Xenotransplantation 2015: 22: 408–410
doi: 10.1111/xen.12197

Literature Update

Xenotransplantation literature update, July–August 2015

Burlak C, Chakrabarti S. Xenotransplantation literature update, July–August 2015. Xenotransplantation 2015: 22: 408–410. © 2015 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd.

Christopher Burlak[1] and Sudipta Chakrabarti[2]

[1]Department of Surgery, Schulze Diabetes Institute, University of Minnesota School of Medicine, Minneapolis, MN, [2]Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA

Key words: renal xenograft – xenotransplantation

Abbreviations: TMB, thrombomodulin; TNF-RFP, TNF-receptor fusion protein; LDH, lactate dehydrogenase enzyme.

Address reprint requests to Christopher Burlak, Department of Surgery, Schulze Diabetes Institute, University of Minnesota School of Medicine, 420 Delaware St. SE, Minneapolis, MN 55455, USA (E-mail: christopherburlak@gmail.com)

Received 19 August 2015;
Accepted 21 August 2015

Review

Zhou et al. [1] reviewed the potential for hepatocyte transplantation. In their review, they assert that porcine hepatocyte transplantation is a potential therapy for certain liver diseases. The literature suggested that isolation techniques necessary for the exclusion of vascular structures leave hepatocytes, even from genetically engineered pig livers, susceptible to some immunological problems but will likely improve xenograft survival. Of particular utility was Table 7 listing the diseases that present an immediate target for the application of porcine hepatocytes. The authors tabulate the studies of hepatocyte transplantation painting a clearer picture of where the field is headed and what we must accomplish to reach clinical success.

In vivo studies

To that end, Ham et al. [2] have dissociated porcine hepatocytes and then facilitated re-aggregation into alginate capsules. The encapsulated pig neonatal liver cells were transplanted into a model of acute liver failure in mice. The aim in this study was to improve the survival mice with acute liver failure by the transplantation of encapsulated neonatal pig re-aggregated liver cells. The authors' strategy for bridge therapy as a treatment for acute liver failure rescued mice, the result of which was normal metabolic function for 30 days. Some capsules initiated fibrotic over growth which may limit long-term use, but as there are no means to support someone in acute liver failure, injection of encapsulated hepatocytes may provide immediate relief to patients. The authors also point out that safety and functional issues need to be addressed before a clinical application could be considered. Luca et al. [3] using similar techniques describe the encapsulation of sertoli cells to be used as a transplantation therapy for immune diseases. Encapsulated sertoli cells produce anti-inflammatory compounds that may enhance long-term stability, functional competence of xenografts with minimum immune suppression. Encapsulated sertoli cells were transplanted into the subcutaneous adipose tissue in mice and allowed to reside for 4 months. An impressive 88% of cells were intact at the end of the study, and anti-inflammatory markers of sertoli cell function were comparable to those measured at 1 h after implantation. The authors believe that cell stability and quality of encapsulation support further studies.

Pre-clinical studies

Iwase et al. [4] reveal pig kidney graft survival in baboon for 136 days claiming the longest life supporting kidney graft survival to date. They suggested that the pig phenotype, costimulation blockade-based immunosuppressive regimen, and anti-inflammatory therapy may all have contributed to the outcome. However, the previous issue of the Xenotransplantation Journal published the work by Higginbotham et al. [5] describing >133 and >126 days of survival. The very exciting "take home" from these studies is that we are ever closer to immune suppression strategies that could be applied to the clinical application of xenotransplantation. Analysis of the variables between these studies will provide insight into how to design future renal xenograft experiments. Of significant interest will be the comparison of immune cell and histological studies upon completion of the Higgenbotham et al.'s experiments.

In vitro studies

Kim et al. [6] describe the in vitro expression of human thrombomodulin (TMB) and the potential to regulate activation and coagulation of the immune response during xenotransplantation. Using well-designed experiments, the authors expressed TMB in aGal-positive cells and reduced coagulation and immune assault. The authors further defined the important domains of TMB through modification of their original genetic construct. Domains 5 and 6 were important for complement inhibition. While outside the scope of this manuscript, stable expression of the active domains of TMB may be healthier for cells and generation of viable pigs while providing all or most of the anti-coagulative effects.

Using a normothermic perfusion circuit, Ramackers et al. [7] evaluated the effect of TNF-alpha blockade using a TNF-receptor fusion protein (TNF-RFP). Perfusions that included the TNF-RFP did not affect coagulator pathways but reduced endothelial cell activation. They demonstrated that the TNF-RFP is able to suppress inflammation occurring after xenoperfusion. This work defines in a quantitative way the development of coagulation by-products as related to kidney endothelium. The persistence of the thrombin–antithrombin complex emphasizes the importance of controlling coagulation in the solid organ xenotransplantation model.

Complement damage to endothelial cells can be measured by the release of lactate dehydrogenase enzyme (LDH) after membrane disruption due to complement binding. Interestingly, cytokines can protect cells from complement-mediated damage but it has been unclear how this is possible. Benson et al. [8] describe that IL-4 and IL-13 can induce protection of endothelial cells in the presence of human serum. The authors describe that endothelial cells are protected despite initial loss of cytoplasmic proteins by enhancing the mechanism of protection and repair of membrane injury. The authors suggest the mechanisms mediating IL-4-induced protection may provide new strategies for preventing complement-mediated vascular injury and vasculopathy as observed in vascularized allograft and xenograft rejection.

Azimzadeh et al. [9] have compiled a large multicenter study that describes the incidence of early graft failure in GalTKO pig organs transplanted into baboons was reduced by expression of a human complement pathway regulatory protein, CD46 or CD55. The authors point out that pigs expressing one of the several human "anti-thrombotic" genes, including thrombomodulin, and endothelial protein C receptor are available and appear to be consistently associated with extended porcine graft survival and may overcome systemic coagulation due to xenotransplantation.

Neonatal pig islets are known to express the xenoantigen galactose-alpha-1, 3-galactose and elicit an antibody response in vitro. Nagaraju et al. [10] have expanded on this knowledge by comparing the survival of wild type and genetically modified pig neonatal islet-like cell clusters to human blood. The authors report that gene knockout or expression did not affect the islet viability and unfortunately enhanced the time of coagulation. They conclude that neonatal islet-like cell clusters may be better protected by enhanced expression of anti-coagulation and anti-complement proteins.

References

1. Zhou H, Liu H, Ezzelarab M et al. Experimental hepatocyte xenotransplantation-a comprehensive review of the literature. Xenotransplantation 2015; 22: 239–248.
2. Ham D-S, Song M-S, Park H-S et al. Successful xenotransplantation with re-aggregated and encapsulated neonatal pig liver cells for treatment of mice with acute liver failure. Xenotransplantation 2015; 22: 249–259.
3. Luca G, Mancuso F, Calvitti M et al. Long-term stability, functional competence, and safety of microencapsulated specific pathogen-free neonatal porcine Sertoli cells: a potential product for cell transplant therapy. Xenotransplantation 2015; 22: 273–283.
4. Iwase H, Liu H, Wijkstrom M et al. Pig kidney graft survival in a baboon for 136 days: longest life-supporting organ graft survival to date. Xenotransplantation 2015; 22: 302–309.

5. Higginbotham L, Mathews D, Breeden CA et al. Pretransplant antibody screening and anti-CD154 costimulation blockade promote long-term xenograft survival in a pig-to-primate kidney transplant model. Xenotransplantation 2015; 22: 221–230.
6. Kim H, Hawthorne WJ, Kang HJ et al. Human thrombomodulin regulates complement activation as well as the coagulation cascade in xeno-immune response. Xenotransplantation 2015; 22: 260–272.
7. Ramackers W, Klose J, Tiede A et al. Effect of TNF-alpha blockade on coagulopathy and endothelial cell activation in xenoperfused porcine kidneys. Xenotransplantation 2015; 22: 284–294.
8. Benson BA, Vercellotti GM, Dalmasso AP. IL-4 and IL-13 induce protection from complement and melittin in endothelial cells despite initial loss of cytoplasmic proteins: membrane resealing impairs quantifying cytotoxicity with the lactate dehydrogenase permeability assay. Xenotransplantation 2015; 22: 295–301.
9. Azimzadeh AM, Kelishadi SS, Ezzelarab MB et al. Early graft failure of GalTKO pig organs in baboons is reduced by expression of a human complement pathway-regulatory protein. Xenotransplantation 2015; 22: 310–316.
10. Nagaraju S, Bertera S, Tanaka T et al. In vitro exposure of pig neonatal isletlike cell clusters to human blood. Xenotransplantation 2015; 22: 317–324.