C. Session "Cardiac Transplantation"

(1) Gene-knockout (GT-KO) heterotopic cardiac xenotransplantation: are GT-KO pigs essential for successful clinical xenotransplantation?

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One of the fundamental advantages of xenotransplantation is that the donor organ, because it is derived from a pig, can be genetically modified to relieve the patient of as much of the immunosuppressive burden as possible. The first example of the utility of this approach was the development of human complement regulatory protein pigs which essentially eliminated hyperacute rejection (HAR) and extended cardiac xenograft survival from less than a few hours to 5–7 days. These donor organs eliminated the need for systemic complement inhibition and thereby significantly reduced the immunosuppressive burden of the recipient. With the advent of nuclear transfer technology, and the extension of that technique to agricultural species, it became possible to produce pigs with targeted mutations. This lead to the disruption of the porcine α-galactosyltransferase gene which catalyzes the terminal addition of UDP-galactose to produce the α-Gal carbohydrate. As optimal control of α-Gal antibodies using daily infusions of Gal-polymers had resulted in median cardiac xenograft survival beyond 3 months, the creation of pigs deficient in the α-Gal antigen was widely anticipated to eliminate the need for α-Gal polymers and perhaps extend graft survival by reducing the intensity of the overall immune response. Before the utility of gene-knockout (GT-KO) donors for clinical xenotransplantation can be determined, there are three major questions that should be addressed: First, will these animals be phenotypically normal and will they reproduce through natural breeding? Secondly, will using GT-KO pig hearts eliminate the need for infusion of α-Gal polymers and achieve similar survival times? Finally, what form of rejection will be observed using GT-KO organs and how will this new hurdle be overcome?

The Mayo group, in collaboration with Nextran, developed GT-KO pigs from porcine fetal fibroblasts containing a neomycin insertion that disrupts exon 9 of the α-galactosyltransferase gene [1]. The initial heterozygous female animals were bred to a series of unrelated male pigs carrying an hDAF transgene and the offspring were backcrossed to produce homozygous GT-KO and GT-KO; hDAF pigs. We presented the lineage of GT heterozygous by heterozygous and GT-KO homozygous boar by heterozygous sow matings. All of the GT-KO animals produced to date were healthy and showed no evidence of cataracts, a phenotype initially reported in GT-KO mice. Production of the GT-KO genotype has been at or near the predicted Mendelian frequencies.

We also reported on a series of eight GT-KO pig to baboon heterotopic heart transplants that used a maintenance immunosuppressive regimen that matched a previous study using GT+ CD46 transplants in which the recipients were successfully treated with daily infusion of α-Gal polymers to block anti-Gal function and induction. These GT-KO transplants were designed to test the efficacy of GT-KO donor hearts as a substitute for α-Gal polymers. The recipients were not pre-selected for low levels of preformed non-Gal antibody as this form of selection was not possible in previous transplants. Unexpectedly, one of these hearts underwent HAR. While immediate graft function after reperfusion was excellent, within 10 min the graft exhibited discoloration which progressed globally until graft failure 90 min after reperfusion. Flow cytometry using GT-KO porcine aortic endothelial cells confirmed the presence of preformed non-Gal IgM and IgG and the cytolytic nature of this preformed antibody in the recipient. Histological analysis of the graft showed typical HAR including widespread edema and hemorrhage with strong vascular deposition of IgM and C5b. The remaining transplants survived for a median of 27 days (range: 2–128 days) with five out of seven grafts exhibiting graft failure and delayed xenograft rejection. All grafts had vascular antibody deposition at the time of explant with variable levels of complement deposition. Survival of these GT-KO organs was comparable with the survival of GT+ CD46 transgenic hearts in recipients treated with an α-Gal polymer to control induction of anti-Gal antibody.

We believe that the GT-KO donors may be clinically useful as they may eliminate the need for intravenous α-Gal polymers, however, the

HAR of one graft and the uniform presence of vascular antibody in the remaining transplants illustrate the pathogenic potential of preformed non-Gal anti-pig antibody and suggest that non-Gal antibody response will dominate contemporary xenograft rejection.

Reference

1. Sharma A, Nazirrudin B, Cui C et al. Pig cells that lack the gene for alpha1-3 galactosyltransferase express low levels of the gal antigen. Transplantation 2002; 75: 430.

(2) α1,3-galactosyltransferase gene-knockout was an essential step towards successful pig organ transplantation in primates

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Current status of pig-to-non-human primate organ transplantation

Recent studies using organs from α1,3-galactosyltransferase gene-knockout (GT-KO) pigs [1–3] transplanted into non-human primates have indicated that, if the immunosuppressive regimen prevents a T cell-dependent elicited antibody response, the organs survive for several weeks or months before graft failure [4–6]. In particular, heterotopic hearts transplanted into baboons functioned from 2 to 6 months before succumbing to a thrombotic microangiopathy (TM) [4,5], the histology of which is quite different from the acute humoral xenograft rejection seen previously [7]. TM had been seen previously in hearts from pigs transgenic for human decay-accelerating factor (hDAF) [8], using the same immunosuppressive regimen, but with an additional continuous intravenous infusion of a Galα1,3Gal (Gal)-conjugate to adsorb and deplete anti-Gal antibodies [9].

Although the survival of the GT-KO hearts was not greatly extended when compared with the hDAF hearts, the lack of necessity of administering a Gal-conjugate represents a significant advance. Clearly, the less suppression of the host immune system that is required, then the fewer will be the complications of the regimen. In the eight experiments performed in which a GT-KO heart was transplanted into a baboon, there were no infectious complications, and no other complications directly attributable to the immunosuppressive regimen. This indicates that this regimen would be tolerated in humans, even though the anti-CD154 monoclonal antibody (mAb) that is an integral part of the regimen is unlikely to be used clinically because of its known association with thrombotic events [10]. In this respect, the TM seen in the transplanted organs was not thought to be associated with the administration of this agent because: (i) TM was not seen in any of the native organs, and (ii) TM has also been reported by others who used a regimen based on conventional pharmacologic immunosuppressive therapy [11,12].

Potential factors contributing to thrombotic microangiopathy

The binding of natural anti-non-Gal antibodies to the vascular endothelial cells of the graft might activate those cells and/or initiate injury that may lead to a local change from an anticoagulant to a procoagulant state. In this regard, we have documented that humans [13], baboons [14], and monkeys [15] produce anti-non-Gal cytotoxic antibodies, and these contribute approximately 50% of the natural anti-pig antibodies in these species. It has also been documented that, if immunosuppression is not adequate, there is a significant elicited anti-non-Gal antibody response after GT-KO pig organ transplantation [14,16]. However, evidence, largely from rodent studies [17–19], suggests that coagulation dysregulation may also play a significant role in the development of TM, possibly secondary to endothelial cell activation from binding of anti-non-Gal antibodies.

Potential solutions

Potential solutions involve identification of the specific antigens to which anti-non-Gal antibodies bind [20,21], which would allow the knockout of the gene(s) responsible for the expression of the antigen(s). However, there may be multiple non-Gal antigens involved, and the knockout approach may be difficult and/or time-consuming. An alternative would be to genetically engineer the GT-KO pig to express high levels of a human complement-regulatory protein, such as hDAF, or increased expression of a pig complement-regulatory protein, to protect the vascular endothelium from complement-mediated injury initiated by the binding of anti-non-Gal antibodies. An equally successful approach may be to genetically engineer the pig to express a human 'anti-coagulant' gene, such as tissue factor pathway inhibitor, thrombomodulin or CD39.