD damage at days 7 and 14 (Figure 4C). No significant difference was found between recipients of WT T cells and recipients of $\beta_7^{-/-}$ T cells on day-7 or -14 samples. Finally, on close histopathologic analysis, we found significantly greater numbers of T cells infiltrating lamina propria, hepatic lobules, and hepatic portal tracts in recipients of WT T cells (Figure 4D-E) on day 7 after transplantation.

**Figure 4. Recipients of $\beta_7^{-/-}$ T cells and of WT T cells have similar serum levels of IFN$\gamma$.**

To assess the cytokine response of alloreactive T cells in vivo, we examined serum from mice with GVHD at days 7 and 14 after transplantation (Figure 5). Serum levels of IFN$\gamma$ were similar in
mice that received $\beta_7^{-/-}$ T cells and mice that received WT T cells, suggesting that the difference in morbidity and mortality did not stem from a difference in IFNγ production. In addition, because IFNγ is produced primarily by alloreactive T cells, these data suggest that $\beta_7^{-/-}$ alloreactive T cells have intact IFNγ production in addition to intact alloreactive proliferation, activation, and cytotoxicity (Figure 2).

$\beta_7^{-/-}$ T cells have intact GVT activity

To assess the effects of $\beta_7$ integrin deficiency on the GVT activity of alloreactive T cells, we performed experiments in a well-characterized GVHD/GVT model: B6→B6D2F1 with the P815 mastocytoma cell line (Figure 6). A low dose of donor T cells ($0.5 \times 10^6$) was used to decrease GVHD mortality, allowing for better measurement of GVT activity. When possible, dead mice underwent necropsy with histopathologic analysis to determine cause of death (GVHD vs tumor). Results of necropsy and histopathologic analysis for all groups are listed in Table 1. Recipients of $\beta_7^{-/-}$ T cells demonstrated overall improvement in survival over recipients of WT T cells. Our earlier finding that GVHD mortality is decreased in recipients of $\beta_7^{-/-}$ T cells was evident in this transplantation model as well, with fewer recipients of $\beta_7^{-/-}$ T cells dying of GVHD. Surprisingly, recipients of $\beta_7^{-/-}$ T cells not only had intact GVT activity but actually had fewer documented tumor deaths, suggesting improved GVT activity in this group.

Further support for these findings was obtained using in vivo bioluminescence imaging. Lethally irradiated (1300 cGy) B6D2F1 mice underwent transplantation with $5 \times 10^6$ WT TCD-BM and either $0.5 \times 10^6$ WT or $\beta_7^{-/-}$ splenic T cells. P815 tumor cells ($0.5 \times 10^9$) that had been transduced with an LTR-HSV1 TK-EGFP-Luc retroviral vector were infused into each mouse at the time of transplantation. Serial imaging (Figure 7) shows that tumor was established in both groups but that recipients of $\beta_7^{-/-}$ donor T cells exhibited significantly delayed tumor growth; in several mice, established tumor even appeared to regress, whereas most recipients of WT donor T cells developed progressively more luminescence and widespread tumor.

Discussion

Several recent studies have examined the role of integrins and their respective ligands in the development of acute GVHD. A number of these have confirmed a central role for the $\alpha_\beta_7$ integrin in lymphocyte homing to gut,3,24-26,32 and a pivotal role for Peyer patches and dendritic cells in the Peyer patches in the imprinting of gut-specific tropism on circulating lymphocytes.3,23,24 Tanaka et al33 used GVHD models to demonstrate a moderate improvement in intestinal GVHD in mice treated with anti-CD62L antibodies. However, the emphasis of this study was the phenotype of gut-infiltrating lymphocytes during GVHD, and relatively little was assessed in terms of therapeutic benefits of blocking antibodies.28 Li et al29 examined the role of $\alpha_4$ integrin and CD62L in the development of acute graft-versus-host reaction (a-GVHR) and showed that incubating donor splenocytes with anti-$\alpha_4$ and anti-CD62L antibodies resulted in delayed death. However, the potential role of the $\alpha_4$ integrin subunit in other processes, such as stem cell homing to the marrow compartment, was not evaluated. Murali et al30 examined the role of Peyer patches in lymphocyte homing in a-GVHR and showed that the development of a-GVHR could be attenuated through the use of anti-MAdCAM-1 antibodies, emphasizing the key role of the $\alpha_4\beta_7$ integrin–MAdCAM-1 interaction in the imprinting of gut-homing specificity on donor lymphocytes. Yet a limitation of some of these earlier studies is the reliance on a-GVHR models, which use nonirradiated or sublethally irradiated hosts. Our previous study31 used GVHD models to demonstrate that the adoptive transfer of selected $\alpha_\beta_7$ donor T cells compared with $\alpha_\beta_7$ selected donor T cells resulted in a delay of intestinal GVHD and a significant decrease in GVHD morbidity and mortality secondary to reduced donor T-cell infiltration of intestine, but we noted that $\alpha_\beta_7$ selected alloreactive T cells up-regulate their $\alpha_\beta$ expression on activation and that $\alpha_\beta_7$ selected alloreactive T cells were, therefore, only delayed in their infiltration of the gut, as reflected by the delayed development of intestinal GVHD.

In this study, using $\beta_7^{-/-}$ donor T cells in murine allogeneic HSCT models, we built on previous findings to assess the role of $\alpha_\beta_7$ in GVHD by permanent inhibition of the $\beta_7$ integrin in alloreactive T cells. This represents a significant departure from our previous study,32 in which we used $\alpha_\beta_7$ selection as a method of adoptive cell transfer by preventing re-expression of $\alpha_\beta_7$, and from previous studies using a-GVHR models, because using lethally

![Image 5](https://example.com/image5.png)

**Figure 5.** Recipients of $\beta_7^{-/-}$ donor T cells and of WT T cells generate similar levels of serum IFNγ. Lethally irradiated (1300 cGy) B10.BR recipients underwent transplantation with $5 \times 10^6$ WT TCD-BM and either $1 \times 10^6$ WT or $\beta_7^{-/-}$ splenic T cells. Serum levels of IFNγ were determined by ELISA at day 7 and 14 after transplantation. Shown are combined results of 2 experiments (n = 9), P = .08 at day 7, and P = .751 at day 14.

![Image 6](https://example.com/image6.png)

**Figure 6.** GVT activity is preserved in recipients of $\beta_7^{-/-}$ T cells. Lethally irradiated (1100 cGy split dose) B6D2F1 mice underwent transplantation on day 0 with $5 \times 10^6$ WT TCD-BM cells with or without the addition of splenic T cells. Recipients were given $1 \times 10^6$ P815 murine mastocytoma cells as a separate intravenous injection at the time of transplantation. Survival is depicted as a Kaplan-Meier curve representing mice that received TCD-BM + P815 (□), TCD-BM + $\beta_7^{-/-}$ T cells + P815 (△), TCD-BM + WT T cells + P815 (●), or TCD-BM + WT T cells + P815 (○). Causes of death (GVHD vs tumor) for all recipients that died during the course of the experiment are shown in Table 1. Statistical analysis: ▲ versus □ (P = .005). Shown are combined results of 3 experiments (n = 30).

### Table 1. Causes of death (GVHD vs tumor) for all recipients who died during the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor</th>
<th>GVHD</th>
<th>Not analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM only</td>
<td>15 of 15</td>
<td>0 of 15</td>
<td>0 of 15</td>
</tr>
<tr>
<td>P815 + WT T cells</td>
<td>11 of 30</td>
<td>11 of 30</td>
<td>4 of 30</td>
</tr>
<tr>
<td>P815 + $\beta_7^{-/-}$ T cells</td>
<td>7 of 30</td>
<td>7 of 30</td>
<td>3 of 30</td>
</tr>
</tbody>
</table>

Values indicate number of mice dead out of total number of mice for the indicated category.
irradiated hosts in GVHD models results in an accurate reproduction of clinical GVHD-associated organ damage. Because conditioning regimen toxicity, especially to the intestines, may itself play a role in the development of GVHD,1,45,46 its replication in studies of intestinal GVHD is essential. In addition to previous work with MAdCAM-1 and the \( \alpha_4 \) integrin subunit, we found that the other significant role of the \( \beta_7 \) integrin subunit is (in conjunction with \( \alpha_4 \)) in lymphocyte localization to the gut intraepithelial compartment15,16 and that the \( \beta_7 \) integrin subunit is also potentially involved in lymphocyte adhesion and infiltration of target tissues through its less specific interactions with VCAM-1 and fibronectin, all addressed here by the use of \( \beta_7^{+/–} \) T cells. From a translational perspective, these studies can serve as a preclinical model for novel drug therapies aimed at inhibition of the \( \beta_7 \) integrin (eg, by a neutralizing antibody) that could be used in the HSCT setting to prevent or ameliorate GVHD.

In seeking to examine the effects of permanent blockade of the \( \alpha_4 \beta_7 \) integrin, we chose not to focus on the \( \alpha_4 \) subunit. Despite the promising early results of strategies targeting the \( \alpha_4 \) integrin subunit in patients with IBD, the \( \alpha_4 \) subunit plays a role in stem cell homing to the marrow compartment and in trafficking of prothymocytes to thymus,43,44 making it a less attractive target in the context of HSCT, where blockade could interfere with engraftment. Experiments with \( \alpha_4^{+/–} \) mice have demonstrated defective migration of prothymocytes from bone marrow to thymus.20 However, though the \( \alpha_4 \beta_7 \) integrin may also play a role in thymocyte adhesion to thymic epithelial cells, the few studies published to date do not support a central role for \( \alpha_4 \beta_7 \) in thymocyte development.17,18

To study the \( \beta_7 \) subunit, we used donor T cells derived from \( \beta_7^{+/–} \) mice. These T cells showed activation, proliferation, and cytotoxicity profiles similar to those of WT donor T cells, so that...
differences in T-cell infiltration of target organs and GVHD morbidity and mortality could reasonably be attributed to a homing defect rather than to a functional defect in the β7−/− T cells.

As expected, recipients of β7+/− T cells developed significantly less GVHD morbidity and mortality than recipients of WT T cells. Interestingly, not only was survival in recipients of β7+/− T cells significantly improved, we also found that onset of disease appeared to be delayed. These findings were further supported by our findings on histopathologic examination that recipients of β7−/− donor T cells have less organ damage and fewer infiltrating T cells, again suggesting that the differences we found throughout our studies were caused by a functional homing defect.

Accordingly, we found on histopathologic examination that β7−/− donor T cells caused less GVHD-related organ pathology, though the difference between recipients of β7−/− donor T cells and WT donor T cells becomes less pronounced over time (Figure 4A-B). Consistent with our previous study with αβ7−/− selected T cells in which we observed similar CD4 and CD8 T cell numbers in the livers of recipients of αβ7− and αβ7+/− selected T cells on day 14 after transplantation and possibly higher CD4 T cell numbers at day 22 after transplantation in the recipients of αβ7− T cells, in this study we found similar histopathologic scores in the livers of recipients of WT and β7−/− donor T cells on day 14 after transplantation (Figure 4B). However, examination of recipients of β7−/− donor T cells at day 7 after transplantation revealed significantly less liver disease (Figure 4A) and fewer infiltrating lymphocytes in the lobular and portal areas of the liver than in recipients of WT donor T cells (Figure 4D-E). Thus, we hypothesize that the β7 subunit plays only a role in the initial T-cell trafficking and infiltration of GVHD target organs, such as intestines and liver. Other regulators of T-cell trafficking, such as inflammatory chemokines, might become more important during the further development of GVHD. Therefore, we postulate that the inhibition of the β7 subunit on donor T cells causes only a delay (not a complete inhibition) of the infiltration by alloreactive donor T cells into the intestines and liver.

Additionally, given that we speculate that T cells are “imprinted” to home to the liver within the Peyer patches and the mesenteric lymphoid tissues, inhibition of the β7 subunit would be expected to disrupt T-cell homing to the GALT and the imprinting process but not necessarily homing to the liver; however, we cannot rule out a direct role for the β7 subunit in liver trafficking.

In addition, we found that cotransplantation of WT and β7−/− donor T cells into the same recipient resulted in a far greater percentage of WT T cells infiltrating intestine. This experimental setup has the advantage of controlling for host expression of chemokines and circulating levels of inflammatory cytokines; both types of T cells are exposed to the same environment so that differences in infiltration may be assumed to be caused solely by the difference in T-cell integrin expression.

These findings are consistent with our hypothesis that the use of β7−/− donor T cells results in less T-cell homing to gut in the early posttransplantation period but that T cells may accumulate later in the posttransplantation period because of other homing signals, such as the interaction of CCR9 and its ligand CCL25 in the small intestine, or to a general milieu of increased inflammatory cytokines. Nonetheless, our data suggest that this early delay in T-cell homing to target organs and the consequent delay in the development of target organ GVHD can have significant effects on the development of systemic GVHD and appears to confer a long-term benefit in morbidity and mortality. It has been well documented that less GVHD is seen when donor lymphocyte infusion is delayed, and it is possible that the use of β7−/− T cells results in a similar phenomenon, whereby even delayed infiltration of target organs causes less GVHD morbidity and mortality. We can only speculate that this delay in onset of GVHD could allow for improved recovery of the intestinal tract from irradiation and conditioning and thus could result in lower levels of inflammatory cytokines (such as TNF and IL-1) and endotoxin, which, in mouse GVHD models, are largely dependent on early intestinal damage, as previously demonstrated by Hill et al.

An intriguing finding in our study was the demonstration that β7−/− T cells not only had intact GVT activity, they actually appeared to have improved GVT activity, with tumor challenge resulting in fewer tumor deaths in recipients of β7−/− T cells than in recipients of WT T cells. These findings are especially significant because, with the exception of our previous study, the effect of manipulation of integrins on GVT activity in HSCT models has been minimally explored. Our results were reinforced by in vivo imaging studies showing that tumor challenge resulted in the establishment of tumor in recipients of WT T cells and in recipients of β7−/− T cells but that the latter displayed significantly delayed tumor growth or complete eradication of tumor, whereas the former experienced rapid tumor growth. Given our findings that β7−/− T cells have activation, proliferation, and cytotoxicity profiles similar to those of WT T cells and the finding that recipients of β7−/− T cells have consistently higher circulating lymphocyte counts, we hypothesize that this difference was likely a result of the decreased infiltration of liver and intestines by the β7−/− T cells. With fewer donor T cells infiltrating target organs, more alloreactive T cells remained in the general circulation, a site where the P815 mastocytoma resides, allowing greater interaction between alloreactive T cells and tumor cells and improved GVT activity. This improved ability to clear circulating tumor cells would be an attractive and important addition to a therapy that successfully reduces intestinal GVHD. Thus, use of a neutralizing monoclonal antibody against the β7 integrin subunit is the logical next step in the preclinical development of a strategy to prevent intestinal homing of alloreactive T cells in patients undergoing HSCT.

In conclusion, the absence of the β7 integrin subunit on alloreactive T cells resulted in less GVHD morbidity and mortality because of decreased intestinal GVHD and intact (and possibly improved) GVT activity. The β7 integrin subunit warrants further study, especially as a potential target for monoclonal antibody therapy, which could lead to successful clinical trials of a novel agent to make allogeneic HSCT safer, less toxic therapy.

References


Absence of β7 integrin results in less graft-versus-host disease because of decreased homing of alloreactive T cells to intestine

Elisha Waldman, Sydney X. Lu, Vanessa M. Hubbard, Adam A. Kochman, Jeffrey M. Eng, Theis H. Terwey, Stephanie J. Muriglan, Theo D. Kim, Glenn Heller, George F. Murphy, Chen Liu, Onder Alpdogan and Marcel R. M. van den Brink