Thrombotic Microangiopathy: The Next Big Hurdle for Xenotransplantation

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Current in the US there are 66,885 patients waiting for a renal transplant (1). This number continues to rise each year and waiting time for renal transplants has increased, due to a lack of donors. In fact, deceased donor rates have fallen 36% since 1995 and the transplant rate in patients <50 yr of age has fallen 13 to 19% over the same time period (2). Xenotransplantation has the potential to solve this problem but rejection remains a formidable obstacle. Whereas allograft rejection is a cellular response mediated by the adaptive immune system, xenograft rejection is mediated by both innate and acquired immune mechanisms (3). Xenograft rejection can be divided into three phases: hyperacute rejection, acute humoral xenograft rejection, and T cell–mediated cellular rejection. The most important features of hyperacute rejection (HAR) are the binding of preformed anti-donor antibody to pig endothelium (EC) and the activation of complement, which occurs not only because of antibody binding but because of incompatibilities between human complement factors in the blood and complement regulatory molecules (CRF) on pig endothelium (3). Hence, either the removal of preformed anti-donor antibody or the prevention of complement activation through the transgenic expression of human CRF on pig EC is sufficient to prevent HAR (4–7). It was this observation that led to the proposition that xenotransplantation could be developed as a clinical therapy.

Circumventing HAR merely lead us to the next hurdle: acute humoral xenograft rejection (AHXR). The key pathogenic events are thought to be endothelial activation, abnormal thromboregulation, and infiltration by host NK cells and macrophages (3). The binding of preformed host antibody with the galactose-α1,3-galactose (αGal) carbohydrate epitope on pig cells is thought to be a major initiating event in this process. However, a proper understanding of the pathogenesis of AHXR has been difficult because of a lack of appropriate animal models and difficulty in completely absorbing anti-αGal antibody from the host or removing the αGal epitope from the donor pig. The article by Shimizu et al. in this issue of the JASN has clarified many of these complex issues, especially as it relates to renal xenotransplantation (8). In their study, HAR was averted by using kidney grafts from human decay accelerating factor (hDAF) transgenic pigs, and treating the donor with cobra venom factor. T cell–mediated rejection was prevented by using a combination of a pig thymus implant, donor thymectomy (the so-called thymokidney), and strong immunosuppression. In previous studies the authors showed that newly derived, donor-reactive T cells are deleted in the “thymokidney model” and rejection averted (9). Finally, attempts were made to delete anti-αGal antibody at the time of transplant, which returned in all recipients despite prior splenectomy and the use of continuous cyclophosphamide and mycophenolate mofetil. Despite this complex and intensive conditioning regimen, the renal xenografts were rejected within one month. All grafts were lost to thrombotic microangiopathy, the pathogenesis of which is the major focus of the article by Shimizu et al. Thrombosis microangiopathy and disseminated intravascular coagulation (DIC) have been described in previous studies. In fact, CRF-transgenic pig renal grafts succumb to DIC and microangiopathy when transplanted into nonimmunosuppressed baboons, suggesting that it is an early and major host response to vascularized xenografts (7,10). This was confirmed by the work of Shimizu et al. (8), where abnormal thromboregulation preceded any cellular response to the graft. This is in contradistinction to prior animal studies that predicted that an innate, immune-mediated accumulation of natural killer (NK) cells and macrophages would be the predominant immune response if HAR could be averted (11).

The study by Shimizu et al. identifies several predisposing factors for thrombotic microangiopathy (8). First and foremost, anti-αGal antibody has long been suspected as a significant factor in this process, and its rapid reappearance correlated strongly with more severe renal injury. This was followed closely by an altered EC phenotype with evidence of EC apoptosis preceding EC activation. This combination of events then lead to upregulation of von Willebrand factor and expression of tissue factor, and hence the creation of a prothrombotic environment, the final consequence being intraglomerular microangiopathy. The impact of these early initiating events was quickly amplified. Intraglomerular deposition of platelet thrombi was associated with loss of hDAF and CD39 on EC cells. This was associated with renewed complement activation (as evidenced by strong C4d staining), a greater shift to a prothrombotic environment, ongoing amplification of the thrombotic microangiopathy, and loss of the graft.

How can these findings help us develop a pig kidney that can be transplanted clinically without microangiopathy and survive beyond one month? First, these data confirm what was already suspected: The αGal epitope needs to be removed, complement activation needs to be prevented, and pig EC need to be maintained in an antithrombotic state. Already, αGal, the major target of preformed anti-pig antibody in humans, has

Published online ahead of print. Publication date available at www.jasn.org.

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been deleted in pigs (12,13). As Shimizu et al. comment, the use of αGal knockout pigs has had a significant impact on the severity of renal thrombotic microangiopathy after pig renal xenotransplantation in baboons (14).

Will the development of αGal knockout pigs mean the end of thrombotic microangiopathy as a major problem for renal xenotransplantation? Current evidence suggests that altered thromboregulation persists despite the elimination of αGal. When hearts from αGal knockout pigs were transplanted into heavily immunosuppressed baboons, graft survival was prolonged but many of the hearts ultimately succumbed to thrombotic microangiopathy (15). This is not surprising as there are several molecular incompatibilities between human clotting factors and thromboregulatory molecules present on pig EC. In particular, thrombomodulin and tissue factor pathway inhibitor are incompatible with human clotting factors, and as a result pig EC is naturally prothrombotic when exposed to the human circulation (16). If we are to overcome this problem and bring renal xenotransplantation to the clinic, then we need to develop a pig whose EC lacks αGal and expresses both human complement regulatory and thromboregulatory molecules. Such animals are already under development to test this hypothesis. Whether they will be sufficient to overcome all the major incompatibilities between humans and pig renal xenografts or merely expose new ones remains a major focus of ongoing research.

References