Immunomodulation of cardiac allograft vasculopathy: beyond immunosuppression

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Introduction

Following heart transplantation a rapid form of atherosclerosis develops known as cardiac allograft vasculopathy (CAV). As a leading cause of graft failure, CAV remains the dominant limiting factor for the long-term survival of human heart transplant recipients [1, 2]. The angiographically determined incidence of CAV is around 30% at 3 years post-transplantation [3] and by 10 years more than half of all surviving recipients have angiographic CAV [4].

Even though CAV appears to be related to the native form of atherosclerosis found in coronary artery disease (CAD), no direct association has been found between standard risk factors for CAD in the general population and the development of CAV in transplanted hearts [1, 5] and its etiology remains unknown. However, several immunological and nonimmunological mechanisms have been proposed, including mechanisms that result in the establishment of a prothrombotic microvasculature within the allografts.

Immunosuppression and CAV

The introduction of effective immunosuppressive regimens, along with a variety of therapies designed to target the immune response, have improved heart transplant outcome. Examples include the use of calcineurin inhibitors, which has been pivotal in reducing the frequency of acute rejection and improving early survival. The use of steroids, cyclosporine, tacrolimus and MMF has also had a significant beneficial impact, although these drugs have strong adverse side effects. Antiproliferative immunosuppressants have been used to inhibit T-cell and B-cell proliferation and proliferation-signal inhibitors have been used to block activation of T cells after autocrine stimulation by IL-2. The best results have been achieved with combined immunotherapy and, of the current commonly used immunosuppressive regimens, tacrolimus in combination with MMF appears to produce the greatest benefit [6].

Although the introduction of new immunosuppressive agents has shown beneficial effects on graft rejection and early survival, the development of CAV continues to be the principal limiting factor for long-term survival of heart transplant recipients, and the incidence of angiographically detected CAV has not changed appreciably over the past two decades [7]. Thus, current research is focused on identifying factors that stimulate or limit CAV in an effort to better understand the mechanisms of this disease and to develop new therapeutic strategies that might inhibit its development and progression.

Microvascular changes and CAV

During the past several years we have shown that the development of CAV in heart transplant patients is associated with several abnormalities within the cardiac microvasculature that are detectable immediately following transplantation [8-16]. These abnormalities are characterized by a heightened activation of the coagulation system, leading to a build-up of fibrin deposits in the arterial microvasculature, coupled with a loss of factors, such as antithrombin and tissue plasminogen activator (tPA), that would normally impede and remove fibrin deposits.

Patients whose cardiac allografts exhibit myocardial fibrin deposits during the first month post-transplant develop significantly more CAV and have a higher incidence of graft failure in the ensuing years than patients whose allografts are without fibrin deposits [10, 11]. Increasing levels of fibrin deposition are also associated with myocardial cell damage as evidenced by detectable levels of cardiac troponin I in the circulation of these patients [17].

To explain why some patients have early fibrin deposition within the cardiac allograft microvasculature, we have analyzed the status of the heparan sulfate proteoglycan-antithrombin anticoagulant pathway and the fibrinolytic pathway in the grafts [12-15]. This research has demonstrated that vascular antithrombin, which is found on arterial and arteriolar smooth muscle cells, arterial intima, and venous endothelium of normal hearts, is associated with the absence of microvascular fibrin deposition when present in transplanted hearts [18-21]. Conversely, we have also observed that

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the early loss of vascular antithrombin following heart transplantation is associated with fibrin deposition in the microvasculature and myocardial cells, particularly in areas of infarction, and with the subsequent development of CAV and cardiac graft failure [10, 11, 14, 15, 17, 21-23]. Importantly, the recovery of vascular antithrombin binding following the development of microinfarction is always associated with new capillary antithrombin binding in areas of vascular remodeling or new vessel growth, and is correlated with improved survival [23].

We have demonstrated further that when allografts lose vascular antithrombin in the first three months post-transplant and, consequently, exhibit fibrin deposits, these allografts also show a significant depletion of tPA from arteriolar smooth muscle cells [14, 15] and tPA depletion is also predictive of CAV and graft failure [13]. It is not clear what causes this depletion of tPA, although it is known that proinflammatory molecules such as C-reactive protein (CRP) can downregulate tPA [24]. It is also possible that the depletion of tPA might be related to the presence of uninhibited thrombin [13, 14]. In any case, all the above changes are known to promote the establishment of a prothrombotic microenvironment and to weaken thromboresistance [21].

Factors that activate the vascular endothelium are also thrombogenic and, therefore, increase the risk of CAV, because activated endothelial cells express tissue factor, the principal activator of the coagulation cascade. We have studied allografts with microvascular abnormalities to determine whether they also show signs of endothelial activation following transplantation, and whether such activation is associated with outcome [9, 15]. What we have observed is that when allografts show signs of fibrin deposits and loss of vascular antithrombin and tPA, they also tend to express arterial endothelial ICAM-1 and HLA-DR [15, 25], and the early expression of these markers on arterial and arteriolar endothelium correlates with the subsequent development of CAV and graft failure following cardiac transplantation [9]. There is also a correlation between the blood concentration of soluble ICAM-1 early after transplantation and the upregulation of ICAM-1 in endomyocardial biopsies of transplanted hearts, with both of these markers predicting subsequent CAV and graft failure [25].

The idea that adhesion molecules contribute to the development of CAV is further supported by studies in animal models [26-30]. It has been shown, for example, that CAV can be prevented by the short-term blockade of ICAM-1 and lymphocyte function-associated antigen-1 adhesion with monoclonal antibodies in a heterotopic heart transplantation mouse model [30]. It has also been shown that ICAM-1 participates in the neutrophil-mediated damage associated with ischemia-reperfusion injury in the heart [31].

Adhesion molecules have been found on arterial endothelium taken from CAV lesions in both animal models and human patients [26-30, 32], and in humans the expression of adhesion molecules on endothelium is considered a risk factor for the development of both native atherosclerosis [33] and CAV [8, 9].

The plasma concentration of soluble ICAM-1 is also considered a risk factor for future coronary occlusion and myocardial infarction in the general population [33]. Further studies are needed to investigate the mechanism(s) responsible for the upregulation of ICAM-1 in cardiac allografts and to explain why these allografts concomitantly show increased prothrombotic activity.

Proinflammatory molecules and CAV

Another line of investigation that we have pursued involves the association between various inflammatory factors and the development of CAV following heart transplantation. One such factor, C-reactive protein (CRP), is known to be an important risk factor for native atherosclerosis and CAD in the general population [34]. Elevated plasma levels of CRP have been shown to be predictive of subsequent cardiovascular events among apparently healthy men [35-38] and women [39], patients with stable and unstable angina [40-44], and patients with a previous history of myocardial infarction [45]. Elevated CRP serum levels may promote atherosclerosis through its effect on adhesion molecule expression, since it has been shown that CRP induces ICAM-1 expression in coronary artery endothelial cells [46, 47]. Proinflammatory molecules, such as CRP, also downregulate tPA [48]. Possible links between CRP and adhesion molecule expression, and between CRP and atherosclerosis, have been reported in the clinical research literature and we and others have demonstrated an association between elevated CRP levels and the subsequent development of CAV [49] and graft failure [49, 50] in cardiac transplant recipients.

Despite the wealth of epidemiological and in vitro evidence suggesting that CRP is a key contributor to the development of atherosclerosis [35, 47, 51-53], mechanistic evidence from experimental mouse models on the role of CRP has been controversial. Some investigators have observed accelerated progression of atherosclerosis in CRP-transgenic apoE$^{-/-}$ mice compared with CRP-nontransgenic controls [54]. Others, however, have reported that CRP is not proatherogenic in either apoE$^{-/-}$ [55-57] or LDL receptor-deficient mice [58]. Schwedler et al. [59] have shown that pentameric native CRP (nCRP) *increases* atherosclerosis, while modified monomeric CRP *reduces* atherosclerosis in apoE$^{-/-}$ mice, and Tennent et al. [60] reported that transgenic human CRP is neither proatherogenic, proatherothrombotic, nor proinflammatory in apoE$^{-/-}$ mice. A recent study by Kovacs et al. [61] showed that CRP is antiatherogenic in CRP-transgenic LDL receptor-deficient apoB100 mice. Our own research has shown that pentameric CRP is not proatherosclerotic or proinflammatory in apoE$^{-/-}$ mice [62]. Thus, the role of CRP in atherosclerosis, whether spontaneous or transplant-associated, needs to be elucidated further before serious consideration can be given to the development of new therapeutic strategies directed to antagonize CRP.

Immunomodulation: Beyond immunosuppression

The role of innate immunity in the prevention or inhibition of transplant-associated CAV and graft failure represents another promising avenue for investigation. We have been especially interested in exploring the possible beneficial role that the early presence of IgM antibodies may play following heart transplantation. Preliminary data from our laboratory reported 15 years ago were the first, and the only data to date, showing a protective effect for IgM antibodies in transplanted human heart patients [63]. Those data indicated that the early presence of these antibodies is associated with reduced morbidity and mortality when compared with patients lacking IgM reactivity. Furthermore, the absence of IgM was significantly related to the deposition of fibrin within the heart, which, as discussed above, has a detrimental effect on allograft and patient survival.

Our initial report was supported by other studies that showed an atheroprotective effect of natural IgM antibodies in mouse models of atherosclerosis [64]. These innate antibodies are present even in naive germ-free mice in the absence of any exogenous antigen exposure [65]. Binder et al. have proposed that these antibodies confer their atheroprotective effect by responding to oxidation-specific epitopes that constitute a "self-altered" danger signal, which occurs as a result of oxidative stress [66]. Oxidative stress may arise from events that occur when cells undergo apoptosis or in association with endothelial accumulation of oxidized low-density lipoproteins (ox-LDL). Either of these events could occur within the allografts of heart transplant recipients. Based on the experience gained from recent animal studies, we hypothesize that the presence of IgM antibodies in human cardiac allografts is associated with reduced CAV and reduced morbidity and mortality because of their capacity to remove the source of oxidative stress related to either the accumulation of cells undergoing apoptosis, the accumulation of endothelial ox-LDL, or both.

Summary and conclusions

Despite the success of immunosuppressive agents in the management of graft rejection in heart transplant recipients, the current leading cause of long-term graft failure, CAV, continues to be a significant problem and the principal factor limiting long-term survival in these patients. Research conducted during the past two decades by us and others suggests several promising avenues for developing new therapeutic approaches to prevent or control the progression of CAV. Some of these avenues are familiar, such as: (1) approaches that seek to block the coagulation system, either directly by preventing activation of the coagulation pathway, or indirectly by promoting factors, such as antithrombin and tPA, that hold it in check; (2) approaches that seek to prevent the activation of arterial endothelium and block the coagulation cascade by suppressing the expression of cell adhesion molecules and tissue factor; and (3) approaches that seek to prevent the chronic inflammation that seems to induce a prothrombotic state by blocking the effects of proinflammatory proteins such as CRP, or its principal activator, interleukin-6. Although all of these factors are familiar and considered to be important disease markers in heart transplantation, the specific biochemical mechanisms responsible for fibrin deposition in the microvessels, activation of the arterial endothelium, promotion of a procoagulant and proinflammatory microenvironment, and, ultimately, the establishment of an atherothrombotic vasculature in heart transplant patients are not completely understood.

Other avenues suggested by the research literature are, perhaps, less familiar, involving approaches that would seek to stimulate the innate immune response in order to counter the adverse effects of oxidative stress arising from cell apoptosis or the accumulation of ox-LDL, thereby establishing an atheroprotective microenvironment. The possible beneficial role of IgM antibodies is especially interesting in this regard. We are currently studying the possibility of reinforcing the immunosuppressive approaches currently used in heart transplantation with an immunomodulation approach designed to favor the production of IgM natural antibodies directed against oxidization-specific epitopes.

The enhancement of atheroprotective factors early after transplantation, when most oxidative and inflammatory processes occur, may be extremely relevant to counteract the effects of proinflammatory and proatherogenic molecules.

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