Critical Role of Donor Tissue Expression of Programmed Death Ligand-1 in Regulating Cardiac Allograft Rejection and Vasculopathy

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Background—Allograft vasculopathy is a major limiting factor in the long-term success of cardiac transplantation. T cells play a critical role in initiation of cardiac allograft rejection and allograft vasculopathy. The negative T-cell costimulatory pathway PD-1:PDL1/PDL2 (programmed death-1:programmed death ligand-1/2) plays an important role in regulating alloimmune responses. We investigated the role of recipient versus donor PD-1 ligands in the pathogenesis of allograft rejection with emphasis on the role of tissue expression in regulating this alloimmune response in vivo.

Methods and Results—We used established major histocompatibility complex class II– and class I–mismatched models of vascularized cardiac allograft rejection, blocking anti-PDL1 and anti-PDL2 antibodies, and PDL1- and PDL2-deficient mice (as donors or recipients) to study the role of the PD-1:PDL1/PDL2 pathway in chronic rejection. We also used PDL1-deficient and wild-type mice and bone marrow transplantation to generate chimeric animals that express PDL1 exclusively on either hematopoietic or parenchymal cells. PDL1 but not PDL2 blockade significantly accelerated cardiac allograft rejection in the bm12-into-B6 and B6-into-bm12 models. Although wild-type cardiac allografts survived long term, PDL1−/− donor hearts transplanted into wild-type bm12 mice exhibited accelerated rejection and vasculopathy associated with enhanced recipient T-cell alloreactivity. Interestingly, PDL1−/− recipients did not exhibit an accelerated tempo of cardiac allograft rejection. Using chimeric animals as donors, we show that PDL1 expression on cardiac tissue alone significantly prolonged graft survival compared with full PDL1+/− donor grafts in transplanted wild-type recipients.

Conclusions—This is the first report to demonstrate that expression of the negative costimulatory molecule PDL1 on donor cardiac tissue regulates recipient alloimmune responses, allograft rejection, and vasculopathy. (Circulation. 2008;117:660-669.)

Key Words: transplantation ■ immunology ■ lymphocytes ■ rejection

Transplantation is now firmly established as the treatment of choice for many forms of end-stage organ failure.1 Despite better short-term outcomes, a similar improvement in long-term allograft survival has not occurred. Chronic allograft dysfunction secondary to an ongoing alloimmune response is a major cause of late allograft loss. T-cell costimulatory signals have been shown to play a critical role in determining the fate of T-cell activation by alloantigen.2 T-cell receptor recognition of an appropriately presented antigen delivers signal 1 to the T cell, and a simultaneously delivered signal 2 (the costimulatory signal) is required to fully activate the T cell. Our understanding of the functions of T-cell costimulatory pathways is undergoing an evolutionary process.3 It is now clear that in addition to provision of positive signals to promote T-cell activation, T-cell costimulatory pathways can provide negative signals that inhibit T-cell activation and terminate immune responses.2 Furthermore, costimulatory ligands expressed on parenchymal cells play important roles in the regulation of immune responses in the target organ.4

Clinical Perspective p 669

Modulation of T-cell costimulatory function has been shown to improve graft survival and function in models of
chronic allograft dysfunction. However, these approaches have been limited to blocking positive T-cell costimulation through treatment of the recipient, rather than modifying the context of antigen presentation by donor tissue. Here, we studied the role of negative costimulatory molecule expression within donor allograft versus recipient in the regulation of the alloimmune response in vivo.

The PD-1 (programmed death-1):PDL1/PDL2 (programmed death ligand-1 and -2) pathway is a novel negative costimulatory pathway that belongs to the CD28-B7 family. PD-1 is expressed on activated T, B, and myeloid cells. PD-1 interacts with its ligands, PDL1 and PDL2. Murine PDL1 and PDL2 share 38% amino acid identity and, like other B7 family members, have characteristic extracellular IgV- and Ig constant-like domains. PDL1 is expressed on resting cells and upregulated on activated B, T, myeloid, and dendritic cells and on many nonhematopoietic tissues such as heart, placenta, pancreas, brain, muscle, and endothelial cells.

Previous in vitro studies have suggested that overexpression of PDL1 on endothelial cells plays a critical and differential role in regulating CD4+ and CD8+ T-cell–mediated immune responses. PD-1-PDL1/PDL2 interaction plays an important role in regulating alloimmune responses in vivo, but the role of recipient versus donor PD-1 ligands, and specifically parenchymal expression of these ligands, has not been explored. In the present report, we describe for the first time that expression of the negative costimulatory ligand PDL1 in the donor graft serves as an important negative regulatory mechanism that limits the alloimmune response in vivo.

Methods

Mice
C57BL/6 (H2b, B6), B6.C-H2h12/KhEgJ (bm12), and B6.C-H2h11/J ByJ (bm1) mice were purchased from The Jackson Laboratory (Bar Harbor, Me). PDL1−/− and PDL2−/− (obtained from T.S.) mice on the B6 background were maintained as a breeding colony in our animal facility. All mice were used at 8 to 12 weeks of age and housed in accordance with institutional and National Institutes of Health guidelines.

Heterotopic Heart Transplantation
Vascularized heart grafts were placed in an intra-abdominal location by use of microsurgical techniques as described by Corry et al. Graft function was assessed by palpation of the heartbeat. Rejection was determined by complete cessation of palpable heartbeat and was confirmed by direct visualization after laparotomy. Graft survival is shown as the median survival time (MST) in days.

In Vivo Monoclonal Antibodies and Treatment Protocol
Anti-PDL1 (MIH6) monoclonal antibody (mAb) was generously provided by M.A. Anti-PDL2 (TY25) mAb was generously provided by H.Y. The antibodies were manufactured and purified from the original hybridomas by a commercial source, Bioexpress Cell Culture (West Lebanon, NH). In vivo use of the anti-mouse PDL1 mAb (MIH6) and PDL2 mAb (TY25) in transplantation models has been described previously by our group. Monoclonal antibodies were administered intraperitoneally according to the following protocol: 0.5 mg of mAb on the day of transplantation and 0.25 mg on days 2, 4, 6, and 8 after transplantation, as described previously.

ELISpot Assay
Splenocytes from transplanted bm12 recipient mice were harvested at 3 weeks after transplantation and stained with fluorescent-labeled mAbs against CD4, CD8, CD62 ligand (CD62L), and CD44 (BD Biosciences, San Jose, Calif). Flow cytometry analysis was performed with a FACSCaliber system (BD Biosciences) and analyzed with CellQuest software. Percentages of effector CD4 and CD8 T cells expressing the CD44high/CD62L− phenotype as described previously.

Flow Cytometry
Splenocytes were obtained from recipient bm12 mice at 3 weeks after transplantation and stained with fluorescein-labeled mAbs against CD4, CD8, CD62 ligand (CD62L), and CD44 (BD Biosciences, San Jose, Calif). Flow cytometry analysis was performed with a FACSCaliber system (BD Biosciences) and analyzed with CellQuest software. Percentages of effector CD4 and CD8 T cells expressing the CD44high/CD62L− phenotype as described previously.

Morphology
Cardiac graft samples from transplanted mice harvested at 3 weeks after transplantation were fixed in 10% formalin, embedded in paraffin, coronally sectioned, and stained with hematoxylin and eosin for evaluation of the degree of rejection according to International Society of Heart and Lung Transplantation (ISHLT) guidelines and for cellular infiltration and vasculopathy by light microscopy. The severity of vasculopathy was graded according to the percentage of luminal occlusion by intimal thickening with a scoring system described previously. Briefly, a vessel score of 0 indicated a normal artery; 1, <10% luminal occlusion; 2, 20% to 50% luminal occlusion; and 3, >50% luminal occlusion. Only vessels that were cut orthogonally and displayed a clear internal elastic lamina were scored. An examiner blinded to the groups read all the samples.

Bone Marrow Chimeric Mice
Bone marrow was isolated from B6 WT and PDL1−/− donor mice. Red blood cells were lysed with ACK lysing buffer, and a single-cell suspension of the bone marrow–derived cells in sterile PBS was prepared at 4×10^7/mL. Four-week-old recipient WT and PDL1−/− mice were lethally irradiated with a single dose of 1100 rad. Twenty-four hours after irradiation, the recipients were infused intravenously with 2×10^7 bone marrow cells. The PDL1−/− recipients were infused with WT (chimera 1), and WT recipients were infused with PDL1−/− bone marrow (chimera 2). Using 4-color flow cytometry analysis, we confirmed chimerism by analyzing the percentage of PDL1 expression on dendritic cells, macrophages, B cells, natural killer cells, CD4+ T cells, and CD8+ T cells in blood and peripheral lymphoid organs. Six weeks after irradiation and bone marrow reconstitution, the mice were euthanized and the hearts harvested into WT bm12 recipients. At the time of transplantation, blood, lymph nodes, and spleens were harvested from the chimeras and analyzed by flow cytometry. The following antibodies were used for fluorescence-activated cell sorting analysis: anti-CD4-peridinin chlorophyll-b protein complex (PerCP), anti-CD8-allophycocyanin (APC), anti-NK1.1 FITC, anti-CD11c APC, anti-CD19 FITC (all from BD Pharmingen, San Diego, Calif); anti-F4/80 APC (Caltag, Carlsbad, Calif); and anti-PDL1 PE (Bioscience, San Diego, Calif).

Statistical Analysis
Graft survival was expressed graphically by the Kaplan-Meier method, and statistical differences in survival between groups were assessed by the log-rank test. Student t test was used for comparison of means. P<0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
Results

PDL1 Blockade Accelerates Cardiac Allograft Rejection in the Major Histocompatibility Complex Class II–Mismatched Transplantation Model
First, we examined the role of the PD-1:PDL1/PDL2 pathway in chronic cardiac allograft rejection using the single major histocompatibility complex (MHC) class II–mismatched bm12-into-B6 and B6-into-bm12 cardiac transplant models. In these established models,26,27 most cardiac allografts survive indefinitely (>8 weeks) but develop T-cell–mediated chronic allograft rejection characterized by cellular infiltration, vasculopathy, and fibrosis. PDL1 blockade by anti-PDL1 mAb accelerated allograft rejection in the bm12-into-B6 (MST = 34.5 days, n = 4, P = 0.0032; Figure 1A) and B6-into-bm12 (MST = 23 days, n = 4, P = 0.0069; Figure 1B) models compared with untreated mice. The bm12 cardiac grafts had comparable survival in both WT B6 and PDL1-deficient recipient mice (C). Cardiac allograft rejection in bm12 recipients was dramatically accelerated when PDL1-deficient B6 donors were used compared with WT B6 donors, whereas PDL1 heterozygous and PDL2-knockout donor grafts did not experience accelerated allograft rejection (D). Furthermore, anti-PDL1 or anti-PDL2 antibody treatment of bm12 recipients did not further shorten graft survival compared with untreated bm12 recipients of PDL1-knockout grafts.

PDL1 Deficiency in Donor but Not Recipient Leads to Accelerated Cardiac Allograft Loss
To dissect the relative role of donor versus recipient PDL1 expression in alloimmune responses in the chronic rejection model, WT or PDL1-deficient mice on a B6 background were used as donors (B6-into-WT bm12) or recipients (WT bm12-into-B6) of cardiac allografts. WT bm12 cardiac allografts had comparable survival in both WT and PDL1-deficient B6 recipients (Figure 1C). In contrast, PDL1-deficient B6 cardiac allografts were rejected in a significantly accelerated manner (MST = 16.5 days, n = 6, P = 0.0015; Figure 1D) compared with B6 cardiac grafts from WT littermates (MST > 56 days, n = 6) in bm12 recipients. PDL2-deficient donor grafts did not experience accelerated rejection (Figure 1C and 1D), which indicates that PDL1 expression on donor grafts serves a negative regulatory mechanism for limiting the alloimmune response in vivo. Furthermore, additional anti-PDL1 or anti-PDL2 antibody treatment did not further shorten graft survival compared with untreated bm12 recipients of PDL1-deficient grafts.

Pathological Evaluation of Rejected PDL1-Deficient Cardiac Allografts
The histology of WT and PDL1-deficient cardiac allografts harvested 2 to 3 weeks after transplantation (time of clinical rejection as defined by cessation of heart beats) was compared (Figure 2). ISHLT grade of rejection (Figure 2A), degree of cellular infiltration (Figure 2B), and vasculopathy...
score (Figure 2C) were all significantly higher in PDL1-deficient heart allografts than in WT allografts. Figure 2D represents an accelerated form of vasculopathy compared with a typical vasculopathy score of H11015/2.0 at 8 weeks.29

Donor PDL1 Deficiency Enhances Recipient Alloreactivity

To address the mechanism by which the absence of donor PDL1 accelerates rejection, we measured the frequency of IFN-γ– and IL-4–producing alloreactive T cells using an established ELISpot assay.18 Responder splenocytes from bm12 recipients were harvested 3 weeks after transplantation and separately stimulated with irradiated naïve B6 WT or PDL1-deficient splenocytes. As shown in Figure 3A and 3B, splenocytes from bm12 mice transplanted with PDL1-deficient hearts exhibited a significantly increased frequency of alloreactive IFN-γ– and IL-4–producing cells compared with bm12 mice transplanted with WT B6 hearts. A similar response was seen irrespective of whether B6 WT or PDL1-deficient stimulators were used.

Next, we compared the generation of effector CD4+ and CD8+ T-cell populations (CD62L<low>CD44<high> phenotype) 3 weeks after transplantation in spleens of bm12 recipients of WT or PDL1-deficient heart allografts (Figure 3C and 3D). Flow cytometry analysis showed that the frequency of CD4+ effector T cells was significantly increased (by ≈48%) in bm12 mice transplanted with PDL1-deficient B6 hearts (49.43±2.47%, P=0.0355) compared with WT (33.23±4.57%). No difference was present in the frequency of CD8+ effector T cells in WT (35.98±5.20%) versus PDL1<−/−> (36.83±8.130%, P=0.9). Taken together, these data indicate for the first time that donor graft PDL1 deficiency enhances recipient alloimmune responses by expansion and activation of alloreactive CD4+ T cells in vivo.

Effect of PDL1 Deficiency in an MHC Class I–Mismatched Heart Allograft Model

Next, using the B6-into-bm1 heart transplantation model, we studied the effect of PDL1 deficiency in donors in the MHC class I–mismatched transplant setting, in which allograft rejection is mediated primarily by CD8+ T cells. Allografts were rejected at an earlier time point (MST=45, n=4, P=0.0011) than with controls (MST≥56, n=6) and PDL2-deficient (MST≥56, n=4) allografts when transplanted into bm1 recipients (Figure 4A). Interestingly, compared with the MHC class II–mismatched model (MST=16.5, n=6), the time of rejection of these grafts was delayed significantly (P=0.0046). These findings suggest that PDL1 expression in donor tissue affects both CD4+– and CD8+–mediated allograft rejection.

The histology of WT and PDL1-deficient cardiac allografts harvested 3 weeks after transplantation was compared (Figure 4B through 4E). There was demonstrable accelerated vasculopathy in PDL1-deficient allografts (PDL1<−/−>) compared with minimal vasculopathy in WT (Figure 4B). ISHLT grade of rejection (Figure 4C), degree of cellular infiltration (Figure 4D), and vasculopathy score (Figure 4E) were all significantly higher in PDL1-deficient heart allografts than in WT
allografts. Although the vasculopathy score was relatively low, hearts harvested at 8 weeks after transplantation have a mean vasculopathy score of 0.55/0.18 (unpublished data), which probably reflects a predominantly CD8+ response.

The frequency of IFN-γ– and IL-4–producing alloreactive T cells was also measured by ELISpot (Figure 5A and 5B). WT bm1 mice were transplanted with WT B6 or PDL1-deficient B6 heart allografts. Splenocytes from bm1 mice were isolated 3 weeks after transplantation and then separately challenged with irradiated WT or PDL1-deficient stimulators. The bm1 mice that received PDL1-deficient B6 heart allografts showed a significant increase in IFN-γ and IL-4 production compared with bm1 mice that received WT B6 heart allografts. This response was seen when both WT and PDL1-deficient splenocytes were used as stimulator cells. A similar response was seen with PDL1-knockout stimulator cells.

Next, we compared the generation of effector CD4+ and CD8+ T-cell populations (CD62LlowCD44high phenotype) 3 weeks after transplantation in spleens of bm1 recipients of WT or PDL1-deficient heart allografts (Figure 5C and 5D). Splenocytes harvested from bm1 mice that had been transplanted previously with PDL1-deficient cardiac allografts had significantly increased CD8+ effector T-cell generation compared with bm1 recipients transplanted with WT cardiac allografts (P<0.05; Figure 5C). In contrast to the MHC II–mismatch model, no significant difference could be found in the generation of CD4+ effector T cells between WT and PDL1-knockout transplanted bm1 mice (data not shown), which indicates that PDL1 can regulate the generation of both CD4 and CD8 effector T cells. The data also suggest that whether CD4+ or CD8+ effector T cells are regulated appears also to be dependent on which cells have a dominant role in a particular experimental model.

PDL1 Deficiency on Nonlymphoid Tissue of Donor Plays a Dominant Role in Accelerating Allograft Rejection

Because PDL1 is expressed on both bone marrow–derived and parenchymal cells, including endothelial cells, the maintenance of graft survival in this model may be mediated by expression of PDL1 on either bone marrow–derived passenger leukocytes, nonlymphoid heart tissue, or both. To determine the role of PDL1 expression on nonlymphoid heart tissues versus passenger leukocytes in regulating the alloimmune response in the present model, we created chimeric mice by reconstituting PDL1-deficient mice with bone marrow from WT mice and WT mice with bone marrow from PDL1-deficient mice, as described previously. In the former resulting bone marrow chimera (chimera 1), PDL1 is ex-
pressed on passenger leukocytes but not on cardiac nonlymphoid tissues. The latter chimera (chimera 2) has the opposite expression pattern of PDL1. Chimerism was confirmed by flow cytometry analysis for percentage of PDL1-positive cells in blood and peripheral lymphoid organs (Figure 6A); as expected, WT and chimera 1 splenocytes showed high levels of PDL1 expression (74.6 ± 4.2% and 71.4 ± 5.2%) in contrast to PDL1-knockout and chimera 2 splenocytes (6.7 ± 1.4% and 10.5 ± 4.5%). The percentages of PDL1-positive dendritic cells (CD11c), macrophages (F4/80), and B cells (CD19) in the spleens of chimera 1 and 2 animals (Figure 6B) were 62.1% versus 11.8%, 71% versus 16%, and 72.1% versus 11.6%, respectively.

Hearts were transplanted from chimeric mice into WT bm12 recipients. Figure 7 shows that PDL1 expression on nonlymphoid heart tissues (chimera 2), but not on passenger leukocytes (chimera 1), resulted in significant prolongation (P = 0.0046) of the survival of full PDL1-deficient allografts transplanted into bm1 recipient 3 weeks after transplantation. In contrast to PDL1-knockout and chimera 2 splenocytes, PDL1-knockout and chimera 2 splenocytes showed high levels of PDL1 expression (74.6 ± 4.2% and 71.4 ± 5.2%) in contrast to PDL1-knockout and chimera 2 splenocytes (6.7 ± 1.4% and 10.5 ± 4.5%). The percentages of PDL1-positive dendritic cells (CD11c), macrophages (F4/80), and B cells (CD19) in the spleens of chimera 1 and 2 animals (Figure 6B) were 62.1% versus 11.8%, 71% versus 16%, and 72.1% versus 11.6%, respectively.

Hearts were transplanted from chimeric mice into WT bm12 recipients. Figure 7 shows that PDL1 expression on nonlymphoid heart tissues (chimera 2), but not on passenger leukocytes (chimera 1), resulted in significant prolongation (P = 0.0046) of the survival of full PDL1-deficient allografts transplanted into WT bm12 recipients. However, survival of cardiac allografts from chimera 2 was not restored to that of WT controls, which suggests that PDL1 expression on both nonlymphoid heart tissues and passenger leukocytes contributes to regulation of alloimmunity, with PDL1 expression on donor nonlymphoid tissue playing a dominant role in regulating the alloimmune response in the present model.

**Discussion**

We first studied the role of the PD-1:PDL1/PDL2 pathway in the pathogenesis of cardiac allograft rejection and vasculopathy using the established single MHC class II–mismatched (bm12-B6) model in which rejection is mediated by CD4+ T cells. In this model, the allografted hearts generally continue to beat for >56 days without immunosuppression but develop allograft vasculopathy. We showed that blockade of PDL1 by administration of anti-PDL1 mAb accelerated cardiac allograft rejection, and in particular, allograft vasculopathy, the sine qua non of chronic cardiac rejection.30,31 Koga et al16 previously demonstrated increased severity of graft arterial disease with anti-PDL1–treated recipients but not accelerated rejection in the same model. These disparities may be reconciled by differences in the dosing regimen of the anti-PDL1 antibodies used in the 2 studies. In the study by Koga et al,16 in which anti-PDL1 mAb was administered in 2 dosing regimens, animals treated with 100 µg/d twice weekly for 4 weeks developed minimal graft arterial disease, whereas those that received 200 µg/d developed severe vascular lesions. In the present study, we treated animals with higher doses of anti-PDL1 mAb early after transplantation (500 µg on day 0 and 250 µg on days 2, 4, 6, and 8 after transplantation). Taken together, the results clearly indicate that in this model, PDL1 plays an important role in regulating CD4+ T-cell–mediated alloimmune responses against cardiac allografts. These results are also consistent with those reported by Ito et al18 in a fully MHC-mismatched model of acute cardiac allograft rejection.

We also used the B6-into-bm1 MHC class I–mismatched model to confirm the universal nature of our observations. In this model, as in the B6-into-bm12 model, accelerated allo-
graft loss occurred in PDL1-deficient donors, with associated allograft vasculopathy. When PDL1-knockout B6 cardiac donors were transplanted into bm1 mice, allograft loss was accelerated to a mean of 45 days, with pathological evidence of cellular infiltration and allograft vasculopathy (Figure 4A), which confirms the role of PDL1 in dampening the aggressive alloimmune response against the allograft.

In support of these findings, we have also recently shown that when PDL1-deficient donors are used in recipients of fully MHC-mismatched cardiac allografts treated with CTLA4Ig to block B7 and induce long-term graft survival, they develop severe allograft vasculopathy compared with WT hearts, even though both heart grafts in both groups survived indefinitely.32 Collectively, these data establish a critical role of donor PDL1 in regulating the cytopathic alloimmune response that results in cardiac allograft rejection and vasculopathy.

The most important and novel finding in the present report is the observation that donor but not recipient deficiency of PDL1 accelerated allograft rejection and vasculopathy in more than 1 transplantation model. This acceleration was associated with enhanced recipient alloreactivity, as evidenced by increased frequency of IL-4– and IFN-γ–producing alloreactive T cells and by expansion of effector T cells.

These results are consistent with recent observations by Keir et al4 in the autoimmune diabetes model in nonobese diabetic mice showing that islet cell expression of PDL1 regulated the autoimmune response against islet autoantigens in vivo. PDL1 is constitutively expressed on lymphocytes, macrophages, and dendritic cells and is further upregulated on activation.2,33 In addition, PDL1 expression has been detected on parenchymal cells, including vascular endothelial cells.22,34–36 Because progressive transplant arteriosclerosis is considered the primary manifestation of chronic rejection, it has been postulated that the donor vascular endothelium is the primary target of the chronic alloimmune response.37,38 This viewpoint is supported by the unique immunologic properties of endothelial cells. Human endothelial cells constitutively express MHC I and MHC II molecules, whereas murine endothelia constitutively express MHC I and express MHC II on stimulation with cytokines such as IFN-γ and therefore can activate recipient T cells both by the direct and indirect pathways of allore cognition.39 A comparison of the T-cell stimulatory effect of endothelial cells and other non–bone marrow–derived cells such as fibroblasts, smooth muscle cells, and epithelial cells that also express MHC-II indicates that the T-cell stimulatory property is unique to endothelium. Therefore, the expression of PDL1 on vascular endothelium

Figure 5. A and B, Frequency of alloreactive cytokine-producing cells in bm1 recipients of cardiac allografts from WT and PDL1-knockout littermates. WT bm1 mice were transplanted with WT B6 or PDL1-deficient B6 grafts. Splenocytes from bm1 recipients were isolated at 3 weeks and then separately challenged with irradiated WT stimulators. Frequencies of IFN-γ–producing (A) and IL-4–producing (B) T cells were then determined by ELISpot assay. Data represent mean±SEM of triplicate wells from 3 mice per group. The bm1 recipients of PDL1-deficient B6 allografts showed a significant increase in both IFN-γ and IL-4 production compared with bm1 mice that received WT B6 grafts (P<0.005 in both groups). A similar response was seen with PDL1-knockout stimulator cells (P=0.18 and P<0.005, respectively). C and D, Flow cytometry analysis of effector memory CD8+ T-cell generation. CD8+ effector T cells were identified by their staining characteristics (CD8+CD44highCD62Llow). Splenocytes harvested from bm1 mice that had been transplanted previously with PDL1-deficient cardiac allografts had significantly increased CD8+ effector memory T-cell generation compared with bm1 recipients transplanted with WT cardiac grafts (P<0.05; C). Panels are representative examples of dot plots demonstrating the percentage of CD8+ effector memory T cells obtained from WT (45.7%) and PDL1 knockouts (66.4%), respectively.
of the donor organ is strategically positioned to downregulate recipient alloreactive T-cell responses by interaction with the PD-1 receptor expressed on alloactivated recipient T cells. Indeed, antibody blockade of endothelial cell PDL1 has been shown to enhance endothelial cell costimulation CD8⁺ T cells. However, because donor organs also contain passenger leukocytes that express PDL1 and can trigger an alloimmune response in the recipient, we set out to dissect the contribution of PDL1 expression on donor tissue parenchyma versus donor passenger leukocyte in regulating T-cell alloreactivity. Interestingly, using bone marrow chimeras, we showed that PDL1 expression on parenchymal cells but not passenger leukocytes prolonged survival of PDL1-deficient cardiac allografts compared with full PDL1-deficient grafts. Another important finding in the present study is that PDL2 does not appear to play an important role in regulating the alloimmune response against cardiac allografts. These results are consistent with previously published data in the fully allogeneic cardiac transplantation model. Importantly, PDL2 expression is restricted to antigen-presenting cells, especially dendritic cells. These results provide further support for our findings, Grabie et al recently demonstrated an important role for PDL1 in protecting against autoimmune injury. Their studies show that PDL1 expressed on cardiac endothelial cells plays an important role in downregulating a cytotoxic T-cell–mediated form of transient myocarditis.

Taken together, these studies demonstrate a novel function of the negative T-cell costimulatory molecule PDL1 expressed on parenchymal (endothelial) cardiac tissue in regulating both autoimmune and alloimmune T-cell–mediated injury. These results have important implications for the development of therapeutic strategies aimed at manipulation of PDL1 expression on heart tissue to prevent or treat immune-mediated diseases, including development of allograft vasculopathy, by manipulating the donor.

Sources of Funding
This study was supported in part by the following grants: National Institutes of Health AI-56299 and AI-51559 (Dr Sayegh), AI-51664 and National Kidney Foundation clinical scientist award (both to Dr Chandraker), Transplantation Society basic science fellowship (Dr Popoola), and American Society of Transplantation basic science faculty grants (Drs Ansari, Guleria, and Yuan).

Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Chronic allograft vasculopathy is characterized by the gradual occlusion of arteries within a transplanted organ and is caused by an ongoing immune response against the allograft. It is also the hallmark of chronic rejection, a leading cause of loss of function of transplanted hearts and all other solid-organ transplants. This immune response is orchestrated by T lymphocytes. In recent years, we have learned more about the pathways that control the activation of T cells, and a number of positive, and more recently negative, costimulatory pathways have been characterized. The PD1-PDL1 (programmed death-1–programmed death ligand-1) pathway is now recognized as an important negative costimulatory pathway capable of downregulating an aggressive T-cell response. Manipulation of T-cell costimulatory pathways has been shown to be an effective means of reducing T-cell activation and inducing immune tolerance to transplanted organs. PDL1 is expressed not only on circulating bone marrow–derived cells but also on tissue parenchyma, which leads to the intriguing possibility that the modification of donor tissue expression of PDL1 might influence graft survival. The present study confirms this idea by showing that the absence of donor tissue expression of PDL1 accelerates transplant rejection in a mouse cardiac transplantation model. The corollary to this would be that an increase of expression of PDL1 on donor tissue might be able to dampen the immune response against the transplanted organ. Targeting of PDL1 could be achieved by gene therapy or by the use of drugs that upregulate expression of this molecule and is attractive because it avoids the need for treatment of the recipient, with all the concomitant nonspecific consequences.
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*Circulation*. 2008;117:660-669; originally published online January 22, 2008; doi: 10.1161/CIRCULATIONAHA.107.741025

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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