Graft

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Preventing Acute Vascular Rejection

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Successful transplantation of organs between discordant species is complicated by multiple immunological and physiological barriers. The primary obstacle to long-term graft survival in today's pig-to-primate model is acute vascular rejection (AVR). Although progress has been made in understanding its mechanism, there are currently no proven and practical strategies to prevent AVR in the pig-to-baboon setting. This review briefly reviews the events leading to AVR, lists the important effectors in its pathogenesis, and describes several approaches to extend xenograft survival beyond this persistent form of rejection.

Development of Acute Vascular Rejection

Pig-to-primate xenograft rejection is initiated by the binding of xenoreactive natural antibody (XNA) to the xenoantigen galactose-α1,3-galactose (Gal) expressed on endothelial cells (EC), triggering activation of the recipient's complement cascade. Renal xenografts provide an excellent example of the strength of the response; the transplantation of a wild type porcine kidney into a baboon is almost invariably followed by hyperacute rejection within minutes to hours. Modification of the donor organ using transgenic technology and/or manipulation of the recipient's immune response can modulate the histological features of rejection and prolong xenograft survival. However, grafts are eventually lost due to a mixed humoral and cellular form of rejection, previously described in rodent models and termed delayed xenograft rejection.[1] The addition of high-dose immunosuppressive therapy, particularly regimens that include cyclophosphamide, significantly extends xenograft survival and reduces the cellular infiltration, but the graft is ultimately lost to a predominantly humoral form of rejection, AVR.[2]

Effectors of Acute Vascular Rejection

XNA

It is becoming evident that the actions of XNA on xenograft endothelium are critical to the development of AVR in the pig-to-primate model. The Gal epitope is expressed on a wide variety of molecules, such as adhesion molecules, linked to the porcine EC membrane. Cross-linking of Gal by either XNA or lectins, even in the absence of complement, is capable of activating EC via tyrosine phosphorylation, leading to transcription of genes for adhesion molecules and inflammatory cytokines.[3]

XNA-binding also results in a conformational change in the Fc region of the immunoglobulin molecule, making it accessible to Fc receptors expressed on NK cells and monocytes. This interaction activates the NK cells[4] and monocytes,[5] leading to secretion of cytokines (TNF, IFN, IL-1, lymphotoxin) and increasing the cytotoxic capacity of human NK cells towards porcine EC.[4]

Complement. Inhibition of complement activation using soluble agents or transgenic expression of CD55 and/or CD59 overcomes hyperacute rejection of xenografts, but does not influence the generation of early activated complement components, such as C1q, which can act as chemoattractants and alter capillary permeability.[6] Incomplete inhibition of complement may cause activation of EC by sublytic levels of the membrane attack complex and by fluid-phase inactive terminal complement complexes.[7]

Monocytes and NK cells. Monocyte and NK cell infiltration and anti-graft activity is potentiated by XNA binding (see above) but, even in the absence of XNA, both have been shown to activate porcine EC in vitro.[4] Prior activation of the EC increases the ability of NK cells/monocytes to adhere, and may enhance the damage inflicted by these cells on the transplanted organ.

Vascular thrombosis and local ischemia. Non-immunological factors such as ischemia-reperfusion injury following transplantation and ischemic EC injury as a consequence of small vessel thrombosis, have been underestimated in their ability to activate EC. Hypoxia/ischemia reduces the anticoagulant properties of the EC, favoring intravascular thrombosis.

Molecular incompatibility. Many of the differences between xenograft rejection and allograft rejection may be attributable to molecular incompatibilities between species. The first and most dramatic is the inability of porcine membrane-bound complement regulatory factors to inhibit human complement activation, resulting in HAR. Additional incompatibilities exist for cytokines, cytokine receptors, adhesion molecules, and platelets. As with hyperacute rejection, these incompatibilities may not be overcome unless the molecule is expressed in the donor organ as a transgene.

Prevention of Acute Vascular Rejection

Removal of XNA and/or elimination of Gal. Depletion of XNA may be achieved by immuno-affinity column adsorption to remove all immuno-globulin[2] or anti-Gal antibodies.[8] In combination with high-dose immunosuppression to inhibit further XNA synthesis, this approach may delay the onset of AVR.[2] However, it has two inherent problems. First, it must be pursued aggressively and repeatedly, with concomitant risk of infectious and other complications in the recipient. Second, it appears to be very difficult to prevent the eventual return of high levels of high-affinity XNA directed against Gal and possibly other epitopes.[9] Among the more promising strategies being pursued to overcome this rebound of XNA are the use of immunotoxins (anti-B cell monoclonal antibodies conjugated to toxins)[8] and the induction of costimulatory blockade.[10]

An alternative approach is to reduce or eliminate expression of Gal, the target of the majority of XNA. Although reduction of Gal by transgenic

expression of H transferase has proved less than satisfactory in the pig,[11] the generation of a Gal-negative pig has moved a step closer with the recent cloning of a pig by nuclear transfer from a fetal fibroblast.[12] Whether such an animal proves to be viable remains to be seen, and it is important to note that antibodies directed against non-Gal epitopes will still need to be addressed.

Improved control of complement activation. None of the pig-to-primate studies reported to date have achieved complete inhibition of complement activation. Studies by the Imutran group suggest that transgenic expression of CD55 in the donor organ may be incapable of fully blocking the complement activation associated with AVR.[13] Higher expression of CD55 and/or other regulators, or treatment of the recipient with soluble complement inhibitors, may solve this problem, but will be ineffective against the generation of C1q and other early components of the complement cascade. Peptide inhibitors of C1q hold some promise,[14] although their efficacy in the pig-to-primate model remains to be determined.

Prevention of graft infiltration and injury by monocytes and NK cells. A number of strategies have been suggested to prevent the destructive action of monocytes and NK cells. These include specific depletion using antibodies or liposome-toxin conjugates, prevention of cellular adhesion using antibodies directed against adhesion molecules, prevention of cytokine production or action using soluble receptors or anti-cytokine antibodies, and reduction of ADCC/direct toxicity by transgenic expression of HLA-G and E.[15] While several of these have shown potential in small animal models, none has yet been proven in the pig-to-primate model.

Inhibition of thrombosis. Intravascular coagulation is a key feature of AVR. The approaches described in the previous sections will undoubtedly reduce the severity of the problem; nevertheless, non-immunological factors such as ischemia-reperfusion injury and molecular incompatibilities between the coagulation control systems of donor and recipient must also be addressed. Clearly immunosuppression will not influence these factors and a more detailed understanding of the physiology of the EC following donor-organ harvesting and during storage is required to reduce the susceptibility of the transplanted

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organ. Selective anticoagulant treatment or transgenic expression of human anticoagulant and/or antioxidant proteins may also be effective.

Inhibition of EC activation. In conjunction with controlling the above factors that activate xenograft EC, a direct approach to blocking the activation process holds great appeal. Cyclophosphamide appears to inhibit EC activation, but is a difficult drug to use long-term. A more attractive possibility is the transgenic expression of a negative-dominant mutant of NF-κB,[16] but this remains untested in vivo.

Conclusion

AVR is a multifactorial process involving immunological and physiological responses of both the donor organ and the recipient. It is our opinion that XNA, predominantly against Gal, is the primary cause of AVR and that, without the elimination of either XNA or Gal, xenograft failure is inevitable. Furthermore, the detection of non-anti-Gal, non-complement-fixing antibodies in primates prior to rejection of CD55-transgenic porcine[9] raises the possibility that antibodies directed to epitopes other than Gal will lead to AVR in the absence of Gal. Thus, prevention of AVR will likely require a more effective protocol to deal with XNA and xenoantigen than is currently available, along with blockade at multiple sites to inhibit EC activation, cellular infiltration, and intravascular thrombosis.

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