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COMMENTARY

Cardiac Xenotransplantation

5 Things Every Cardiologist Should Know

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An estimated 300,000 patients live with advanced heart failure in the United States, and 3,817 patients underwent allogenic orthotopic heart transplant (HT) in 2021, an historical high. However, more than 3,400 patients still remain on the transplant waitlist. Despite recent advances in using hepatitis C-positive donors and donation after cardiac death, there remains a vast imbalance of supply and demand in suitable donor organs. Thus, expanding the donor pool is essential to reduce waitlist mortality and improve clinical outcomes in the advanced heart failure population. The recent announcement of the early success of a pig-to-human HT at the University of Maryland (UMD), demonstrates the potential promise of xenotransplantation in the 21st century. Insofar as the use of genetically modified organs for xenotransplantation is relatively new, here, we briefly review 5 basic facets of cardiac xenotransplantation that cardiologists should understand so they are better prepared to address the questions that their patients may ask them.

WHAT IS THE HISTORY OF CARDIAC XENOTRANSPLANTATION?

Xenotransplantation is the process of transplanting an organ from one species to another. The practice of xenotransplantation existed as early as 1667 when a 15-year-old boy received a blood transfusion from a lamb. However, it wasn't until the turn of the 20th century that advances in surgical techniques facilitated solid-organ xenotransplantation research. The first xenotransplant was in 1905, with transplantation of slices of rabbit kidney into a human child.[1] Since then, there have been over 30 attempts at solid-organ xenotransplants from nonhuman primates (NHP) and nonprimates into humans. The first cardiac xenotransplantation occurred in 1964 at the University of Mississippi, when a chimpanzee heart was transplanted into a human recipient and lasted for 90 minutes. Unfortunately, early experiences were fraught with hyperacute rejection and death. To date, there have been a total of 10 cardiac xenotransplants and until recently, the longest reported survival was 20 days, which occurred after a baboon heart was transplanted into a neonate with hypoplastic left heart syndrome. With advances in immunotherapy and gene editing technology, a genetically modified porcine heart (UHeart [xenoheart]) developed by Revivicor was transplanted into a 57-year-old man on January 7, 2022, at the UMD. He lived for 60 days after surgery, setting a new benchmark in the pursuit of durable xenotransplantation.

WHY USE PIGS AS THE DONOR SPECIES?

An ideal xenogenic donor heart should have similar physiology, be immunologically compatible, and have the potential to be upscaled for widespread adaptation. From a phylogenic standpoint, NHP are the preferred donor species for xenotransplantation. However, this is fraught with practical and ethical issues. NHP require large breeding facilities and significant resources. They also take a long time to reach maturity, reproduce slowly, and often produce undersized organs. Furthermore, the concept of capturing and breeding NHP for purpose of harvesting organs is ethically and morally challenging. In addition, the spread of viruses between similar species is also a major concern. On the other hand,

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porcine hearts are structurally similar to human hearts and compared with NHP, breeding is faster, more cost-efficient, and creates less moral conundrum. For these reasons, pigs remain the most viable donor species for human xenotransplantation.[2]

WHAT ARE THE MAJOR IMMUNOLOGICAL BARRIERS TO XENOTRANSPLANTATION?

HYPERACUTE REJECTION. Hyperacute rejection refers to immediate recognition of the transplanted organ by preformed antibodies against donor cells, leading to endothelial injury and destruction of the graft within hours. In porcine xenotransplantation, a major mechanism of hyperacute rejection is driven by human antibodies against carbohydrates expressed on the surface of pig cells, specifically galactose α-1,3-galactose (α-Gal). These pre-existing antibodies are much like those formed against the A and B antigens that determine our blood type. A second major component of hyperacute rejection involves complement activation. Under physiological conditions, complement proteins circulate in the bloodstream where they play an important role in recognizing and killing blood-borne pathogens. As a protective mechanism, humans express complement regulatory proteins (CRP) that prevent inadvertent complement activation at the organ-blood interface. However, pig CRP are unable to fully inhibit human complement proteins, resulting in excessive activation of the complement cascade, which contributes to hyperacute rejection. With the advent of CRISPR (clustered regularly interspaced short palindromic repeats) gene editing technology, scientists have been able to engineer pigs in which the enzyme responsible for α-Gal is deleted and where human CRP (CD46, CD55, CD59) are transgenically expressed on pig cells to reduce hyperacute rejection.

ACUTE CELLULAR AND ANTIBODY-MEDIATED REJECTION. Cellular and antibody-mediated rejection occurs weeks to months after transplant. The human immune system recognizes "self" and "foreign" molecules through the expression of cell surface proteins encoded by the major histocompatibility complex (MHC) genes, also called human leukocyte antigens (HLA). When there are HLA mismatches between donor and recipient, the recipient's immune system may identify the donor organ as foreign and attack it. Although this can occur with allogenic transplants, it is especially significant in xenotransplantation due to differences in MHC genes and proteins across species. To control our body's immune responses against pig antigens, aggressive immunosuppression therapy is necessary, involving thymoglobulin to deplete T cells, rituximab to suppress B-cell antibody production, and anti-CD40 antibodies to block costimulation of immune cells.[3] In addition, natural killer (NK) cells and macrophages play an important role in xenogenic graft rejection. NK cells normally kill virally infected or tumor cells that have down-regulated MHC molecules to avoid T-cell recognition. Macrophages contribute to xenograft rejection due to loss of inhibitory interaction between SIRPa on macrophages with CD47 on porcine cells. Again, CRISPR technology has been used to create pig endothelial cells that express human inhibitor molecules such as MHC I, CD33-related Siglecs, CD47, and CD200, which reduce the activation of NK cells and macrophages.

UNCONTROLLED THROMBOSIS. Hematological incompatibility between pigs and humans can also trigger uncontrolled thrombosis and donor organ damage. Although pigs express anti-thrombotic proteins such as thrombomodulin, endothelial protein C receptor (EPCR), and thrombin-activatable fibrinolysis inhibitor, they fail to inhibit human coagulation factors. Therefore, transgenic expression of these antithrombotic proteins has been employed to minimize thrombotic complications.

BESIDES IMMUNOCOMPATIBILITY, WHAT OTHER CHALLENGES FACE PORCINE XENOTRANSPLANTATION?

Xenotransplantation carries a risk of spreading infectious diseases. Due to previous studies showing porcine endogenous retrovirus (PERV) was able to infect susceptible human cell lines, this led to a concern that pig-derived pathogens might cause a global pandemic. This issue dampened enthusiasm for xenotransplantation in the late 1990s and 2000s, especially coming on the heels of the HIV epidemic. A major breakthrough occurred in 2016, when Niu et al[4] used CRISPR technology and successfully disabled all PERV genes to generate fertile pigs for clinical application. Beyond the infectious concerns, there are also challenges related to early heart function and organ growth that occur with pig xenotransplantation. Insight into these phenomena came from the landmark, proof-of-concept study by Langin et al,[5] who performed orthotopic heart transplants into baboons using α-Gal knockout, human CD46- and thrombomodulin-expressing pigs. The investigators found that pig hearts could not be stored on ice, as frequently occurs for human HT, but rather needed continuous perfusion to maintain function

TABLE 1 Barriers to Pig-to-Human Cardiac Xenotransplantation

Barriers to Porcine Xenotransplantation	Clinical Manifestation	Strategy
Pig surface carbohydrate xenoantigens	Hyperacute rejection	• α-Gal knockout • Triple knockout (a-Gal, Neu5Gc, and SDa)
Excessive complement activation	Hyperacute rejection	• ↑Human CD46, CD55, and CD59
B- and T- cell activation	Acute rejection	• Induction immunotherapy with steroids, thymoglobulin, rituximab, and anti-CD40 antibodies
NK cell and macrophage activation	Acute rejection	• ↑Human MHC I, CD33-related Siglecs, CD47, and CD200
Uncontrolled thrombosis	Graft dysfunction	• ↑Human thrombomodulin, EPCR, and TAFI
Endogenous retroviruses	Zoonotic infection	• Deletion of PERV genes
Primary graft dysfunction	Perioperative xenograft failure	• Ex vivo preservation and perfusion
Organ overgrowth	Massive cardiac hypertrophy	• Blood pressure control, steroid taper, and mTOR inhibitor use • Knockout of porcine growth receptor

α-Gal = α-1,3-galactose; EPCR = endothelial protein C receptor; MHC = major histocompatibility complex; mTOR = mechanistic target of rapamycin; Neu5Gc = N-glycolylneuraminic acid; PERV = porcine endogenous retrovirus; TAFI = thrombin-activatable fibrinolysis inhibitor.

post-transplantation. Porcine hearts also demonstrated accelerated cardiac growth that eventually compromised heart function. A multitiered approach was needed to overcome this problem that included aggressive management of blood pressure, rapid and early steroid taper, and treatment with mechanistic target of rapamycin (mTOR) inhibitor to blunt growth signals. With this approach, survival increased to 195 days. The barriers to xenotransplant are summarized in Table 1.

WILL XENOTRANSPLANTATION BECOME ROUTINE PRACTICE IN THE NEAR FUTURE?

The Revivicor xenoheart that was surgically implanted at the UMD was performed under emergency use authorization by the Food and Drug Administration and is not yet undergoing evaluation in clinical trials at the time of this writing. Nonetheless, the early success of the recent porcine heart xenotransplant has many people wondering if the time for xenotransplantation has arrived. Although this strategy has tantalizing potential to help solve the shortage of donor hearts, many questions about long-term durability remain. The Revivicor xenoheart used in the patient at UMD harbors a total of 10 gene modifications, with triple knockouts of pig carbohydrate antigens and transgenic expression of human genes that down-regulate the complement cascade, NK cells/macrophages, thrombosis, and deletion of a porcine growth receptor. Additional research in NHP is needed to evaluate the most optimal combination of genetic modifications for maximal long-term graft function and survival. The recent scientific advances and human experiences should foster future investigations. Moreover, the intensive immunosuppressive regimen necessary for acceptance of the xenograft increases the risk of infection and malignancy. Finally, improvements in durable mechanical support also impacts the future of xenotransplant. Contemporary survival with the HeartMate 3 left ventricular assist device (Abbott Cardiovascular) now approaches that of allogenic HT for the first 2 years and is >50% at 5 years. As such, this benchmark must also be achieved with xenotransplantation for it to become an acceptable alternative therapy. In the short term, porcine heart transplants could be considered on a case-by-case basis for patients who do not qualify for left ventricular assist device or allogenic HT as was the case for the patient at UMD.

CONCLUSIONS

Xenotransplantation offers an avenue to greatly increase the donor organ pool for HT. Recent scientific advances, specifically in gene editing using CRISPR technology, have rekindled the field of xenotransplantation, and initial experiences in NHP and in select patients are encouraging. Although not yet ready for clinical use, xenotransplantation is an exciting and active area of research, and there will likely be human clinical trials in the next 10 to 15 years.

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