Reducing the Threshold for Clinical Renal Xenotransplantation

Peter J. Cowan, PhD[1,2] and Francesco L. Ierino, MBBS, PhD[2,3]

Preexisting antibodies have long been recognized as a major barrier to successful xenotransplantation of porcine organs. The primary manifestation of this problem is the binding of natural human or nonhuman primate (NHP) antibodies to carbohydrate epitopes on pig endothelial cells, which if not addressed results in hyperacute rejection or accelerated acute rejection. A wealth of data, collected over more than 2 decades, identify galactose-α1,3-galactose (αGal) as the most important carbohydrate xenoantigen. More recent studies have also implicated *N*-glycolylneuraminic acid (Neu5Gc)[1] and an as yet unidentified moiety synthesized by β1, 4*N*-acetylgalactosaminyl transferase 2 (B4GalNT2).[2] Recent advances in genome editing have paved the way for the elimination of carbohydrate xenoantigens by single-step deletion of multiple glycosyltransferase genes.[3] In this issue of *Transplantation*, Martens et al[4] report the impact of the deletion of the *GGTA1* (αGal), *CMAH* (Neu5Gc) and *B4GalNT2* genes on the xenoantigenicity of porcine peripheral blood mononuclear cells (PBMC). Flow cytometric crossmatch was performed using sera from renal transplant waitlisted patients and PBMC from wild type and genetically modified pigs. The results were clear: the addition of *CMAH* knockout (KO) and then *B4GalNT2* KO to *GGTA1* KO progressively reduced human antibody binding to the porcine cells. Indeed, 31% of patient sera showed a negative crossmatch (IgG and IgM) to the "triple KO" PBMC. This may prove to be a significant step forward in advancing renal xenotransplantation to the clinic.

By substantially reducing the background contribution of carbohydrate xenoantigens to human antipig antibody binding, the authors attempted to examine a second important question: whether sensitized individuals on the waiting list possess anti-HLA class I antibodies that will cross-react with swine leukocyte antigen (SLA) class I, thus potentially pathogenic in a clinical xenotransplantation setting. Previous studies have reached conflicting conclusions on this issue. Here, Martens et al performed a rather convoluted series of experiments involving adsorption of sera on wild type pig erythrocytes to remove non-SLA antibodies, incubation with triple KO (SLA class I-positive) or *GGTA1*/SLA-I double KO PBMC, elution of antibodies and binding to HLA class I single antigen beads.[4] One could question some aspects of the methodology; for example, transfectants expressing SLA class I might have been used to more convincing effect. With this caveat, the results indicated that a subset of highly sensitized patients have HLA class I antibodies that recognized antigens (most likely SLA class I) on pig cells. Importantly, though, more than half of the highly sensitized patients tested did NOT have significant levels of these antibodies, and had only a weak or negative crossmatch with triple KO PBMC. This raises a further question: are there now sufficient data to proceed cautiously with clinical trials of renal xenotransplantation, provided that safety concerns can be adequately addressed?

The impetus for clinical trials has increased with the recent prolongation of life-supporting function of renal xenografts in preclinical NHP models to up to 10 months,[5] albeit in relatively few animals so far, and the demonstration that it may be possible to eliminate endogenous retroviruses from the porcine genome[6] with the same technology used to knock out the glycosyltransferases and SLA class I. The likely genetic makeup of the donor pigs that might be used in an initial clinical trial is still not decided. Tector's group has stressed the importance of a negative crossmatch,[7] and the current article[4] suggests the potential value of the *GGTA1*/*CMAH*/*B4GalNT2* triple KO modification in this regard. However, the field must still proceed with caution. Although the in vitro data support the use of the triple KO pigs, the testing of their organs in standard NHP models may not be fully informative because Old World primates (unlike humans) express Neu5Gc and possess negligible anti-Neu5Gc natural antibodies.[8] Furthermore, crossmatching is mainly relevant to relatively early events posttransplant, whereas other mechanisms independent of preexisting antibodies are likely to play a role in long-term xenograft survival. It should be noted that negative antibody crossmatching does not test for the capacity of induced anti-donor antibodies, which will also determine short- and long-term graft survival. Nevertheless, the triple KO pig may be a useful platform on which to build additional proven beneficial modifications, for example to regulate the activation of complement and coagulation.[9]

Martens et al[4] further propose that deletion or manipulation of SLA class I, even to the point of tailoring donor pigs

Received 5 January 2017.

Accepted 6 January 2017.

[1] *Immunology Research Centre, St Vincent's Hospital Melbourne, Victoria, Australia.*

[2] *Department of Medicine, University of Melbourne, St Vincent's Hospital Melbourne, Victoria, Australia.*

[3] *Department of Nephrology, St Vincent's Hospital Melbourne, Victoria, Australia.*

P.J.C. is a member of the Scientific Advisory Board of Hunan Xeno Life Sciences Inc. F.L.I. received Educational Grants from Novartis and honorariums for Medical Advisory Meetings from Novartis, MSD and Astellas.

Correspondence: Peter Cowan, PhD, Immunology Research Centre St Vincent's Hospital Melbourne PO Box 2900, Fitzroy 3065, Victoria, Australia. (peter.cowan@svha.org.au).

ISSN: 0041-1337/17/10104-692

DOI: 10.1097/TP.0000000000001641

to individual patients, will expand the potential recipient pool to include patients with cross-reacting anti-HLA antibodies. However, as mentioned earlier, it is clear from this study that there are already numerous highly sensitized patients with a negative crossmatch to existing donor pigs. We would therefore argue that a logical first step is to demonstrate success in a clinical trial using these recipients and donors (probably further modified as described above). This would be an extraordinary achievement, and may be sufficient to justify an expansion to include less sensitized patients. Finetuning of SLA class I, if it can be done without being cost-prohibitive, is something that could be considered further down the track.

REFERENCES

1. Saethre M, Baumann BC, Fung M, et al. Characterization of natural human anti-non-gal antibodies and their effect on activation of porcine gal-deficient endothelial cells. *Transplantation*. 2007;84:244–250.
2. Byrne GW, Du Z, Stalboerger P, et al. Cloning and expression of porcine β1, 4N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. *Xenotransplantation*. 2014;21:543–554.
3. Butler JR, Martens GR, Estrada JL, et al. Silencing porcine genes significantly reduces human-anti-pig cytotoxicity profiles: an alternative to direct complement regulation. *Transgenic Res*. 2016;25:751–759.
4. Martens GR, Reyes LM, Butler JR, et al. Humoral reactivity of renal transplant-waitlisted patients to cells from GGTA1/CMAH/B4GalNT2, and SLA Class I knockout pigs. *Transplantation*. 2017;101:e86–e93.
5. Wijkstrom M, Iwase H, Paris W, et al. Renal xenotransplantation: experimental progress and clinical prospects. *Kidney Int*. 2016;pii: S0085-2538: 30569–5.
6. Yang L, Güell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;350:1101–1104.
7. Higginbotham L, Mathews D, Breeden CA, et al. Pre-transplant 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.
8. Yeh P, Ezzelarab M, Bovin N, et al. Investigation of potential carbohydrate antigen targets for human and baboon antibodies. *Xenotransplantation*. 2010;17:197–206.
9. Cowan PJ, Cooper DK, d'Apice AJ. Kidney xenotransplantation. *Kidney Int*. 2014;85:265–275.