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Absence of P-Selectin in Recipients of Allogeneic Bone Marrow Transplantation Ameliorates Experimental Graft-versus-Host Disease

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Alloreactive T cells are crucial for graft-versus-host disease (GVHD) pathophysiology, and modulating their trafficking patterns has been efficacious in ameliorating experimental disease. We report in this paper that P-selectin, a glycoprotein found on resting and inflamed endothelium, is important for donor alloreactive T cells trafficking into GVHD target organs, such as the intestines and skin. Compared with wild-type (WT) recipients of allogeneic bone marrow transplantation, P-selectin−/− recipients exhibit decreased GVHD mortality and decreased GVHD of the skin, liver, and small bowels. This was associated with diminished infiltration of alloactivated T cells into the Peyer’s patches and small bowels, coupled with increased numbers of donor T cells in the spleen and secondary lymphoid organs (SLOs). Surprisingly, however, donor T cells deficient for P-selectin glycoprotein ligand 1, the most well described P-selectin ligand, mediated GVHD similar to WT T cells and accumulated in SLO and target organs in similar numbers as WT T cells. This suggests that P-selectin may be required for trafficking into inflamed tissues but not SLO and that donor T cells may use multiple P-selectin ligands apart from P-selectin glycoprotein ligand 1 to interact with P-selectin and traffic into inflamed tissues during GVHD. We conclude that targeting P-selectin may be a viable strategy for GVHD prophylaxis or treatment. The Journal of Immunology, 2010, 185: 1912–1919.

Alloreactive donor T cells play an important role in the pathophysiology of GVHD, which is a systemic T cell-mediated disease with specific involvement of the intestines, liver, and skin. The infiltration of alloreactive T cells into target organs is an important step in GVHD pathophysiology, and modulation of T cell trafficking represents a promising strategy for GVHD prophylaxis or treatment (1–8).

T cells and other leukocytes exit the bloodstream and enter into tissues via a series of regulated steps. In a simplified model, these can be divided into 1) tethering and rolling via the selectins and their ligands; 2) integrin activation upon chemokine ligand–receptor interactions; 3) firm adhesion via high-affinity integrins; and 4) extravasation via molecules including CD31, CD99, and the junctional adhesion molecules (9).

P-selectin is one of a family of three glycosylated lectins (E-, L-, and P-selectin). It is constitutively expressed on vascular endothelium of the skin and bone marrow and inducibly expressed on other endothelial cells during inflammation. P-selectin is a receptor for P-selectin glycoprotein ligand 1 (PSGL1), a glycoprotein highly expressed on all leukocytes (9), as well as other less well defined ligands, generally believed to be sialyl Lewis x-bearing glycoproteins (10–12). Leukocyte interactions with P-selectin are important for tethering and rolling along endothelium, and interference with leukocyte–P-selectin interactions have shown benefit in treatment.

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Abbreviations used in this paper: allogeneic bone marrow transplantation; allo-BMT; IEL, intraepithelial lymphocyte; MFI, median fluorescence intensity; MLN, mesenteric lymph node; PLN, peripheral lymph node; Peyer’s patch; PSGL1, P-selectin glycoprotein ligand 1; SLO, secondary lymphoid organ; TCD-BM, T cell-depleted bone marrow; WT, wild-type.

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experimental models of ischemia-reperfusion injury, allergic airway disease, and bleomycin-induced pulmonary fibrosis (13–15). This led us to hypothesize that P-selectin–ligand interactions may be relevant for leukocyte trafficking in acute GVHD.

PSGL1 mRNA is upregulated during GVHD in multiple models (16–18). In addition, donor T cells localizing into GVHD target organs can upregulate PSGL1 (19), although one report by Sykes and colleagues (20) indicated that adoptive transfer of whole splenocytes lacking functional PSGL1 appears to result in intact GVHD, underlining the complexity and potential redundancy of interactions between P-selectin and its multiple ligands.

In this study, we showed that compared with wild-type (WT) recipients, P-selectin−/− recipients of allogeneic bone marrow transplantation (allo-BMT) have improved survival and diminished clinical GVHD morbidity and mortality, as well as attenuated target organ GVHD. Donor T cells in P-selectin−/− recipients were found in greater numbers in the spleen and lymph nodes but in diminished numbers in the Peyer’s patches (PPs) and small bowels. However, cognate experiments with the transfer of PSGL1−/− donor T cells into irradiated allo-BMT recipients resulted in intact GVHD responses, indicating that other P-selectin ligands on donor alloreactive T cells may also be important for trafficking during GVHD.

Our results suggest a requirement for vessel P-selectin for the infiltration of GVHD target tissues by donor alloactivated T cells and that donor alloreactive T cells during GVHD may use multiple P-selectin ligands in addition to PSGL1.

Materials and Methods

Bone marrow transplantation

LP, B10.BR, C57BL/6 (B6), B6 Ly5.1+, B6D2F1, BALB/c mice, and PSGL1−/− and P-selectin−/− mice on the B6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). Memorial Sloan-Kettering Cancer Center’s Institutional Animal Care and Use Committee approved all animal protocols. Mice were transplanted as previously described (1) and housed in the Memorial Sloan-Kettering Cancer Center-specific pathogen-free barrier facilities. All bone marrow transplant recipients were monitored daily for survival and scored weekly for weight loss and signs of clinical GVHD (1).

B6 and P-selectin−/− mice received 11 Gy total body irradiation as a split dose 3 h apart from a 137 Cs source. B6D2F1 mice received 13 Gy total body irradiation as a split dose 3 h apart from a 137 Cs source. B6D2F1 mice received 13 Gy total body irradiation as a split dose.

Markers for donor and host cells

We used Ly9.1 (CD229.1) to differentiate donor LP T cells (Ly9.1+) from B6-recipient cells (Ly9.1−). Ly5.1 (CD45.1) was used to identify B6 Ly5.1+ cells. The marker H-2D6 was used to identify B6D2F1 host cells. In experiments with B10.BR donor T cells, the marker used was H-2K6.

Abs and flow cytometry

Leukocytes were washed and resuspended in staining buffer (PBS plus 0.5% BSA plus 2 mM EDTA). Cells were stained with DAPI, Ly9.1-FITC, Ly5.1-biotin, H-2D6-PE, H-2K6-PE, H-2K6-biotin, Ly5.1-PE, Ly5.1-biotin, CD3e-allophycocyanin-Cy7, CD4-Pacific Blue, CD8-PerCP, CD25-PE-Cy7, CD44-Alexa 700, L-selectin (CD62L)-PE-Texas Red, CD45-PerCP-Cy5.5, Annexin V-PE, Ki67-PE, PSGL1-PE, and FoxP3-PE and analyzed on an LSR II with DivA 6.1 (BD Biosciences, San Jose, CA).

To study the role of P-selectin in GVHD, we first assessed P-selectin expression in vessels of the skin and liver in mice with and without GVHD (allologenic: LP→B6; syngeneic: B6→B6). This revealed that on day 7 posttransplant, endothelial cells (CD45 Ter119 CD11b CD31−) from the spleen and liver in both recipients of syngeneic and allogeneic bone marrow transplantation (BMT) displayed P-selectin (Fig. 1A, 1B). By contrast, endothelium from nontransplanted B6 mice displayed less P-selectin (Fig. 1C).

CFSE labeling of T cells and their adoptive transfer

Magnetically purified splenic T cells (>90% purity by CD3 staining) were labeled with 1 µm CFSE from Invitrogen for 20 min, washed twice, and infused i.v. into irradiated recipients.

MLRs

Stimulator cells were obtained via CD5+ magnetic bead selection (Miltenyi Biotec, Auburn, CA) and verified to be >90% pure for T cells by flow cytometry after staining with CD3-FITC. Responder cells were obtained from the spleens of nontransplanted young female BALB/c and B10.BR mice and were depleted of RBCs by hypotonic lysis and were depleted of T cells via CD5+ magnetic bead selection. Responders were irradiated with 20 Gy from a 60Co source. A total of 105 stimulators were plated with 103 responders in RPMI 1640 plus 20% FCS plus glutamine plus nonessential amino acids plus penicillin and streptomycin and incubated at 5% CO2 for 6 d, and 1 µCi [3H]thymidine was added to each well for 24 h before reading.

Enrichment for endothelial cells from the liver and spleen

Livers were flushed in situ with 5 ml PBS, and livers and spleens were transferred into 10 ml PBS with 2 mg/ml collagenase D, cut into small pieces, and incubated for 45 min at 37°C on a shaker. The pieces were then gently mashed through a 70-µm cell strainer in PBS with 0.5% BSA and centrifuged, and the pellet was resuspended in 7 ml 30% Histodenz (Sigma-Aldrich, St. Louis, MO) and layered on top of 2 ml RPMI 1640. This gradient was then centrifuged at 1500 × g for 20 min, and the endothelial cell-rich interphase was then obtained and washed before use.

Lymphocyte isolation from liver

Livers were washed in PBS plus 0.5% BSA and centrifuged at 300 × g for 5 min, and the pellets were resuspended in 40% Percoll in PBS (Sigma-Aldrich) and layered on top of 70% Percoll in PBS for centrifugation at 400 × g for 30 min at 4°C. The lymphocyte-rich infranatant was then obtained and washed with PBS plus 0.5% BSA before use.

Serum cytokines

Cytokines were analyzed by Cytometric Bead Array (BD Biosciences) per the manufacturer’s directions. Linear regression curves were analyzed in Microsoft Excel.

Complete blood counts

Blood was analyzed on a Hemavet (Drew Scientific, Waterbury, CT).

Statistics

Survival was calculated with the Mantle-Cox log-rank test. We made all other comparisons with the Mann-Whitney U test. Calculations were performed in Prism (GraphPad, La Jolla, CA).

Results

Both recipients of syngeneic and allogeneic bone marrow transplant upregulate expression of P-selectin and E-selectin on vessel endothelium of the liver and spleen

We chose a clinically relevant, well-defined MHC-matched minor Agmismatched strain combination LP→B6 (H-2d) to study the role of P-selectin in GVHD. We first assessed P-selectin expression in vessels of the skin and liver in mice with and without GVHD (allologenic: LP→B6; syngeneic: B6→B6). This revealed that on day 7 posttransplant, endothelial cells (CD45 Ter119 CD11b CD31−) from the spleen and liver in both recipients of syngeneic and allogeneic bone marrow transplantation (BMT) expressed P-selectin (Fig. 1A, 1B). By contrast, endothelium from nontransplanted B6 mice displayed less P-selectin (Fig. 1C). Moreover, hepatic endothelium also expressed E-selectin, whereas splenic endothelium did not, on day 7 posttransplant (Fig. 1D). These data suggest that BMT-associated inflammatory processes, potentially related to the conditioning regimen, can upregulate selectin molecules on vessel endothelium post-transplant.

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P-selectin−/− allo-BMT recipients display less clinical GVHD than WT recipients, but donor T cells deficient for PSGL1 mediate GVHD similar to WT T cells

To test the importance of P-selectin in GVHD development, we transplanted WT or P-selectin−/− recipients with LP T cell-depleted bone marrow (TCD-BM) and T cells and observed that P-selectin−/− recipients had attenuated GVHD morbidity and mortality (Fig. 2A, 2B), suggesting that P-selectin in allo-BMT recipients could be important for GVHD pathophysiology. We also noted intact engraftment of TCD-BM in P-selectin−/− recipients (Fig. 2A and data not shown).

PSGL1 is an important (though not sole) ligand for both E- and P-selectin (9, 11). We therefore assessed the ability of PSGL1−/− donor alloreactive T cells to cause GVHD in WT allo-BMT recipients. When we performed cognate experiments with WT or PSGL1−/− donor T cells in the parent→F1 model B6 (H-2b)→B6D2F1 (H-2b/d) with 5 × 10^6 B6 TCD-BM + 2 × 10^6 WT or PSGL1−/− T cells, we were surprised to note similar survival and GVHD morbidity between recipients of WT and PSGL1−/− donor T cells (Fig. 2C, 2D). These observations, however, correspond well to a report suggesting that functional inactivation of PSGL1 in donor splenocyte allografts does not significantly ameliorate GVHD (20).

PSGL1−/− T cells display significant levels of P-selectin ligands

We next tested whether PSGL1−/− T cells could still bind P-selectin. When we stained WT and PSGL1−/− splenic T cells from

FIGURE 1. Recipients of both syngeneic and allogeneic BMT express E- and P-selectin on endothelium of the liver and spleen. A, Lethally irradiated B6 mice received either 5 × 10^6 LP TCD-BM + 10^6 LP splenic T cells (allogeneic group) or 5 × 10^6 B6 TCD-BM + 10^6 B6 splenic T cells (syngeneic group). Spleens and livers were analyzed on day 7 posttransplant. Endothelial cells were identified as DAPIloCD45−CD11b−CD31+ cells. The percentage of cells positive for P-selectin was identified, based on gates from the isotype control, set at 1%. n = 5–6/group. B, Representative histogram of endothelial cell P-selectin staining in the liver from allo-BMT recipients on day 7, as from A above. Shaded, isotype control; open histogram, specific staining. C, Spleen and liver were harvested from nontransplanted B6 mice. Endothelial cells were analyzed for P-selectin staining as above in A; shaded, isotype control; open histogram, specific staining. D, Mice were transplanted as in A, and endothelial cells in the spleen and liver were assessed for the expression of E-selectin on day 7 posttransplant. n = 5–6/group.

FIGURE 2. P-selectin−/− recipients of allo-BMT experience attenuated GVHD in comparison with WT recipients, but PSGL1−/− donor T cells cause similar GVHD as WT T cells. A and B, Lethally irradiated (11 Gy) B6 Ly5.1+ and P-selectin−/− mice received 5 × 10^6 LP TCD-BM with or without 10^6 LP splenic T cells. BM only groups, n = 5; BM+T groups, n = 10. Data from two combined experiments. Allo-BMT hosts are denoted by parentheses in the legend. C and D, Lethally irradiated (13 Gy) B6 D2F1 recipients received 5 × 10^6 B6 or PSGL1−/− splenic T cells. BM only groups, n = 5; BM+T groups, n = 10. Representative data from one of two independent experiments.
FIGURE 3. P-selectin deficiency in allo-BMT recipients does not influence the activation, proliferation, apoptosis, or PSGL1 expression of donor alloreactive T cells; however, P-selectin−/− recipients of allo-BMT have more donor T cells in the spleen, MLN, PLN, and peripheral blood but fewer donor T cells in the PPs and small bowels. A, Lethally irradiated WT or P-selectin−/− recipients received $10^7$ CFSE-labeled magnetically purified LP splenic T cells. Spleens were harvested from recipients on day 5 after adoptive transfer and analyzed by flow cytometry. Histogram overlays for the proliferation of CD4 and CD8 T cells in WT and P-selectin−/− recipients are shown. Two independent experiments, total n = 4/group. Shaded, P-selectin−/− hosts; open histograms, WT hosts. B, Lethally irradiated WT or P-selectin−/− recipients received CFSE-labeled T cells and were harvested on day 5 as above. Histogram overlays are shown for the activation markers CD25, CD44, and CD62L on donor CFSElo fast-proliferating alloactivated CD4 and CD8 T cells. 

E – GVHD mice, Day 14

F – GVHD mice, histopathologic infiltrates, Day 35

FIGURE 3. P-selectin deficiency in allo-BMT recipients does not influence the activation, proliferation, apoptosis, or PSGL1 expression of donor alloreactive T cells; however, P-selectin−/− recipients of allo-BMT have more donor T cells in the spleen, MLN, PLN, and peripheral blood but fewer donor T cells in the PPs and small bowels. A, Lethally irradiated WT or P-selectin−/− recipients received $10^7$ CFSE-labeled magnetically purified LP splenic T cells. Spleens were harvested from recipients on day 5 after adoptive transfer and analyzed by flow cytometry. Histogram overlays for the proliferation of CD4 and CD8 T cells in WT and P-selectin−/− recipients are shown. Two independent experiments, total n = 4/group. Shaded, P-selectin−/− hosts; open histograms, WT hosts. B, Lethally irradiated WT or P-selectin−/− recipients received CFSE-labeled T cells and were harvested on day 5 as above. Histogram overlays are shown for the activation markers CD25, CD44, and CD62L on donor CFSElo fast-proliferating alloactivated CD4 and CD8 T cells.
donor mice for P- and E-selectin ligands, we found that PSGL1\textsuperscript{−/−} donor T cells (as expected) did not express PSGL1 but still expressed substantial levels of P-selectin ligands at levels comparable with those of WT T cells (Supplemental Fig. 1). By contrast, we found no appreciable levels of E-selectin ligands (Supplemental Fig. 1).

**Donor alloreactive T cells in WT and P-selectin\textsuperscript{−/−} allo-BMT recipients have similar levels of alloactivation and apoptosis in vivo**

We assessed the functionality of donor T cells in WT and P-selectin\textsuperscript{−/−} allo-BMT recipients by adoptively transfusing purified (CD5\textsuperscript{+}) CFSE-labeled LP splenic T cells into irradiated WT and P-selectin\textsuperscript{−/−} recipients. On day 5, we observed increased numbers of CFSE\textsuperscript{lo} fast-proliferating allogeneic-activated CD4 T cells in the spleens of P-selectin\textsuperscript{−/−} recipients as compared with WT recipients (Fig. 3A), suggesting that alloreactive T cells selectively accumulate in the spleens of P-selectin\textsuperscript{−/−} recipients. Rapidly proliferating CFSE\textsuperscript{lo} alloactivated T cells in P-selectin\textsuperscript{−/−} and WT recipients displayed similar levels of CD25, CD44, and CD62L (Fig. 3B), suggesting that T cell alloactivation is intact in P-selectin\textsuperscript{−/−} recipients.

We evaluated donor T cell apoptosis in WT and P-selectin\textsuperscript{−/−} recipients in two models, B10.BR (H-2\textsuperscript{b})→B6 (H-2\textsuperscript{b}) as well as LP→B6. We adoptively transferred CFSE-labeled LP or B10.BR donor-splenic T cells into irradiated allogeneic B6 or P-selectin\textsuperscript{−/−} recipients and then analyzed T cell apoptosis on day 3 (B10.BR T) or day 5 (LP T). These experiments revealed similar levels of T cell apoptosis (data not shown).

**Donor alloreactive T cells in WT and P-selectin\textsuperscript{−/−} allo-BMT recipients have similar alloactivation in vitro**

To directly assess the alloreactivity of donor T cells in WT versus P-selectin allo-BMT recipients, we recovered purified T cells from the spleens and livers of mice with GVHD on day 28 posttransplant (5×10\textsuperscript{5} LP TCD-BM + 1×10\textsuperscript{6} LP T→B6 or P-selectin\textsuperscript{−/−}) and performed MLRs with irradiated B6 (allogenic) and BALB/c (third party) T cell-depleted stimulators. Donor alloreactive T cells in WT versus P-selectin\textsuperscript{−/−} transplant recipients had similar patterns of proliferation and activation (CD25, IFN-\gamma, FoxP3\textsuperscript{+} regulatory T cells on day 14 after allo-BMT in WT and P-selectin\textsuperscript{−/−} recipients) (data not shown).

We also found similar numbers of splenic donor CD4\textsuperscript{+}CD25\textsuperscript{−}FoxP3\textsuperscript{+} regulatory T cells on day 14 after allo-BMT in WT and P-selectin\textsuperscript{−/−} recipients (data not shown). In addition, serum TNF and IFN-\gamma levels were similar on day 14 posttransplant (data not shown).

**Donor alloreactive T cells in WT and P-selectin\textsuperscript{−/−} allo-BMT recipients have comparable levels of PSGL1 expression**

PSGL1 may interact with other receptors in addition to P-selectin, and we therefore assessed PSGL1 expression on donor CFSE\textsuperscript{lo} alloactivated T cells in WT and P-selectin\textsuperscript{−/−} recipients to evaluate whether donor T cells may have a compensatory upregulation of PSGL1 when placed into a P-selectin\textsuperscript{−/−} recipient. This revealed that donor CFSE\textsuperscript{lo} fast-proliferating alloactivated T cells in P-selectin\textsuperscript{−/−} recipients had similar or decreased levels of surface PSGL1 compared with those in WT recipients (Fig. 3D).

**WT and PSGL1\textsuperscript{−/−} T cells have similar patterns of activation and proliferation after adoptive transfer into lethally irradiated allogeneic recipients**

To assess the alloreactivity and proliferation of WT versus PSGL1\textsuperscript{−/−} T cells, we adoptively transferred WT and PSGL1\textsuperscript{−/−} splenic T cells into irradiated allogeneic (B6D2F1) recipients. We observed that WT and PSGL1\textsuperscript{−/−} alloreactive T cells in the spleen showed similar patterns of proliferation and activation (CD25, CD44, and CD62L) (data not shown).

We also assessed the in vivo expansion and trafficking of PSGL1\textsuperscript{−/−} T cells in GVHD in the model B6 TCD-BM + B6 WT versus PSGL1\textsuperscript{−/−} donor T→B6D2F1. On day 14, we found similar numbers of donor CD4 and CD8 T cells in the spleen, liver, mesenteric lymph node (MLN), and peripheral lymph node (PLN) in recipients of WT versus PSGL1\textsuperscript{−/−} T cells (data not shown).

**Allocorrelated PSGL1\textsuperscript{−/−} T cells express P-selectin ligands during GVHD**

We also evaluated levels of P-selectin ligand on donor WT and PSGL1\textsuperscript{−/−} T cells on day 14 posttransplant and noted that PSGL1\textsuperscript{−/−} alloreactive T cells in the spleen had a moderate decrease in levels of cell surface P-selectin ligand but that PSGL1\textsuperscript{−/−} alloreactive T cells in the liver, MLN, and PLN had similar levels of cell surface P-selectin ligand as WT T cells (Supplemental Fig. 2). These P-selectin ligands may be relevant for the trafficking of PSGL1\textsuperscript{−/−} T cells during GVHD.

**Donor T cell numbers are increased in the spleen, MLN, PLN, and peripheral blood of P-selectin\textsuperscript{−/−} recipients after allo-BMT and concomitantly decreased in the PP and intraepithelial lymphocyte**

As donor alloreactive T cells appeared to display similar activation, proliferation, apoptosis, and PSGL1 expression in irradiated WT and P-selectin\textsuperscript{−/−} allo-BMT recipients, we next assessed their accumulation in lymphoid and nonlymphoid tissues. Nontransplanted P-selectin\textsuperscript{−/−} mice have similar numbers of CD4 and CD8 T cells in the spleen, MLN, PLN, and PPs as corresponding WT animals but decreased numbers of CD4 T cells in the liver (data not shown). Twenty-four hours after lethal radiation
(11 Gy, split dose), we observed comparable numbers of T cells in the spleen, liver, MLN, PLN, and PP of WT and P-selectin−/− animals (data not shown).

Finally, upon enumerating donor infiltrating T cells in lymphoid tissues and GVHD target organs of allo-BMT recipients on day 14 posttransplant, we found increased numbers of CD4 and CD8 effector (CD4+CD62L−) and central memory (CD4+CD62L+) T cells in the spleen, MLNs, and PLNs of P-selectin−/− recipients (Fig. 3E). This was associated with decreased numbers of donor T cells in the PPs and epithelium of the small bowels (intraepithelial lymphocyte [IEL]) in P-selectin−/− recipients (Fig. 3E). Complete blood counts also revealed increased numbers of circulating lymphocytes in the blood of P-selectin−/− recipients on days 7 and 14 posttransplant (Fig. 3E).

These findings were confirmed via histopathological analysis at a later time point (day 35). When we analyzed the pathological subscores for lymphocytic infiltrates, P-selectin−/− recipients exhibited significantly decreased lymphocytic infiltrates into the small bowels (Fig. 3F, left panel). At this later time point, we also observed a nonsignificant trend toward decreased lymphocytic infiltrates in the liver. Finally, we also observed decreased neutrophilic infiltrates into the small bowel, and a trend toward decreased neutrophilic infiltrates into the liver at day 35 after allo-BMT (Fig. 3F, right panel), which may be due to the function of P-selectin in neutrophil tethering and rolling (21); consequently, P-selectin-deficient endothelium may also impair neutrophil trafficking.

P-selectin−/− recipients of allo-BMT have decreased GVHD of the liver, small bowel, and skin on day 35 posttransplant

We assessed damage to GVHD target organs in WT and P-selectin−/− allo-BMT recipients by histopathology and observed a decrease in hepatic and small-bowel GVHD on day 35 posttransplant (Fig. 4A–C). On day 35 posttransplant, P-selectin−/− recipients also showed significantly decreased numbers of apoptotic cells in the skin, indicating diminished cutaneous GVHD (Fig. 4D, 4E).

**Discussion**

In this paper, we demonstrate that P-selectin−/− allo-BMT recipients are resistant to the development of GVHD, which suggests that P-selectin of recipient origin is an important molecule for GVHD pathophysiology. Furthermore, in cognate experiments, we observe that donor T cells deficient for PSGL1, the most well described ligand for P-selectin, had a surprisingly intact potential to cause GVHD, suggesting that donor T cells can use multiple ligands, in addition to PSGL1, to mediate their interactions with P-selectin on vessel endothelium during the inflammatory processes associated with acute GVHD.

**FIGURE 4.** P-selectin−/− allo-BMT recipients have decreased GVHD damage to the liver, skin, and small intestines. A, WT or P-selectin−/− recipients received TCD-BMT supplemented with splenic T cells (5 × 10⁶ LP BM + 1 × 10⁶ LP T cells). At day 35 posttransplant, the liver and small bowels were harvested, paraffin-embedded, analyzed by H&E-stained slides, and scored for histopathological damage. WT group, n = 12; P-selectin−/− group, n = 7. B and C, Lethally irradiated WT or P-selectin−/− recipients received T cell-replete BMT as in A. Small and large bowels were fixed, embedded in paraffin, sectioned, stained with H&E, and analyzed by microscopy (original magnification ×100). Small blue cells in the crypts and lamina propria are lymphocytes. Representative sections are shown. WT group, n = 12; P-selectin−/− group, n = 7. D, WT or P-selectin−/− recipients received T cell-replete BMT as above, and on day 35 posttransplant, the skin from ears and tongue was harvested, paraffin embedded, and analyzed for numbers of apoptotic cells. Circles represent number of apoptotic cells per 10 mm² for an individual animal. WT group, n = 12; P-selectin−/− group, n = 7. E, Representative micrographs of damage to the skin in WT and P-selectin−/− allo-BMT recipients on days 28 and 35 posttransplant (original magnification ×400). Experiment as in D. Arrows indicate apoptotic keratinocytes.
First, we observed that alloreactive T cells in WT versus P-selectin \(^{-/-}\) allo-BMT recipients demonstrate comparable levels of alloreactivity (activation markers in vivo and T cell proliferation in vitro), apoptosis, and expression of PSGL1. However, upon enumerating donor T cells in mice with GVHD, we noted that compared with the situation in WT recipients, donor alloreactive T cells in P-selectin \(^{-/-}\) recipients accumulated in increased numbers in lymphoid tissues and decreased numbers in GVHD target tissues such as the small intestine. By contrast, nontransplanted WT B6 and P-selectin \(^{-/-}\) animals have fairly similar numbers of T cells in lymphoid and nonlymphoid organs.

The observation that P-selectin \(^{-/-}\) recipients had more donor-alloactivated T cells in the spleen, secondary lymphoid organ (SLO), and peripheral blood after allo-BMT was initially surprising, in light of the enhanced survival of these mice. Yet although P-selectin has been implicated in the trafficking of T cells into inflamed organs (11, 22, 23), no data exist regarding its involvement in lymphoid tissue trafficking, which is instead mediated by the PLN addressins (24), CD62L (25), CCR7 (26), and LFA-1 (27). We therefore believe that P-selectin may be important for the trafficking of alloreactive T cells into nonhematopoietic tissues such as the gut or liver during GVHD but relatively dispensable for the trafficking of T cells into lymphoid organs.

We were surprised to note that despite a difference in overall mortality, there was no difference in colonic GVHD pathology or degree of lymphocytic or neutrophilic infiltrates in the colons of WT and P-selectin \(^{-/-}\) allo-BMT recipients. However, a number of reports may explain this finding. The first is the observation that in murine models of chronic colitis, T cells require CD18, but not PSGL1, to cause disease, suggesting that in our model systems, there simply may not be a direct requirement for P-selectin for its involvement in lymphoid tissue trafficking, which is instead mediated by the PLN addressins (24), CD62L (25), CCR7 (26), and LFA-1 (27). Indeed, nontransplanted WT and PSGL1 \(^{-/-}\) mice displayed similar leukocyte cellularity in lymphoid and nonlymphoid tissues, and numbers of WT and PSGL1 \(^{-/-}\) donor T cells were also comparable in lymphoid and nonlymphoid tissues posttransplant in mice with GVHD.

In conclusion, our paper suggests that although recipient P-selectin is an important molecule for the pathophysiology of GVHD, multiple P-selectin ligands on donor T cells may be important for their trafficking and tissue infiltration. Consequently, ablation of PSGL1 alone on donor T cells may not be sufficient to abrogate their interactions with recipient P-selectin. Our results suggest that targeting P-selectin in allo-BMT recipients, or multiple P-selectin ligands on donor T cells and leukocytes, may represent a novel therapeutic strategy for GVHD prophylaxis or treatment.

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Disclosures

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References


