European Heart Journal (1997) **18**, 1712–1717

Review Article

Endothelial dysfunction and accelerated coronary artery disease in cardiac transplant recipients

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Introduction

Important advances in the field of heart transplantation have been accomplished during the last two decades. As a result, current survival rates are 85% and 70% after 1 and 5 years, respectively. The most conspicuous improvement in survival was associated with the introduction of cyclosporine treatment in the 1980s, which reduced mortality from infectious complications. Despite these advances, rejection and infection remain the leading causes of mortality during the first year after transplantation. In contrast, accelerated coronary artery disease has emerged as the major determinant of long-term survival. The clinical importance of graft atherosclerosis is attested to by the fact that up to 50% of cardiac transplant recipients have angiographically detectable coronary artery lesions 5 years after transplantation[1], and 50% of these will develop graft failure. Virtually all patients who survive beyond the 5th year after transplantation have histopathological evidence of coronary artery disease[2].

Graft atherosclerosis is characterized by diffuse intimal thickening that results in an accelerated narrowing of the coronary vessels. Intimal thickening is detectable by intracoronary ultrasound in a substantial proportion of transplant recipients with no angiographic abnormalities[3]. This discrepancy between ultrasonographic and angiographic findings may be due to the diffuse pattern of intimal thickening in graft atherosclerosis and the compensatory enlargement of the affected vessels[4].

The underlying mechanisms of graft atherosclerosis have not yet been fully elucidated, but, immunological mechanisms certainly play a crucial role[5,6]. This is already suggested by the simple observation that, while transplanted vessels develop atherosclerotic changes, the host's native arteries are spared. Graft endothelium is the primary target of the immunological responses to the allograft due to the expression of alloantigens on its surface, which activate host helper T cells[5]. Immune-mediated endothelial injury causes endothelial dysfunction with abnormal vasodilation. The present paper reviews recent reports that have shed new light on the pathogenesis and clinical significance of endothelial dysfunction as an early manifestation of graft vasculopathy.

Clinical evidence of graft endothelial dysfunction

Intact endothelium releases a number of mediators that regulate vascular tone and growth in response to changes in shear stress and other haemodynamic and metabolic factors[7]. One important endothelial product is nitric oxide, which is formed from L-arginine by the enzyme nitric oxide synthase. Damage to the endothelium may result in reduced production and/or nitric oxide and other vasoactive substances such as prostacyclines. Functional endothelial disturbances are clinically detectable as abnormal vasomotor responses to endothelium-dependent vasodilators such as acetylcholine or substance P. Reduced coronary vasodilation, or vasoconstruction, in response to these agents has been observed in a substantial proportion of cardiac transplant recipients[8–15]. In contrast, endothelium-independent vasodilators such as nitroglycerine, papaverine, and adenosine usually elicit normal vasodilator responses[13–17].

A bicycle exercise test can be used instead of pharmacological agents to assess the endothelium-dependent vasomotor response of the coronary arteries[17]. Exercise-induced coronary vasodilation was reversibly lost after endothelial denudation in an experimental study[18]. Both epicardial coronary vasodilation and coronary flow reserve during exercise were normal

Key Words: Heart transplantation, graft vasculopathy, endothelial dysfunction, coronary vasomotion.

Revision submitted 26 November 1996, and accepted 27 November 1996.

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0195/668X/97/111712+06 $18.00/0

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2–3 months after transplantation, but decreased 1–5 years later in heart transplant recipients[17]. Coronary blood flow measurements with positron-emission tomography confirmed that exercise-dependent flow reserve is reduced in transplant recipients, possibly due to increased blood flow at rest rather than to an abnormal increase of flow during exercise.[19]. Abnormal acetylcholine-induced coronary vasodilation was improved by administration of L-arginine in transplant recipients, suggesting that endothelial dysfunction may initially be reversible[12]. The responses of the endothelium to different stimuli may become impaired at different time points after transplantation: the vasomotor response to acetylcholine[8,11] and to the cold-pressor test[20] may be abnormal a few weeks to months after transplantation, whereas exercise-induced vasodilation is typically maintained for several months or even a few years[17]. Similar vasomotor abnormalities have been shown in non-transplant patients with coronary artery disease[21].

Pathogenesis of graft endothelial dysfunction and atherosclerosis

The 'response to injury' hypothesis, first formulated by Ross[22] as an explanation for atherogenesis in non-transplant patients, also provides a conceptual framework for understanding graft vasculopathy[23,24]. According to this model, a number of factors, including allograft rejection, peri-operative hypoxia, viral infections, cyclosporine toxicity, and traditional cardiovascular risk factors, may cause endothelial injury. In response to the variety of perturbing agents, the endothelium displays a quite uniform pattern of reaction. Activated host helper T cells release interleukin-2 that leads to the proliferation of other alloreactive cells, which secrete additional cytokines onto the intimal surface. In response, the endothelium expresses cell surface antigens and adhesion molecules and secretes macrophage chemoattractant factor. Recruited macrophages and activated lymphocytes enter the vessel wall and release cytokines and mitogenic factors such as interleukin-1, fibroblast growth factor, platelet-derived growth factors, transforming growth factor-α (TGF-α) and tumour necrosis factor-α (TNF-α)[25,27]. Cytokines, in turn, enhance macrophage and vascular smooth muscle cell migration and proliferation as well as extracellular matrix deposition. A role for TNF-α and vascular cell adhesion molecules[28–30] in the pathogenesis of graft vasculopathy is supported by the observation that blockade of TNF-α prevents graft coronary atherosclerosis in cholesterol-fed rabbits, while anti-adhesion molecules have similar effects in transplanted mice.[31]. Furthermore, increased circulating levels of intercellular adhesion molecule-1 (ICAM-1) are associated with reduced survival in transplanted patients[32].

The participation of specific immune mediators and inflammatory cell types in the genesis of graft vasculopathy was examined in a recent study in which carotid arteries were transplanted between pairs of inbred mice in both syngeneic and allogeneic combinations[33,34]. Seven mutant strains of mice, each with a specific immunological defect, were used for these experiments. While an acquired immune response involving $CD4^+$ (helper) T cells, antibody, and macrophages was essential to concentric neointimal proliferation and luminal narrowing, $CD8^+$ (cytotoxic) T cells and natural killer cells were not involved in this process.

Experimental data suggest a link between acute cellular rejection and graft vasculopathy[35], but clinical data are controversial in this regard[36,37]. However, the vascular (or humoral) pattern of allograft rejection, which lacks large myocardial inflammatory infiltrates on histologic examination, is clearly associated with an increased risk of subsequent graft atherosclerosis[38,39]. Antibodies against a doubling of endothelial antigens (molecular mass 60 and 62 kDa) have been detected in the serum of patients in whom accelerated atherosclerosis developed during the first 2 years after transplantation[40]. Lower levels of the antibodies were also found in a minority of patients in whom graft vasculopathy developed after the second year after transplantation and in rare cases of non-transplant patients with coronary artery disease. It is not clear whether such antibodies are primarily responsible for endothelial injury or whether they are secondarily produced as a result of prior damage to the endothelium.

Cyclosporine treatment may cause endothelial dysfunction with decreased prostacyclin production[41–43], resulting in arterial vasoconstriction that is synergistically potentiated by angiotensin II[44]. Cyclosporine also upregulates the expression of major histocompatibility complex antigens[45,46] and vascular adhesion molecules[47], while inducing vascular inflammatory cell infiltrates[46,48] and smooth muscle cell proliferation[49]. Allograft rejection in cyclosporine-treated animals is characterized by a vascular rather than myocardial inflammatory pattern[46], which is associated with accelerated graft arteriosclerosis[50]. In contrast to these experimental data, the analysis of large cohorts of heart transplant recipients showed that the incidence of graft atherosclerosis has remained substantially unchanged after the introduction of cyclosporine-based immunosuppression[51–53]. Cyclosporine dose reduction, started 1 year after heart transplantation, had no beneficial effect on coronary artery narrowing compared with the conventional dosage in a prospective study[54].

Cytomegalovirus infection, the most frequent infectious complication in transplanted patients, can induce vascular inflammation and graft atherosclerosis[55–57]. Experimental data suggest a protective effect of ganciclovir prophylaxis against graft vasculopathy[58].

Finally, conventional vascular risk factors such as hyperlipidaemia and hypertension also induce endothelial dysfunction and may enhance graft atherosclerosis[59,60]. High plasma LDL cholesterol levels and the presence of multiple vascular risk factors were

associated with impaired coronary artery vasodilation during exercise in transplant recipients[61].

In summary, the pathogenesis of graft endothelial dysfunction and atherosclerosis is multifactorial: immune-mediated mechanisms are probably crucial in initiating endothelial injury, while cyclosporine treatment, cytomegalovirus infection, and vascular risk factors may contribute to the progression of the disease.

Prognostic implications of endothelial dysfunction

Discordant findings have been reported on the predictive value of endothelial dysfunction for later development of graft atherosclerosis. Angiographic studies have shown that acetylcholine-induced coronary vasodilation 2 months after transplantation may be impaired to the same extent in patients with and without angiographic evidence of graft vasculopathy at 1-year follow-up[62]. An intravascular ultrasound study showed impaired vasodilation in 13 of 22 normal coronary segments 1 year after transplantation, whereas it was maintained in 9 of 11 segments with intimal thickening 5 years after transplantation[63]. A recent study using serial intravascular ultrasound showed that coronary segments with endothelial dysfunction had a greater increase in intimal thickness than normally dilating segments 1 year after transplantation[64]. These data suggest that endothelial dysfunction is an early, potentially reversible manifestation of graft vasculopathy and that it has a moderate predictive value for the subsequent development of arterial lesions. Intimal thickening, which represents a more advanced stage of the disease, has stronger prognostic implications than vasomotor abnormalities[65]. For this reason, serial intracoronary ultrasound imaging has become the gold standard for the monitoring of the progression of graft vasculopathy[66].

Potential therapeutic approaches to graft vasculopathy

The prevention of graft vasculopathy is one of the major challenges facing cardiovascular research today. Potential therapeutic approaches include new immunosuppressive agents, corticosteroid-free regimens, cytomegalovirus prophylaxis, and the use of vasodilators and lipid-lowering drugs.

Rapamycin, an inhibitor of T-cell proliferation, was associated with a protective effect against graft atherosclerosis in a recent clinical trial[67]. Elafin, an inhibitor of elastolytic activity (which is increased in graft vasculopathy), and monoclonal antibodies against vascular adhesion molecules also inhibited graft vasculopathy in experimental models[31,68]. Despite these promising results, none of the new immunosuppressive agents has clearly improved on cyclosporine treatment. Cyclosporine-based, corticosteroid-free immunosuppression is associated with a reduced atherogenetic risk and is feasible in a majority of transplant recipients[69]. Cyclosporine monotherapy with suppression of steroid and azathioprine treatment within 12 and 18 months respectively, after transplantation reduced the incidence of infectious complications with no increased risk of rejection[70].

Although ganciclovir prophylaxis prevented graft atherosclerosis in an experimental model[58] and reduced the overall incidence of cytomegalovirus disease in transplanted patients, it failed to protect cytomegalovirus-seronegative patients receiving hearts from seropositive donors[71]. The best prophylactic effect might be achieved with a combination of ganciclovir and cytomegalovirus hyperimmune globulin, which reduces the incidence of acute rejection through independent humoral mechanisms[72]. The effect of gancyclovir prophylaxis on the development of graft atherosclerosis still needs to be examined in a prospective clinical trial.

Another experimental approach to the treatment of graft vasculopathy has focused on nitric oxide, an endogenous vasodilator that also inhibits vascular smooth muscle cell proliferation[73] and T-cell activation[74]. Administration of L-arginine, the precursor of nitric oxide, inhibited the development of atherosclerosis in hypercholesterolaemic rabbits[75]. Intracoronary infusion of L-arginine to cardiac transplant recipients normalized the vasomotor response to acetylcholine in a majority of patients[17]. However, oral administration of L-arginine failed to prevent myointimal proliferation, although it enhanced vascular nitric oxide production, in an animal model of alloimmune injury[76].

Diltiazem, a calcium channel blocker, has recently attracted attention as a possible treatment for graft vasculopathy. Calcium channel blockers reduced the progression of atherosclerotic lesions in cholesterol-fed animals[77] and in humans with native coronary artery disease[78,79]. Treatment with diltiazem reduced coronary artery narrowing and decreased both mortality from graft atherosclerosis and overall mortality in the first year after transplantation[80]. Another calcium antagonist, amlodipine, and angiotensin-coverting enzyme inhibitors had a protective effect against graft vasculopathy in experimental models[81,82]. The mechanisms that are responsible for these effects are not fully understood. Suppression of calcium-dependent smooth muscle cell migration and proliferation and/or chronic vasodilation with an increase in coronary blood flow and, thus, flow-dependent production of nitric oxide by the endothelium have been postulated[83]. However, other studies have questioned the efficacy of diltiazem in preventing graft vasculopathy because differences in coronary artery diameter appeared to be due to acute vasodilation rather than to structural vascular changes[84,85].

A new potential approach to the treatment of graft vasculopathy is the administration of pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, which prevented coronary artery narrowing in a recent prospective study[86]. Surprisingly, the protective

effect of pravastatin was independent of its lipid-lowering effect and was associated with a decreased incidence of haemodynamically relevant rejection and improved 1-year survival after transplantation. These findings were explained with a pravastatin-induced decrease in the cytotoxicity of natural killer-cells.

Based on these observations, treatment with diltiazem and pravastatin may be considered in transplant recipients with ultrasonographic or angiographic evidence of coronary artery disease. Prophylactic administration of these agents in the first year after transplantation is also being debated due to their beneficial effect on short-term survival[80,86]. However, the efficacy of diltiazem and pravastatin in preventing graft atherosclerosis needs to be confirmed in additional prospective trials before these treatments are universally accepted.

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