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Prothrombogenic Microvasculature and Transplant Outcome

Carlos A. Labarrere, David R. Nelson, and Steven J. Miller

A major obstacle to the long-term success of transplanted solid organs is the development of transplant-associated vasculopathy. A prime risk factor for the development of vascular disease is the conversion of the microvasculature from a thromboresistant to a prothrombogenic state, leading to fibrin deposition. A thromboresistant microvasculature is characterized by vessels in which the endothelium has intact natural anticoagulant and fibrinolytic pathways and by the lack of activated endothelial cells. A prothrombogenic microvasculature results when there are failures in anticoagulant pathways, such as the protein C and the vascular antithrombin pathways, or when there is an absence of fibrinolytic activity, as when tissue plasminogen activator is depleted. Activated endothelium, characterized by increased arterial expression of moieties such as intercellular adhesion molecule-1 and human leukocyte antigen-DR, also is associated with a prothrombogenic microvasculature. The presence of a prothrombogenic microvasculature early after transplantation is associated with an increased incidence of both coronary artery disease and chronic graft failure. Although the mechanisms responsible for the loss of thromboresistant endothelium are unclear, the fact that changes in the anticoagulant, fibrinolytic, and activational status of endothelial cells may occur early after transplantation suggests a peritransplant phenomenon as an initiating event. The use of both new and established therapies to inhibit the formation of a prothrombogenic microvasculature could slow the development of transplant coronary artery disease and significantly improve allograft survival.

Introduction
The development of transplant-associated vasculopathy within the allograft is one of the major impediments, if not the major obstacle, to the long-term success of a transplanted solid organ. Indeed, it seems that in the long-term acceptance of a transplanted organ, the donor’s vessels, not the recipient’s immune cells, are the final determinants of outcome. During the past several years, efforts were made to identify changes within the allograft microvasculature that could be risk factors for transplant failure. It was shown that the status of the microvasculature (i.e., thromboresistant vs. prothrombogenic) is directly associated with the development of transplant-associated vasculopathy and finally allograft failure. Investigations performed using the cardiac transplant model, in which patients are followed with serial endomyocardial biopsies, allow for the identification of these factors immediately after transplantation and for their evaluation during the entire posttransplant period. The evaluation of serial tissue samples in cardiac allografts enables the generation of a moving picture of the developing pathological events within the allograft microvasculature.

Thromboresistant Microvasculature
The normal microvasculature of an organ is characterized by the presence of thromboresistant endothelial cells. Thromboresistance is mediated by mechanisms involving anticoagulation and fibrinolysis, and these mechanisms are associated with a lack of endothelial activation (Fig. 1). Two principal anticoagulation mechanisms are present on the
Thromboresistant Microvasculature

a. Absence of Microvascular Fibrin
b. Presence of Tissue Plasminogen Activator in Arteriolar Smooth Muscle Cells
c. Absence of Arterial and Arteriolar Endothelial Activation (ICAM-1/HLA-DR)
d. Presence of Vascular Antithrombin (Arteries and Veins)

Figure 1. Characteristics of a thromboresistant microvasculature.

plasma membranes of normal human endothelial cells. One of these mechanisms involves the protein C anticoagulant pathway and the other the heparan sulfate proteoglycan-antithrombin anticoagulant pathway. Indeed, components of these pathways, such as thrombomodulin and antithrombin, are normally identified in the vasculature of different organs such as the kidney, heart, and placenta. The thromboresistant microvasculature also shows particular characteristics of expression of endothelial activation markers such as intercellular adhesion molecule-1 (ICAM-1) and major histocompatibility class II antigen, human leukocyte antigen-DR (HLA-DR). It has been demonstrated that ICAM-1 and HLA-DR are constitutively expressed in the capillaries and veins of normal hearts and kidneys, but the thromboresistant arterial tree does not express immunohistochemically detectable levels of these endothelial activation markers. Obviously, if the microvasculature of any organ is thromboresistant, fibrin deposits will be absent from the microvessels of that organ (Fig. 1).

Characteristics of a Prothrombogenic Microvasculature

Microvascular Fibrin

Conversely, the principal characteristic of a prothrombogenic microvasculature is the presence of microvascular fibrin. Fibrin could be deposited within the allograft microvasculature through different mechanisms, such as failure of microvascular anticoagulation and fibrinolysis and endothelial tissue factor expression associated with endothelial cell activation (Fig. 2). The presence of microvascular fibrin can be detected within a week after cardiac transplantation, and the microvascular changes promoting the deposition of fibrin early after transplantation directly affect the long-term outcome of those patients. Indeed, the presence of microvascular fibrin during the 1st month posttransplantation is directly associated with the subsequent development of transplant coronary artery disease (CAD) and allograft failure years after the procedure. The association of microvascular fibrin with subsequent outcome becomes more important when we consider that the presence of fibrin within the allograft microvasculature is associated with concomitant myocardial cell damage. Indeed, microvascular fibrin within the allograft is significantly associated with the presence of circulating levels of troponin I, a marker of myocardial cell injury. Each of these factors—the immunohistochemical detection of microvascular fibrin within the endomyocardial biopsies and the presence of elevated levels of circulating troponin I—is an independent risk factor for transplant CAD (Table 1) and allograft failure.
Interestingly, the importance of fibrin for the outcome of solid organ transplantation is not limited to cardiac transplantation. The presence of fibrin within the microvasculature of renal allografts is directly associated with renal allograft dysfunction and early allograft failure.15,16 The relevance of a prothrombogenic microvasculature for allograft survival is clearly recognized in a naturally occurring transplant, the feto-placental unit. As in any other organ of the economy, the normal characteristic of the placental microvasculature is to show thromboresistance. Thromboresistance is mainly through the expression of components of the protein C pathway such as thrombomodulin and the presence of fibrinolytic pathways (depletion of vascular tissue plasminogen activator).

Loss of Vascular Antithrombin

One of the characteristics of a prothrombogenic microvasculature within the allografts is the loss of endogenous anticoagulation (Fig. 3). Indeed, the loss of vascular antithrombin in renal and cardiac allografts is associated with the development of transplant-associated vasculopathy and increased failure.8,15,16 Therefore, the status of vascular antithrombin after transplantation is directly associated with subsequent allograft outcome. Early depletion of vascular antithrombin during the 1st 3 months after transplantation is associated with development of transplant CAD7 (Table 1). Patients with depleted vascular antithrombin develop the disease earlier, have more rapid progression of the disease, and develop more severe disease than patients with normal levels of vascular antithrombin in the arterial and venous microvasculature.8

Several mechanisms could be involved in the loss of vascular antithrombin binding within the allograft microvasculature. It is possible that the recipient’s macrophages and T-lymphocytes within the allograft could release growth factors, cytokines, or enzymes such as heparinase that could directly affect the expression or availability of antithrombin-binding molecules such as cellular or extracellular heparan sulfate proteoglycans.19-22 Although this possibility cannot be completely excluded as a trigger of the events leading to loss of vascular antithrombin binding, it is the loss of vascular antithrombin and not the presence of cellular rejection episodes that is associated with transplant CAD and outcome, suggesting that another mechanism could be involved.8

Because the loss of vascular antithrombin is associated with a prothrombogenic microvasculature and the final deposition of microvascular fibrin, it is possible that components of the coagulation cascade directly affect the expression or availability of heparan sulfate proteoglycan molecules. Interestingly, thrombin can accelerate shedding of syndecan-1 and syndecan-4 molecules and reduce the synthesis of perlecan by endothelial cells,23,24 and changes in these molecules in the allograft microvasculature could explain the reduced ability to bind antithrombin.

The loss of vascular antithrombin binding could be explained by alterations in the expression or availability of the heparan sulfate proteoglycan core protein with or without changes in the polysaccharide chain length, inadequate sulfation of the heparan moieties, or release of heparan sulfate proteoglycan.
glycan molecules secondary to antibody-mediated complement activation.25-29

Because the loss of vascular antithrombin occurs during the immediate posttransplant period, the possibility of ischemia and reperfusion as the trigger for these microvascular changes needs to be considered. This possible trigger is particularly relevant because ischemia and reperfusion are associated with neutrophil recruitment, which could lead to cleavage of heparan sulfate molecules through the release of enzymes such as neutrophil elastase or heparanase.30,31

The importance of vascular antithrombin binding for allograft outcome is strengthened by the finding that recovery of antithrombin binding after initial loss, which is always associated with novel capillary antithrombin binding (never found in normal circumstances), is associated with reduced incidence of transplant CAD and improved survival.3 Patients showing these characteristics within the allograft microvasculature not only have less incidence of transplant CAD, but they develop the disease later, show less disease progression, and have less severe disease compared to patients that never recover vascular antithrombin binding after initial loss.4 Because the binding of antithrombin within the capillary network occurs after the deposition of microvascular fibrin and develops mainly around areas of microinfarction, it has been suggested that these microvascular changes could be the result of new vessel formation or vascular remodeling.32 This idea is particularly tempting when we consider that these changes are associated with increased synthesis and expression of growth factors such as vascular endothelial growth factor within the cardiomyocytes.32

Although the recovery of vascular antithrombin binding associated with novel capillary antithrombin binding reduces transplant CAD and improves survival, the incidence of transplant CAD and allograft failure in the group of patients who initially lose vascular antithrombin is significantly higher than in patients who never lose vascular antithrombin after transplantation.5 This suggests that capillary antithrombin binding is perhaps a healing mechanism in response to ischemia in a group of patients whose baseline characteristic is the presence of a prothrombogenic microvasculature. The final outcome will be determined by the presence of such a prothrombogenic microvasculature.

Figure 3. Characteristics of a prothrombogenic microvasculature.
Endothelial Activation

A prothrombogenic microvasculature shows other peculiar characteristics (Fig. 3). Endothelial activation is associated with expression of tissue factor, which initiates the extrinsic pathway of coagulation by serving as a cofactor and receptor for factor VIIa. The microvasculature of transplanted human hearts with deposits of microvascular fibrin shows evidence of endothelial activation (Fig. 3). These allografts show up-regulation of ICAM-1 and HLA-DR expression on endothelium of capillaries and veins and novel expression of these endothelial activation markers on endothelium of arteries and arterioles. These findings are particularly relevant because expression of ICAM-1 and HLA-DR is not immunohistochemically detected in the arterial microvasculature of the normal heart.

The expression of endothelial activation markers ICAM-1 and HLA-DR is detected immediately following the transplant procedure within the 1st week after transplantation, and the early expression of these markers (during the 1st 3 months after transplantation) seems to be relevant for the long-term outcome of the patients. Allografts with early expression of arterial and arteriolar ICAM-1 and HLA-DR also concomitantly show increased levels of serum soluble ICAM-1. Both increased expression of endothelial activation markers within the allografts and increased ICAM-1 levels in circulation are independently related to development of transplant CAD (Table 1) and subsequent allograft failure.

Increased expression of endothelial activation markers within the allograft microvasculature could be the result of increased cytokine release. Cytokines released by immune cells such as macrophages and T-lymphocytes could promote such activation, but this does not seem to be the mechanism of endothelial activation in these allografts because patients showing activated endothelium do not have increased incidence of cellular rejection episodes. Endothelial cell injury can be mediated by immune cells, antibodies, or perhaps ischemia and reperfusion. All of these different mechanisms could explain increased endothelial activation. It has been shown that tissue hypoxia can enhance induction of endothelial ICAM-1 and that these changes can be inhibited by antisense oligodeoxynucleotides or antibodies to ICAM-1. The importance of endothelial adhesion molecule expression as a risk factor for subsequent CAD also has been shown in animal models of heart transplantation, because the use of monoclonal antibodies to ICAM-1 and lymphocyte-functional-associated

### Table 1 | Relationship between Early Risk Factors (First 3 Months Posttransplant) and Subsequent Transplant CAD

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>HAZARD RATIO*</th>
<th>95% CI</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial fibrin</td>
<td>6.9</td>
<td>3.6-13.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Detectable serum cardiac troponin I</td>
<td>1.8</td>
<td>1.1-2.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Depleted arteriolar tissue plasminogen activator</td>
<td>4.9</td>
<td>2.7-8.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Arterial endothelial ICAM-1/HLA-DR</td>
<td>6.1</td>
<td>3.2-11.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vascular antithrombin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative antithrombin</td>
<td>2.6</td>
<td>1.4-4.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Capillary antithrombin</td>
<td>2.0</td>
<td>1.2-3.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Elevated serum soluble ICAM-1</td>
<td>2.7</td>
<td>1.3-5.6</td>
<td>0.009</td>
</tr>
<tr>
<td>Cellular rejection episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grades 1 and 2</td>
<td>1.3</td>
<td>0.8-2.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Grades 3 and 4</td>
<td>1.2</td>
<td>0.6-2.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Number of nonimmunologic risk factors†</td>
<td>1.9</td>
<td>1.6-2.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Hazard ratios and 95% confidence intervals (CI) from Cox proportional hazard regression. Hazard ratios for “Number of nonimmunologic risk factors” calculated for each increase of one risk factor.
†Number of nonimmunologic risk factors includes myocardial fibrin, detectable serum cardiac troponin I, depleted arteriolar tissue plasminogen activator, arterial endothelial ICAM-1/HLA-DR, vascular antithrombin, and elevated serum soluble ICAM-1. ICAM-1 = intercellular adhesion molecule-1; HLA-DR = human leukocyte antigen-DR.
antigen-1 inhibits subsequent development of the disease.35

Lack of Fibrinolytic Activation

Another relevant characteristic of a prothrombogenic microvasculature is the lack of fibrinolytic activation within the microvessels (Fig. 3). The microvasculature of the normal heart contains the principal activator of the fibrinolytic pathway, tissue plasminogen activator, within the smooth muscle cells of arteries and arterioles, and the presence of this activator is associated with absence of microvascular fibrin deposits.35 Depletion of arteriolar smooth muscle cell–associated tissue plasminogen activator early after transplantation (within 3 months of the procedure) is associated with increased incidence of transplant CAD (Table 1) and allograft failure.1 Allografts with depletion of microvascular tissue plasminogen activator show earlier development of transplant CAD, more severe disease, and more rapid progression of the disease than allografts that never lost microvascular tissue plasminogen activator. The fibrinolytic system seems to be dysfunctional in allografts prone to developing transplant CAD because overexpression of plasminogen activator inhibitor-1 has been found in the arterial walls of those allografts.36 Interestingly, allografts that show depletion of arteriolar tissue plasminogen activator also show increased tissue plasminogen activator complexed to plasminogen activator inhibitor-1 within the whole allograft microvasculature, suggesting that these allografts perhaps have increased expression of endothelial plasminogen activator inhibitor-1, which is able to inhibit the activity of tissue plasminogen activator released from the microvasculature. The relevance of plasminogen activator inhibitor-1 in the microvasculature of transplanted human hearts is emphasized by the finding that specific genotypes for this molecule in the allografts are associated with increased risk for transplant CAD.37 These particular genotypes could be associated with increased expression of the inhibitor and decreased fibrinolytic activation. The fact that patients with transplant CAD have elevated circulating levels of tissue plasminogen activator and plasminogen activator inhibitor-1 and low fibrinolytic activity supports the presence of a dysfunctional fibrinolytic system within the allograft microvasculature.38,39

Therapies to Reduce Microvascular Prothrombogenicity

The presence of a procoagulant microvasculature is a condition directly associated with transplant CAD and allograft failure. However, it is not yet clear what sequence of events leads to a procoagulant microvasculature, nor is it clear what trigger(s) initiate(s) the development of a procoagulant microvasculature. The detection of procoagulant changes in the allograft microvasculature within 1 week of transplantation strongly suggests that peri-transplant phenomena could trigger these changes. Whether the trigger(s) is(are) ischemia and reperfusion, immune cells or antibodies, endothelial injury associated with donor’s brain death or donor age, or any other factor needs to be proven. However, the fact that a microvasculature that is prothrombogenic develops in a significant number of allografts and that this microvasculature is associated with subsequent vascular disease has been clearly established.

These findings suggest that therapies directed to block or impede the generation of a prothrombogenic microvasculature (Table 2), together with conventional immunosuppressive therapies directed to control the activity of the recipient's immune cells, could perhaps retard the development of vascular disease and subsequently prolong allograft survival.

The Role of Statins

Microvascular changes that promote thrombogenicity could be affected by lipid-lowering drugs such as statins, which inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step in cholesterol synthesis. The ability of these inhibitors to lower plasma cholesterol effectively and reduce cardiovascular morbidity and mortality in patients with and without cardiovascular disease has been well documented.40-42 However, the beneficial effects of statins on vascular function independent of their ability to lower serum cholesterol have been noted.43-46 These so-called pleiotropic vascular effects include improved endothelial function, decreased coagulation, increased fibrinolysis, decreased inflammation, and increased plaque stability, among other effects. Statins can reduce the incidence of acute rejection with hemodynamic
compromise after heart transplantation and the incidence and progression of CAD. Statins may then affect the status of the cardiac microvasculature independently of their lipid-lowering effects. Statins may be useful in reducing the incidence of graft vascular disease by minimizing thrombogenic events. Simvastatin has been shown to depress blood clotting by inhibiting activation of prothrombin and factor XIII, reducing rates of factor Va generation, and enhancing factor Va inactivation. These effects were unrelated to lipid-lowering effects.

Tissue plasminogen activator activity was increased 3-fold in aortas isolated from rats receiving lovastatin for 2 days. Lovastatin treatment also increased tissue plasminogen activator activity and protein in human umbilical vein endothelial cells (HUVEC) and rat aortic endothelial cells (SVARECs) in a time- and concentration-dependent manner, whereas it inhibited plasminogen activator inhibitor-1 activity and mRNA in SVARECs. These effects could be reversed by mevalonate and geranylgeranyl pyrophosphate, but not by low-density lipoprotein-cholesterol (LDL-C) and farnesyl pyrophosphate. Interestingly, an inhibitor of Rho mimicked effects of lovastatin on tissue plasminogen activator and plasminogen activator inhibitor-1.

In another study, atorvastatin and fluvastatin decreased plasminogen activator inhibitor-1 protein and mRNA in HUVEC, while increasing tissue plasminogen activator in cells grown in low serum. Both statins slightly decreased plasminogen activator inhibitor-1 synthesis in cells stimulated with tumor necrosis factor-α, but not with interleukin-1α. Both statins also inhibited a decrease in tissue plasminogen activator release mediated by tumor necrosis factor-α. Pravastatin therapy caused a reduction in thrombogenicity in hyperlipidemic patients through tissue plasminogen activator- and plasminogen activator inhibitor-1-mediated effects. Interestingly, these fibrinolytic/antithrombotic actions were found not to be proportional to the magnitude of LDL-C reduction. Based on evidence to date and considering the prothrombotic nature of the microvasculature in a high proportion of cardiac allografts, it seems likely that statin therapy may prove effective in ameliorating this condition, thereby improving outcome.

The Role of Anticoagulants
Another way to regulate the deposition of microvascular fibrin within the allografts is to use recombinant antithrombin to inhibit thrombin-mediated cleavage of fibrinogen. The administration of antithrombin either to replete depleted stores or to achieve supraphysiological concentrations could be a novel approach during cardiopulmonary bypass. Microvascular fibrin can also be reduced

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Pathway Affected</th>
<th>Therapeutic Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statins</td>
<td>Anticoagulant, fibrinolytic, &amp; EC activation</td>
<td>↓ Fibrin, ↑ IPA, ↓ PAI-1, ↓ inflammation</td>
</tr>
<tr>
<td>Recombinant AT on pump</td>
<td>Anticoagulant</td>
<td>↑ AT in plasma during surgery</td>
</tr>
<tr>
<td>Low-molecular-weight heparin</td>
<td>Anticoagulant</td>
<td>↓ Fibrin</td>
</tr>
<tr>
<td>Hirudin</td>
<td>Anticoagulant</td>
<td>↓ Fibrin, ↓ tissue factor</td>
</tr>
<tr>
<td>Overexpress HSPG (perlecans)</td>
<td>Anticoagulant</td>
<td>↑ AT binding</td>
</tr>
<tr>
<td>HELP/apheresis treatment</td>
<td>Anticoagulant</td>
<td>↓ Fibrinogen</td>
</tr>
<tr>
<td>Decrease steroid use</td>
<td>Fibrinolytic</td>
<td>↓ PAI-1</td>
</tr>
<tr>
<td>Overexpress IPA</td>
<td>Fibrinolytic</td>
<td>↑ IPA</td>
</tr>
<tr>
<td>Mab/antisense to ICAM-1</td>
<td>EC activation</td>
<td>↓ Inflammation, ↓ Fibrin</td>
</tr>
<tr>
<td>Omega-3 fatty acids</td>
<td>EC activation</td>
<td>↓ Inflammation</td>
</tr>
<tr>
<td>Reactive oxygen species inhibitors &amp; antioxidants</td>
<td>EC activation</td>
<td>↓ Inflammation</td>
</tr>
</tbody>
</table>

EC = endothelial cell; IPA = tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor-1; AT = antithrombin; HSPG = heparan sulfate proteoglycans; HELP = heparin-induced extracorporeal low-density lipoprotein-cholesterol/fibrinogen precipitation; ICAM-1 = intercellular adhesion molecule-1.

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with the use of other anticoagulant molecules such as low-molecular-weight heparins or hirudin. The use of these molecules may be beneficial not only for acute early events during the peritransplant period but also for long-term outcome of the allografts. Indeed, it has been demonstrated that low-molecular-weight heparins prevent the development of transplant CAD in animals. Hirudin also was able to decrease neointimal hyperplasia in a rat animal model of heart transplantation not only because of its direct effect on thrombin but also by inhibiting tissue factor expression within the cardiac microvasculature. Interestingly, in patients with unstable angina treated with heparin and having subnormal antithrombin levels, the use of antithrombin supplementation resulted in less activation of coagulation and a tendency toward less stenosis. Anticoagulation can also be affected within the allograft microvasculature, perhaps by using novel approaches that could deliver genes able to promote angiogenesis, such as perlecan. The overexpression of perlecan molecules may also allow increased binding of antithrombin within the allograft microvasculature and may impede further deposition of fibrin by inhibiting thrombin.

The Role of Plasma Fibrinogen Reduction

The reduction of plasma fibrinogen also could affect the generation of microvascular fibrin. It has been shown that early aggressive lowering of fibrinogen after cardiac transplantation using heparin-induced extracorporeal low-density lipoprotein cholesterol/fibrinogen precipitation (HELP)-apheresis significantly reduced the risk of developing CAD. Interestingly, HELP-apheresis not only can reduce the development of transplant CAD but also can provoke regression of preexisting CAD.

The Role of Fibrinolysis Improvement

Because decreased fibrinolytic activity is a characteristic of a prothrombogenic microvasculature, therapies to benefit outcome should be directed to improving fibrinolysis within the allografts. It has been shown that treatment with steroids is associated with a hypo-fibrinolytic state due to increased plasminogen activator inhibitor-1 antigen and activity levels, suggesting that perhaps decreasing steroid use in transplantation would improve fibrinolytic activity. Another approach directed to correcting decreased fibrinolytic activity in patients with a prothrombogenic allograft microvasculature involves in vivo intracoronary transfer of the tissue plasminogen activator gene to promote the synthesis of tissue plasminogen activator in the arterial wall. It has been demonstrated recently that intracoronary delivery of human tissue plasminogen activator gene at the time of transplantation results in significant early transgene expression and significantly inhibits the development of transplant CAD in an animal model of heterotopic heart transplantation.

The Role of Reducing Expression of Endothelial Activation

Because a prothrombogenic microvasculature shows evidence of endothelial activation, any intent to reduce the expression of endothelial activation markers also would favor transplant outcome. Antibodies to adhesion molecules or antisense oligodeoxynucleotides to reduce adhesion molecule expression within the allograft microvasculature could be used to reduce microvascular disease and prolong allograft survival.

Perhaps simple approaches, such as the use of omega-3 fatty acids in dietary fish oil, could be used since it has been demonstrated that fish oil decreases endothelial cell activation. As previously mentioned, statins could directly affect endothelial activation because it has been shown recently that simvastatin inhibits interferon gamma-induced major histocompatibility class II expression in human vascular endothelial cells and that pravastatin reduces plasma markers of inflammation and improves peripheral endothelial function in heart transplant recipients.

The Role of Ischemia and Reperfusion

The development of a prothrombogenic microvasculature occurs during the 1st week after transplantation. It has been suggested that the ischemia and reperfusion phenomenon could be one of the possible triggers of these changes. Several therapeutic approaches directed toward reducing endothelial cell injury during the peritransplant period could be used.

The use of recombinant superoxide dismutase during surgery could mitigate not only the peri-transplant acute events but also long-term compli-
cations such as development of CAD. Nitric oxide precursors like L-arginine could improve myocardial recovery and the recovery of endothelial functions after reperfusion and could perhaps prevent chronic changes such as the development of CAD.

Indeed, it has been demonstrated recently that the ex vivo administration of L-arginine polymers to cardiac allografts enhanced vascular nitric oxide production and decreased intimal hyperplasia in a rat animal model of heterotopic heart transplantation.

The introduction of antioxidants such as vitamins E and C and beta-carotene soon after transplantation could have an impact because it has recently been demonstrated that these vitamins impede the progression of CAD in cardiac allografts. Because thrombin seems to be a pivotal player in the generation of lesions of ischemia and reperfusion through the recruitment of neutrophils within the lesions and also is the principal player in the generation of fibrin, the use of serine protease inhibitors like antithrombin could be beneficial. This is a promising possibility when we consider that antithrombin reduces tissue damage after ischemia and reperfusion.

Conclusion

A thromboresistant microvasculature is associated with delayed transplant CAD and improved survival. Development of a prothrombogenic microvasculature early after transplantation is directly associated with development of transplant CAD and allograft failure, and any treatment directed to countering prothrombogenicity (Table 2) could reduce the incidence of disease and prolong survival. Therapies need to be introduced during the peritransplant period to impede or delay development of this disease and prolong allograft and patient survival. Efforts may need to be concentrated on better protection of the donor organ during preservation and/or better care of the donor patients to reduce the risks of microvascular damage within the allografts.

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