On cardiac xenotransplantation and the role of xenogeneic tolerance

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Feature Editor’s Introduction—The year 2022 marked the first pig-to-human heart transplantation and sparked renewed interest in clinical cardiac xenotransplantation. Indeed, the potential of genetically modified bioengineered pig hearts appears very promising, especially for infants with an immature immune system. The immature immune system in infants has allowed us to perform successful ABO-incompatible heart transplantation with excellent results. Could immune tolerance to bioengineered hearts be achieved? Could immunologic barriers to xenotransplantation be overcome by advances in genome editing? What would be the ideal design for an emerging clinical trial? These and many more questions remain unanswered. Herein a group of experts from Columbia University discuss the current status of cardiac xenotransplantation and the role of tolerance that may pave the way to sustained clinical success.

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An increasing number of patients die without a life-saving transplant as the gap between individuals awaiting transplantation and available human donor hearts widens. Cross-species transplantation (xenotransplantation) of pig hearts into human recipients offers the prospect of an unlimited supply of organs and would permit elective transplantation using quality-controlled organs. Although initial results in the 1960s and 1980s using nonhuman primate (NHP) donors for cardiac xenotransplantation were poor, several advances in genetic engineering and immunosuppression have helped mitigate previously insurmountable obstacles. The initial success of the first pig-to-human heart transplantation this year1 ushers in newfound excitement for the promise of xenotransplantation but also highlights several uncertainties that require further investigation.

PEDIATRIC VERSUS ADULT RECIPIENTS

Probably the best known cardiac xenotransplant was that by Leonard Bailey, who transplanted a baboon heart into an infant girl, known as Baby Fae, in 1983. At that time infant organs were nearly impossible to obtain, and xenotransplantation offered a potential solution. The surgical procedure was technically successful, but the patient died from acute rejection 20 days later.2 Since then, advances in mechanical circulatory support have markedly improved survival to transplantation as well as survival among noncandidates for transplantation, and today 2-year survival free from disabling stroke or reoperation exceeds 75% in adults.3 Although satisfactory outcomes are achievable for larger
children and young adults with mechanical circulatory sup-
port devices, neonates and infants represent a particularly 
challenging group. Even in experienced centers, mortality 
after ventricular assist device implantation in patients 
with a single ventricle approaches 50% to 75% in neonates, 
and only 30% to 50% survive to discharge. However, with 
wait times of 4 to 6 months, and wait-list mortality rates 
exceeding 30%, we believe this to be the population most 
in need of a viable alternative such as xenotransplantation. 

Neonates and infants pose unique challenges but also 
offer important advantages. Heart size and growth rate 
become very important considerations for translatability; 
pigs reach adult human size within 6 months. Miniature 
swine or growth hormone receptor knockout pigs likely 
offer advantages for transplantation to humans, including 
young children. On the other hand, neonates and infants 
do not yet produce antibodies to T cell–independent anti-
gens, which may mitigate organ rejection. As a result, graft 
 survival after heart transplantation is significantly longer in 
neonates and infants than in older individuals, even after 
ABO-incompatible heart transplantation. The potential to 
achieve tolerance—long-term graft survival without immu-
nosuppression—will be fundamental to the ultimate success 
of xenotransplantation. The increased susceptibility of 
neonatal and infant immune systems to tolerance induction 
may facilitate this goal.

IMMUNOLOGIC BARRIERS TO 
XENOTRANSPLANTATION

The earliest human xenotransplants—including car-
diac—used NHP organs because of their phylogenetic prox-
imity. However, the very limited survival, coupled with 
ethical and virologic concerns, rendered this approach 
impractical. Pigs were subsequently chosen as organ 
sources owing to their comparable size, anatomy, and phys-
iology. But hyperacute rejection ensued in NHP recipients, 
whereby preexisting natural antibodies (NAb) to epitopes 
on porcine endothelial cells activated complement and 
coagulation cascades, leading to organ ischemia and death. 
NAb exists irrespective of a previous exposure to pig 
antigens; instead, they exist because of cross-reactivity 
with shared antigens on common microbes. Most human 
and NHP anti-pig NAb recognize a single carbohydrate, 
galactose-α1,3-galactose (Gal), and adsorption of these 
NAb helped prolong pig organ survival from hours to 
days in NHP recipients in the 1990s. A decade later, nuclear transfer–based cloning methods permitted 
the creation of pigs deficient in the enzyme that produces 
Gal, further prolonging pig organ survival in NHP 
recipients to weeks or even months (Figure 1).

Over the last 20 years, CRISPR/Cas9-mediated gene 
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FIGURE 1. Chronology of xenotransplantation. General timescales of obstacles and advances in pig-to-primate xenotransplantation are shown. NHPs, Nonhuman Primates; HAR, hyperacute rejection; DXR, delayed xenograft rejection; AMR, antibody-mediated rejection; CR, chronic rejection; Ab, antibody; CRP, complement regulatory protein; GalT, α1,3 galactosyl transferase; KO, knockout; costim, costimulatory.
It is important to note that the need for each individual genetic modification remains unproven, and it is possible that certain modifications (or combinations thereof) may be detrimental. Successful immunosuppression regimens typically include depletion of recipient T and B lymphocytes and post-transplantation maintenance with mycophenolate mofetil and anti-inflammatory agents. Monoclonal antibody blockade of the CD40–CD154 “costimulation” pathway is also important. Using these approaches, Langin and colleagues performed life-sustaining orthotopic heart transplants from Gal-knockout pigs that express human thrombomodulin and CD46 into baboons, which survived up to 195 days. It is important to note that Langin and colleagues also used continuous ex vivo perfusion to ensure donor organ preservation and found that continuous mTOR inhibition was necessary to prevent pathologic myocardial hypertrophy and diastolic heart failure.

**HUMAN INVESTIGATIONS**

The first pig-to-human solid organ xenotransplants were performed last year (Figure 1). Two research groups, one from New York University (NYU) and one from University of Alabama Birmingham (UAB), used brain-dead recipients to support pig kidney xenografts. The NYU group used a Gal knockout donor, and the UAB group used a donor with multiple genetic modifications. Both experiments were short-term, lasting 3 days or less, owing to the experimental design. Early antibody-mediated rejection, cytokine storm, and coagulopathy did not occur, nor did transmission of zoonotic infections. The kidneys in the NYU experiments functioned. Although the information gathered from these investigations is limited by the short duration of the studies, they do help substantiate the case for clinical trials.

In January 2022, physicians and scientists at the University of Maryland performed the first pig-to-human orthotopic heart transplantation. The recipient was reliant on venoarterial extracorporeal membrane oxygenation and was deemed ineligible for human heart transplantation or durable ventricular assist device implantation. The heart originated from a pig with 10 genetic modifications (10-GE), akin to that from the UAB group; the heart supported the recipient for 49 days, and the patient died on post-transplantation day 60. The team used a T cell- and B cell–depleting induction strategy with maintenance monoclonal antibody blockade of the CD40 pathway and mycophenolate mofetil (which ultimately transitioned to tacrolimus owing to neutropenia). No acute cellular or antibody-mediated rejection was detected on myocardial biopsy by International Society for Heart and Lung Transplantation criteria, but the patient was treated empirically for possible atypical antibody-mediated rejection. Levels of donor-specific antibodies remained low but rose after administration of intravenous immune globulin (which in laboratory studies contains anti-pig NAbs). It is still unclear what precipitated the recipient’s demise, but reactivation of latent porcine cytomegalovirus, which is known to accelerate xenograft rejection in NHPs, is one theory at present. If so, improved screening and husbandry practices could alleviate such risks. Despite the numerous genetic modifications, requisite immunosuppression remains significant and highlights the need to better understand mechanisms to induce tolerance.

It remains unclear how many of the 10 genetic modifications are beneficial and whether any are actually harmful. Preclinical studies using NHPs as recipients have been instrumental in advancing human organ transplantation; however, biological differences between NHPs and humans limit the extent to which NHP xenotransplant models can predict outcomes in humans. The array of human anti-pig NAbs does not completely overlap with NHP anti-pig NAbs; for example, anti-pig NAbs to the carbohydrate NeuGc are present in humans but not in NHPs, because both NHPs and pigs express NeuGc. Knocking out NeuGc in pigs increases NAb binding in NHPs and increases xenograft rejection. Variable amounts of data support each modification in preclinical models (some only in mice), but equivalent survival has been achieved in NHP models of cardiac xenotransplantation with fewer modifications. Therefore, human investigations, such as the early trials in brain-dead recipients, are essential for identifying the ideal donor organ. Additionally, more detailed NHP studies may reveal unexpected and possibly organ-specific effects of certain genetic modifications. For example, a human CD47 transgene was present in the pig heart transplanted into a patient. However, this modification was associated with systemic inflammation, possibly related to increased levels of thrombospondin 1, an alternate CD47 ligand, in NHPs receiving pig kidney transplants with widespread tubular hCD47 expression.

**TOLERANCE**

Despite advances in genetic engineering of donor organs to mitigate the innate immune response, many xenografts in NHPs continue to demonstrate rejection despite chronic immunosuppression. Herein lies a major opportunity; inducing tolerance, or reeducating the host immune system to recognize the donor organ as “self,” offers substantial theoretical benefits toward successful and durable xenotransplantation. Methods to induce xenograft tolerance currently under investigation include thymic transplantation, wherein recipient T lymphocytes are educated within the pig thymus in recipients treated with T cell–depleting antibodies, and mixed chimerism, wherein bone marrow–derived cells from both pig and recipient coexist.

Thymic transplantation supports recipient thymopoiesis in the presence of both donor and recipient antigen-presenting cells, thereby resulting in T cell tolerance of xenograft and self (reviewed by Sykes and Sachs and Gressemer and colleagues). Early investigations demonstrated...
that pig thymus transplantation into thymectomized, T cell–depleted mice generated mature murine T cells that were tolerant of pig skin grafts.16 This tolerance reflected a combination of intrathymic deletion of donor-reactive T cells and production of regulatory cells recognizing pig antigens.18 In large animal models, nonvascularized thymic xenografts did not survive long term but did achieve initial donor-specific hyporesponsiveness (reviewed by Sykes and Sachs8). Vascularized thymic grafts, such as vascularized thymic lobe grafts and thymokidney composite grafts, have been more successful in large animals. Concomitant heterotopic transplantation of fully major histocompatibility complex–mismatched cardiac allografts with vascularized thymic lobes facilitated long-term graft acceptance after 4 weeks of tacrolimus.20 En-bloc heterotopic heart–thymus allografts also demonstrated survival without rejection until the study endpoint (200 days).21 In xenotransplantation models, NHPs receiving pig vascularized thymus plus kidney xenotransplants have survived beyond 6 months and were found to harbor new thymic emigrants with donornpecific T cell unresponsiveness (reviewed by Sykes and Sachs8). Despite these very encouraging results, complete withdrawal of immunosuppression has not yet been attempted in this model.

Cultured thymus tissue implantation, wherein slices of thymic tissue are cultured and then implanted into the quadriiceps muscle as nonvascularized grafts following heart transplantation, is an alternative to vascularized thymic transplantation.22 In August 2021, a team at Duke University performed the first orthotopic human heart transplantation followed by cultured thymus tissue implantation in a recipient with heart failure and T cell deficiency. Whether this recipient will tolerate withdrawal of immunosuppression is unknown, but perhaps similar methods for tolerance should be investigated for xenotransplantation.

Although thymic transplantation can induce T cell tolerance across xenogeneic barriers, it does not directly induce B cell or natural killer cell tolerance in pig-to-NHP models. An alternative approach is mixed hematopoietic chimerism, wherein a recipient produces both self and donor hematopoietic cells through hematopoietic stem cell transplantation following nonmyeloablative conditioning. Transient mixed chimerism combined with donor kidney transplantation has achieved renal allograft tolerance across HLA barriers in humans.23 In xenotransplantation models, even low levels of more durable mixed chimerism have been shown not only to tolerate recipient T cells, but also to induce B cell and natural killer cell tolerance in a rat-to-mouse model (reviewed by Griesemer and colleagues18) and more recently among human lymphocytes generated in immunodeficient mice with human immune systems.24–26 However, sustained mixed chimerism has not been achieved between pigs and NHPs. Different genetic modifications, such as the addition of human CD47, may prolong chimerism,27 but the extent and duration of chimerism necessary to achieve tolerance for cardiac xenotransplantation is unknown, and this may vary with the age of human recipients. For example, B cell tolerance might not be necessary prior to cardiac xenotransplantation in infants who have not yet established adult NAb repertoires and who may spontaneously develop B cell tolerance, as observed in ABO-mismatched heart transplantation. Indeed, vascularized thymic transplantation, with its ability to tolerate T lymphocytes, might prove sufficient to achieve cardiac xenograft tolerance in this subgroup of patients.

### PARTIAL HEART XENOTRANSPLANTATION

Many donor hearts are declined because of poor ventricular function, and fewer are declined due to valve disease. Transplanting a component of a heart, such as the semilunar or atrioventricular valves, may offer the potential of a living, growing valve substitute for patients with valve disease. Of course, the immunologic considerations that underpin solid organ transplantation also exist with partial heart transplantation, but the extent to which immunosuppression is required to maintain a functional valve as opposed to a functional ventricle is unknown. Valve disease is far more prevalent than end-stage heart failure, so the potential for shortages in donor valve availability is high should this therapy become widely adopted. Herein lies a major opportunity for xenotransplantation: transplantation of genetically engineered pig valves (or other cardiovascular components, such as pulmonary arteries, pulmonary veins, and systemic arteries) to avoid the drawbacks of conventional valve prostheses, with possibly fewer immunosuppression requirements than conventional total heart replacement.

### CONCLUSIONS

Numerous immunologic and developmental biology questions remain to be answered before xenotransplantation can be truly optimized. Efforts spanning from mechanistic to preclinical translational studies must continue to improve this potentially groundbreaking solution to end-stage heart failure. Yet some questions may be answerable in only humans, and the rapid progress and durable success of orthotopic heart xenotransplantation in pig-to-NHP preclinical models justify continuation of human clinical testing in both children and adults.

### Conflict of Interest Statement

Dr Sykes was supported by National Institutes of Health Grant P01 AI045897 and a Xeno Holdings/ChoironeX Sponsored Research Agreement. All the other authors reported no conflicts of interest.

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of interest. The editors and reviewers of this article have no conflicts of interest.

We thank Julissa Cabrera for assistance with the manuscript.

References

Key Words: heart transplantation, human, kidney transplantation, non-human primate, pig, tolerance, xenotransplantation