LPAM ($\alpha_4\beta_7$ integrin) is an important homing integrin on alloreactive T cells in the development of intestinal graft-versus-host disease

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Lymphocyte Peyer patch adhesion molecule (LPAM) or $\alpha_4\beta_7$ integrin is expressed on lymphocytes and is responsible for T-cell homing into gut-associated lymphoid tissues through its binding to mucosal addressin cell adhesion molecule (MAdCAM), which is present on high endothelial venules of mucosal lymphoid organs. We found in murine allogeneic bone marrow transplantation (BMT) models that recipients of $\alpha_4\beta_7$- donor T cells had significantly less graft-versus-host disease (GVHD) morbidity and mortality compared with recipients of $\alpha_4\beta_7^+$ donor T cells. A kinetic posttransplantation analysis of lymphocytes in the intestines and mesenteric lymph nodes demonstrated a delayed invasion of lower numbers of $\alpha_4\beta_7^+$ T cells in recipients of $\alpha_4\beta_7$- T cells compared with recipients of $\alpha_4\beta_7^+$ T cells. Histopathologic analysis of GVHD target organs revealed that recipients of $\alpha_4\beta_7^+$ T cells developed less GVHD of the intestines and liver, whereas there was no difference in cutaneous and thymic GVHD between recipients of $\alpha_4\beta_7^+$ or $\alpha_4\beta_7^-$ T cells. Finally, we found that in vivo GVT activity of $\alpha_4\beta_7^+$ donor T cells was preserved. We conclude that the $\alpha_4\beta_7$ integrin is important for the invasion of alloreactive donor T cells into the gut and the subsequent development of intestinal GVHD and overall GVHD morbidity and mortality. (Blood. 2004;103:1542-1547)

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an important therapeutic modality for malignancies of hematopoietic origin, some metastatic solid tumors, as well as for a variety of nonmalignant diseases. Donor T lymphocytes are primarily responsible for both the beneficial graft-versus-tumor (GVT) effect following HSCT, as well as graft-versus-host disease (GVHD), which remains an important cause of posttransplantation morbidity and mortality. Although potential alloantigens are expressed on all host tissues, acute GVHD develops only in certain organs (skin, intestine, liver, and thymus), whereas other organs remain unaffected (eg, heart and kidney). The reasons for this GVHD target organ specificity remain largely unknown but could be related to organ specific differences in (1) susceptibility to tissue damage by the conditioning regimen and GVHD-associated cytokines and effector cells; (2) the inflammatory cytokine response; (3) numbers, types, and activation of antigen-presenting cells, and (4) activation, proliferation, and infiltration of T cells (and other effector cells).

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 by cells in the tissues is responsible for the tissue tropism of T-cell migration. Recirculation begins with a tethering and rolling phase, during which T cells in the blood transiently and reversibly interact with vascular adhesion receptors (including selectins, selectin ligands, and integrins) and sample the endothelium for activating factors (often chemokines). On activation, a combination of additional adhesion molecules, chemokines, and other signals will lead to an arrest of the T cell, followed by transmigration across the endothelium and further localization directed by tissue-associated chemokine gradients.

Naive T cells express receptors, such as CCR7 (a receptor for CCL19 and 21), which allow them to recirculate through secondary lymphoid organs (spleen, lymph nodes, and Peyer patches). On encountering antigen presented by antigen-presenting cells in the secondary lymphoid organs, T cells become activated, begin to proliferate and differentiate, and reprogram their homing receptors so that they can migrate to specific extra-lymphoid tissues.

The $\alpha_4\beta_7$ or LPAM (lymphocyte Peyer patch adhesion molecule) integrin is expressed on T (and B) cells and acts as an intestinal homing receptor. $\alpha_4\beta_7$ interacts with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on high endothelial venules in the Peyer patches and intestinal lamina propria. Lympocytes in spleen and mesenteric lymph nodes (MLNs) have low levels of $\alpha_4\beta_7$ expression but can up-regulate their $\alpha_4\beta_7$ expression on activation.

Previous studies have demonstrated that inhibition of L-selectin, MAdCAM-1, or $\alpha_4$ and $\alpha_6$ integrin can ameliorate the development of acute GVHD. Therefore, we hypothesized that $\alpha_4\beta_7$ expression on donor T cells would be important for the...
development of intestinal GVHD. In this study, we analyzed the ability of αβ+ and αβ− selected donor T cells to infiltrate the intestinal mucosa of the recipient, cause systemic and organ-specific GVHD, and exert GVT activity.

Materials and methods

Cell line and antibodies

EL-4 is a murine T-cell leukemia/lymphoma derived originally from the C57BL/6 mouse and was obtained from ATCC (Manassas, VA). Cell culture medium consisted of Dulbecco modified essential medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine.

Antimurine CD16/CD32 Fc block (2.4G2) and fluorochrome-labeled antimurine antibodies against CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD62L (MEL-14), Ly-9.1 (30C7), and αβT (DATK32) were all obtained from PharMingen (San Diego, CA).

Mice and BMT

Female CBA, B10.BR (H2k), C57BL/6 (Ly5.1), and B6.PL (H-2b) mice were pathogen-free facility of Memorial Sloan-Kettering Cancer Center. Mice were used for the experiments between 8 and 12 weeks of age. BMT protocols were approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee. Briefly, BM cells were removed aseptically from the femurs and tibias and were T-cell depleted with anti-Thy-1.2 and low–TOX-M rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada). Purified splenic T cells were obtained in 2 different ways. The first method consisted of passage of splenocytes over a nylon wool column (to remove monocytes/macrophages and B cells), followed by RBC lysis and staining with anti-CD3–fluorescein isothiocyanate (FITC) and anti-αβ−–phycoerythrin (PE) antibodies. These cells were then sorted into αβ+ and αβ− CD3+ populations with the use of FACSvantage (BD Biosciences, San Jose, CA) or MoFlo (DakoCytometry, Fort Collins, CO) cell sorters in the Flow Cytometry Core Facility of the Memorial Sloan-Kettering Cancer Center. We opted for gating windows close to each other, so that we would capture sufficient αβ+ and αβ− cells for in vivo experiments. We considered our highest priority to obtain sufficient numbers of αβ− cells with as few as possible αβ+ T cells. Our αβ− fraction purity after sorting was 98% ± 1.6% and our αβ+ fraction purity after sorting was 86.7% ± 4.9%. The second method of T-cell purification consisted of natural killer (NK) cell, B-cell, monocyte, granulocyte, and dendritic cell depletion of whole splenocytes by negative magnetic cell separation (Miltenyi Biotech, Auburn, CA), followed by anti-αβ− PE antibody labeling and cell sorting into αβ− and αβ+ populations. The allograft consisted of 5 × 106 T-cell-depleted BM cells with or without 0.5 to 1 × 106 unselected or αβ− selected T cells. These cells were reinfused in DMEM and infused into the tail vein of lethally irradiated recipients on day 0. Prior to transplantation, recipients received on day 0 90% total body irradiation (Cesium source) of 1300 cGy (CBA) or 1100 to 1200 cGy (C57BL/6, B10.BR) as a split dose with 3 hours between doses. Mice were housed in the specific pathogen-free facility of Memorial Sloan-Kettering Cancer Center in sterilized micro-isolator cages and received normal chow and autoclaved hyperchlorinated drinking water (pH 3).

Leukemia/lymphoma induction, assessment of GVHD, and determination of leukemic death

Animals received 1 × 106 EL-4 cells intravenously in a separate injection on day 0 of BMT after irradiation. Survival was monitored daily, and ear-punched mice were individually scored weekly for 5 clinical parameters (weight loss, hunched posture, activity level, fur ruffling, and skin lesions) on a scale from 0 to 2. A clinical GVHD score was generated by the summation of the 5 criteria scores as first described by Cooke et al,16 and mice scoring 5 or greater were killed. The cause of death (tumor versus GVHD) was determined by necropsy and histopathology. Briefly, all animals with macroscopic liver/spleen metastasis at autopsy or grossly visible abdominal masses were recorded as tumor deaths. Animals that did not exhibit any gross evidence of tumor had their liver and spleen histopathologically examined for microscopic evidence of leukemia/lymphoma by a veterinary pathologist (Dr Hai Nguyen, Cornell University Medical College, New York, NY), and cause of death was subsequently determined.

Histopathologic analysis

GVHD target organ pathology for small and large bowel, liver, and skin was assessed by experts (C.L., G.J.M.) in a blinded fashion. Formalin-preserved target organs were paraffin-embedded, sectioned, hematoxylin/eosin-stained, and scored with a semiquantitative scoring system as described before.15 In short, bowel and liver were scored for 19 to 22 different parameters associated with GVHD as previously described,17 and skin was evaluated for the number of apoptotic cells per millimeter of epidermis as previously described.18

IEL, LPL, and Peyer patch preparation

Mice were killed, and small intestine was dissected from the gastric-duodenal junction to the ileocecal junction. Intestines were washed/flushed by using a solution containing 10% HEPES (N-2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid), 10% HBSS (Hanks balanced salt solution), and 80% ddH2O, cut into 1-cm–long pieces with luminal side exposed and placed in 50-ML conical tubes. Intestinal pieces were then incubated with DTE (dithioerythritol) for 20 minutes at 37°C, being continuously stirred. Following the incubation, the intestinal pieces were vortexed for 15 seconds, and the supernatant was collected. Incubation and collection of supernatant was repeated 2 more times, and then the supernatants were pooled and centrifuged at 1500 rpm for 5 minutes. The pellet was resuspended in 1 to 2 mL RPMI (without additives) and subsequently passed through a 0.3-g nylon wool column to enrich the T-cell population.

Flow cytometric analysis

Splenocytes and intraepithelial lymphocytes (IELs), lamina propria lymphocytes (LPLs), and Peyer patch lymphocytes were washed, incubated with anti-CD16/CD32 Fc block, and subsequently incubated with fluorochrome-labeled primary antibodies at saturating concentrations for 20 minutes at 4°C. Flow cytometric analysis of these cells was performed on a FACSCalibur (Becton Dickinson) with CellQuest software. Appropriate isotype controls were used with each experiment.

Intracellular staining

Cells were incubated for 5 hours with phorbol myristate acetate (PMA; 10 ng/mL) + ionomycin (2 μM). Brefeldin A (10 μg/mL) was added after 2 hours of incubation. The cells were then harvested, washed, and stained with fluorochrome-conjugated antibodies against cell surface antigens. Subsequently, cells were fixed and permeabilized with Cytofix/Cytoperm Kit reagents (PharMingen) and stained with PE-conjugated anti-interferon γ (IFNγ; XMG1.2) antibodies from PharMingen.

Carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling

Cells were labeled with CFSE as described previously.19 Briefly, cells (thymocytes or splenocytes) were incubated with CFSE at a final concentration of 2.5 μM in HBSS at 37°C for 10 minutes. Cells were then washed 3 times with HBSS before intraocular injection.

Statistics

Histopathologic scores and thymocyte counts were compared between groups using the Wilcoxon rank sum statistics. The log-rank statistic was applied for comparison of survival data between groups. The area under the curve (AUC) was used to summarize the GVHD trajectory of each mouse under study. The statistic used to test whether a differential GVHD change occurred between treatment groups was the pairwise difference in the AUC between groups, using all possible pairwise contrasts. Not all the mice were followed for the full length of the study. The primary reason for censoring was death or sacrifice. To account for informative dropouts, the AUCs were calculated up to the minimum follow-up time for each pairwise difference in the double sum above. A permutation distribution was used to compute the achieved significance level.
Results

Recipients of $\alpha\beta^{-}$ donor T cells develop significantly less GVHD morbidity and mortality compared with recipients of LPAM$^+$ donor T cells

To study the role of $\alpha\beta^{-}$ expression on donor T cells in the development of intestinal GVHD, we used well-described clinically relevant major histocompatibility complex (MHC)-matched and -mismatched murine allogeneic BMT models. We chose as an MHC-matched model with a disparity in minor histocompatibility antigens B10.BR $\rightarrow$ CBA.\(^{20}\) As an MHC-mismatched model, we used the B10.BR $\rightarrow$ B6 strain combination.\(^{21}\) In both models, lethally irradiated recipients were infused with $5 \times 10^6$ T-cell–depleted donor bone marrow (TCD-BM) cells, and GVHD was induced by the addition of donor splenic T cells to the allograft.

To determine the GVHD activity of $\alpha\beta^{-}$ and $\alpha\beta^{-}$ donor T cells, we obtained purified populations of $\alpha\beta^{-}$ or $\alpha\beta^{-}$ splenic donor T cells by flow cytometric cell sorting. We first analyzed if these $\alpha\beta^{-}$ or $\alpha\beta^{-}$ selected cells differed in their expression of CD62L, which plays an important role in the initial interactions of naive T cells with the endothelium in secondary lymphoid organs, and we found no differences ($\alpha\beta^{-}$CD4$^+$CD62L$^+$, 78%; $\alpha\beta^{-}$CD4$^+$CD62L$^+$, 66%; $\alpha\beta^{-}$CD8$^+$CD62L$^+$, 89%; $\alpha\beta^{-}$CD8$^+$CD62L$^+$, 86%; Figure 1A). We then assessed the alloreactive proliferation in vivo of the $\alpha\beta^{-}$ or $\alpha\beta^{-}$ selected T cells by adoptive transfer after labeling with CFSE into irradiated allogeneic recipients and found no differences in the percentage of dividing cells and the number of divisions between the 2 selected populations (Figure 1B).

To detect potential differences in cytokine expression on alloactivation in vivo, we transferred $\alpha\beta^{-}$ and $\alpha\beta^{-}$ sorted B10.BR T cells and B10.BR TCD-BM into irradiated (750 cGy) CBA recipients and 3 days later determined intracellular IFN-$\gamma$ expression in the splenocytes from these recipients (Figure 1C). We could not detect any profound differences in IFN-$\gamma$ expression of donor CD4$^+$ or CD8$^+$ T cells in these recipients.

Finally, we determined that alloreactive donor T cells upregulate their $\alpha\beta^{-}$ expression on alloactivation by adoptive transfer of unselected CFSE-labeled donor T cells into irradiated allogeneic recipients (Figure 1D).

We then performed GVHD experiments by adding $0.5 \times 10^6$ (B10.BR $\rightarrow$ CBA) or $0.5 \times 10^6$ (B10.BR $\rightarrow$ B6) selected $\alpha\beta^{-}$ or $\alpha\beta^{-}$ T cells to the TCD-BM containing allograft. Allografts with only TCD-BM or TCD-BM + unsorted T cells were injected as control groups. CBA recipients of $\alpha\beta^{-}$ donor T cells had a significant delay and decrease in GVHD mortality and morbidity (Figure 2A) compared with recipients of $\alpha\beta^{-}$ or unsorted T cells. A similar delay in GVHD mortality and morbidity (Figure 2B) was observed when B6 recipients of $\alpha\beta^{-}$ donor T cells were compared with recipients of $\alpha\beta^{-}$ T cells. From these results we conclude that $\alpha\beta^{-}$ donor T cells have significantly less potential to induce GVHD than $\alpha\beta^{-}$ or unselected donor T cells.

Recipients of $\alpha\beta^{-}$ donor T cells have higher numbers of $\alpha\beta^{-}$ donor T cells in their intestinal mucosa

To analyze whether $\alpha\beta^{-}$ or $\alpha\beta^{-}$ donor T cells differ in their capability to infiltrate into the intestinal mucosa of the recipient, we determined the numbers of B10.BR donor CD4$^+$ or CD8$^+$ $\alpha\beta^{-}$ T cells at 14 and 22 days after transplantation into the intestinal mucosa of the gut, spleen, peripheral lymph nodes, mesenteric lymph nodes, and liver in CBA recipients of $\alpha\beta^{-}$ or $\alpha\beta^{-}$ donor T cells (Figure 3). As expected, we found significantly higher numbers of $\alpha\beta^{-}$ T cells (both CD4$^+$ and CD8$^+$) in the mesenteric lymph nodes and gut of recipients of $\alpha\beta^{-}$ T cells at both time points. Interestingly, we also observed more $\alpha\beta^{-}$ T cells in the spleens of recipients of $\alpha\beta^{-}$ T cells on day 22, whereas $\alpha\beta^{-}$ T cells were increased in the peripheral lymph nodes of recipients of $\alpha\beta^{-}$ T cells on day 22.

Recipients of $\alpha\beta^{-}$ T cells develop intestinal and liver GVHD more rapidly than recipients of $\alpha\beta^{-}$ T cells

Mortality in our mouse models is primarily determined by intestinal GVHD and mucositis-associated infections related to intestinal GVHD. To analyze whether recipients of $\alpha\beta^{-}$ T cells incurred more severe intestinal GVHD, we analyzed GVHD-associated organ damage in terminal ileum, colon, liver, skin (ear and tongue), and thymus. We performed semiquantitative histopathologic analysis in a blinded fashion on the terminal ileum, colon, and liver. Dyskeratotic indices were calculated to determine the number of apoptotic keratinocytes per millimeter of epithelium. Thymic GVHD was assessed by thymic cellularity as we have described previously.\(^{22}\)

We found significantly higher histopathologic scores of terminal ileum, colon, and liver in recipients of $\alpha\beta^{-}$ T cells on days 14 and 22 after transplantation compared with recipients of $\alpha\beta^{-}$ T cells (Figure 4A). The development of intestinal and liver GVHD...
Recipients of αβ7+ T cells have intact GVT activity

To assess if αβ7+ expression of alloreactive T cells had any effect on GVT activity, we used the B10.BR → B6 strain combination and challenged recipients of an allograft containing αβ7+ or αβ7- T cells with EL-4 lymphoma cells on day 0. We performed autopsies on all animals that died after transplantation and determined the cause of death (GVHD or lymphoma). We observed again a significant higher GVHD mortality in recipients of αβ7+ T cells compared with recipients of αβ7- T cells, whereas GVT activity in recipients of αβ7- T cells was not diminished (Figure 5; Table 1). We conclude, therefore, that αβ7+ expression of alloreactive T cells does not affect their GVT activity.

Discussion

Few studies have examined the role of integrin receptors in the development of GVHD. Tanaka et al12 found in a parent-into-F1 allogeneic HSCT model with a full MHC class I and II disparity that the treatment of lethally irradiated recipients with anti-α4, -α3, or -β7 antibodies resulted in a moderate amelioration of intestinal GVHD (as measured by histopathologic criteria: reduction of the villous-crypt ratio and mononuclear infiltrate), whereas the combination treatment with anti-α4 and -α3 antibodies completely prevented intestinal GVHD by histopathologic criteria. These results suggested that these 2 integrins, which both can associate with β7, are required for lymphocyte infiltration into the inflamed intestine during the development of GVHD. However, no data were provided regarding the effects of anti-α4 and -α3 antibody treatment on GVHD morbidity and mortality.

Li et al14 used an acute GVH reaction (a-GVHR) model and transferred B6 splenocytes into nonirradiated C.B-17 severe combined immunodeficient (SCID) recipients. Preincubation of donor splenocytes with anti-α4 and anti-CD62L antibodies resulted in a delay in a-GVHR mortality with 3 of 10 long-term survivors.

Murai et al15 recently demonstrated, using a-GVHR models, that the Peyer patches in the gut are required for the activation of donor antithost T cells. Their findings suggested that host dendritic cells in the subepithelial dome of Peyer patches express regulated upon activation, normal T cell expressed and secreted (RANTES)/CCL5 during the development of intestinal a-GVHR, which results

**Figure 2.** Recipients of αβ7+ T cells have increased GVHD mortality and morbidity. Lethally irradiated (1300 cGy) CBA (A) and (1100-1200 cGy) C57BL/6 (B) recipients received transplants with B10.BR TCD-BM (5 × 10⁶) and splenic T cells (0.5-1 × 10⁶). T cells were included in the allograft as unsorted or αβ7+CD3+ and αβ7+CD3- sorted populations from B10.BR donors. Kaplan-Meier and clinical GVHD score (± SEM) curves are shown that represent 4 (BM only), 8 to 11 (BM + T cells), and 13 to 16 (BM + αβ7+ or αβ7- T cells) recipients per group from 2 combined experiments. Statistical analysis is as follows: (A) left, ○ versus □: P < .007; □ versus ■: P < .001; (B) Left, ○ versus □: P < .017; right, ○ versus □: P < .01. T cells have significantly higher numbers of CD4+ and CD8+ T cells compared with recipients of αβ7- T cells, whereas GVT activity in recipients of αβ7- T cells was not diminished (Figure 5; Table 1). We conclude, therefore, that αβ7+ expression of alloreactive T cells does not affect their GVT activity.

**Figure 3.** Recipients of αβ7+ T cells have significantly higher numbers of αβ7+ donor T cells in their intestinal mucosa and mesenteric lymph nodes. CBA mice received transplants as described in Figure 2. Recipients of αβ7+ and αβ7- T cells were killed on days 14 and 22 after BMT. Infiltrating αβ7+ T cells of donor origin were determined by multicolor flow cytometry in multiple organs. Top panels represent averages of absolute cell numbers (± SE) of αβ7+ CD4+ donor cells in spleen (day +14, n = 8; day +22, n = 9), peripheral lymph nodes (PLNs; day +14, n = 4; day +22, n = 4), mesenteric lymph nodes (MLNs; day +14, n = 3-4; day +22, n = 3-5), small intestine (gut, day +14, n = 3-4; day +22, n = 4-5) and liver (day +14, n = 6-7; day +22, n = 8-9). Bottom panels represent averages of absolute cell numbers of αβ7+CD8+ donor cells in the above mentioned organs. *Signifies statistically significant P values.
in the attraction of CCR5+CD8+ donor T cells. The infiltration of donor splenocytes into the Peyer patches of the recipient and the development of a-GVHR could be prevented by disruption of CCR5 in donor T cells, by inhibition of the interaction between αβ+ and MadCAM-1 with neutralizing anti-MadCAM-1 antibodies, or by using recipients, which lack Peyer patches.

These studies established the importance of αβ+ in the development of intestinal GVHD and demonstrated that the inhibition of the α4 integrin with neutralizing antibodies can ameliorate intestinal GVHD. Our experiments using true GVHD (not a-GVHR) models confirm that αβ+ is important for the development of intestinal GVHD and demonstrate that intestinal GVHD can be delayed by depleting αβ+ T cells from the allograft. This delay in intestinal GVHD is associated with a significant decrease in GVHD morbidity and mortality. The association between a delay in the invasion of an epithelial target tissue by alloreactive T cells and a decrease in GVHD morbidity and mortality is reminiscent of the well-documented decrease in the GVHD alloresponse seen with delayed posttransplantation infusion of donor lymphocytes. This relationship between the timing of donor lymphocyte infusion and the severity of the GVHD alloresponse could be related to the degree of inflammation, tissue damage, and levels of inflammatory cytokines ("cytokine storm") early after transplantation, as well as the turnover of host-derived antigen-presenting cells.

Naïve T cells have a uniform intermediate cell surface expression of αβ+, whereas the expression of αβ+ on CD4+ activated/memory T cells follows a bimodal pattern with a negative and a positive population (Williams and Butcher and A.P., M.R.M.v.d.B., our unpublished observations, February 27, 2003). For practical reasons, we selected in our experiments donor T cells only on their αβ+ expression. Therefore, both αβ+ and αβ- populations contained naïve and activated/memory T cells, and we found equal percentages of CD62L expression in both populations. Several investigators have proposed that naïve T cells express a variety of homing molecules, which allow them to recirculate through secondary lymphoid organs (spleen, lymph nodes, and Peyer patches). In support of this theory, Williams and Butcher and A.P. found that naive CD44+ T cells can become activated after encountering alloantigens in the mesenteric lymph nodes of the allograft. This delay in intestinal GVHD is associated with a significant decrease in GVHD morbidity and mortality. Therefore, we hypothesize that the naïve T cells in our experiments donor T cells only on their αβ+ expression. Therefore, both αβ+ and αβ- populations contained naïve and activated/memory T cells, and we found equal percentages of CD62L expression in both populations. Several investigators have proposed that naïve T cells express a variety of homing molecules, which allow them to recirculate through secondary lymphoid organs (spleen, lymph nodes, and Peyer patches).

On encountering antigen presented by antigen-presenting cells in the secondary lymphoid organs, T cells become activated, begin to proliferate and differentiate, and reprogram their homing receptors so that they can migrate to specific extra-lymphoid tissues. Therefore, we hypothesize that the naïve T cells in our experiments donor T cells only on their αβ+ expression. Therefore, both αβ+ and αβ- populations contained naïve and activated/memory T cells, and we found equal percentages of CD62L expression in both populations. Several investigators have proposed that naïve T cells express a variety of homing molecules, which allow them to recirculate through secondary lymphoid organs (spleen, lymph nodes, and Peyer patches).

Table 1. Cause of death (GVHD versus tumor) for all recipients that died during the course of the experiment

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A similar delay between recipients of αβ⁺ T and αβ⁻ T cells was observed in the development of intestinal and liver GVHD. Recipients of αβ⁺ T cells had developed extensive GVHD in liver and intestines at day +14 after transplantation, whereas recipients of αβ⁻ T cells developed maximal liver and intestinal GVHD by day +22 (Figure 4). Interestingly, the severity and kinetics of skin and thymic GVHD did not differ between recipients of αβ⁺ and αβ⁻ T cells, which argues against a general decrease in alloreactivity of αβ⁻ T cells compared with αβ⁺ T cells as an explanation for the differences in GVHD morbidity and mortality between recipients of αβ⁺ and αβ⁻ T cells.

The higher numbers of αβ⁺ donor T cells in the spleens of recipients of αβ⁺ donor T cells at day +22 could be due to greaterallostimulation at this later time point, when the recipients of αβ⁺ donor T cells have developed more severe GVHD (see GVHD morbidity data in Figure 2A-B). This difference in overall GVHD morbidity could result in higher levels of immunostimulatory cytokines and chemokines, as well as more activated antigen-presenting cells. The higher numbers of αβ⁺ T cells in the peripheral lymph nodes of recipients of αβ⁻ T cells reflect a more rapid decline in αβ⁺ T cells in recipients of αβ⁺ donor T cells. This could also be due to greaterallostimulation in recipients of αβ⁺ donor T cells, resulting in more rapid trafficking of alloactivated T cells from the peripheral lymph nodes into the target organs.

αβ⁺ has been demonstrated to play a role in homing of lymphocytes to the liver sinusoids28 as well as migration of αβ⁺ lymphocytes in primary sclerosing cholangitis in humans.29 Therefore, αβ⁺ could be involved in the homing of alloreactive T cells to the liver; however, we did not find significantly increased numbers of αβ⁺ T cells in the livers of recipients of αβ⁺ donor T cells. We did detect increased liver GVHD in recipients of αβ⁺ T cells, which could be due to other mechanisms, such as non-T-effector cells or cytokines.

Finally, our findings that αβ⁺ alloreactive T cells have intact GVMT activity is intriguing. The αβ⁺ alloreactive T cells are capable of inducing cutaneous and thymic GVHD comparable to αβ⁻ alloreactive T cells, and this finding suggests that their alloreactivity is intact in all tissues except the intestines. Most leukemias and lymphomas are largely confined to the lymphohematopoietic niche in the early stages of their development, and we hypothesize, therefore, that the impaired migration of αβ⁻ alloreactive T cells has no effect on their GVMT activity against leukemias and lymphomas. This hypothesis is supported by a recent study, which demonstrated reduced GVHD and intact GVMT in recipients treated with a sphingosine-1-phosphate receptor agonist (FTY720).30 FTY720 binds to T-cell G protein-coupled receptors, which affects migration of lymphocytes and traps them in secondary lymphoid tissues.

In conclusion, this study demonstrates the importance of the expression of αβ⁺ by alloreactive donor T cells in the development of intestinal GVHD and overall GVHD morbidity and mortality. It supports the notion that strategies aimed at the inhibition of homing of alloreactive T cells to gut, including neutralizing antibodies against αβ⁺, could result in significant improvement in the prevention and/or treatment of intestinal GVHD and overall morbidity and mortality from GVHD.

References

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