

Alive and Kicking

Endothelium at the Geographic Nexus of Vascular Rejection

Sean P. Mazer, David J. Pinsky

Transplantation of vascularized cardiac grafts across an allogeneic barrier has revolutionized the treatment of organ failure, including heart failure. With current immunosuppressive therapy, acute vascular rejection is an uncommon complication of allograft transplantation. However, the shortage of donor organs continues to limit the benefit of organ replacement therapy. The United Network of Organ Sharing (UNOS) registry data indicate that although 2202 heart transplants were performed in 2001, approximately 4000 patients remained on the waiting list and 622 patients died before receiving an organ. Many times this number died without even being listed for transplant at all.

Xenotransplantation, transplantation of organs between different species, could theoretically provide an unlimited source of organs. However, a number of barriers remain to the clinical implementation of xenotransplantation. One of the most important of these barriers, acute vascular rejection, develops in the continuum of delayed xenograft rejection (DXR). DXR occurs shortly after implantation of a vascularized xenograft and remains a major impediment to clinical application of xenotransplantation of vascularized organs. Acute vascular rejection occurs within a time frame of one to several days after transplantation, as a consequence of immune activation. Histologically, it is characterized by platelet aggregation, fibrin deposition, and cellular infiltration by host natural killer cells and monocytes. Small vessels are typically involved in DXR, and eventually, focal infarcts and interstitial hemorrhage develop in the transplanted organ.

As a tissue, the endothelium sits at the geographic nexus of blood and tissue. It regulates homeostasis (nutrient delivery and waste removal) and modulates pathophysiology (inflammation, thrombosis, fibrinolysis, and platelet aggregation). In solid-organ transplantation, the development of diffuse, concentric narrowing of small blood vessels (called transplant vasculopathy), provides evidence that the vascular endothelium is indeed an allogeneic target organ. In DXR, inciting events remain unclear, particularly the relevance and timing of endothelial cell apoptosis and activation. Apoptotic endothelial cell death would cripple critical endothelial homeostatic functions, such as inhibition of thrombosis and suppression of cellular activation cascades, and theoretically could result in DXR. Furthermore, processed antigens from apoptotic endothelial cells could amplify the host versus graft immune reaction. Endothelial cell activation is an equally plausible mechanism for initiating DXR. In this scenario, not only are endothelial cells alive during the development of DXR, but their metabolic machinery is revved up for maximal disruption of basal vascular homeostasis. In this state, endothelial cells are capable of expressing glycoprotein adhesion receptors, synthesizing cytokines, enhancing immune costimulation, altering the thrombotic/antithrombotic balance, and permitting unchecked platelet aggregation. Which of these two mutually exclusive mechanisms, endothelial apoptosis or endothelial activation, dominates the vascular milieu during DXR?

In this issue of Circulation Research, Holzknecht et al deliver a clear answer to this question, providing an overdue description of the inciting events in the pathogenesis of DXR after heart transplantation. Using a pig to baboon model of heart transplantation, these authors show that vascular fibrin deposition precedes the appearance of apoptotic endothelial cells by TUNEL staining. In addition, they demonstrate that markers of necrosis (membrane attack complex and human C4 immunoreactivity) precede TUNEL positivity by at least 4 days. Focal areas of necrosis can also be seen by gross histological examination to precede evidence of apoptosis. In the same model system, mRNA levels for potential pro- and antiapoptotic genes increase, but lag significantly behind the histological development of DXR. An active role for endothelial cells in DXR is supported by high levels of immune staining for the metabolic marker ribosomal P-antigen in endothelium on the first day after cardiac xenotransplantation. A supporting electron micrograph shows a nonapoptotic endothelial cell with expanded rough endoplasmic reticulum, indicative of cellular activation. These data show clearly that the endothelium is nonapoptotic during early critical stages of DXR. Taken together, this would suggest that active endothelial cell processes rather than apoptotic death underlie DXR at the earliest stages. These data do not exclude the possibility that endothelial cells undergo apoptosis during DXR at later stages; in fact, the data would suggest just the opposite. The salient message is that endothelial apoptosis does not initiate DXR.

Instead, DXR is probably the outcome of a complex process that begins at the molecular level in the moments after vascular anastomosis and reperfusion of the xenograft. Immune and nonimmune factors present in these first moments critically influence the development of delayed organ...
rejection in the ensuing days. Data from both animal and human studies suggest that preservation injury represents the initial cause of endothelial activation, contributing to a subsequent increase in vascular rejection.5–7 Other critical initiating events include platelet, monocyte, and natural killer cell (NK cell) interactions with the endothelium. These interactions contribute to endothelial cell activation. Naïve human NK cells can directly activate xenogeneic endothelial cell in an α-gal–independent manner.8 Platelets and monocytes can activate endothelial cells and each other through a variety of contact-dependent and independent mechanisms, which probably play a propagating and amplifying role in the early stages of DXR.

In the context of DXR, activated endothelial cells respond to both cytokines and direct contact with leukocytes and platelets with a stereotypical pattern of gene expression (see Figure). Chemokines such as MCP-1 and IL-8 are synthesized and secreted, attracting and activating monocytes and natural killer cells. Increased expression of glycoprotein adhesion receptors such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin at the endothelial surface represents an important mechanism for leukocyte retention to sites of vascular activation. Proinflammatory mediators such as tumor necrosis factor-α (TNF-α) are synthesized by both the activated endothelium as well as the recruited leukocytes, amplifying cellular activation and tissue destruction in the vicinity. In this toxic environment, oxygen radicals rapidly quench endothelium-derived nitric oxide and endothelial surface apyrase CD39 declines.9,10 These changes alter the major homeostatic mechanisms preventing platelets from activation, aggregation, and degranulation. The activated platelets, monocytes, and endothelium induce local tissue factor expression. Declining levels of thrombomodulin on endothelial cells prevent the generation of the profibrinolytic and anti-inflammatory activated protein C.11

The events in DXR are quite similar to those seen in microvessels after an inflammatory stimulus (eg, ischemia/reperfusion injury or endotoxemia). In an in vitro model of ischemia, hypoxic exposure of endothelial cells results in P-selectin translocation to the cell surface from preformed subplasmalemmal storage vesicles (Weibel-Palade bodies), which also release prothrombotic von Willebrand factor.12 These very early events proceed without the requirement for de novo protein synthesis, as they proceed in the presence of cycloheximide. In isogenic models of cardiac transplantation, vascular P-selectin expression represents an important cause of early inflammation and microvascular failure.13 E-selectin, the expression of which requires de novo transcription of mRNA and its translation into functional protein, is also an important participant in the generalized inflammatory upregulation characteristic of endothelial cell activation. Vascular expression of ICAM-1, exacerbated by a positive feedback loop wherein both endothelial cells and cardiac myocytes themselves synthesize and secrete interleukin-1, further contributes to graft leukocyte accumulation and tissue damage.14,15 Similar events occur in other vascularized transplants, such as the kidneys16 and lungs,17 suggesting that endothelial cells in different transplanted vascular beds react to the transplantation procedure (severance from native blood supply followed by implantation/reperfusion) in much the same way.

Another characteristic facet of the rejecting vascular milieu is the widespread detection of microvascular thrombosis. In the work of Holzknecht et al,3 acute vascular rejection in the porcine to baboon model of cardiac xenotransplantation was associated with a specific increase in immunoreactivity for fibrin, detected using a FITC-conjugated antibody against human fibrin monomer.3 These data indicate that in DXR, the precarious coagulant/anticoagulant balance that exists within the quiescent vessel wall tips in favor of thrombosis. Not surprisingly, platelet accumulation represents an important
feature of this same model, in this case, detected with an antibody to CD41. It is not surprising that there is a concordance between evidence of humoral coagulation (ie, fibrin accumulation) and platelet deposition because the two are linked at multiple levels. Accumulating thrombin directly activates platelets, while the presence of platelet aggregates provides an intravascular phospholipid framework to perpetuate further fibrin generation and coagulation.

Abundant evidence suggests that activation of coagulation can itself trigger immune inflammatory mechanisms, via thrombin (PAR) receptor–mediated endothelial cell activation, or via the EPR receptor on endothelial cells, a receptor for activated factor X. Induction of tissue factor mRNA provides another important clue as to the pathogenesis of the thrombotic component of acute vascular rejection. Recent data in a hypoxic cell culture model system suggests that mononuclear phagocytes may be key players in the initiation and propagation of vascular thrombosis in response to a diverse set of inflammatory or ischemic stimuli, through their ability to markedly augment their synthesis of tissue factor and plasminogen activator inhibitor-1, the major serine protease inhibitor of fibrinolysis.

The CD40-CD154 pathway represents an emerging paradigm for endothelial-platelet-monocyte crosstalk at the inflamed vessel wall. Endothelial cells and platelets express both CD40 constitutively and CD154 upon activation. Monocytes express CD40 upon activation with a variety of proinflammatory stimuli. CD40-CD154 signaling increases endothelial tissue factor production and decreases thrombomodulin expression. Platelet CD154 can provide the costimulatory signal to CD40 on adherent monocytes or endothelial cells, resulting in the increased cell surface expression of tissue factor from preformed stores and chemokine production. Biopsies from human cardiac allografts demonstrate marked CD40 expression on arterial endothelial and smooth muscle cells. In murine vascularized cardiac allografts, blockade of CD154 with a monoclonal antibody suppresses vascular rejection and prolongs graft function. Receptor-ligand dyads, like CD40-CD154, contribute to the convergence of coagulation, inflammation, and platelet activation at the vascular wall, and their proinflammatory and procoagulant signaling likely have a critical role in inciting acute vascular rejection.

Not only are posttranscriptional mechanisms likely to be of relevance to acute vascular rejection, but transcriptional mechanisms may be equally important. Early growth response gene-1 (Egr-1), a member of the zinc finger family of transcription factors, has been recently shown to trigger transcription of diverse families of genes, leading to expression of cytokines, chemokines, coagulant molecules, and permeability-enhancing gene products. In the context of lung transplantation, Egr-1 expression has been implicated in the development of primary lung graft failure. In cardiac allotransplantation, mice null for the Egr-1 gene develop significantly less chronic rejection, marked by reduction of neointimal formation as determined by histomorphometric vascular analysis at 60 days. However, tissue analysis reveals that Egr-1 induction by the transplantation procedure is an early event, indirectly suggesting that it may also participate in a causal way to the earlier-occurring DXR in which characteristic Egr-1 target genes (such as tissue factor) are known to be induced.

Taken together, these data show that patterns of endothelial activation are conserved even when inciting stimuli diverge. Characteristic repertoires of endothelial behavior may be programmed by common underlying transcriptional mechanisms, or common triggers for preformed activation cascades. Although the endothelial executioner, in the form of caspase 3, arrives at the scene in DXR, it is a latecomer to the party and therefore only a bystander to the infernal tumult that culminates in the vascular rejection of xenotransplanted organs.

Acknowledgments
This work was supported by the USPHS (NIH RO1 grants HL60900, HL55397, and HL69448 to D.J.P. and T32 HL007854 to S.P.M.).

References


KEY WORDS: xenotransplantation vascular biology rejection
Alive and Kicking: Endothelium at the Geographic Nexus of Vascular Rejection
Sean P. Mazer and David J. Pinsky

Circ Res. 2002;91:1085-1088
doi: 10.1161/01.RES.0000047883.93904.AA

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/91/12/1085

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org//subscriptions/