Cardiac Allograft Vasculopathy: The silent, long-suffering enemy

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Cardiac transplantation is currently the preferred choice of treatment for end-stage cardiac disease. Despite great advances in controlling rejection and infection episodes, cardiac allograft vasculopathy (CAV) remains the leading cause of death after the first year of transplantation. CAV is an accelerated form of obliterative coronary artery disease that occurs in the heart transplant recipient. Currently, retransplantation is the only definitive treatment for patients with CAV and bioethical concerns of the growing donor shortage and increasing demand have served as a major obstacle in the long-term care for these patients. This short review will discuss the various molecular etiologies of CAV, emphasizing the endothelium as a critical site of injury that is capable of inducing CAV pathogenesis. Potential preventative, existing and novel immunosuppression regimens for treating CAV will also be reviewed.

Heart transplantation is scarcely recognizable from what it was when Dr. Christiaan Barnard performed the world’s first heart transplant on December 3, 1967 in Cape Town, South Africa (1). Advances in surgical, preservation protocols and pharmacological treatments in the past two decades have seen considerable improvement in short-term patient survival following cardiac transplantation, with reported survival rates of 80%-90% at 1 year (2). These favourable outcomes have been largely attributed to the introduction of more potent and specific immunosuppressants, such as cyclosporine and tacrolimus (3), and improvements in the understanding of the immunology of transplantation, tissue typing, organ preservation, management of acute rejection and opportunistic infections (4). However, despite these advances, long-term survival is substantially hindered by the prevalent incidence of cardiac allograft vasculopathy (CAV) (1,5,6-9).

Though the incidence of CAV is 5% to 10% per year in the postoperative period, intimal thickening, a classic early sign of CAV genesis, is present in around 58% of transplant arteries during the same year after transplantation (10). By 5 years, diagnosis of CAV reaches 50% in heart transplant recipients (10,11). Patients with CAV have silent ischemia due to the denervated heart. Other classical symptoms include congestive heart failure, myocardial infarction, and sudden cardiac death (12). Because of the nature of the symptoms, and given that some patients may be asymptomatic, follow-up with periodic coronary angiogram and intravascular ultrasound (IVUS) is imperative to detect CAV in patients. Currently, retransplantation is the only cure for patients with CAV, though the feasibility of this option is substantially hindered by organ shortage. The problem is exasperated in Canada, where the number of organ donors remains fairly constant each year (14-15 donors per million population) (13).

1. Cardiac Allograft Vasculopathy and Atherosclerosis

CAV is a type of cardiovascular disease that occurs uniquely in transplant recipients and is a rapidly progressive form of atherosclerosis. In its early stages, it is characterized by intimal proliferation and in later stages, by luminal stenosis of epicardial branches, occlusion of smaller arteries and myocardial infarction (6).
Cardiac Allograft Vasculopathy – REVIEW

Myocardial ischemia and infarction secondary to CAV in transplant patients are usually silent, due to a lack of cardiac innervation. Instead of chest pain, more serious events, such as congestive heart failure, ventricular arrhythmias and sudden death, are commonly the first clinical manifestations (11).

Although CAV resembles atherosclerosis (14), there are some important differences. In CAV, intimal proliferation is concentric rather than eccentric and the lesions are diffuse, involving both distal and proximal portions of the coronary tree (Figure 1). Calcification is uncommon and the elastic lamina remains intact. This contrasts with atherosclerosis, in which calcification is common and the internal elastic lamina is often severely disrupted. Though focal eccentric lesions associated with lipid deposition are found and may represent underlying, native (donor) atherosclerotic disease, lipid infiltration and calcification within the coronary vessels can occur in late grafts and typically represents manifestations of ‘host-versus-graft disease’ (14,15). The presence of moderate-to-severe proximal or mid-vessel disease is associated with a predictive mortality of 50% at 2 years (16). Finally, CAV develops rapidly, even over a matter of months (14,15), pathophysiological patterns in a normal, atheromatous and a graft vasculopathic vessel.

2. Pathogenesis and Diagnosis

Though the pathogenesis of CAV is poorly understood, there is considerable evidence to support a primarily immunologically mediated injury. The consensus however is that CAV is caused by immunologic mechanisms (e.g., alloreactive T cells and the humoral immune system), non-immunologic mechanisms relating to the transplant itself or the recipient (e.g., donor age, hypertension, hyperlipidemia and pre-existing diabetes) or to the side effects often associated with immunosuppression by calcineurin inhibitors or corticosteroids (e.g., cytomegalovirus infection, nephrotoxicity and new-onset diabetes) (6,15). Allograft vasculopathy is confined to the vasculature of the ‘donor’ segment only, sparing recipient vessels. Veins as well as arteries are also susceptible to the development of CAV.

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Figure 1: Typical Atherosclerosis and Allograft Vasculopathy. Allograft vasculopathy involves diffuse narrowing and occlusion of proximal and distal coronary arteries, rather than the more focal lesions seen in atherosclerosis.
Recent findings illustrate the pivotal role of the endothelium, resulting in release of pro-fibrotic cytokines, recruitment of circulating leukocytes, proliferation of vascular smooth muscle cells resulting in intimal thickening, and deposition of extracellular matrix proteins (ECMs).

**The Endothelium in CAV**

It is likely that the initiating event of CAV is sub-clinical endothelial cell injury in the coronary graft, which leads to a cascade of immunologic processes involving cytokines, inflammatory mediators, complement activation and leukocyte adhesion molecules (17). These changes can produce inflammation and subsequent thrombosis, smooth muscle cell proliferation and vessel constriction. The initial endothelial injury may be the result of ischemia–reperfusion damage or the host-vs-graft immune response. Activated endothelial cells show increased uptake of circulating cytokines and become more permeable to lipids (18). Activated endothelial cells and macrophages produce oxygen-free radicals that react with low-density lipoproteins and increase monocyte adhesion and T-cell activation (19). This ‘inflammatory response’ is perpetuated by lymphocytic cytokine secretion of interleukin-2 (IL-2), which stimulates proliferation of alloreactive lymphocytes, and interferon-c; this in turn upregulates intercellular adhesion molecule-1 (ICAM-1), thus recruiting more lymphocytes and macrophages to the vessel wall. These macrophages also release a host of cytokines and growth factors (e.g., IL-1, IL-2, transforming growth factor-β, basic fibroblast growth factor) that are potent mitogens of vascular smooth muscle cells. The result is proliferation of smooth muscle cells and deposition of extracellular matrix proteins, and, ultimately, encroachment of the coronary vasculature.

**Brain Death and Endothelial Injury**

Recently, efforts to understand the precise causes of endothelial injury in experimental transplantation studies have been made. In what proportion neurologic and hormonal pathways mediate endothelial dysfunction, and therefore cardiovascular dysfunction, after donor brain death is not known. A few recent studies have involved the inflammatory response in the equation, with far-reaching implications. Two independent experiments in the rat showed that donor

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**Figure 2:** Normal, Atherosclerotic, and Allograft Vasculopathic Vessels. Immunologic and non-immunologic factors play a part in initiating endothelial injury and inflammatory responses in both CAV and atherosclerosis. Intimal hyperplasia and proliferation of vascular smooth muscle cells is much more pronounced in CAV than in atherosclerosis. The presence of a lipid core is also often absent in CAV, though lipid infiltration in CAV vessels can occur, and is usually indicative of native (donor) disease or ‘host-versus-graft’ disease in late grafts.

Hypothesis - 21
brain death is a dynamic process associated with endothelial activation and end organ dysfunction. Solid organs upregulated major histocompatibility complex (MHC) molecules, expressed selectins and integrins, and attracted leukocytes in the interstitium, which corresponded with an increase in circulating activation factors (20-22). Pretreatment with selectin antagonists and blockade of T-cell activation resulted in suppression of both T-cell and macrophage-related cytokines in end organs (20). When hearts and kidneys went on to be transplanted, there was accelerated rejection of rat allografts from brain-dead donors compared to controls (21,23). Koo and colleagues (24) studied pre-transplant biopsies from human cadaveric kidney donors and demonstrated an increase in E-selectin, DR locus of human leukocyte antigen (HLA-DR) and ICAM-1 when compared to biopsies from living-related donors. This finding may explain the higher graft survival in living-related clinical kidney transplantation. A similar scenario in human cardiac transplantation is possible. This may help also explain the excellent long-term outcomes, both in terms of an absence from CAV and overall survival, seen with transplants performed with live donor organs and short ischemic times (22).

Molecular Modulators of Inflammation and CAV
In chronic rejection and during the development of CAV, which begins in the first year posttransplant, the endothelium is kept in a chronic state of low inflammation and mediates proliferative events that take place in the surrounding smooth muscle and matrix. Andreassen and colleagues (23) showed that serum levels of tumor necrosis factor-α (TNF-α, P-selectin, and vascular cellular adhesion molecule-1 (VCAM-1) were persistently elevated in patients followed up for up to 2 years after transplantation. These, and most of the proinflammatory genes, are expressed in CAV, in addition to evidence of Fas-mediated cytotoxicity (23-25). In a rat model of accelerated CAV, P-selectin and VCAM-1 were significantly upregulated during acute and chronic rejection (24). Furthermore, in a mouse model of transplantation, it was demonstrated that ischemia-reperfusion injury is sufficient alone to induce CAV in isografts and that CAV was greatly accelerated in allografted mice (26). Moreover, cyclic adenosine monophosphate (cAMP) pulse therapy during preservation was able to inhibit CAV, but the mechanism remains unknown.

The pathogenesis of CAV also depends on cytokine and growth factor genotypes, a good example being the reduced incidence of CAV in donors or recipients who are low producers of TGF-β (27,28). The recipient may also influence the degree of allograft loss by production of anti-endothelial and anti-human leukocyte antigen (HLA) antibodies, although the precise mechanism is not yet elucidated (26–28). In any case, there is substantial evidence to suggest that early endothelial dysfunction is predictive of development of CAV 1 year postoperatively (29).

With the accumulating evidence that endothelial injury, particularly in the early phase, can alone induce and/or contribute to the rapid progression of CAV, many efforts have become directed towards inhibiting the enhanced inflammatory state during the posttransplant period and toward predicting CAV before it is clinically apparent (23,29-31). Weis and colleagues established a relationship between endothelial vasomotion and perturbation of endothelin physiology early after transplantation (29). Another study from the same group showed that 26% of their patients had microvascular endothelial dysfunction within 1 month of transplantation and suggested that abnormalities in the nitric oxide (NO) synthesis pathways and increased levels of circulating cytokines might be partly responsible (30). To date it is unclear whether inducible NO synthase (iNOS) has a protective or a causative role in the development of CAV (31).

Cytomegalovirus infection and CAV
A number of clinical studies have shown an association between cytomegalovirus (CMV) infection and accelerated arteriosclerosis in both transplanted and native coronary vessels (32, 33). The possible mechanisms of CMV-associated vasculopathy have been only partly elucidated. CMV has the potential to infect endothelial cells and cause a cytopathic effect, resulting in increased
endothelial adherence of granulocytes (34). Increased expression of inflammatory cytokines such as IL-1 and TNF-β initiates vascular smooth muscle cell proliferation (35). Work by Lemstrom et al. has demonstrated an increase in TGF-β and platelet derived growth factor-B levels in rat aortic allografts infected with cytomegalovirus. Valantine et al. later showed a significant reduction in the severity of transplant vasculopathy in those patients randomized to ganciclovir (36).

Diagnosing CAV
Given its silent nature, it is important to identify asymptomatic patients early during the development of CAV to prevent irreversible reductions in graft survival. While both coronary angiography and intravascular ultrasound (IVUS) have been used to detect CAV, IVUS is currently the most sensitive tool to study early transplant vasculopathy and is the only valid method to study donor atherosclerotic lesions in vivo (37). IVUS uses a catheter with miniature transducer at the end, which is advanced into coronary arteries. It can image both the lumen and the intima, and can define a number of parameters by reflecting the structure of the arterial wall (36,38,39). IVUS can be used to measure intimal thickness, lumen cross-sectional area and external elastic membrane cross-sectional area, although only intimal thickening is related to patient outcome. IVUS is more likely than coronary angiography to identify CAV (37). IVUS soon after transplant demonstrates donor atherosclerosis and subsequent serial imaging shows progression of this atherosclerosis. Serial imaging also allows early identification of de novo CAV lesions and assessment of the response to therapy. The increase in IVUS-detected intimal thickness is greater in the first year post-transplant than in other early posttransplant years (40). An increase in intimal thickness of at least 0.5 mm in the first year after transplant is a reliable indicator of both CAV development and 5-year mortality.

3. Treatment Modalities
Because CAV affects between 30% and 60% of cardiac transplant recipients within 5 years of surgery, and because morbidity and mortality rates following retransplantation are much greater than that of primary transplantation, prevention is a key focus for cardiac transplant teams today (37,41-44). Furthermore, the therapeutic options in CAV are very limited and the outcome is universally fatal (9). Therefore, prevention and slowing down the progression of disease appear to be the best strategies.

Prophylaxis often includes risk factor modification. Although data are lacking in regards to their efficacy, therapies target the treatment of hypertension, hyperlipidemia, obesity, diabetes, and promote exercise and smoking cessation. Despite the proven fact that modifications of these risk factors has an impact on the development and progression of native coronary artery disease, the true benefits in posttransplant CAV patients are not known. The current therapeutic modalities are discussed below.

Pharmacological Therapies
a. Lipid-lowering drugs
Therapies for CAV are focused on alleviating or limiting the most prevalent metabolic risk factors in transplant patients. Hyperlipidemia is observed in 60–80% of heart transplant recipients. There is now convincing evidence to show a direct correlation between hypercholesterolemia and CAV (37, 45). The development of obesity, which is common after transplantation, may be an additional factor (46). Winters et al. have shown a correlation between body mass index and the degree of intimal narrowing in failed cardiac transplants (45). The cause of hyperlipidemia after transplantation is multi-factorial, though immunosuppressive therapy seems to be an important contributing factor. Indeed, the dose of prednisolone is a strong predictor of increased levels of cholesterol and low-density lipoprotein post-transplantation (47). Co-administration of cyclosporine increases the steroid-induced effect on hyperlipidemia by decreasing hepatic lipoprotein lipase activity P1 (48), resulting in a further rise of 20–30% total and low-density lipoprotein (LDL) cholesterol. Treatment with HMG-CoA reductase inhibitors (statins) has been shown to reduce LDL-cholesterol levels, to significantly prolong long-term survival, and to reduce the incidence of angiographically detectable CAV (49). Furthermore, studies assessing the effect of HMG-CoA reductase
Cardiac Allograft Vasculopathy – REVIEW

inhibitors on native coronary atherosclerosis have shown significant regression of pre-existing lesions (50). The beneficial effects of statins extend beyond their directly lipid-lowering effect. Simvastatin has been shown to inhibit both rodent and human vascular smooth muscle proliferation in vitro (51), inhibit monocyte chemotaxis, and regulate cytotoxicity of T lymphocytes (52). Kobashigawa et al. have previously shown similar findings with pravastatin. The clinical data from these two trials, as well as the experimental data, advocate the use of routine HMG-Co reductase as a therapy immediately following heart transplantation (37).

b. Anti-hypertensive agents
Similar to the incidence of hypolipidemia, post-transplant hypertension develops in 60–80% of patients in the immediate postoperative period. This is largely attributed to the hypertensive effects induced by the prescribed immunosuppressants prednisolone and cyclosporine (53). Research into the use of an anti-hypertensive agent that may also have a vascular protective role is ostensibly contradictory. The use of calcium channel blockers and angiotensin-converting enzyme inhibitors (ACEI) have been advocated in experiments from balloon injury (54), hypercholesterolemic and aortic/cardiac allograft models of intimal hyperplasia and vasculopathy (55). In the clinical setting, several controlled trials have demonstrated a significant reduction in CAV following treatment with calcium channel blockers, (56-58), but no difference in intimal thickening following prophylactic use of ACEI (57). Other studies also purport the limited efficacy of ACEI (59,60).

c. Proliferation inhibitors
Mammalian target of rapamycin (mTOR) inhibitors, known anti-proliferative agents, inhibit vascular smooth muscle proliferation and have been successful in inhibiting restenosis after angioplasty in studies using rapamycin (sirolimus)-eluting stents as compared with bare metal stents (61). Patients receiving sirolimus had fewer major adverse cardiovascular events or less rapid progression of CAV compared with controls. This demonstrated that cardiac transplant recipients with established CAV might benefit from the addition of proliferation inhibitors. Further studies are needed to define the benefits of these drugs in patients with established CAV.

To date, there is no evidence to support the use of methotrexate to enhance immunosuppression by affecting the immune-mediated factors involved in CAV (7). Despite the lack of data, it is not uncommon to see the use of methotrexate as part of the posttransplant medical regimen.

Surgical Treatments

a. Percutaneous transluminal coronary angioplasty (PTCA)
The diffuse obliteration of distal vessels in CAV is often not amenable to revascularization (see Figure 1). In spite of this, PTCA and directional coronary atherectomy have been studied in patients with single-vessel CAV (62,63). This has a success rate of 93% immediately after the procedure with a restenosis rate of more than 55% at 6 to 15 months (63). Survival in the largest cohort was 61% at 19 months. PTCA cannot address the distal disease of CAV. The utility of stents and, in particular, the new drug-eluting stents, is less clear. Nonetheless, one study, looking at bare stents in 26 patients with PTCA alone showed an improvement in vessel patency (64).

b. Retransplantation
Coronary bypass surgery has not been successful for this disease (65). Instead, the definitive therapy is retransplantation. Data from the Registry of the International Society of Heart and Lung Transplantation shows the survival rate at 1 year for 449 patients who underwent retransplantation to be significantly less than those who had primary transplants (48% compared with 79%), although the survival rate was higher at 1 year in patients who underwent retransplantation for CAV (60%).

Other treatments

a. Mycophenolate mofetil
Current studies are in place to determine the outcome effects of mycophenolate mofetil (MMF) and rapamycin on CAV. Recent studies in animal models have shown a decrease in the severity of CAV with the use
Cardiac Allograft Vasculopathy – REVIEW

of both MMF and rapamycin. Although the mechanisms are not known, it is speculated that blocking purine synthesis and inhibiting the proliferation of both T- and B lymphocytes, thereby blocking cellular and humoral responses, may be the mode of action (7).

b. Photopheresis
Photopheresis is a newer therapy that is being used to modulate the immune response. Photopheresis involves administration of oral 8-methoxy-psoralen, which binds white blood cells (wbc). The wbc are then harvested by apheresis, irradiated with ultraviolet light, and reinfused into the patient. The process of photopheresis results in autoregulation of the response of the T-cell, which affects the development of intimal thickening, as evidenced by IVUS measurements (7). Limitations to photopheresis include cost and time constraints.

c. Total lymphoid irradiation
Frequent episodes of rejection are associated with an increased incidence of CAV. Therefore, total lymphoid irradiation (TLI) is being studied for its potential benefits in preventing the development and progression of CAV. It is well known that TLI is effective in decreasing recurrent rejection episodes in patients who are refractory to the anti-rejection regimens of corticosteroids, muromonab-CD3 (OKT3), and antithymocyte globulin (66). Although studies suggest that TLI has a protective effect on the coronary endothelium, leukopenia and thrombocytopenia, as well as the unknown long-term effect and benefits, prevent TLI from being a widely accepted therapeutic modality in the treatment of CAV (66).

Conclusion
Cardiac allograft vasculopathy is a major obstacle for long-term survival in heart transplant patients. The accruing evidence is strong to postulate endothelial dysfunction and associated inflammation - upregulation of immune cells, cytokines, adhesion molecules, proliferative factors, and immune cell infiltration - as likely causes of intimal thickening and advanced atherosclerosis seen in CAV patients (67). Efforts to establish preventative measures to abolish or limit the earliest type of endothelial injury from brain death onward, as well targeting inflammatory mediators should, in theory, serve as the best prophylaxis for this disease especially and immediately following transplant.

Clinically, only a small number of patients will show signs of the disease in the first year but almost half will show clinical manifestations of CAV after 5 years as diagnosed by periodic coronary angiogram and IVUS (37,40). Survival after retransplantation is bleak and ethical concerns with the shortage of viable donor organs, particularly in Canada, has severely limited this option. Though generally current treatment options for patients with CAV are not satisfactory, statins and calcium channel blockers show the most promise in treating hyperlipidemia and hypertension – the common side effects of immunosuppressive regimens. Early detection is still a challenge and prevention should be emphasized, though this will likely require adjunct testing to IVUS, such as monitoring available serum markers predictive of early CAV development and progression.

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