Xenotransplantation, the transplantation of viable organs, tissues and cells between species, has been proposed as a solution to the shortage of human organs for the treatment of organ failure. Although hyperacute rejection (HAR) in experimental discordant combinations can be now effectively managed, vascularized xenografts are still subject to acute vascular rejection, alternatively referred to as delayed xenograft rejection (AVR/DXR). This latter mode of rejection is associated with vascular inflammation, thrombo-cytopenia and the consumption of coagulation factors, that may evolve to disseminated intravascular coagulation (DIC). There are historical precedents for the development of coagulation abnormalities and thrombocytopenia in association with solid organ xenograft rejection. In a recent clinical context, hemoperfusion of porcine renal explants by blood from human volunteers has resulted in significant thrombocytopenia with rapid onset of vascular injury; similar events have been described with ex vivo porcine liver hemoperfusion (reviewed in ref. 4). We have also demonstrated that the infusion of discordant (porcine) hematopoietic cells in primates to induce tolerance by mixed chimerism is also associated with widespread thrombotic vascular injury with deleterious consequences for the recipient.

How Does the Thrombotic Process Relate to Inflammation and to the Proposed Molecular Barriers?

The mechanisms underlying DIC and thrombotic microangiopathy in these settings are still unclear. It is possible that varying levels of immune mediators within the vascularized xenograft could promote vascular thrombosis, as a component of the inflammatory response ab initio with endothelial cell (EC) activation. However, thrombosis may also relate to non-immunological molecular barriers that we and other groups have identified.

**EC activation.** EC activation processes, with the accompanying vascular prothrombotic and inflammatory changes, are important manifestations of experimental xenograft rejection. Natural xenoreactive antibodies (XNA) directed at α-galactosyl (Gal) residues of pig epitopes and associated complement activation by the classical pathway, appear to be the major immediate mediators of the immediate EC injury, vascular thrombosis and xenograft infarction in the discordant swine-to-primate combination.

**Complement activation and humoral immunity.** Primate recipients may be treated prophylactically by depletion of XNA and/or complement inhibition to ameliorate these events. Transplantation of transgenic pig organs that express human complement regulatory proteins (e.g., hDAF and hCD59) into primates provides an alternative approach to overcoming HAR. Complement inhibition following the grafting of these transgenic organs appears to be very effective in blocking HAR and the immediate complement-mediated activation of platelets and coagulation. Unfortunately, the duration of this beneficial effect is limited and the prolongation of experimental porcine xenograft survival in primate models can be still measured only in days to weeks. These delayed rejection events are generally associated with the deposition of XNA, local generation of procoagulants, vascular thrombosis and ultimate graft loss.
Perturbation of coagulation with thrombosis. The demonstrated loss of the vascular EC natural anticoagulants such as tissue factor pathway inhibitor (TFPI) and/or platelet regulators such as CD39 ATP diphosphohydrolase activity following activation responses in vivo would potentiate procoagulant changes within the rejecting xenograft. Such developments could exacerbate vascular damage from whatever cause and further potentiate the activation of platelets and coagulation pathways resulting in graft infarction. In addition, there are also increasingly well-defined incompatibilities between activated primate coagulation factors, e.g., thrombin or factor Xa, and the natural anticoagulants e.g., thrombomodulin (TBM) or TFPI, expressed on xenogeneic leukocytes and endothelium. This proposed primary biological dysfunction of xenografts or xenogeneic cells with respect to regulation of clotting could primarily amplify vascular injury, promote immunological responses and/or independently contribute to graft failure or cellular non-engraftment. Platelet activation and vascular injury. EC-platelet interactions and development of platelet aggregates also appear to be prominent factors in xenograft rejection. The enhanced potential of porcine von Willebrand factor (vWF) to associate with human platelet glycoprotein (GP)Ib through the porcine vWF A1 domain suggests that this may represent an important barrier to xenograft viability. Exposure and expression of vWF in the xenogeneic sub-endothelium following EC retraction or injury could result in massive activation of circulating platelets with formation of aggregates even prior to activation of coagulation.

Consequences of the Disordered Regulation of Coagulation and Platelet Activation

We have recently determined that the activation of coagulation factors by the xenograft vasculature has the potential to generate serious systemic hemostatic abnormalities with localized injury progressing to a form of DIC. Our observations are that coagulopathies do not develop in similarly conditioned primates exposed to allografts or discordant xenografts. The data suggest that low levels of complement activation, XNA deposition and putative molecular barriers, individually or together, are critical for the development of DIC. Recent studies by d'Apice and colleagues presented in Nagoya have confirmed our own assumptions that the process of DIC is a consequence of the xenograft rejection and not an artifact induced by the extensive conditioning regimen used to induce immunological tolerance. Yet, DIC has not been reported by other groups using cardiac or renal CD55 or CD55/CD59 grafts. One apparent discriminating feature is the use of high doses of the cytotoxic agent cyclophosphamide in groups that have not observed coagulopathy. It is not clear whether this apparent benefit is associated with direct inhibition of platelet activation or via attenuation of xenoantibody-mediated responses.

Therapeutic Implications

Further evidence for the importance of coagulation mediators in xenograft rejection can be inferred by the beneficial effects of the inhibition of thrombin/serine proteases. These are largely comparable to benefits seen with complement inhibition. Inhibition of platelet aggregation by treatment of xenograft recipients with an antagonist to the platelet fibrinogen receptor, GPIIbIIIa, by the use of P-selectin or PAF antagonists, or by administration of a soluble NTPDase have been generally shown to prolong graft survival in several discordant xenotransplantation models. The use of these anticoagulants and anti-thrombotic agents following surgery is challenging and hence we are initially addressing their application in the thrombotic disturbance associated with the infusion of xenogeneic cells. The targeted expression of important thromboregulatory factors in the vasculature of transgenic pigs already over-expressing human complement regulators may facilitate the ultimate application in vascularized xenotransplantation.

Conclusions

Disordered thromboregulation could have deleterious effects, comparable to unregulated complement activation, in the pathogenesis of xenograft rejection and may represent a unique, substantiative barrier to xenotransplantation. Therapeutic avenues under consideration will address both the primary incompatibility and inflammation-mediated loss of what we believe to be the key thromboregulatory components, namely TBM, TFPI and CD39.
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**TABLE OF CONTENTS**

**Ligands—Small Molecules**
1.1 Non-Peptidic “Small Molecules”
   - Insert: Representation of Proteins
   - TGFβ
   - hCG-Receptor
1.2 Peptidic and Peptidohormones
1.3 Peptidic Toxins

**Ligand-Binding Proteins—Large Molecules**
2 Enzymes
3 Membrane Receptors
4 Signal Transduction Proteins
5 Multimodular Adhesion Proteins
6 DNA-Binding Proteins
7 Immunological Proteins
8 Miscellaneous Macromolecules

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