Increased Tissue Factor Expression Predicts Development of Cardiac Allograft Vasculopathy

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Background — Cardiac allograft vasculopathy (CAV) limits the long-term success of cardiac transplantation. The incidence of CAV is increased in patients with elevated plasma levels of oxidized lipids or fibrin deposition within right heart biopsy (RHB) specimens. The present study investigated whether tissue factor (TF), the expression of which is regulated by oxidized lipids, is upregulated in patients with CAV.

Methods and Results — A TF score was developed to quantify TF expression in RHB specimens from 63 consecutive patients undergoing routine annual posttransplantation RHB and coronary angiography. In patients >2 years (3.0 ± 0.8 years) posttransplantation (n = 35), a high TF score was observed with greater frequency (75% versus 26%, P < 0.004) in patients with CAV than those without CAV. In patients <2 years (0.87 ± 0.48 years) posttransplantation (n = 28) without evidence of CAV, the TF score was determined and patients were followed up prospectively. A high TF score had a positive predictive value of 78.6% for the development of CAV, and a low TF score had a negative predictive value of 100%.

Conclusions — These data demonstrate that early TF expression predicts subsequent development of CAV. Increased TF expression could link the elevated levels of oxidized LDL and fibrin deposition known to precede CAV. These findings suggest that TF may play a role in the pathophysiology of CAV and could offer a potential prognostic tool and a novel target for the prevention of CAV, possibly with antioxidants or inhibitors of the TF pathway. (Circulation. 2002;106: 1379-1383.)

Key Words: lipoproteins • thrombosis • transplantation • atherosclerosis

Cardiac allograft vasculopathy (CAV) remains one of the leading causes of mortality for heart transplantation patients who survive more than 3 to 5 years after transplantation, accounting for almost 20% of all deaths. It is characterized by proliferative, diffuse, concentric intimal thickening with a well-preserved internal elastic lamina that involves not only the major allograft coronary arteries, but also branch and intramyocardial vessels. The incidence of angiographically determined CAV after 1 and 5 years ranges from 8% to 10% and 21% to 42%, respectively, in large patient cohort studies.

Although the pathology of CAV remains unknown, studies suggest a strong association between its development and an imbalance of the coagulation and fibrinolytic pathways that favors coagulation. The presence of early fibrin deposition in biopsy samples after the first posttransplantation month has been significantly correlated with the subsequent development of CAV as well as with clinical outcome. Fibrin deposition implies an increased local production of thrombin, which can also lead to proliferation of smooth muscle cells and activation of platelets and endothelial cells.

Other factors that have been shown to predict the development of CAV are the plasma levels of oxidized low-density lipoprotein (oxLDL) and C-reactive protein (CRP). Our group and others have demonstrated in vascular cells that tissue factor (TF), a 47-kd cell membrane-bound glycoprotein that initiates the extrinsic coagulation cascade leading to thrombin formation by binding factor VII, is upregulated in response to oxLDL. CRP has also been shown to induce TF expression in monocytes. In the present study, we tested the hypothesis that TF expression may be increased in patients with CAV, and that TF expression early after transplantation could predict which patients subsequently developed angiographically significant CAV.

Methods

Patient Population

We studied 63 consecutive adult heart transplantation patients who underwent routine right heart biopsies at the time of surveillance angiography between December 1999 and February 2000. These patients were divided into 2 groups, those >2 years (3.0 ± 0.8 years, n = 35) posttransplantation and those <2 years (0.87 ± 0.48 years,
n=28) posttransplantation at the time of biopsy. Patients <2 years posttransplantation were prospectively followed up until at least 2 subsequent annual coronary angiograms were performed. Information about donor and recipient age, sex, ischemic time, immunosuppressive regimen, pathogenesis of cardiac dysfunction leading to transplantation, blood pressure, lipid levels, and use of other concurrent medications were obtained from the Heart Transplant database of the Cleveland Clinic Foundation, which is approved by the Institutional Review Board of the Cleveland Clinic Foundation. Biopsy score was determined by the sum of the numerical value given to each International Society of Heart and Lung Transplantation grade of rejection divided by the total number of biopsies within the first year after transplantation. Written informed consent was obtained from all patients with regard to access to their information from the Cardiac Transplant Research Database (CTRD) at the Cleveland Clinic Foundation.

Definition of Cardiac Allograft Vasculopathy
Annual left heart catheterization with selective coronary angiography was performed in all transplant recipients with the percutaneous femoral approach and standard angiographic techniques. All patients had at least 2 coronary angiograms for comparison. An independent angiographer unaware of any study parameters evaluated the coronary angiograms serially to detect the presence, absence, or progression of CAV. A diagnosis of CAV was made if there were any evidence of angiographic coronary artery narrowing or luminal irregularities in either the proximal or distal branches.

Tissue Factor Staining

Three 5-micron serial sections were obtained from a flash-frozen right heart biopsy specimen from each patient. The sections were first treated with 3% hydrogen peroxide to eliminate native peroxidase activity and were then blocked with 1% bovine serum albumin. Sections were stained for TF protein with a primary monoclonal anti-human TF antibody (4509, American Diagnostica) at a dilution of 1:1000, and a biotin-labeled rabbit anti-mouse antibody. The sections were treated with streptavidin bound to horseradish peroxidase, followed by diaminobenzidine and hydrogen peroxide following the manufacturer’s protocol (Vector Laboratories), which led to the development of a brown reaction product at sites of TF expression. Specificity of TF staining was verified by staining serial sections with an irrelevant primary or secondary antibody.

We developed a TF score ranging from 0 to 4, depending on the amount and frequency of TF-positive vessels in the biopsy specimen. A TF score of 0 indicated that no TF was detected; 1, TF staining in a single vessel confined to the luminal portion of the vessel wall; 2, TF in a single vessel with evidence of TF beyond the vessel wall; 3, multiple TF positive vessels with staining limited to the luminal portion of the vessel wall; and 4, TF in multiple vessels with evidence of TF beyond the vessel wall. Representative examples of each score are presented in Figure 1. Photomicrographs of each section were obtained, and 2 independent observers blinded to all clinical data scored each section. Similar results were obtained from each serial section from a given patient. Importantly, all TF scores were obtained before any investigator knew any clinical data about the patients. Furthermore, for those patients followed up prospectively, the TF score was determined at the time of the index biopsy, 2 years before the patient underwent the final coronary angiogram used in the study.

Statistical Analysis

All results are presented as mean values±SD. Differences among categorical variables were analyzed with the χ² or Fisher’s exact test. For continuous variables, two-tailed t tests were used to assess differences. A probability value <0.05 was considered statistically significant.

Results

Tissue Factor and Existing CAV
The data in Figure 2 show the percentage of patients >2 years posttransplantation within each TF score who had either evidence of CAV or no CAV at the time of surveillance angiography. These data demonstrate that higher TF scores were significantly associated with the presence of CAV (P<0.01). On the basis of these data, we simplified our subsequent analyses by defining a low and high TF score. A low TF score was defined as a TF score of 0 or 1, and a high TF score was defined as a TF score of 2, 3, or 4. Figure 3 demonstrates that in those patients who had biopsies >2 years after transplantation, a low TF score was associated
with CAV in a 22.2% of patients, whereas a high TF score was associated with a 70.6% incidence of CAV. These data revealed the strong correlation between the presence of CAV and TF expression (P<0.004). No correlation with biopsy rejection grade was found (data not shown).

### Tissue Factor Expression Predicts the Development of CAV

TF score was also predictive of the subsequent development of CAV in the patient cohort who had their index biopsy <2 years after transplantation (Figure 4). In this cohort, TF score significantly predicted the development of CAV (P<0.0001). Of the 14 patients with a low TF score at the time of biopsy, none developed CAV with subsequent surveillance angiography over 2 years (negative predictive value: 100%). In contrast, a high TF score had a positive predictive value of 78.6% for the development of CAV within 2 years. The demographic and clinical characteristics of this cohort <2 years after transplantation are compared in the Table. Donor cytomegalovirus (CMV) positivity (P<0.002) and donor age (P<0.02) were the only parameters that correlated with the development of CAV. Ischemic time, lipid levels, immunosuppression regimens, average biopsy score during the first year after transplantation, and use of other medications such as hydroxymethyl glutaryl coenzyme A reductase inhibitors and angiotensin-converting enzyme inhibitors were similar between the 2 groups and did not predict the development of CAV in a univariate model.

### Discussion

In the present study, we demonstrate that increased TF expression is associated with the development of CAV. This is the first report to show elevated levels of TF in the setting of human CAV, and extends to humans the previous finding of increased TF expression in a rat model of CAV.13 Because TF expression is highly regulated by inflammatory mediators,14 its elevation in CAV provides further evidence that upregulation of the inflammatory and coagulation pathways in response to vascular injury seems to be of paramount importance in the development of not only native atherosclerosis, but also of CAV.

We observed TF staining from the luminal surface to beyond the medial layers of small intramyocardial arteries in biopsy specimens from transplanted hearts with or at risk for transplant arteriopathy. This pattern of TF staining is similar to that observed in a rat transplantation model of cardiac allograft vasculopathy.13,15 Interestingly, this TF staining pattern is distinct from that observed in normal myocardium,16 in which it has been postulated that TF may have a structural role in the maintenance of cardiac muscle.17 Of note is the fact that TF expression is significantly decreased in the myocardium of patients with sepsis,16 perhaps suggesting distinct pathways of myocardial TF regulation in inflammatory and noninflammatory states.

TF plays a central role in hemostasis and thrombosis by initiating the process of coagulation through binding and activating coagulation factor VII. The resultant TF:VIIa complex can then activate both the intrinsic and extrinsic
blood coagulation pathways via cleaving factor X to Xa and factor IX to IXa, respectively, ultimately leading to the formation of thrombin and the conversion of fibrinogen to fibrin. In addition to its fundamental role in the activation of coagulation, the TF:VIIa complex also possesses the ability to mediate smooth muscle cell migration and induce smooth muscle cell proliferation via increased local thrombin production.

Although the pathogenesis of CAV is still poorly understood, its development is thought to be related to early damage to the endothelium, which leads to endothelial dysfunction. Posttransplantation patients who have an early constrictor response to acetylcholine, a marker of endothelial dysfunction, have been shown to be at a higher risk of developing CAV within the first year of transplantation. Possible initiators of early endothelial damage include ischemic injury that occurs before harvest (eg, endothelial cell injury associated with intracerebral hemorrhage) or during the harvest and cold ischemic period, during implantation and reperfusion, or after implantation in response to circulating oxidized LDL and viral infections such as CMV.

The correlation of TF expression with the development or presence of CAV could be an epiphenomenon; however, it is also intriguing to consider whether TF could be involved in the pathophysiology of CAV. Increased TF expression leads to increased levels of local thrombin that can lead to endothelial cell activation and smooth muscle cell proliferation. By mediating the activation of blood coagulation, the increased expression of TF in CAV leading to thrombin formation could provide a mechanism by which previous studies have correlated increased fibrin deposition, depletion of tissue plasminogen activator, and increased levels of α2 plasmin-inhibitor with the development of CAV. The ability of CRP to induce TF expression may explain the recently reported association between elevated levels of CRP and decreased graft survival in cardiac transplantation patients.

The association between elevated TF expression and CAV could lead to several potential therapeutic strategies to either retard or prevent the progression of CAV. We have shown that TF activity is increased on the smooth muscle and endothelial cell surface in response to oxidant stress, such as that initiated by hydrogen peroxide and lipid hydroperoxides. It has been postulated that oxLDL is proatherogenic in native atherosclerosis via multiple mechanisms, one of which is endothelial dysfunction, which is dependent on the degree of protection from oxidation of LDL. Oxidant stress also seems to be increased in CAV, as shown by the association between elevated levels of oxLDL and the subsequent development and severity of CAV. Increased levels of nitric oxide synthase in smooth muscle cells and macrophages of patients with CAV.

Increased TF expression could potentially be inhibited by mechanisms that reduce oxidative stress, such as antioxidants. Fang et al recently demonstrated that treatment of cardiac transplantation patients with vitamin E and C retarded the progression of CAV. Moreover, one could postulate that the modulation of TF is a potential mechanism of how increased LDL levels worsen the severity of CAV, and treatment with hydroxymethyl glutaryl coenzyme A-reductase inhibitors reduce the incidence of CAV. Lastly, the development of direct inhibitors of TF such as anti-TF antibodies, factor VIIa inhibitors, or recombinant TF pathway inhibitor would reduce TF expression. Such treatments would allow the determination of whether impeding TF expression also blunted the progression of CAV.

The present study also shows that CAV was correlated with increasing donor age and donors who were seropositive for CMV. These factors may have played a role in the pathogenesis of CAV, thus leading to elevated TF expression. Several investigations have shown that cardiac allograft recipients seem to be at a higher risk of developing CAV when transplanted with hearts from donors that are >35 years of age. Similarly, CMV has been implicated in the pathogenesis of CAV in several studies. In a retrospective analysis, cardiac transplantation patients who were treated prophylactically for 28 days with ganciclovir after heart transplantation had a reduced incidence of angiographically determined CAV at a mean follow-up of 4.7 years compared with placebo. On the basis of the results of the present study, one could postulate that inflammation in response to the presence of CMV may result in increased vascular expression of TF in transplanted hearts leading to endothelial cell activation and smooth muscle cell proliferation.

Limitations of the present study suggest the future studies that should be performed. First, TF expression was assessed only at 1 biopsy with the use of a semiquantitative method. A more quantitative approach with an enzyme-linked immunosassay on protein extracts from biopsy specimens may be useful. A more precise chronological assessment of TF expression and its association with the development of CAV is an important avenue to pursue. The fact that we observed a particularly strong correlation between TF expression in a single right heart biopsy specimen with the presence or development of CAV, however, suggests that CAV may involve the entire coronary vasculature. It would also be meaningful to assess circulating levels of other mediators of inflammation and coagulation such as fibrin, tissue plasminogen activator, oxLDL, and CRP. Because serial intravascular ultrasound examinations were not consistently performed in our study population, we may have underestimated the proportion of patients who developed CAV. Finally, we defined the development of CAV by coronary angiography, which may have underestimated the occurrence of CAV. Future studies should define and follow the progression of CAV with the use of intravascular ultrasound.

In summary, we have shown for the first time that early TF expression in cardiac allografts predicts the development of CAV. This finding potentially offers a mechanistic link between lipid oxidation, inflammation, and the coagulation cascade in cardiac allografts, and supports the current concept that the development of atherosclerosis in the transplantation population is dependent on the upregulation of inflammatory and thrombotic pathways. It also offers a potential method (heart biopsy and TF staining) for identifying patients at risk for the development of CAV, who may benefit from aggressive novel therapies.
Additional studies of CAV that address the chronological expression of TF, the pathological changes seen with increased TF expression, and the clinical outcomes of patients who receive treatment strategies that lead to decreased TF expression (ie, antioxidants, anticoagulants,15 or VIIa mimetics) are needed to determine whether TF synthesis is an epiphenomenon or a primary regulator of CAV.

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