XENOTRANSPLANTATION: OVERCOMING IMMUNE BARRIERS

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IMMUNE BARRIERS OF XENOTRANSPLANTATION AND CURRENT APPROACHES TO OVERCOME THEM

In this section, we will briefly introduce xenotransplantation and the immune barriers to xenotransplantation. Then we will summarize the current approaches to overcome these immune barriers, including genetic engineering and tolerance induction.

RECENT ADVANCES: XENOTRANSPLANTATION

This section will first go through the technological developments in xenotransplantation since the 1980s. Then move on to recent xenotransplantation studies in humans to have a deeper insight into the latest advancements and finally discuss the limitations and hurdles for its future development.

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IMMUNE BARRIERS IN XENOTRANSPLANTATION AND CURRENT APPROACHES TO OVERCOME THEM

Currently, there is a massive gap between organ supply and demand, and several patients die every year while waiting for a transplant. Several attempts of using individual recipient stem cells to bioengineer transplantable organs have been made. However, these approaches are expensive and face substantial challenges in forming a complex functional organ (1). Xenotransplantation, that is transplantation of organs, tissues, or cells from one species to another could help resolve this challenge. Pigs are the most used donors for xenotransplantation in humans because of several reasons including adequate organ size, a higher number of offspring, and rapid growth compared to other non-human primates (NHPs). Moreover, there is considerable knowledge and experience available with pig's tissue typing and genetic engineering (2). This recent progress in xenotransplantation with huge advancements in technology has led to renewed hope to alleviate the organ shortage (3).

Immune barriers

With successful cross-matching, hyperacute rejection (HAR) is a rare observation in allotransplantation although rejection still exists despite immunosuppressive medications (4). However, in xenotransplantation, the major immunologic barrier is the presence of, preformed natural xenoreactive antibodies (XNA) against porcine's alpha-gal (galactose-α 1,3-galactose) antigen to which humans and several NHPs react (5). During antibody-mediated response, these XNAs react with the xenograft and activate the complement system, causing lysis of endothelial cells and vascular disruption, eventually leading to antibody-mediated rejection of the graft (6). Current approaches to overcome these immune barriers for successful xenotransplantation include gene editing, immunosuppression, and tolerance induction (1).

Gene Editing

The identification of alpha-gal as the major antigen causing porcine graft rejection via preformed XNA in humans in 1991, directed genetic engineering towards targeting complement cascade-mediated cytotoxicity and thrombotic microangiopathy (TMA), as well as antibody-mediated response (2,7). Moreover, with the advancements in DNA editing technologies such as CRISPR, gene editing has been used to delete key porcine antigens (e.g., GGTA-1 deletion to prevent natural antibody-mediated rejection) and insert human transgenes (e.g., Human CD46 transgene insertion to protect from human complement and coagulation activity) (2).

	Function	Genes
Transgenes	Complement inhibition	Human DAF
		Human CD46
		Human CD59
	Coagulation inhibition	Human CD39
		Human thrombomodulin (hTBM)
		Human endothelial protein C receptor
	NK cell inhibition	Class I MHC
	Immunosuppressive molecules	Anti-CD2
		CTLA4Ig
		PD-L1
		FasL
	Anti-inflammatory genes	HO-1
		A20
	Prevention of infection	Porcine endogenous retrovirus (PERVs) short interfering RNA
Knock-outs	Prevention of natural antibody-mediated rejection	α1,3-galactosyl transferase (GGTA-1) or α-gal antigen
		CMAH
		B4GalNT2
	Prevention of infection	PERVs
	Limitation of organ size	Growth Hormone receptor (GHR)
Knock-ins	Prevention of graft thrombosis	Human vWF

Fig 1. Current transgenes, knock-outs and knock-ins [adapted from Nat Rev Nephrol. 2022 Dec;18(12):745-761]

Several strategies such as introducing transgenes (integrated into the genome at random locations), knock-outs, and knock-ins (inserted at a specific genomic location) for successful xenotransplantation are in progress, and some examples are listed in the table (Fig 1) (1).

Immunosuppression

The immune rejection of graft remains a major challenge in successful xenotransplantation of kidneys in humans. Long-term graft and patient survival are distressed by calcineurin inhibitors (CNI) usage in traditional immunosuppressive methods. Recently, the usage of TNX-1500 [anti-CD154 monoclonal antibody (mAB)] targeting the CD154-CD40 pathway has been shown to prevent allograft and xenograft rejection in NHPs and graft survival by targeting the adaptive immune response (8,9).

Tolerance Induction

Xenograft tolerance is introduced to silence the recipient's destructive T-cell response to the graft but meanwhile maintain a functioning immune system to resist infection. Tolerance induction strategies, including mixed chimerism (bone marrow extraction from the donor pig and injected into a T cell-depleted baboon) and thymic transplantation (transplanting donor pig thymus as a vascularized thymic lobe or as part of a composite thymokidney into a T cell-depleted, thymectomized baboon), have been shown to reduce T cell activation and achieve desirable graft fate in the recipient (1).

RECENT ADVANCES: XENOTRANSPLANTATION

Graft survival has prolonged from minutes to months or even years with breakthroughs in genetic engineering, immunosuppression, and tolerance induction. In the 1980s, Cooper et al. showed that pre-transplant adsorption of anti-pig antibodies from a baboon's blood prolonged the cardiac graft survival to hours and even days (10) (Fig 2). Then the period of transgenic pigs expressing human transgenes to prevent complement cascade activation started in the 1990s (11). The development of nuclear transfer technology in the early 2000s enabled the knockout of the GGTA1 gene from pigs that prevented natural antibody-mediated rejection (12,13). Entering the 2010s, new transgenes and knockouts were introduced to donor pigs with the advancements in CRISPR, prolonging graft survival to more than 2 years (14-17). Since 2020, several human xenotransplantation studies have been in the pipeline. In the coming sections, we will have a closer look at two of the xenotransplantation studies.

The First study on brain-dead recipient

The first clinical-grade pig kidney xenotransplant used 10-GE (genetically engineered) pigs that harbor 10 genetic modifications (insertion of hDAF, hCD46, hTBM, hEPCR, hCD47, hHO1, and triple knock-out of GGTA-1, CMAH and B4GalNT2 and deletion of the GHR) as donors and brain-dead human decedent as the recipient. Results showed that no HAR was observed, and the kidneys remained viable, producing variable amounts of urine until termination, 74 hours later. However, TMA was observed without any clear etiology, and it did not further progress to necrosis. This means that TMA could have been either due to complement-mediated cytotoxicity or disseminated intravascular coagulation (DIC) or a mix of both. Hence for future studies, the researchers were inspired to include an immunosuppressant in their regimen, called eculizumab (anti-C5 antibody) to improve graft survival by preventing complement-mediated cytotoxicity. Furthermore, no recovery in creatinine clearance was observed and creatinine levels remained over 2.5 mg/dL. Whether this was influenced by brain death and microvascular injury remains unknown (18).

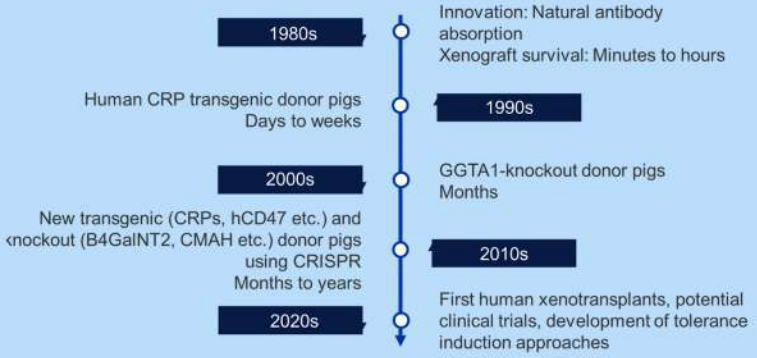

Fig 2. Chronology of xenotransplantation progress [adapted from Nat Rev Nephrol. 2022 Dec;18(12):745-761]

The second study on 2 brain-dead recipients

Another group, transplanted kidneys from GGTA-1 knockout pigs to two brain-dead humans. The grafts remained viable and functioning for 54 hours with no signs of HAR. The xenografts continued to make urine throughout the study, and the hourly urine output with the xenograft was more than double the output with the native kidneys. The creatinine levels in the recipients decreased after implantation, from 1.97 to 0.82 mg/dL in Recipient 1 and from 1.10 to 0.57 mg/dL in Recipient 2. The results suggested that eliminating GGTA-1 alone can prevent HAR (19).

Discussion

Despite the advancements in human experiments, the above two studies were not able to eliminate the possibility of delayed and chronic rejection because of their short durations. Furthermore, concerns about brain-dead recipients and ethical challenges such as religious conflicts, equity, and human dignity remain to be discussed (20).

Additionally, a Phase I clinical trial on 20 patients with ESKD dependent on dialysis, and on the waiting list for transplantation, has been registered and is estimated to start on January 31, 2024, in the US. According to the disclosed information, 10 GE pigs will be used as donors. Recruitment and xenotransplantation will occur over 5 years with a study follow-up of another one year (21).

EXPERT COMMENTARY (with Dr. Benjamin E Hippen, SVP, Head of transplant medicine and emerging capabilities, Fresenius Medical Care)

What are the current and future perspectives on xenotransplantation?

The promise of porcine to human xenotransplantation has been nurtured for decades, with some recent breakthroughs giving cause for a glimmer of optimism and caution. Gene edits targeting the alpha-gal antigen and key steps in the complement cascade, along with the development and testing of a mAB targeting the CD40/CD154 co-stimulation pathway suggest a clinical path towards a phase I safety trial in humans.

It remains to be proven whether the relative inefficacy of CNI based immunosuppression in NHP xenotransplant recipients is equally ineffective in human recipients. This premise will be put to the test in the first proposed Phase 1 trial (21), which proposes to use a traditional CNI-based approach supplemented with eculizumab. This approach does open the door to further/separate studies in humans using CD154 blockade, should the CNI-based approach yield poor immunologic outcomes.

Identifying the "right" study candidate for a xenograft will be difficult, after all, human-to-human allografts have an excellent record of safety and efficacy. Identifying patients who might rationally take on the risks of early xenograft research will involve splitting the difference between those who are unlikely to benefit from and/or receive a human kidney, but also not too sick/too frail to be able to physiologically withstand the unknown risks of a novel immunosuppression regimen and the possibility of zoonotic disease transmission.

References:

1.Sykes M et al. *Nat Rev Nephrol*. 2022 Dec;18(12):745-761. 2. Cooper DKC et al. *British Med Bulletin.* 2018 Mar 1;125(1):5-14. 3. Zhou Q et al. *Front Immunol*. 2022 Jul 28;13:928173. 4. Naik RH, Shawar SH. Available from: *https://www.ncbi.nlm.nih.gov/books/NBK553074/* 5. Igaz P. *Yale J Biol Med*. 2001;74(5):329-340. 6. Fischer K et al. *Transgenic Res.* 2022 Jun;31(3):391-398. 7. Cooper DKC et al. *Am J Transplant*. 2023 Mar: 23(3):326-335. 8. Cooper DKC et al. *J Am Soc Nephrol.* 2020. Jan;31(1):12-21 9. Lassiter G. et al. *Am J Transplant*. 2023 August;23(8):1171-81 10. Cooper DK et al. *J Heart Transplant*. 1988 May-Jun;7(3):238-46. 11. Cozzi E et al. *Nat Med*. 1995 Sep;1(9):964-6. 12. Yamada K et al. *Nat Med*. 2005 Jan;11(1):32-4. 13. Kuwaki K et al. *Nat Med*. 2005 Jan;11(1):29-31. 14. Mohiuddin MM et al. *Nat Commun*. 2016 Apr 5;7:11138. 15. Estrada JL et al. *Xenotransplantation*. 2015 May-Jun;22(3):194-202. 16. Singh AK et al. *Xenotransplantation*. 2019 Mar;26(2):e12465. 17. Fischer K et al. *Sci Rep*. 2016 Jun 29;6:29081. 18. Porrett PM et al. *Am J Transplant*. 2022 Apr;22(4):1037-1053. 19. Montgomery RA et al. *N Engl J Med*. 2022 May 19;386(20):1889-1898. 20. Ganchiku Y et al. *Xenotransplantation*. 2022 May;29(3):e12748. 21. *https://clinicaltrials.gov/ct2/show/NCT05340426.*