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Review article

Alloimmunity and nonimmunologic risk factors in cardiac allograft vasculopathy

G. Vassalli[a]*, A. Gallino[b], M. Weis[c], W. von Scheidt[c], L. Kappenberger[a], L.K. von Segesser[a], J.-J. Goy[a], on behalf of the Working Group Microcirculation of the European Society of Cardiology

[a] *Divisions of Cardiology, Experimental Surgery, and Cardiovascular Surgery, University Hospital, Lausanne, Switzerland*
[b] *Ospedale San Giovanni, Bellinzona, Switzerland*
[c] *Medizinische Klinik und Poliklinik I, University Hospital Grosshadern, Munich, Germany*

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Graft vasculopathy is an accelerated form of coronary artery disease that occurs in transplanted hearts. Despite major advances in immunosuppression, the prevalence of the disease has remained substantially unchanged during the last two decades. According to the 'response to injury' paradigm, graft vasculopathy is the result of a continuous inflammatory response to tissue injury initiated by both alloantigen-dependent and independent stress responses. Experimental evidence suggests that these responses may become self-sustaining, as allograft re-transplantation into the donor strain at a later stage fails to prevent disease progression. Histological evidence of endothelitis and arteritis, in association with intima fibrosis and atherosclerosis, reflects the central role of alloimmunity and inflammation in the development of arterial lesions. Experimental results in gene-targeted mouse models indicate that cellular and humoral immune responses are both involved in the pathogenesis of graft vasculopathy. Circulating antibodies against donor endothelium are found in a significant number of patients, but their pathogenic role is still controversial. Alloantigen-independent factors include donor-transmitted coronary artery disease, surgical trauma, ischaemia-reperfusion injury, viral infections, hyperlipidaemia, hypertension, and glucose intolerance. Recent therapeutic advances include the use of novel immunosuppressive agents such as sirolimus (rapamycin), HMG-CoA reductase inhibitors, calcium channel blockers, and angiotensin converting enzyme inhibitors. Optimal treatment of cardiovascular risk factors remains of paramount importance.

Introduction

Accelerated vascular disease is a characteristic feature of chronic graft dysfunction in organ transplantation. As such, accelerated coronary artery disease is one of the most discouraging aspects in heart transplantation.[1,2] The disease is the third leading cause of death, after infection and rejection, in the first year after heart transplantation, and the first leading cause of death in subsequent years. In a multi-institutional study, 42% of heart transplanted patients had abnormal coronary angiograms, and 7% of patients had severe coronary artery disease by 5 years post-transplant.[3] Disease-related events including death and re-transplantation had an actuarial incidence of 7% at 5 years, and occurred in two of three patients with angiographically severe coronary artery disease. It should be noted that coronary angiography, which has been the main modality of invasive assessment of graft vasculopathy, is insensitive. Intravascular ultrasound (IVUS), which can detect early intimal

* Correspondence to: Dr Giuseppe Vassalli, Cardiologie BH-10, CHUV, 1011 Lausanne, Switzerland. Tel: +41-21-3140076; fax: +41-21-3140076
E-mail address: gvassall@hospvd.ch (G. Vassalli).

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thickening, has revealed a much higher incidence of the disease (up to 75% at 1 year after transplantation).[4,5] Virtually all 5-year survivors have histological evidence of graft vasculopathy.[6]

The first section of the present review briefly discusses the clinical aspects of cardiac allograft vasculopathy, with particular emphasis on endothelial dysfunction as an early manifestation of the disease. The main focus of the review is the pathogenesis of graft coronary vasculopathy, which involves both immunologic and nonimmunologic risk factors. Therapeutic approaches are briefly discussed in the final section.

Clinical aspects of graft vasculopathy

Classic angina as a symptom of graft coronary vasculopathy is rarely perceived because the transplanted heart is denervated. Consequently, the disease may be manifested by shortness of breath, decreased exercise capacity, syncope, arrhythmias, or heart failure. Unfortunately, the first manifestation may be acute myocardial infarction or sudden death. Therefore, patients should be evaluated on an annual basis for the presence of coronary artery disease. IVUS is more sensitive than coronary angiography with respect to early arterial lesions. Serial echocardiography is useful to detect progressive deterioration of left ventricular function as a result of myocardial ischaemia. Dobutamine stress echocardiography early after transplantation has been shown to predict development of graft vasculopathy and clinical events.[7]

Angiographic and histological findings

Angiographic features in patients with graft coronary vasculopathy are variable. Arterial lesions are typically concentric and involve the entire coronary tree, including the small intramyocardial branches. However, isolated lesions closely resembling atherosclerotic lesions in native vessels also occur. A classification system for luminal narrowing based on angiography was proposed by Gao and coworkers.[8] Category A referred to discrete, tubular lesions. Histological analysis showed that nearly half of type A lesions consisted of atheroma.[9] Category B denoted distal lesions that are usually characterized by fibrous intimal thickening, while category C referred to diffusely irregular patterns of narrowing. Fibrous intimal hyperplasia can be associated with arteritis with mononuclear cell infiltrates and obliterating endothelitis, eventually leading to destruction of elastic fibres and thinning of the muscular media.

The basic determinants of coronary narrowing are intimal thickening and vascular remodelling. Serial IVUS has shown that remodelling of the arterial wall can compensate for moderate intimal thickening.[10,11] Compensatory remodelling is greater in excentric than concentric lesions, possibly due to greater compliance of the normal portion of the vessel wall.[11] Besides compensatory enlargement, constrictive changes (negative remodelling) has also been identified and shown to be the most important predictor of the severity of focal stenoses.[10] Constrictive remodelling with a decrease in total vessel volume predominates in the first year after transplantation, whereas a slight compensatory enlargement can be observed at later time points. Recent data in a mouse model show that matrix metalloproteinases (MMPs) involved in the degradation and remodelling of extracellular matrix play an important role in constrictive vascular remodelling.[12]

Endothelial dysfunction as an early stage of graft vasculopathy

Endothelial dysfunction can be clinically detected as abnormal vasomotion in response to endothelium-dependent stimuli such as acetylcholine, substance P, the cold-pressure test, and dynamic exercise.[13–15] Reduced vasodilation, or paradoxical vasoconstriction, in response to acetylcholine occurs even in the absence of angiographic abnormalities.[16] Its predictive value is controversial. One study showed that acetylcholine-induced vasoconstriction early after transplantation may resolve over the following months, suggesting that it reflects a reversible insult to the endothelium around the time of transplantation.[17] This early insult involves ischaemia-induced release of cytokines such as interleukin (IL)-2 and IL-6, and the potent vasoconstrictor endothelin-1.[18–20] In another study, however, early vasomotor abnormalities predicted the development of graft vasculopathy at 1 year post-transplant.[21] Moreover, in a recent study using serial IVUS and Doppler flow-wire measurements, annual decrements in coronary endothelial function were associated with progressive intimal thickening, while abnormal vasomotor response to acetylcholine preceded the development of clinical endpoints.[22] Together, these observations suggest that endothelial dysfunction may represent an early and potentially reversible stage of graft vasculopathy. However, sustained endothelial dysfunction may reflect permanent vascular injury, with or without structural abnormalities.

Vasodilation during dynamic exercise depends on endothelial NO release. Both epicardial coronary vasodilation and coronary flow reserve during exercise were progressively impaired in cardiac transplant recipients 2–3 years post-transplant.[23] In contrast, pharmacological flow reserve was maintained. These results suggested progressive graft microvascular endothelial dysfunction in heart transplanted patients. However, coronary blood flow measurements by positron-emission tomography showed that coronary blood flow at rest is increased in transplanted patients and is responsible, at least in part, for decreased coronary flow reserve during exercise.[24]

Role of alloimmunity in the pathogenesis of graft vasculopathy

The simple observation that while graft coronary arteries develop vascular lesions, the host's native arteries are spared suggests a crucial role for alloimmunity in graft vasculopathy. This is consistent with experimental evidence that hearts transplanted into a genetically different recipient are affected, whereas those transplanted into a genetically identical recipient are spared. Furthermore, protocols that induce donor-specific immune tolerance prevent graft vasculopathy in experimental models.[25]

The number of HLA mismatches, as well as the number of rejection episodes and their duration have been identified as alloantigen-dependent risk factors.[26,27] However, differences in the long-term outcome between patients who experienced an acute rejection in the first year after heart transplantation and those who did not are small.[28] Thus, the relationship between acute cellular rejection and cardiac allograft vasculopathy remains controversial.[29–31]

Role of the endothelium in alloimmunity

Endothelial cells of coronary arteries are the first biological interface between the transplanted donor heart and circulating immunocompetent cells of the recipient. A variety of insults including brain death,[32] organ preservation and surgical trauma,[33] ischemia-reperfusion,[34,35] alloimmune responses to donor antigens,[36] cytomegalovirus infection,[37] cyclosporine toxicity,[38,39] hyperlipidaemia and glucose intolerance[40] can 'activate' endothelial cells. The concept of 'activated' endothelium is not precisely defined, but it includes expression of major histocompatibility complex (MHC) molecules and adhesion molecules (e.g., E-selectin), secretion of cytokines and chemokines, and expression of molecules that provide costimulatory signals for T and B cell activation. In turn, activated $CD4^+$ T cells stimulate the expression of human leukocyte antigens (HLA) class-II molecules on endothelial cells, subsequent to HLA class-I detection by $CD8^+$ T cells. HLA and other donor antigens expressed on graft endothelial cells can elicit a sustained alloimmune reaction.[41] Clinical evidence suggests that HLA donor-recipient matching is an independent predictor of survival in heart transplantation.[26] In the Collaborative Transplant Study, cardiac transplant recipients receiving a graft with no or only one HLA-A, B, or DR mismatch (n=128 out of 8331; 1.5%) had a 83±4% 3-year graft survival rate as compared to 76±2% for those with two mismatches (n=439) and 71±1% for those with three to six mismatches (n=7764).[42] Although this association was highly significant (P<0.001), the impact on the clinical outcome was limited (6.6% difference in 3-year graft survival rates between two and three or more mismatches). In the clinical setting, donor organ shortage and the need for rapid transplantation in many patients with terminal heart failure do not allow for allocation of donor organs based on HLA matching. Nevertheless, information about HLA matching may be of some usefulness to guide early immunosuppression. Prospective HLA matching should be considered for retransplantation or in presensitized recipients.

Besides the well-established activation of the direct pathway of allorecognition,[43] endothelial cells can activate T cells via the indirect pathway, which involves processing and presentation of peptides derived from allogeneic HLA molecules by recipient antigen presenting cells (APCs) such as dendritic cells.[41] Importantly, dendritic cell invasion has been shown to be increased on activated endothelial cells and after blocking of the endothelial nitric oxide synthase.[44] Recently, it has been shown that direct $CD8^+$ T cell activation by endothelial cells can trigger acute cardiac allograft rejection.[45]

Cellular alloimmune responses

Experiments in rodent models of allograft vasculopathy have shown that arterial lesions first develop as endothelitis and subsequently progress to smooth muscle cell-rich fibrosis.[46] T cells ($CD4^+$, $CD8^+$, NK cells), macrophages, and dendritic cells have been evidenced in arterial lesions. These cells secrete a myriad inflammatory mediators including cell adhesion molecules, cytokines, chemokines, growth factors and macrophage activators, as well

as proteases that degrade extracellular matrix proteins.

Depletion of $CD4^+$, but not $CD8^+$, T cells prevented arterial lesion formation in an experimental model.[47] Functional $CD4^+$ T cell responses have been subdivided in Th1- and Th2-type responses. Their respective roles were studied using mice deficient in interferon-γ (IFN-γ) or Stat4 (which regulate Th1-type responses) or in IL-10 (which mediates Th2-type responses) as graft recipients. Cardiac allografts placed in IFN-γ or Stat4-deficient recipients had decreased graft vasculopathy, whereas those placed in IL-10-deficient recipients had increased vascular occlusion.[48–50] Increased vascular lesions were observed in recipient mice deficient in inducible nitric oxide synthase (iNOS) or transforming growth factor-β (TGF-β), suggesting an immunomodulatory role for these molecules.[51,52]

Humoral alloimmune responses

Both experimental and clinical studies have reported an association between antibody production after heart transplantation and graft vasculopathy. Heterotopic heart transplantation between mice bearing MHC class-I alone, class-II alone or minor mismatches, in combination with short immunosuppression, resulted in vascular lesions within 4–8 weeks.[53] Transplantation of B10.A mouse hearts into B10.BR recipients (MHC class-I mismatch only) resulted in high-titer anti-donor antibody production and severe graft vasculopathy.[54] Conversely, low-titer anti-donor antibodies and mild arterial disease were observed in the reciprocal donor-recipient strain combination. However, graft vasculopathy in this combination was induced by treating recipients with an anti-donor antiserum made in a separate strain. Furthermore, hearts transplanted into B cell-deficient mice that are incapable of producing immunoglobulins developed only minor disease.[55] Hearts transplanted into immunodeficient SCID mice survived indefinitely with no significant vasculopathy. However, vascular lesions rich in macrophages and NK cells developed in SCID mice upon treatment with an antiserum reactive with donor antigens. Together, these findings demonstrate that humoral responses play a key role in the pathogenesis of graft vasculopathy, but cellular responses are also involved.

In clinical heart transplantation, circulating anti-HLA antibodies have been associated with poor survival.[56,57] Anti-endothelial antibodies detected at 1 and 2 years post-transplant were found in patients who had developed graft vasculopathy at this time.[58] These antibodies were reactive with as many as 40 peptides from endothelial cells.[59] The most abundant of these antibodies were reactive with the intermediate filament vimentin and were associated with graft vasculopathy. Antibodies against endothelial cell surface molecules such as MHC class-I and intercellular adhesion molecule (ICAM)-1 were associated with endothelial activation and release of growth factors.[60,61] Increasing evidence indicates that a large number of potential antigens are released from the allograft to be processed and presented via the indirect pathway by recipient APCs.[62] Antibody responses against non-HLA and minor antigens have been reported.[58,59,63]

Role of alloantigen-independent factors in the pathogenesis of graft vasculopathy

According to the 'immunologic view', centred on the overwhelming importance of alloimmunity, graft vasculopathy has long been referred to as chronic rejection. However, this term is misleading because it does not adequately take alloantigen-independent factors into account. Alternatively, the 'response to injury' concept, first formulated as an explanation for naturally occurring atherosclerosis,[64] may provide a theoretical framework for understanding graft vasculopathy as well. This concept dictates that vascular lesions are the result of cumulative vessel injury induced both by alloimmune responses and by nonspecific insults. Endothelial injury triggers a cascade of 'tissue repair' mechanisms that involve vascular cell proliferation, fibrosis, and vascular remodelling. However, exuberant 'tissue repair' leads to intimal thickening and luminal narrowing. At a later stage, nonspecific mechanisms may become self-sustaining and promote arterial lesion progression even in an immunologically friendly environment, as experimental late allograft re-transplantation back into the donor strain does not prevent progression of the disease.[65,66]

Nonimmunologic risk factors

Studies evaluating the contribution of nonimmunologic risk factors to graft vasculopathy have reported variable results. A multi-institutional coronary angiography study identified donor characteristics such as older age, male gender and hypertension, as well as recipient characteristics such as older age and male gender as risk factors.[3] Moreover, multicentre IVUS study identified older donor age and recipient body mass index as risk factors for coronary intimal thickening.[67]

Input injury

'Input injury' designates the burden in the organ at the time of transplantation. It includes both donor-transmitted disease and graft injury as a result of brain death, donor maintenance, organ removal and preservation, and surgical trauma.[33] Studies on kidney transplantation have shown that organs from live donors, even in an immunologic mismatch setting, have a significant advantage over cadaveric donors.[32] Pre-existing coronary artery lesions are not infrequent, even in young donors (56% of donors aged 32±12 years, as assessed by IVUS). However, donor-transmitted lesions do not seem to enhance accelerated graft vasculopathy after heart transplantation.[68]

Ischaemia-reperfusion injury

Interruption of blood supply during ischaemia leads to anaerobic metabolism, depletion of adenosine-triphosphate (ATP), dysfunction of membrane ionic ATP-dependent pumps, entry of calcium and sodium into the cell. Upon reperfusion, highly reactive hydroxil radicals are generated, which stimulate P-selectin expression on endothelial cells. Activated endothelial cells also produce platelet activating factor (PAF) that interacts with the PAF receptor on neutrophils.[34] Acting in concert, PAF and P-selectin promote neutrophil activation and induce β_2-integrin expression, resulting in β_2-integrin binding to ICAM, which mediates firm attachment of neutrophils to endothelial cells. Together, these observations suggest that ischaemia/reperfusion injury plays a major role in graft vasculopathy.

Cytokines and chemokines

Cytokines are secreted pro-inflammatory molecules that regulate leukocyte activation. The Th1 subset of $CD4^+$ cells produces IFN-γ, IL-2, and TGF-β_1, whereas the Th2 subset produces IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. As mentioned, IFN-γ seems to promote, whereas IL-10 protects against graft vasculopathy.[48,50] Chemokines (chemoattractant cytokines) regulate the recruitment of leukocytes to the allograft.[69] Expression of several chemokines including monocyte chemoattractant protein (MCP)-1, MCP-3, IL-8 and RANTES was demonstrated in transplanted human hearts. RANTES production promoted monocyte adhesion to activated endothelium and arterial lesion formation in a graft vasculopathy model.[70] Mice deficient in the CC chemokine receptors (CCRs)-1 and 5 had markedly attenuated cardiac allograft rejection in the absence of immunosuppression.[71] Short-term treatment with low-dose cyclosporine, which failed to prevent rejection in wildtype mice, induced indefinite allograft acceptance with no significant vasculopathy in CCR-1-deficient mice. Consistently, administration of a CCR-1 antagonist delayed heart transplant rejection in an experimental model.[72] These results suggest an important role for chemokines in allograft rejection and vasculopathy.

Cytomegalovirus (CMV) infection

CMV infection, the most frequent infectious complication in transplanted patients, can cause vascular inflammation (endothelitis and arteritis). Both clinical and experimental evidence points toward a role for CMV in the pathogenesis of graft atherosclerosis.[73–76] Prophylactic therapy with ganciclovir has reduced the incidence of CMV disease, but not in the group at highest risk (i.e., seronegative recipients of grafts from seropositive donors). In a post-hoc analysis, ganciclovir prophylaxis was associated with decreased intimal thickening, reduced coronary artery disease and improved survival.[77] Moreover, CMV hyperimmune globulin in combination with ganciclovir may be more protective than ganciclovir alone.[78] These issues need to be further addressed in prospective, randomized trials.

Metabolic factors

Last but not least, conventional vascular risk factors such as hyperlipidaemia, glucose intolerance and hypertension, which are highly prevalent in transplanted patients on current immunosuppressive regimens, play contributory roles in graft endothelial dysfunction and transplant vasculopathy.[79] Presence of multiple vascular risk factors was associated with severe impairment of coronary vasodilation during exercise in cardiac transplant recipients.[80] At necropsy, histological analysis of transplanted hearts showed frequent atheromata with diffuse intra- and extra-cellular lipid accumulation in both intimal and medial walls.[81] Mean total cholesterol content in coronary arteries was more than 10-fold higher than in comparable donor age-matched native vessels. Extent of lipids in the arterial walls was highly correlated with mean cumulative cyclosporine and prednisone doses.[81] Thus, the importance of metabolic factors in the development of graft vasculopathy should be emphasized, particularly because effective treatments for these risk factors are available.

Therapeutic approaches to graft vasculopathy

The optimal treatment prophylaxis for graft vasculopathy has not been established. Current treatments are moderately effective in delaying the development of arterial lesions, but they do not completely prevent it. Experimental evidence that improved immunosuppression can prevent graft vasculopathy suggests that optimized immunosuppression is a primary goal in treatment prophylaxis of the disease.[25]

Most current protocols are based on cyclosporine-A or tacrolimus (FK506), calcineurin inhibitors that block early T-cell activation, and on the purine synthesis inhibitors azathioprine and mycophenolate mofetil. In most centres, steroids are weaned when endomyocardial biopsies show low or negative rejection scores. Most cardiac transplant recipients can be maintained on steroid-free regimens, thus avoiding the detrimental metabolic effects of these drugs.[82] Low-dose cyclosporine protocols have been evaluated in an attempt to reduce endothelial toxicity; however cyclosporine dose reduction at 1 year post-transplant was not associated with improved coronary artery diameter at 2 and 3 year follow-up.[83] These negative results may be due to the late onset of low-dose cyclosporine, as initial lesions develop during the first year post-transplant. This is consistent with another report showing that augmented immunosuppression started in the first year post-transplant is associated with arterial lesion regression in 92% of patients, as compared to 40% when started in the second year post-transplant.[84] Daclizumab, a hybrid humanized interleukin-2 receptor antibody, has been effective in preventing allograft rejection in clinical heart transplantation.[85] The immunosuppressive and anti-proliferative drug sirolimus (rapamycin), an inhibitor of interleukin-2-mediated signal transduction pathways, can be used as a rescue drug when cyclosporine is contraindicated or ineffective.[86] Sirolimus has been shown to reverse graft vasculopathy in experimental studies and initial clinical trials.[87–89] Preliminary results of an ongoing multicentre trial suggest that sirolimus treatment is associated with reduced coronary artery disease in cardiac transplant recipients.[89] If the beneficial effects of sirolimus are confirmed by definitive results, this drug may represent a major advance in the prevention of graft vasculopathy.

Only a few small trials have compared different immunosuppressive regimens for prevention of graft vasculopathy. The combination of tacrolimus and mycophenolate mofetil was superior to tacrolimus and azathioprine, and comparable to cyclosporine and azathioprine, with respect to coronary vasomotor function early after transplantation; however, this combination failed to reduce coronary intimal thickening.[90] Another study showed no significant differences in vascular occlusion between mycophenolate mofetil and azathioprine immunosuppression.[91] In a prospective IVUS trial, cyclosporine was slightly, although not significantly, more effective than tacrolimus in preventing graft vasculopathy in the first year post-transplant.[92]

The calcium channel blocker diltiazem started early after transplantation was associated with reduced coronary artery narrowing, reduced mortality from graft arteriosclerosis, and reduced overall mortality at 1 year post-transplant.[93] Angiotensin-converting enzyme (ACE) inhibitors may also be effective in delaying development of graft vasculopathy.[94]

The only established clinical treatment is routine therapy with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors (statins). Pravastatin improved 1-year survival in a prospective study.[95] Notably, protection was independent of changes in lipid levels, probably reflecting immunomodulation such as reduced natural killer cell cytotoxicity,[95] decreased cytokine activity and/or improved coronary endothelial function.[96] Simvastatin reduced graft coronary artery disease and overall mortality after heart transplantation in a 4-year follow-up trial.[97]

Once transplant vasculopathy is manifest, percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass grafting (CABG) offer palliative options in cases characterized by a single or a few focal lesions, as opposed to diffuse luminal narrowing.[98,99] Although restenosis was typically more frequent in dilated graft versus native coronary lesions, IVUS studies have identified extensive early recoil as a major cause for restenosis in cardiac transplant recipients.[99] Consequently, intravascular stent implantation recently resulted in improved outcome in patients with focal coronary obstruction. In a single centre experience, treatment of 41 patients (75 lesions, 85 stents) resulted in a success rate of 98% with a 6-month restenosis rate of 14%.[99] A similar restenosis rate (18%) after stent implantation was reported by another one-centre study, which favourably compared to a 60% restenosis rate with PTCA in the absence of stenting.[100] Although only limited experience is available regarding the surgical approach with CABG, this should be considered in

selected patients with focal coronary lesions not suitable for PTCA.

In summary, both alloantigen-dependent and independent factors are involved in the development of cardiac allograft arteriopathy. Treatment prophylaxis with HMG-CoA reductase inhibitors and, possibly, calcium channel blockers and ACE inhibitors may delay arterial occlusion. Preliminary clinical results using sirolimus are very encouraging,[89] and long-term results of an ongoing multicentre trial with this drug are eagerly awaited. Novel immunomodulating approaches (e.g., T-cell costimulatory blockade with CTLA4-Ig or CD40-Ig) have shown promising effects in experimental models. Optimized treatment of non-immunologic cardiovascular risk factors remains of paramount importance.

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