Abstract—Cardiac allograft vasculopathy (CAV) is a form of coronary arterial stenosis and a leading cause of death in patients who survive beyond the first year after heart transplantation. Histopathologically, this lesion is concentric diffuse intimal hyperplasia of the arterial wall that is accompanied by extensive infiltration of inflammatory cells, including T cells. Many studies have explored the potential risk factors related to this arterial lesion and its pathogenesis. Continuous minor endothelial cell damage evokes inflammatory processes including T cell activation. Costimulatory molecules play crucial roles in this T cell activation. Many costimulatory pathways have been described, and some are involved in the pathogenesis of CAV, atherogenesis, and subsequent plaque formation. In this review, we summarize the present knowledge of the role of these pathways in CAV development and the possibility of manipulating these pathways as means to treat heart allograft vascular disease and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2006; 26:1447-1456.)

Key Words: transplantation ♦ rejection ♦ T cell–mediated immunity ♦ arteriosclerosis ♦ atherosclerosis ♦ smooth-muscle cell

Cardiac transplantation provides long-term survival for patients with end-stage heart disease. The percentage of patients surviving to 1 year after heart transplantation continues to increase; however, the percentage of patients surviving beyond 1 year has not changed significantly over the past 20 years.1 Long-term functional deterioration of allografts is caused by chronic rejection. The pathologic features of chronic rejection include reduced vessel size and parenchymal fibrosis. Development of cardiac allograft vasculopathy (CAV) has been a major cause of morbidity and mortality following heart transplantation.1 The mechanism of CAV is not known despite extensive basic and clinical studies; however, inflammation and immunity are known to be associated with the pathogenesis of CAV. CAV lesions contain immune competent cells; among these cells, activated T lymphocytes are the most conspicuous. Therefore, T cell-mediated immunity and subsequent inflammation appear to be an important feature of initiation and progression of CAV. There are similarity and difference among CAV, atherosclerosis, and restenosis after balloon angioplasty as shown in Table 1. The pathophysiology of CAV should be recognized in a spectrum of wide range of arterial lesions.

Risk Factors for and Treatment of Graft Vasculopathy
One of the major risk factors for CAV is the episodic frequency and severity of acute rejection. Donor–recipient differences in major histocompatibility complexes and ineffective immunosuppression increase the risk.2,3 Nonimmunologic factors are also known to confer risk. In cases of kidney transplantation, cadaveric donor kidneys are more likely to have stenotic arterial lesions than living-related donor kidneys, suggesting the importance of ischemia/reperfusion injury in the pathophysiology of allograft vasculopathy.4 Both clinical and experimental studies have indicated that cytomegalovirus infection promotes CAV.5 Factors influencing endothelial function, such as hyperlipidemia, diabetes, hypertension, and high donor age, are also known to increase the risk of CAV.6 (Figure 1).

Treatment of CAV is controversial. Although a variety of pharmacological interventions has been applied,7–11 their effects are limited and these agents have not achieved popularity. Catheter-based coronary interventions have been reported,12 but the results are not satisfactory because the coronary lesions are not segmental; they are diffuse. To save a patient’s life, cardiac re-transplantation is sometimes performed, but the survival rate is worse than that for first-time transplantation.

Immunomodulatory Agents and CAV
The effects of immunosuppressive drugs have been investigated experimentally and clinically. Cyclosporine and tacrolimus bind to the intracellular cytosolic immunophilins, cyclo-
philin and FK binding protein 12, respectively, inhibiting calcineurin phosphatase. This prevents transcription of cytokines such as IL-2 and progression of the T cell cycle from G0 to G1. Early experimental studies demonstrated that cyclosporine inhibits smooth muscle proliferation. Clinically, triple-drug therapy with cyclosporine, steroid, and azathioprine has been a standard immunosuppressive treatment for 20 years and is effective in suppressing acute rejection. However, this drug combination has little effect on the development of CAV. Observation of 256 patients revealed a positive correlation between coronary intimal thickness and low daily doses of cyclosporine dose, but the clinical usefulness of cyclosporine in cases of chronic cardiac allograft rejection remains controversial. The role of tacrolimus in preventing CAV is also unclear. A randomized prospective trial in which 160 recipients were followed-up for 4 years failed to detect any statistical difference in the development of CAV between patients given cyclosporine and those given tacrolimus.

Sirolimus (rapamycin) interferes with DNA and protein synthesis and arrests the cell cycle of T cells in G1 phase. A significant dose-dependent reduction in intimal thickening in rat cardiac allografts after sirolimus treatment was reported. This interesting result can be explained by the potent inhibitory effects of sirolimus on growth factor-mediated proliferation of smooth muscle cells. Administration of 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors (statins) has been associated with a reduced incidence of severe rejection episodes and reduced progression of CAV in patients. Animal experiments showed that this effect was independent of cholesterol reduction and may be associated with inhibition of major histocompatibility complex class II antigens or leukocyte function associated-antigen (LFA)-1 expression. The precise mechanism is yet to be determined. Peroxisome proliferator-activated receptor γ is expressed in macrophages, T cells, endothelial cells, and smooth muscle cells. Our recent observation revealed a potent effect of its agonist, pioglitazone, in the suppression of acute as well as chronic rejection of cardiac allografts in animal models.

Pathology of CAV

In cases of CAV, the stenotic coronary artery in the allograft shows concentric diffuse thickening of the intima (Figure 2). Experimental models of this condition have revealed that the cellular component of the thickened neointima comprises

<table>
<thead>
<tr>
<th>TABLE 1. Comparison Among 3 Types of Coronary Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
</tr>
<tr>
<td>Longitudinal Diffuse lesion</td>
</tr>
<tr>
<td>Circumferential Concentric</td>
</tr>
<tr>
<td>Type of lesion</td>
</tr>
<tr>
<td>Intimal hyperplasia</td>
</tr>
<tr>
<td>Plaque (rupture)</td>
</tr>
<tr>
<td>Rare</td>
</tr>
<tr>
<td>Calcification</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Time course</td>
</tr>
<tr>
<td>Months to years</td>
</tr>
<tr>
<td>Chest pain</td>
</tr>
<tr>
<td>Rare</td>
</tr>
<tr>
<td>Risk factors</td>
</tr>
<tr>
<td>Cytomegalovirus infection, acute rejection, cold ischemia</td>
</tr>
<tr>
<td>Prevention/treatment</td>
</tr>
<tr>
<td>None (retransplantation)</td>
</tr>
</tbody>
</table>

![Figure 1. Schematic representation of our model for the pathogenesis of cardiac allograft vasculopathy (CAV) illustrating the central role of immune activation. CMV indicates cytomegalovirus.](Image)

![Figure 2. Pathology of severe cardiac allograft vasculopathy affecting 3 major coronary arteries and small intramyocardial arterioles from an 8-year-old boy who received a heart allograft at age 1 year. Elastica van Gieson staining. A, Right coronary artery (RCA); B, Left circumflex artery (LCX); C, Left anterior descending artery (LAD); D, Small arterioles in the myocardium.](Image)
smooth muscle cells. These cells express the embryonic-type smooth muscle myosin heavy chain. This increase in expression of the synthetic myosin heavy chain isozyme is accompanied by a decrease in the contractile myosin isozyme (SM2). Recent studies revealed that some of the proliferated smooth muscle cells in the thickened neointima originate from smooth muscle cell progenitor cells from the recipient’s bone marrow. Whatever the origin of the smooth muscle cells, cell cycle regulatory genes are activated to promote their proliferation. In the early stages of CAV, there are macrophages that sometimes contain lipid deposits, and these macrophages resemble the foam cells found in atherosclerosis. There is a significant infiltration of T lymphocytes of various subsets expressing CD4 and CD8. These lymphocytes are found not only in the thickened intima but also in the perivascular areas. Expression of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 is enhanced in endothelial cells in the area of CAV. In animal models of CAV, expression of matrix metalloproteinase (MMP)-2 is enhanced in smooth muscle cells in the thickened neointima and media, and that of tissue inhibitors of MMP is decreased. In the later phases of clinical CAV, focal atherosclerotic plaques develop in the coronary arteries. These plaques can destabilize and rupture, like atheromatous plaques.

Similar chronic changes in renal allografts are known as chronic allograft nephropathy. Pathologically this condition includes tubular atrophy, interstitial fibrosis, and fibrous intimal thickening of the vessel lumen. Fibrous intimal thickening involves smooth muscle cell proliferation and increased lipid-rich matrix in the intima of the arterial lumen, changes that are quite similar to the pathological changes that characterize CAV.

Hypothesis for the Mechanism Underlying Development of CAV: Involvement of T Cell–Mediated Immunity

The risk factors and pathohistological features of clinical and experimental CAV strongly suggest that immune responses are involved in development of CAV (Figure 1). The initiation of this process includes endothelial damage and dysfunction. Ischemia/reperfusion injury, acute rejection episodes, and cytomegalovirus infection can cause vascular inflammation (endothelitis and arteritis). Immunohistological studies showed both humoral and cell-mediated immunity are involved in the development of CAV. It has been reported that development of CAV is minimal in allografts transplanted into B cell-deficient mice that cannot produce immunoglobulins. The infiltration by T lymphocytes in the transplanted into B cell-deficient mice that cannot produce immunoglobulins. The infiltration by T lymphocytes in the thickened neointima originate from smooth muscle cell progenitor cells from the recipient’s bone marrow. Whatever the origin of the smooth muscle cells, cell cycle regulatory genes are activated to promote their proliferation. In the early stages of CAV, there are macrophages that sometimes contain lipid deposits, and these macrophages resemble the foam cells found in atherosclerosis. There is a significant infiltration of T lymphocytes of various subsets expressing CD4 and CD8. These lymphocytes are found not only in the thickened intima but also in the perivascular areas. Expression of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 is enhanced in endothelial cells in the area of CAV. In animal models of CAV, expression of matrix metalloproteinase (MMP)-2 is enhanced in smooth muscle cells in the thickened neointima and media, and that of tissue inhibitors of MMP is decreased. In the later phases of clinical CAV, focal atherosclerotic plaques develop in the coronary arteries. These plaques can destabilize and rupture, like atheromatous plaques.

Similar chronic changes in renal allografts are known as chronic allograft nephropathy. Pathologically this condition includes tubular atrophy, interstitial fibrosis, and fibrous intimal thickening of the vessel lumen. Fibrous intimal thickening involves smooth muscle cell proliferation and increased lipid-rich matrix in the intima of the arterial lumen, changes that are quite similar to the pathological changes that characterize CAV.

Hypothesis for the Mechanism Underlying Development of CAV: Involvement of T Cell–Mediated Immunity

The risk factors and pathohistological features of clinical and experimental CAV strongly suggest that immune responses are involved in development of CAV (Figure 1). The initiation of this process includes endothelial damage and dysfunction. Ischemia/reperfusion injury, acute rejection episodes, and cytomegalovirus infection can cause vascular inflammation (endothelitis and arteritis). Immunohistological studies showed both humoral and cell-mediated immunity are involved in the development of CAV. It has been reported that development of CAV is minimal in allografts transplanted into B cell-deficient mice that cannot produce immunoglobulins. The infiltration by T lymphocytes in the early stages of CAV suggests that these cells interact with damaged graft endothelial cells and sustain the chronic immune response to the injured vessel wall. Many cytokines, chemokines, and other humoral factors play important roles in these processes, and during these processes, T lymphocytes are activated.

There are data that support the involvement of T lymphocytes and the interaction of T lymphocytes with human leukocyte antigen (HLA) in the progression of CAV. The indirect pathway of antigen recognition by T cells in CAV development has been described. In this pathway, T cells recognize processed peptides derived from the recipient’s antigen-presenting cells. In contrast, in the direct pathway, T cells recognize alloantigen directly without antigen presentation. In experimental models, isografted hearts seldom develop intimal hyperplasia, and the degree of major histocompatibility complex difference is crucial in the extent of CAV development in murine models of cardiac allografts. Depletion of CD4 but not CD8 T lymphocytes prevents development of CAV. One interesting experimental model is re-transplantation of allografts to the donor strain at an early stage after allografting. These allografts showed continuous progression of CAV even after retransplantation of the heart graft into the donor strain. These results suggest that initial allogeneic stimulation at an early stage after transplantation is crucial for the development of CAV. In murine models of cardiac allografts, it is possible to induce immunologic tolerance by treatment with anti-LFA-1 and anti–ICAM-1 monoclonal antibodies or anti-CD154 antibodies and CTLA4Ig. In these animals, T lymphocytes become anergic against alloantigens and cannot respond to allostimulation. Cardiac allografts in these animals are reported to be free from CAV. These data also suggest that activation of T lymphocytes is crucial in the development of CAV.

Once activated, T lymphocytes produce a variety of cytokines including IL-2, interferon-γ (IFNγ), and tumor necrosis factor-α (TNF-α). IL-2 promotes proliferation of T lymphocytes, and IFNγ acts on endothelial cells and other potential antigen-presenting cells to express major histocompatibility complex class II antigens. Among these cytokines, IFNγ appears to be particularly important. Mice deficient in IFNγ or treated with antibody to IFNγ do not develop CAV, even though these recipients can reject parenchymal tissues. IFNγ can also induce artherosclerotic changes in the absence of detectable T cells by acting on vascular smooth muscle cells to potentiate growth-factor-induced mitogenesis. However, administration of recombinant IFNγ in experimental models of vascular injury inhibits cell proliferation, as does IFNγ addition to vascular smooth muscle cell cultures unless serum-free conditions are used. A major effect of IFNγ in eliciting vascular remodeling is to prime macrophages for activation. Therefore, development of CAV, but not parenchymal rejection, requires IFNγ. These cytokines also activate donor endothelial cells and promote expression of adhesion molecules such as ICAM-1 and VCAM-1. These adhesion molecules facilitate recruitment of T lymphocytes and macrophages to the site of CAV. In the presence of these cytokines and adhesion molecules, a variety of growth factors which include platelet-derived growth factor, fibroblast growth factor (FGF), TGF, insulin-like growth factor, and others, are secreted from activated endothelial cells and infiltrating cells. However, a recent study shows that the only significant effect of platelet-derived growth factor on atherosclerotic lesions is to inhibit T cell activation in the lesions. These factors stimulate the proliferation and migration of smooth muscle cells to promote intimal thickening. Recent investigations using apolipoprotein E (apoE) or low-density lipoprotein receptor knockout mice demonstrated that abrogation of
TGFβ signaling increased the size of atheroma and reduced the content of smooth muscle cells and collagen in the lesion. Accumulation of extracellular matrix is involved in this process, and endothelial thrombogenic activity increases. Therefore, activation of T lymphocytes and interaction of T lymphocytes with endothelial cells and smooth muscle cells are involved in the initiation and development of CAV.

**T Lymphocyte Activation and Costimulatory Signals**

Optimal activation of T lymphocytes requires costimulatory signals from antigen-presenting cells in addition to the interaction of T cell receptor with major histocompatibility complex antigen on antigen-presenting cells (Figure 3). Signaling through the T-cell receptor without an appropriate costimulation leads to T cell anergy or apoptosis. The costimulatory signal is not antigen specific and is derived from cell surface molecules on antigen-presenting cells and on T lymphocytes. Simultaneous engagement of the T cell receptor and costimulatory receptor–ligand interaction results in the activation of NFκB and leads to production of IL-2 that allows expansion of a specific T lymphocyte clone, and promotes survival of T cells. The interaction between the costimulatory molecules and antigen-presenting cells is not a single event. Many costimulatory factors are involved in various facets of T lymphocyte activation and inactivation. CD28-mediated signaling has been investigated as a major costimulatory signal for T cells; however, mice without CD28 signaling have normal immune responses suggesting that other costimulatory molecules can substitute for CD28.

Other molecules that have been examined for costimulatory activity belong to the B7 family or the TNF/TNFR family (Tables 2 and 3). The B7 family includes CD28, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), inducible costimulator (ICOS), programmed death-1 (PD-1), and B and T lymphocytes attenuator. The TNF/TNFR group includes CD40, OX40, herpes virus entry mediator (HVEM), 4-1BB (CD137), CD27, CD30, and glucocorticoid-induced TNFR-related gene. These molecules provide positive secondary signals for T cell activation or transduce negative signals that downregulate T cell responses. The functions of these signals are differentially regulated depending on the disease and situation. Therefore, stimulation and/or blockade of these molecules show promise as therapeutic applications for control of pathological situations, including cancer, infection, transplantation, autoimmunity, and vascular diseases.

**Costimulatory Molecules and CAV**

Evidence for the effects of T cell activation in CAV and atherosclerosis has been reported. A considerable effort has focused on CD28-B7 and/or CD40-CD154 mediated T cell costimulation. Also, a small number of recent investigations

**TABLE 2. Features of CD28, B7 Family Costimulatory Receptors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>CD28</th>
<th>CTLA-4</th>
<th>ICOS</th>
<th>PD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>T</td>
<td>T</td>
<td>T, NK</td>
<td>T, B, M</td>
</tr>
<tr>
<td>Non-lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular signal</td>
<td>PI3K</td>
<td>SHP-2</td>
<td>PI3K</td>
<td>SHP-1</td>
</tr>
<tr>
<td></td>
<td>Grb2</td>
<td></td>
<td></td>
<td>SHP-2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ligand</th>
<th>B7–1 (CD80)</th>
<th>B7–2 (CD86)</th>
<th>ICOSL</th>
<th>PD-L1 (B7-H1)</th>
<th>PD-L2 (B7-DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>B, M, DC, T</td>
<td>B, DC, T, monocyte</td>
<td>DC, M, T, B</td>
<td>DC, M</td>
<td></td>
</tr>
<tr>
<td>Non-lymphoid</td>
<td>Fibroblast</td>
<td>SMC</td>
<td>SMC</td>
<td>SMC</td>
<td></td>
</tr>
</tbody>
</table>

| | Endothelium | Endothelium | Endothelium | Endothelium |
| | Epithelium | Epithelium | Epithelium | Epithelium |

B indicates B cell; DC, dendritic cell; M, macrophage; NK, natural killer cell; SMC, smooth muscle cell; T, T cell.
are showing involvement of ICOS-ICOS ligand, PD-1-PD-Ligand, and HVEM-LIGHT pathways in arterial lesions.

**CD28-B7 Pathway**

The B7-1/B7-2:CD28/CTLA-4 pathway is the best characterized T cell costimulatory pathway. CD28 transduces positive signal for T cell activation and survival, whereas CTLA-4 delivers a negative signal for T cell responses.\(^6^9\) It was reported that CD28-mediated signal is inhibited by CTLA-4Ig, a recombinant fusion protein that contains the extracellular domain of human CTLA-4 fused to a human IgG heavy chain. In a rat model of cardiac transplantation, CTLA-4Ig reduced the frequency and severity of CAV in comparison with cyclosporine A-treated rats. This attenuation was accompanied by a reduction in IFN\(\gamma\), monocyte chemoattractant protein, inducible nitric oxide synthase, galactose/N-acetylgalactosamine macrophage lectin, and TGF\(\beta\).\(^7^0\) Similar results with CTLA-4Ig\(^7^1,7^2\) and anti-CD28 monoclonal antibody\(^7^3\) have been reported in chronic cardiac or renal allograft rejection in small animals. These investigations are the initial reports showing that T cell recognition of alloantigens is a central event in initiation of chronic rejection and that T cell costimulation could be a target to prevent chronic rejection. Interestingly, blockade of this pathway by CTLA-4Ig late after transplantation is also an effective means to attenuate CAV. Rat cardiac transplant recipients treated with a short course of cyclosporine followed by injection of CTLA-4Ig at 1 to 2 months after transplantation showed reduced CAV, infiltration of mononuclear cell infiltration, and parenchymal fibrosis.\(^7^4\) This observation supports the idea that continuous T cell recognition of alloantigens and T cell activation are mediators of intimal hyperplastic changes in chronic allograft rejection.

The importance of the CD28 pathway in atherogenesis has been shown in mice lacking both apoE and CD28\(^7^5\) and in humans.\(^7^6\) Double knockout mice lacking both B7-1/B7-2 and low-density lipoprotein receptors show significant reduction in early development of diet-induced atherosclerotic lesions.\(^7^7\)

**CD40-CD154 Pathway**

CD40 is expressed on a variety of cell types including macrophages, dendritic cells, B cells, and endothelial cells.\(^7^8\) CD154 is expressed on activated T cells and platelets. Blockade of this pathway either alone or together with the B7-CD28 pathway inhibits autoimmune diseases\(^7^9\) and leads to long-term allograft survival in small and large animal transplantation models.\(^4^4,8^0\) There is evidence that CAV is attenuated when this pathway is inhibited in animal models of cardiac transplantation.\(^8^1,8^2\) Clinical analysis of CAV lesions in heart transplant recipients revealed that CD154 was expressed by infiltrating lymphocytes and that CD40 was expressed by intimal endothelial cells, foam cells, macrophages, and smooth muscle cells.\(^8^3\) This overexpression of CD40 was accompanied by ICAM-1 and VCAM-1 expression in endothelial cells.

The role of the CD40-CD154 pathway in CAV development is still controversial. CD154 monoclonal antibody therapy alone fails to prevent development of CAV in some models.\(^8^4,8^5\) CD154\(^-/-\) transplant recipients develop allo-specific tolerance to the donor hearts, but these allografts show significant CAV by 8 to 12 weeks after transplantation.\(^8^6\) Thus, low-level alloresponses may trigger vascular responses that ultimately result in graft failure even in recipients in whom donor-specific tolerance is induced. Other investigators have reported that blockade of the CD40-CD154 pathway targets predominantly CD4\(^+\) T cells and does not prevent CD8\(^+\) T cell-mediated immune responses. However, even in the absence of CD8\(^+\) T cells, CD154 blockade did not prevent formation of CAV.\(^8^7\) Using this situation, the role of IL-4 in the CAV in absence of CD40-CD154 costimulation is shown in a model of murine abdominal aortic allografts.\(^8^5,8^8\)

The role of the CD40-CD154 interaction in atherogenesis has been the focus of much research.\(^8^9-9^1\) T lymphocytes within the atherosclerotic vessel wall express CD154 and functional CD40. Low-density lipoprotein receptor knockout mice treated with anti-CD154 antibody for 12 weeks showed profound reduction in the areas of atherosclerotic lesions.\(^8^9\) CD154 /apoE double-knockout mice exhibited a decrease in plaque area.\(^9^0,9^2\) This signaling pathway is involved in upregulation of expression of matrix metalloproteinases and procoagulant tissue factors and subsequent development of plaque rupture and thrombosis.\(^9^3-9^5\) However, it should be noted that recent attempts

---

**TABLE 3. Features of TNFR Family Costimulatory Receptors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>OX40</th>
<th>CD27</th>
<th>CD30</th>
<th>4-1BB</th>
<th>LIGHT</th>
<th>GITR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>T</td>
<td>T, B, NK</td>
<td>T, B, NK, eosinophil</td>
<td>T, DC, NK, monocyte</td>
<td>T, NK, monocyte</td>
<td>T</td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medulla of thymus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intracellular signal</strong></td>
<td>TRAF2, 3, 5</td>
<td>TRAF2, 3, 5</td>
<td>TRAF1, 2, 3, 5</td>
<td>TRAF1, 2</td>
<td>TRAF1, 2, 3, 5</td>
<td>TRAF1, 2, 3</td>
</tr>
<tr>
<td><strong>Ligand</strong></td>
<td>OX40L</td>
<td>CD70</td>
<td>CD30L</td>
<td>4-1BBL</td>
<td>HVEM</td>
<td>GITRL</td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>B, DC, T</td>
<td>B, DC, T</td>
<td>B, T</td>
<td>B, DC, M</td>
<td>T, DC, monocyte</td>
<td>B, M, DC</td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
<td>Human neuron</td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-lymphoid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
to treat large animals and patients with anti-CD154 led to thrombotic side effects probably because of the dense expression of CD154 on platelets.

ICOS/ICOS Ligand Pathway

ICOS is a member of the CD28/CTLA-4 family and is expressed on activated T cells. Stimulation of the ICOS pathway promotes secretion of IFNγ, IL-4, and IL-10. Inhibition of the ICOS pathway with anti-ICOS antibody or the soluble form of ICOS (ICOSIg) prolongs cardiac allograft survival in a murine model, and combined treatment with anti-ICOS antibody and cyclosporine A or ICOSIg and CTLA-4Ig prolongs cardiac allograft survival indefinitely and prevents development of CAV. ICOS ligand, also known as B7-related protein 1 (B7RP-1), is expressed constitutively on B cells and in peripheral lymphoid tissues. In vitro studies revealed that ICOS ligand expression is induced on fibroblasts treated with TNF-α and that it is expressed constitutively on endothelial cells and is upregulated by treatment with IL-1β or TNF-α. Although ICOS and CD28 signaling upregulate Th1 and Th2 cytokines, ICOS does not upregulate IL-2 production. Therefore, ICOS stimulates T cell effector function but not T cell clonal expansion.

Treatment of cardiac allografts with ICOSIg with blockade of the CD40 ligand/CD40 pathway attenuates development of CAV in mice. Our experiments revealed that ICOS ligand expression is induced on smooth muscle cells of thickened intima in CAV and treatment of recipient mice with either ICOSIg or anti-ICOS antibody suppresses development of CAV. Similar findings showing the importance of delayed blockade of this pathway have been reported by another laboratory. The authors speculate that delayed blockade of this pathway allows generation of regulatory mechanisms while inhibiting activation of effector/memory T cells. Because ICOS and ICOS ligand are not expressed in normal tissues and expression is induced during immune activation, this pathway may be a suitable target for prevention of CAV and other arterial lesions.

HVEM-LIGHT Pathway

LIGHT (homologous to lymphotoxins, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator [HVEM], a receptor expressed on T lymphocytes) was described as a member of the TNF superfamily. LIGHT is expressed in peripheral blood mononuclear cells, including T and B cells, natural killer cells, monocytes, and granulocytes, and binds to HVEM and lymphotoxin β receptor (LTβR). Although LTβR is not expressed by T or B cells, HVEM is expressed by lymphocytes and endothelial cells. In vitro studies revealed that the interaction of LIGHT with HVEM is involved in T cell proliferation, cytokine production, and activation of NF-κB. In a murine cardiac transplantation model, LIGHT-deficient recipient mice showed prolonged allograft survival. Our recent studies have shown that the LIGHT pathway is important in regulating development of CAV in organ transplant recipients. Blockade of the LIGHT pathway with HVEMIg significantly attenuates the development of CAV.

Interactions between activated T cells and smooth muscle cells are complex. Previous in vitro studies showed that T cells promote smooth muscle cell proliferation via IFNγ. However, other studies show that IFNγ potently inhibits smooth muscle cell proliferation under standard cell culture conditions. Another study has demonstrated bidirectional effects of IFNγ on smooth muscle cells depending on culture conditions. In addition, production of basic fibroblast growth factor and heparin-binding epidermal growth factor-like growth factor, which are potent growth stimuli for smooth muscle cells, in response to T cells is reported. We cocultured smooth muscle cells from a Bm12 donor and sensitized T cells from B/6 mice that reject cardiac allografts from Bm12 mice. Smooth muscle cells proliferated in response to IL-1β stimulations, and this response was enhanced by coculture with the sensitized T cells. HVEMIg suppressed in vitro smooth muscle cell proliferation in response to activated T cells from rejected cardiac allografts, and this suppression is accompanied by reduced transcription of IFNγ and IL-6.

Negative Regulators of T Cell Activation

Costimulatory molecules that negatively regulate T cells have been described. These inhibitory receptors include CTLA-4, PD-1, and B and T lymphocytes attenuator, which are all expressed on lymphocytes. PD-1 is a member of the CD28 family and was identified in a T cell line undergoing programmed cell death; however subsequent studies have shown that its expression is associated with lymphocyte activation rather than cell death. PD-1 activation leads to downregulation of immune responses, and deficiency results in loss of peripheral tolerance to self antigens. In contrast to CTLA-4, which plays central roles in lymphoid organs, PD-1 regulates inflammatory responses in peripheral tissues. PD-L1 (ligand for PD-1) and PD-1 negatively regulate CD8 T cell responses. The role of PD-1 in the development of CAV is still controversial. PD-L1Ig promotes long-term graft survival in CD28− recipients and markedly reduces CAV when given in conjunction with anti-CD154 monoclonal antibody. Expression of PD-L1 is also induced in endothelial cells and smooth muscle cells in response to IFNγ. We observed that administration of anti-PD-L1 monoclonal antibody into mice with a cardiac allograft enhanced the progression of CAV. IFNγ expression by cardiac allografts was increased in response to anti-PD-L1 monoclonal antibody treatment. An in vitro study revealed that activated T cells from recipient mice bearing rejecting allografts increased proliferation of smooth muscle cells, and that anti-PD-L1 monoclonal antibody increased this proliferation. Further studies are needed to clarify the differential roles of this and other costimulatory pathways.

Other Pathways

There are many other costimulatory pathways important for T cell activation. 4-1BB (CD137) is a costimulatory molecule of T cells and a member of the TNFR family; 4-1BB is expressed on activated T cells. In conjunction with a strong signal through the T cell receptor, 4-1BB/4-1BB ligand interactions can provide critical costimulatory signals either
in the absence or presence of CD28.\textsuperscript{119} In a murine model of cardiac transplantation, administration of anti–4-1BB ligand antibody modestly prolonged allograft survival.\textsuperscript{120} Recent investigations have revealed the involvement of OX40, which is expressed primarily on CD4\textsuperscript{+} T cells, in the development of atherosclerosis.\textsuperscript{121} OX40 ligand is found to be present on mouse atherosclerotic lesions; however, their role in CAV has not been investigated.\textsuperscript{122} Other costimulatory molecules include glucocorticoid-induced TNFR-related gene and B and T lymphocytes attenuator. Their important roles in T cell costimulation, peripheral tolerance, inflammation, both pro- and anti-apoptotic effects, and development of immune system have been described; however, their effects on vascular immunology and CAV have not been reported to date.

**Clinical Implications**

These costimulatory pathways play a pivotal role not only in T cell activation but also in regulating smooth muscle cell proliferation. The contributions of these pathways to other acute and chronic inflammatory cardiovascular diseases should be the subject of future studies. Although these findings support the idea that these pathways may be targets for clinical therapeutic interventions for attenuating development of vascular lesions, several issues should be addressed. Because there are many pathways and because the individual roles of these pathways are unclear, future studies should focus on the differential and collaborative roles of these pathways in regulating T cell activation and subsequent CAV development. The roles of these costimulatory molecules in the human immune system have been a matter of investigation because majority of the data have been obtained with murine models. It is reasonable to assume that the processes of activation and inactivation of T cells in humans are more redundant and complex than those in mice.

Another future approach to use the T cell costimulatory pathways is tolerance induction to immunogens in arterial lesions. Blockade of some of these costimulatory pathways has been shown to induce tolerance to alloantigens. If the autoantigens in atherosclerosis and alloantigens in CAV were identified, blockade of specific pathways could serve as a novel therapeutic strategy to prevent or treat CAV.

In conclusion, increasing evidence suggests the importance of costimulatory pathways for T cell activation in vascular biology. As mentioned in this review, these pathways are involved in the pathogenesis of not only CAV but also atherogenesis and restenosis after vascular injury. In this respect, investigation of T cell costimulation in CAV could provide important insights into the pathophysiology of a wide range of vascular diseases and could aid in the development of novel therapeutic interventions for vascular diseases.

**Acknowledgments**

The authors thank Professor Toshimitsu Uede of Hokkaido University for providing us invaluable materials and his stimulating discussions and Professor Jun Amano of Shinsha University for his collaboration. We also appreciate the secretarial assistance of Ma-hoko Watarai.

**Sources of Funding**

Our investigation was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), a grant-in-aid from the Japanese Ministry of Education, Science, and Culture, and a grant-in-aid from the Japanese Ministry of Welfare.

**Disclosures**

None.

**References**


90. Koseog A, Suzuki J, Gotoh R, Koga N, Ito H, Isobe M, Inobe M. Ude T. Induction of immunologic tolerance to cardiac allograft by simulta-


102. Marsters SA, Ayres TM, Skubatch M, Gray CL, Rothe M, Ashkenazi A. Inducible co-stimulator, a novel member of the B7 family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF-κB and AP-1.


107. T cell response.


112. Peoples GE, Blotnick S, Takahashi K, Freeman MR, Klagsbrun M. Blockade of 4–1BB (CD137)/4–1BB ligand interactions increases alloreactive T cell response.


T Cell Costimulation in the Development of Cardiac Allograft Vasculopathy: Potential Targets for Therapeutic Interventions
Mitsuaki Isobe, Hisanori Kosuge and Jun-ichi Suzuki

Arterioscler Thromb Vasc Biol. 2006;26:1447-1456; originally published online April 20, 2006; doi: 10.1161/01.ATV.0000222906.78307.7b
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/7/1447

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/