Cardiac Allograft Vasculopathy and Dysregulation of the NO Synthase Pathway

Michael Weis, John P. Cooke

Abstract—Cardiac allograft vasculopathy is the most aggressive form of atherosclerosis in humans and is the leading cause of death after the first year of heart transplantation. Endothelial dysfunction is a major contributing factor to the acceleration of coronary vascular disease in these individuals. A reflection of this endothelial dysfunction is the severe impairment in endothelium-dependent vasodilation that occurs early after transplantation. The etiology of this allograft endothelial alteration is multifactorial and may include preexisting atherosclerosis of the graft vessels, reperfusion injury during transplantation, denervation, disruption of the lymphatic system, and acute and chronic immune injury, as well as traditional risk factors for coronary artery disease (hyperlipidemia, diabetes, hypertension, or hyperhomocysteinemia) and pathogens, such as cytomegalovirus. The alteration in endothelial function affects vasomotor tone of the coronary arteries. Evidence indicates that there may be an impairment of endothelial production and/or activity of NO. Because NO is a potent vasodilator, its deficiency would explain the abnormal vasomotor tone in these individuals. In addition, because NO inhibits key processes in vascular inflammation and atherosclerosis, its absence may contribute to the acceleration of transplant vascular disease. Recent studies from our group and others have shed light on the mechanisms of endothelial dysfunction and its importance in cardiac allograft vasculopathy. In addition, the alteration in endothelial function contributes to vascular inflammation and progression of the disease. (Arterioscler Thromb Vasc Biol. 2003; 23:567-575.)

Key Words: endothelium ■ coronary ■ transplantation ■ heart ■ inflammation

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graft vasculature contribute to leukocyte invasion. A number of conditions occurring in the context of transplantation stimulate the expression of adhesion molecules and chemokines/cytokines, which participate in the inflammatory process.4,10–12 These predisposing conditions may include preservation/injury, ischemia/reperfusion, acute rejection, antibody deposition and complement fixation, hyperglycemia, hyperlipidemia, and pathogens such as cytomegalovirus (CMV).2,11–17 As a consequence, endothelial adhesion molecule and chemokine expression is upregulated, and vascular growth factors and thrombogenic molecules are expressed.7,18,19 Indirect alloresponse is likely to be permanently active because of the traffic of recipient dendritic cells through the graft.20 Disruption of the cardiac lymphatic system may result in decreased clearance of graft-infiltrating cells. The ongoing inflammation promotes allograft endothelial dysfunction and accelerates structural changes.12,21

**Endothelial Dysfunction: An Early Determinant of CAV**

Coronary endothelial vasodilator dysfunction is a common finding in cardiac transplant recipients and is an early marker for the development of intimal thickening and graft atherosclerosis. In 1988, Fish et al22 observed a paradoxical coronary vasoconstriction to acetylcholine in allograft recipients with and without angiographic evidence of CAV. Subsequently, other investigators have observed abnormal responses (vasoconstriction and/or impairment in coronary blood flow response) to serotonin, substance P, cold-pressor testing, and exercise.23–28 The impairment of endothelial function is time dependent. Early after transplantation, epicardial vasodilatation is preserved in response to tachycardia (reflecting the flow-mediated vasomotor response).29 By contrast, the vasomotor response to acetylcholine and cold-pressor test are often abnormal in the earliest period after transplantation.25,28 During follow-up, exercise-induced flow-mediated endothelial vasodilatation may become impaired.30 The prevalence of epicardial endothelial dysfunction (defined as a paradoxical vasoconstriction of >10% in response to acetylcholine) is ∼30% to 40% of patients during the first year and 35% to 45% at long term follow-up.31 Notably, endothelial function in any one subject may not be diffusely disturbed after cardiac transplantation.28 The existence of coronary segments with functioning endothelium indicates that the coronary endothelium is not globally impaired in all cardiac transplant recipients and that endothelial function may not be irreversibly damaged.28 Indeed, we have reported that intravenous administration of L-arginine acutely improves endothelial vasodilator function of coronary conduit vessels if given at an early stage of graft atherosclerosis.32 The prevalence of microvascular endothelial dysfunction is also time dependent.24,30,33 Early after transplantation, microvascular dysfunction is prominent in 20% of the patients and increases to ∼30% of the patients during long-term follow-up.31 Intriguingly, there is no significant correlation between the degree of epicardial dysfunction and that of microvascular dysfunction.34 Microvascular endothelial dysfunction occurs even in patients with angiographically normal epicardial coronary arteries. Thus, there appear to be some independent determinants of endothelial dysfunction in the epicardial and resistance vessels of the cardiac allograft.35

**Consequences of Allograft Vasomotor Dysfunction**

The endothelium is the maestro of the circulation, a major determinant of vascular tone and blood flow. The healthy endothelium releases a panoply of vasodilator substances, such as NO, prostacyclin, atrial natriuretic peptide, endothelium-derived hyperpolarizing factor, and adrenomedullin. Thus, the healthy endothelium increases vessel diameter and reduces resistance to blood flow. When the endothelium becomes diseased, the synthesis and bioactivity of the vasodilators are reduced, and the balance tips in favor of endothelium-derived vasoconstrictors, such as endothelin and thromboxane.36 This derangement in endothelial function has clinical consequences. As a result of the impairment in endothelial vasodilator function, there is an increase in coronary vascular resistance, which can result in ischemia. Hasdai et al37 have found that coronary endothelial dysfunction in humans is associated with reversible myocardial perfusion defects. In transplant recipients, an impaired coronary flow reserve has been associated with subsequent reduction in left ventricular ejection fraction during a 2-year follow-up,38 suggesting that repetitive subendocardial ischemia during myocardial stress can cause a deterioration of ventricular function. Consistently, heterogeneity of coronary vasodilator reserve is correlated with a significantly increased risk of cardiovascular events.39

As discussed below, endothelial vasodilator dysfunction is associated with other vascular abnormalities (eg, expression of adhesion molecules, leukocyte adherence and infiltration, and smooth muscle cell proliferation). Several groups have reported changes in allograft endothelial functions (vasomotor independent) that are correlated with allograft failure.19,40–44 Thus, endothelial vasomotor dysfunction might be a marker of endothelial activation, increased adhesiveness and thrombogenicity, and the risk of atherogenesis. Indeed, early epicardial endothelial vasodilator dysfunction predicts the development of visible vasculopathy (as imaged by intravascular ultrasound) 1 year after transplantation.45 This is consistent with reports that coronary endothelial dysfunction in transplant and nontransplant patients is predictive of adverse cardiovascular events.45,46 It is also possible that the loss of the vasodilator contributes directly to the progression of transplant vasculopathy.47 As it turns out, most of the endothelium-derived vasodilators oppose key processes involved in atherogenesis, ie, cell adhesion, proliferation, and inflammation.48 Endothelium-derived NO is paradigmatic of an endothelium-derived antiatherogenic molecule.

**Role of Endothelial NOS in Maintaining Vascular Homeostasis**

Endothelium-derived NO is the most potent endogenous vasodilator known.49 NO induces vasodilation by stimulating soluble guanylate cyclase to produce cGMP.50 NO has a short half-life and avidly interacts with sulfhydryl-containing proteins, heme proteins, and oxygen-derived free radicals. By virtue of its ability to nitrosylate proteins, it may change their activity or behavior.50 The physiological importance of this
endothelium-derived vasodilator is reflected by the significant increase in vascular resistance that is induced in animals and humans exposed to pharmacological antagonists of NO synthase (NOS).51,52

Endothelium-derived NO also inhibits platelet and leukocyte adherence to the vessel wall.53,54 This effect of NO is mediated in part by the activation of cGMP and phosphorylation of intracellular signaling proteins, such as vasodilator-stimulated phosphoprotein.55 In addition, NO suppresses the expression of adhesion molecules and chemokines regulating endothelial interaction with circulating blood elements. Finally, endothelium-derived NO also inhibits vascular smooth muscle cell proliferation.56 This is in part mediated by an effect of NO, an increase in vascular smooth muscle cell apoptosis.57 In contrast, NO is a survival factor for endothelial cells.58 These observations are consistent with the view that NO is an endogenous antiatherogenic molecule.

Impairment of endothelial NOS (eNOS) contributes to the pathological alterations in vascular reactivity and structure that are observed in atherosclerosis.59,60 Pharmacological inhibition or genetic deficiency of NOS inhibits endothelium-dependent vasodilation, impairs tissue blood flow, and raises the blood pressure.59 Furthermore, NO deficiency promotes the adherence and intimal accumulation of mononuclear cells and accelerates lesion formation in animal models of atherosclerosis.59,61 By contrast, enhancing NO production in the vessel wall slows or even reverses atherosclerosis or restenosis.62–64 As discussed below, endothelial NO bioactivity is also a modulator of CAV.

**Does NO Deficiency Play a Role in the Progression of Transplant Vasculopathy?**

In organ transplantation, allograft eNOS expression and activity can be impaired by a number of mechanisms, including preexisting arteriosclerotic disease in the graft, graft ischemia before transplantation, immunosuppressive agents such as cyclosporin A and tacrolimus, classic risk factors (hyperlipidemia, hypertension, diabetes, and hyperhomocysteinemia), and, possibly, infectious diseases such as CMV.

In preclinical models of transplant vasculopathy, NO deficiency accelerates the disorder. The inducible form of NOS (iNOS) is expressed in the vessel wall of the aortic allograft. Inhibition of iNOS activity in the aortic allograft significantly increases intimal hyperplasia at 4 weeks.65 Furthermore, early overexpression of iNOS by the use of ex vivo gene transfer completely prevents the development of structural changes in rejecting grafts.65,66 The protective effects of iNOS in these studies may be due to an effect of NO, ie, the inhibition of SMC proliferation and suppression of the adhesion of platelets and leukocytes to the endothelium.65,67 In the context of these observations, it should be noted that there has been some controversy regarding what role iNOS may have in the development of vascular lesions. We hold the view that under the right conditions, iNOS suppresses inflammation and atherogenesis. Specifically, under circumstances in which l-arginine is not rate limiting, the product of iNOS is NO. NO is a survival factor for endothelial cells, but it induces apoptosis of macrophages and proliferating vascular smooth muscle cells.68 Indeed, by increasing vascular NO generation with supplemental l-arginine, apoptosis of macrophages and regression of preexisting lesions is observed in the fat-fed New Zealand White rabbit.69 In this case, the major source of NO in the vessel wall is iNOS. However, under circumstances in which l-arginine becomes rate limiting, the product of iNOS is the superoxide anion (O2·−), which can increase local oxidative stress and exacerbate the inflammatory process. In human cardiac allografts, microvascular endothelial dysfunction is associated with an enhanced endomyocardial iNOS mRNA expression and is accompanied by the expression of nitrotyrosine protein, suggesting peroxynitrite-mediated vessel damage.70 Importantly, dietary l-arginine has been shown to attenuate the structural changes of transplant vasculopathy in vivo associated with downregulation of insulin-like growth factor-I and interleukin-6.71

The literature supports a protective role for eNOS. In a murine chronic-rejection model, transplant atherosclerosis is accelerated in aortic allografts of eNOS-deficient mice.72 Iwata et al73 have demonstrated that intraoperative liposome-mediated gene delivery of eNOS to rabbit donor hearts results in early gene expression sufficient to reduce ischemia/reperfusion injury by inhibiting nuclear factor-κB activation, adhesion molecule expression (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), and leukocyte infiltration.73 Enhanced eNOS expression extends graft survival without immunosuppression. Most important, eNOS immunoreactivity is gradually lost after human heart transplantation,74 and a reduced myocardial eNOS gene expression has been associated with coronary endothelial dysfunction.75 Thus, eNOS in the endothelium appears to protect allografts from endothelial activation and structural changes.

**Is Impairment of the NOS Pathway a Risk Factor for Vascular Disease in Humans?**

Do alterations in the NOS pathway contribute to the initiation and/or progression of atherosclerosis in humans? Several lines of evidence indicate that impairment of the NOS pathway does cause human coronary artery disease and vascular events. Genomic studies of the NOS pathway indicate that genetic alterations in NO or O2·− generation contribute to human vascular disease. Certain eNOS gene polymorphisms are predictive of coronary artery disease.76,77 The eNOS gene Glu298Asp polymorphism is more prevalent in patients with variant angina, essential hypertension, and acute myocardial infarction.78–80 Similarly, a quadruple repeat of a 27-bp sequence in intron 4 of the eNOS gene (allele a) is associated with the risk of coronary artery disease and acute myocardial infarction.81 There is an interaction with smoking, with homozygotes for the eNOSa allele at risk for greater severity of coronary artery disease. Of note, it has been reported that the eNOSa allele is associated with a decrease in the level of plasma nitrogen oxides.82 These studies suggest that genetically determined alterations in the NOS pathway could predispose an individual to atherosclerosis. Recently, several groups have independently offered...
evidence that endothelial vasodilator dysfunction is an independent predictor of vascular events.83–85 These data provide a compelling rationale for understanding the mechanisms of NO deficiency, with a view toward developing new therapeutic avenues to prevent coronary artery disease and its progression.

Mechanisms of NO Deficiency in CAV
Hypercholesterolemia, hypertriglyceridemia, hyperhomocysteinemia, hypertension, and hyperglycemia are all conditions that are associated with the posttransplant state, mainly triggered by the use of cyclosporine or tacrolimus and steroids for immunosuppression.13,15,86 Each of these conditions is associated with endothelial vasodilator dysfunction.

Of note, different immunosuppressive strategies have been shown to elicit different expression patterns of vasoactive mediators in the allograft.79 Specifically, the combination of tacrolimus and azathioprine is associated with decreased myocardial eNOS gene expression and endothelial dysfunction early after transplantation.75 In addition, coronary endothelial vasomotor dysfunction is associated with an increased myocardial expression and elaboration of the vasoconstrictor endothelin.87 Significantly, endothelin immunostaining is increased in coronary arteries affected by CAV.88 The early administration of an endothelin antagonist was associated with lower prevalence of functional and morphological abnormalities in a rat transplant model.89 Moreover, inhibition of the endothelin-1-converting enzyme significantly improved survival.90 Inasmuch as endothelial dysfunction appears to be a predictor of morphological changes,25 one might conclude that an imbalance between NO and endothelin bioactivity in the allograft may contribute to the development of CAV.

Cardiac cytokine release, a hallmark of allograft inflammatory activity and a common phenomenon early after heart transplantation, is related, at least in part, to endothelial vasomotor dysfunction of the epicardial and microvascular compartment.12 We found an association between an early elevation of coronary sinus levels of inflammatory cytokines and endothelial vasomotor dysfunction during a 1-year follow-up (M. Weis, unpublished data, 2002).

With respect to inflammation and vascular disease, increasing attention has been focused on the role of human CMV, a member of the herpesviruses.91–95 CMV can infect human vascular endothelial cells and induce changes relevant to atherogenesis.94 CMV infection increases the expression of endothelial surface adhesion molecules, which upregulate the recruitment of granulocytes.94 Furthermore, CMV infection promotes mononuclear adhesion, activation, and transendothelial migration within the allograft vasculature.96 CMV infection also shifts the balance between endothelial factors mediating blood fluidity so that a procoagulant state is favored.96 It appears that CMV infection of the endothelium promotes processes that favor atherogenesis and vascular lesion formation. Indeed, human CMV is associated with transplant vasculopathy.96–98 The most direct evidence of a link between CMV and transplant atherosclerosis was recently produced by Valantine et al.99 In their study, prophylactic treatment of cardiac transplant recipients with ganciclovir reduced the incidence of vasculopathy.99 Thus, a therapy directed toward CMV infection dramatically improves the outcome of patients after transplantation.

These data suggest that CMV may contribute to the initiation and/or progression of transplant vasculopathy. However, the mechanisms by which CMV may trigger atherogenesis are incompletely defined. The immediate-early gene of human CMV can code for a protein that has sequence homology and immunologic cross-reactivity with a domain of human leukocyte antigen-DR.97 Additionally, CMV interferes with the action of p53, a protein that inhibits proliferation and induces apoptosis of vascular smooth muscle cells.98 One of the major mechanisms by which CMV could initiate and/or accelerate transplant vasculopathy is by impairing the NOS pathway. Inflammation impairs endothelium-dependent vasodilation in humans,12,100 and the virus-induced impairment of the eNOS pathway might accelerate atherosclerosis.101 Indeed, in the hypercholesterolemic mouse, infection with murine forms of chlamydia accelerates plaque growth.102

It is possible that CMV infection could downregulate eNOS expression as well as activity. Tumor necrosis factor (TNF)-α has been reported to destabilize mRNA message for eNOS, possibly by inducing the expression of a binding protein for the 3′ untranslated region of eNOS mRNA.103 CMV infection of cells stimulates the expression of TNF-α104,105 as well as transcription factors (such as nuclear factor-κB) that stimulate the expression of TNF-α. Parenthetically, NO may inhibit viral replication in the THP-1 monocytic cell line.106 In addition to the effects of TNF-α on eNOS message stability, we and others have found that exposure of endothelial cells to TNF-α reduces NO synthesis and bioactivity.107 These effects appear to be due to the increased elaboration of asymmetric dimethylarginine (ADMA) and O₂⁻, respectively (see discussion below). Furthermore, TNF-α may induce the endothelial expression of iNOS. The induction of iNOS does not necessarily increase endothelial NO production. This is because in the setting of inflammation and/or metabolic disturbances associated with vascular disease (eg, hypercholesterolemia), there are deficiencies of the cofactor tetrahydrobiopterin as well as the precursor, l-arginine.108,109 In the absence of tetrahydrobiopterin, NO is no longer capable of transferring electrons to l-arginine to produce NO; instead, the preferred electron acceptor becomes oxygen, to form O₂⁻, a phenomenon known as eNOS uncoupling.110 These data support the hypothesis that human CMV infection, one of the most common infectious complications in allograft recipients, may contribute to the development of CAV via interaction with the NOS system.

A Role for ADMA?
The impairment in endothelium-dependent vasodilation occurs early in the course of vascular disease and affects both conduit and resistance vessels.111–113 The impairment in endothelium-dependent vasodilation is multifactorial and dependent on the vessel and species studied, the stage of atherosclerosis, and the associated metabolic disorders. The mechanism of impairment may include endothelial generation of O₂⁻ and increased degradation of NO, elaboration of
vascular prostaglandins, reduced NO synthase activity, and endothelin, reduced elaboration of prostacyclin, and/or impaired biosynthesis of NO.\textsuperscript{60,114}

Impaired biosynthesis of NO may be due to alterations in the activity of enzymes responsible for its synthesis, such as nitric oxide synthase (NOS), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS).\textsuperscript{104,115} Another important factor is the availability of the cofactor tetrahydrobiopterin, or to inactivating enzymes such as dimethylarginine dimethylaminohydrolase (DDAH).\textsuperscript{107,128,129}

Recently, Weis and Cooke have shown that ADMA is a major regulator of NOS activity.\textsuperscript{571} They found that ADMA binds to NOS and inhibits its activity, which results in reduced NO production.

Central role of impaired NO bioactivity and endothelial dysfunction in allograft failure. Reduced endothelial NO bioactivity is mediated by impaired DDAH activity and peroxynitrite formation. Allograft coronary endothelial dysfunction and/or intimal thickening, as well as donor-derived coronary artery disease, may trigger cardiovascular events. AT II indicates angiotensin II; IP3, inositol 1,4,5-tris-phosphate; DAG, diacylglycerol; MAPK, mitogen-activated protein kinase; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemo tactic protein-1; ET-1, endothelin-1; PDGF, platelet-derived growth factor; and IGF-1, insulin-like growth factor-1; DMA, dimethylarginine; Citr, citrulline.

Therapeutic Options
These recent insights indicate a significant contribution of endothelial dysfunction to the initiation and progression of atherosclerosis and CAV. Strategies to preserve normal endothelial function are likely to be useful therapies after heart transplantation.\textsuperscript{133} For coronary atherosclerosis, endothelial protective agents such as the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) and ACE inhibitors have already been proven to reduce cardiac events.}
mortality in patients with coronary atherosclerosis. Statin therapy may be associated with a lower prevalence of graft endothelial dysfunction in humans, independent of lipid-lowering effects.\textsuperscript{134} Enhancement of myocardial eNOS expression, as well as anti-inflammatory actions of statins, may be particularly beneficial in the transplant setting.\textsuperscript{133} ACE inhibitors have been shown to reverse coronary endothelial dysfunction in nontransplant atherosclerosis\textsuperscript{135} and partially improve allograft microvascular endothelial dysfunction.\textsuperscript{136} Calcium channel blockers improve endothelial function in preclinical models but have not been convincingly shown to reduce the progression of coronary artery disease in humans. However, the situation is different in cardiac transplant recipients, inasmuch as the calcium channel blocker diltiazem appears to slow the progression of transplant coronary artery disease.\textsuperscript{137} Moreover, enhanced nifedipine-induced coronary vasorelaxation occurs in transplant recipients with coronary endothelial dysfunction.\textsuperscript{138} Other drugs that might have the potential to restore endothelial vasodilator dysfunction under circumstances relevant to CAV include antioxidants such as probucol and vitamins C and E,\textsuperscript{139} insulin sensitizers such as rosiglitazone, phosphodiesterase inhibitors such as cilostazol, and modifiers of NO synthesis, including L-arginine and tetrahydrobiopterin.

Another potential strategy is to inhibit the proliferation of infectious particles, such as CMV, that may affect endothelial function. Indeed, ganciclovir treatment appears to reduce the progression of CAV.\textsuperscript{139} Improvement of cardioplegic solutions and reperfusion strategies could decrease early myocardial oxidative stress and inflammation-induced alterations in the NOS pathway. Moreover, the explanted donor heart may offer a potential for direct vascular/myocardial gene therapy to supplement eNOS or other protective molecules. In the future, therapeutic manipulation of circulating endothelial or smooth muscle progenitor cells\textsuperscript{140–142} or pharmacological induction of tolerance\textsuperscript{143} and targeting local or circulating dendritic cells\textsuperscript{9,144} as well as endothelin inactivation\textsuperscript{145–148} may increase our therapeutic options to prevent allograft endothelial dysfunction and structural changes.

Summary

CAV remains the most troublesome long-term complication of heart transplantation. Allograft coronary endothelial cells, the allogeneic barrier between a recipient’s circulating immuneactive cells and the transplanted organ, can serve as stimulators as well as targets of inflammatory reactivity. Immunologic and nonimmunologic factors likely influence the evolution and progression of transplant vasculopathy, mediated in part by dysregulation of the eNOS pathway. Activation and dysfunction of the arterial endothelium predict the development of CAV and may increase the risk of graft failure.

References

28. Weis M, Wolf WP, Mazzini N, Olbrich HG, Burger W, Hartmann A. Variations of segmental endothelium dependent and endothelium inde-
dependent vasomotor tone in the long term follow up after cardiac trans-
37. Drexler H, Fischell TA, Pinto FJ, Chenzbraun A, Botas J, Cooke JP, Alderman EL. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients: relation to vessel wall morphology. Circula-
53. Morishita R, Yamada T, Horuchi M, Gibbons GH. Vasoactive sub-


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