Comparative Medicine
Copyright 2024
by the American Association for Laboratory Animal Science

Vol 74, No 2
April 2024
Pages 33–48

Pigs in Transplantation Research and Their Potential as Sources of Organs in Clinical Xenotransplantation

S Sikandar Raza, MD,[1] Hidetaka Hara, MD, PhD,[2] Willard Eyestone, PhD,[3] David Ayares, PhD,[3] David C Cleveland, MD, MBA,[4] and David K C Cooper, MD, PhD, FRCS[5,*]

The pig has long been used as a research animal and has now gained importance as a potential source of organs for clinical xenotransplantation. When an organ from a wild-type (i.e., genetically unmodified) pig is transplanted into an immunosuppressed nonhuman primate, a vigorous host immune response causes hyperacute rejection (within minutes or hours). This response has been largely overcome by 1) extensive gene editing of the organ-source pig and 2) the administration to the recipient of novel immunosuppressive therapy based on blockade of the CD40/CD154 T cell costimulation pathway. Gene editing has consisted of 1) deletion of expression of the 3 known carbohydrate xenoantigens against which humans have natural (preformed) antibodies and 2) the introduction of human 'protective' genes. The combination of gene editing and novel immunosuppressive therapy has extended life-supporting pig kidney graft survival to greater than 1 y and of pig heart survival to up to 9 mo. This review briefly describes the techniques of gene editing, the potential risks of transfer of porcine endogenous retroviruses with the organ, and the need for breeding and housing of donor pigs under biosecure conditions.

Abbreviations and Acronyms: CRP, complement-regulatory protein; EPCR, endothelial protein C receptor; Gal, galactose-α1, 3-galactose; GTKO, α1,3-galactosyltransferase gene-knockout; HERV, human endogenous retrovirus; Neu5Gc, N-glycolylneuraminic acid; NHP, nonhuman primates; PERV, porcine endogenous retrovirus; TKO, triple knockout; WT, wild-type

DOI: 10.30802/AALAS-CM-23-000030

Introduction

Pigs have become increasingly important animals in surgical research, particularly in the field of transplantation. In more recent years, in an effort to provide an alternative source of organs to offset the limited number of organs available from deceased human donors each year, pigs have been investigated as potential sources of organs, tissues, and cells for clinical transplantation. Their choice as an alternative source was based on a number of anatomic, physiologic, logistical, and ethical reasons.

Here, we will first very briefly review 4 areas of transplantation research in which pigs have played a major role and will follow this with a more detailed review of progress in the field of xenotransplantation, largely in the pig-to-NHP model.

Pigs in Transplantation Research

Based on research in rodents, miniature swine were developed as a large animal model of inducing immunologic tolerance to a kidney allograft by various methods, including hematopoietic stem cell allotransplantation.[66,90,104,105,112-114,117,124,132] An advantage of inbred miniature swine was that they could be 'tissue typed' so that organ transplantation between pigs of known matched or mismatched histocompatibility could be performed. A limitation of this approach, however, is that it is much easier to induce a state of tolerance between pigs with closely matching histocompatibility profiles than between humans with disparate MHC profiles. Nevertheless, the availability of MHC-characterized miniature swine has permitted detailed investigation of the problems associated with the induction of immunologic tolerance, which has been beneficial to the successful induction of tolerance in NHPs and in a low number of patients with kidney allografts.

This model was also used to explore tolerance induced by transplantation of the thymus gland or of thymic tissue together with a kidney graft.[7,9,87,95,106,138,145-156,158] The approach has also been used to study xenotransplantation but, because of the greater immune barriers associated with cross-species transplantation, with less success.

Spleen transplantation was also carried out in the pig-to-NHP model and, although tolerance to a donor-specific kidney graft could be achieved, this approach carried a high risk of graft-versus-host disease,[41-50,64,119] and so this approach was abandoned, at least temporarily.

The early stages of research also determined that, in outbred pigs, liver allotransplantation could be followed by the spontaneous development of host tolerance to the transplanted liver, allowing all immunosuppressive therapy to be discontinued.[12-26,40,52,53,66,67,73,102,107,129,140] Furthermore, a liver transplant could also induce tolerance to a simultaneous kidney transplant from the same donor. Unfortunately, the exact mechanism that allowed 'liver tolerance' to develop in pigs was never clarified, and, although the liver is perhaps the least

Submitted: 03 May 2023. Revision requested: 20 Jun 2023. Accepted: 29 Oct 2023.
[1]Department of Cardiac Surgery, University of Michigan, Ann Arbor, Michigan; [2]College of Veterinary Medicine, Yunnan Agricultural University, Kunming, Yunnan, China; [3]Revivicor, Blacksburg, Virginia; [4]Department of Cardiothoracic Surgery, Children's Hospital of Los Angeles, Los Angeles, California; and [5]Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts
**Corresponding author. Email: dkcooper@mgh.harvard.edu*

immunogenic of the major organs in clinical transplantation, liver tolerance has not been achieved consistently in humans.

Some of the approaches to allotolerance achieved in pigs were subsequently reproduced in NHPs and humans, though with more difficulty. Mainly for logistic reasons (e.g., ease of breeding large numbers, etc.), in more recent years the pig has become the center of attention as a potential source of organs for xenotransplantation.

The Genetically Engineered Pig as a Source of Organs for Humans

Currently, in the United States, approximately 120,000 patients are on the waiting list for organ donation from deceased humans. The majority (approximately 100,000) are waiting for a kidney, with smaller numbers waiting for a heart, liver, pancreas, or lungs.[74] A significant number of deaths occur each year among those on the organ waiting list. For example, approximately 45% of those waiting for a kidney transplant die within 5 y (or are removed from the waiting list because they are no longer suitable to undergo the operation, possibly from the development of comorbidities).[86] Similar data can be obtained from other nations.

An alternative source of organs might be from gene-edited pigs. Advances in the techniques of genetic engineering of pigs have allowed researchers to make significant strides in achieving increased survival of NHPs with life-supporting pig kidney or heart transplants.[1,82,88,100,122] Pig kidney grafts in NHP have functioned for greater than 1 y[1,88] and hearts for less than 9 mo.[27,100,108] Progress has been sufficiently encouraging that some groups are slowly preparing for initial clinical trials, and one patient underwent pig heart transplantation on 'compassionate' grounds, surviving for 60 d.[32,33,65] To date, pig liver and lung transplants have been much less successful.

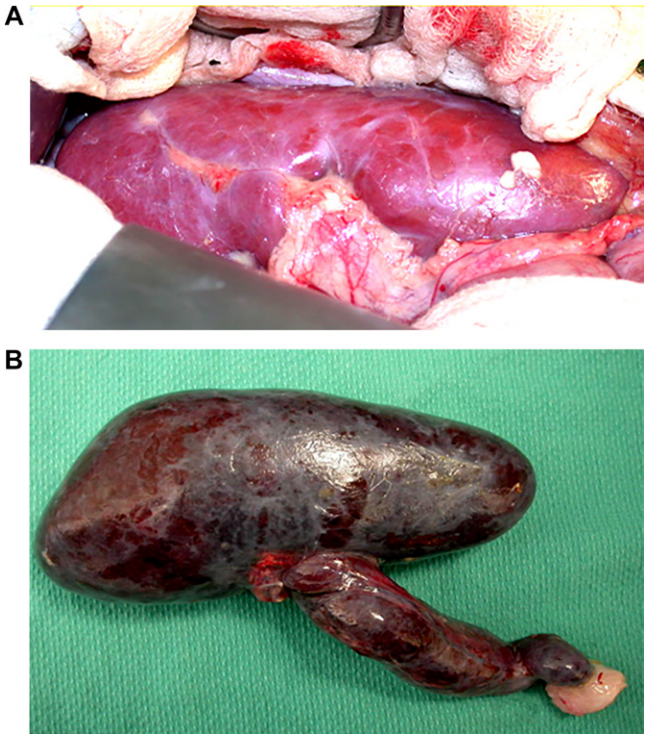

Figure 1. (A) WT (genetically unmodified) pig kidney immediately after blood reperfusion after transplantation into a baboon. (B) Same kidney excised 5 min later, having undergone hyperacute rejection.

Genetic engineering of organ-source pigs. The transplantation of organs from wild-type (WT, that is, genetically *un*modified) pigs into NHPs results in rapid antibody-dependent, complement-mediated rejection (hyperacute rejection) (Figure 1).[30] This mechanism is similar to that of the early rejection that can occur after the transplantation of an ABO-incompatible organ allograft in humans. Progress in pig graft survival in the NHP model depends largely on genetic engineering of the pig. Two major approaches have been followed: 1) deletion of expression of the known pig carbohydrate xenoantigens against which humans have natural (preformed) antibodies, and 2) the introduction of human transgenes that result in expression of 'protective' proteins on the surface of the pig cells.

Deletion of expression of pig carbohydrate xenoantigens. To date, 3 carbohydrate xenoantigens have been identified against which humans have natural antibodies (Table 1).

Gal antigens. The most important of these is galactose-α1, 3-galactose (Gal), which is expressed on many pig cells, most notably on vascular endothelial cells.[89] Gal is added to underlying carbohydrates by the enzyme α1,3-galactosyltransferase.[89] Humans and NHPs do not have this enzyme and subsequently do not express Gal, which results in the production of natural anti-Gal antibodies.[30,63] Like anti-AB blood type antibodies, anti-Gal antibodies are believed to develop during infancy as a response to colonization of the gastrointestinal tract by various Gal-expressing bacterial and viral flora.[62]

To overcome the barrier to successful xenotransplantation associated with the expression of Gal in the pig, initial studies used plasmapheresis or antibody immunoadsorption columns to remove anti-Gal antibodies from the potential organ recipient. Although these approaches delayed graft rejection, they did not result in truly prolonged graft survival.[29,96] Once the technology became possible, attention shifted to genetic engineering of pigs by deleting the gene for α1,3-galactosyltransferase, resulting in GTKO pigs[28,115] (Table 1). The absence of Gal expression delayed graft rejection.

Non-Gal antigens. Some naturally occurring human 'antinon-Gal' antibodies act against 2 additional pig antigens that have a significant, though less important, role in mediating rejection; these are N-glycolylneuraminic acid (Neu5Gc) and Sda (Table 1).[10,11] Although the cytotoxicity associated with human antinonGal antibodies is less than that of anti-Gal antibodies[56,123] (Figure 2), these target antigens on pig organs pose a barrier to the transplantation of pig organs into humans.

Neu5Gc is present in all mammals (including the great apes and Old-World monkeys), with the notable exception of humans.[3,10,139] and New World monkeys.[97] Therefore, the NHPs that are the experimental models for xenotransplantation do not produce anti-Neu5Gc antibodies against pig organs (Figure 3),

Table 1. Carbohydrate xenoantigens that have been deleted in genetically engineered pigs

Carbohydrate (abbreviation)	Responsible enzyme	Gene knockout pig
Galactose-α1,3-galactose (Gal)	α1,3-galactosyltransferase	GTKO
N-glycolylneuraminic acid (Neu5Gc)	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH)	CMAH-KO
Sda	β-1,4N-acetylgalactosaminyltransferase	β4GalNT2-KO

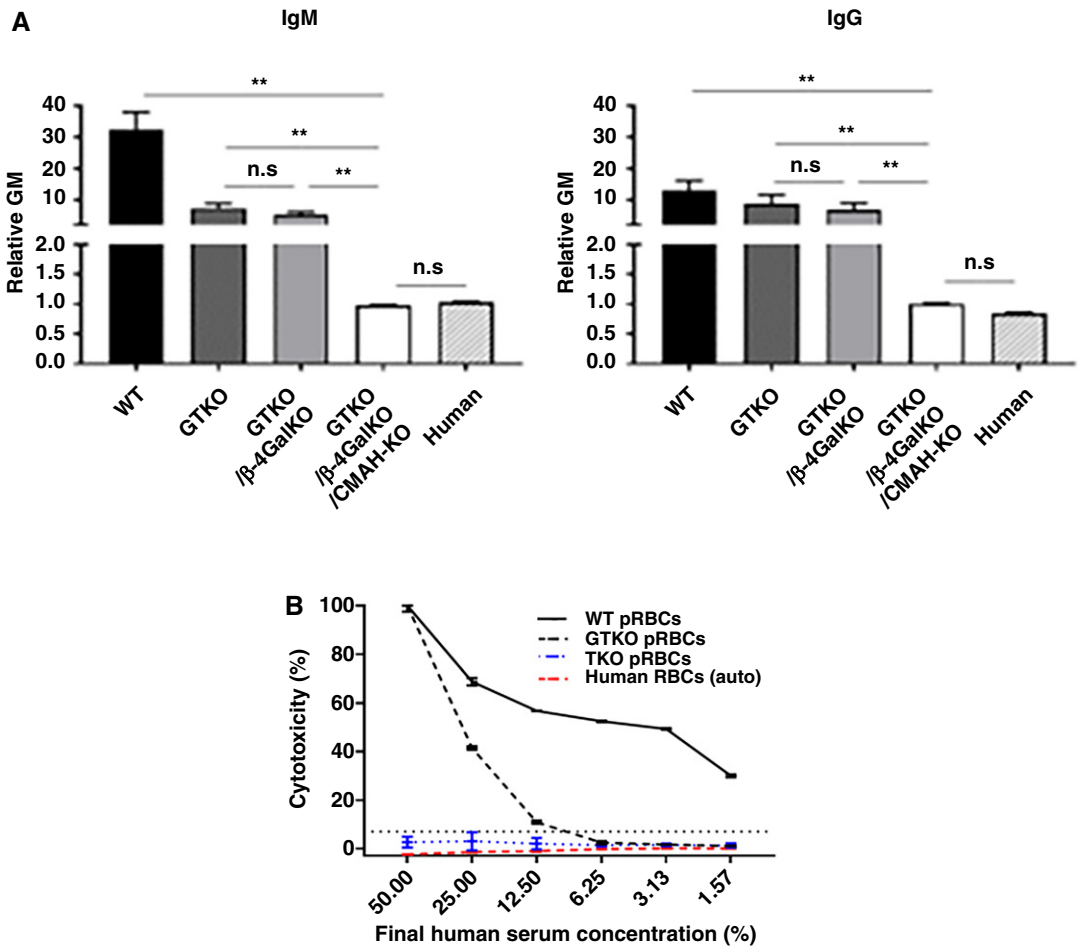

Figure 2. (A) Human serum (n = 14) IgM (left) and IgG (right) antibody binding to WT, GTKO, double-knockout (i.e., deletion of expression of Gal and Sd^a), and TKO (i.e., with additional deletion of expression of Neu5Gc) pig red blood cells (pRBCs). Human serum antibody binding to pRBCs (n = 14) was measured by flow cytometry using the relative geometric mean (rGM), which was calculated by dividing the GM value for each sample by the negative control, as previously described. Negative controls were obtained by incubating the cells with secondary antihuman antibodies only (with no serum). Binding to TKO pig RBCs was not significantly different from human IgM and IgG binding to human RBCs of blood type O. (*P < 0.05, **P < 0.01; ns, not significant). (B) Pooled human serum complement-dependent cytotoxicity (hemolysis) to WT, GTKO, and TKO pig RBCs. Cytotoxicity of the same serum to human blood type O RBCs was tested as a control. Human serum cytotoxicity is significantly less to GTKO pig RBCs than to WT RBCs. Cytotoxicity to TKO RBCs is not significantly different from that to human blood type O RBCs. (Used with permission from John Wiley & Sons, from reference 31; conveyed through Copyright Clearance Center)

though humans do (Figure 2). Thus, in clinical xenotransplantation, humans will have preformed anti-Neu5Gc antibodies against the pig organ.

In humans, the effect of expression of Sda on pig cells is weaker than of Gal (Figures 2 and 3). However, Sda has a greater role in the pig-to-NHP model.[59] If pig kidneys or hearts are to be transplanted successfully into humans, the pig cell membranes must lack the Gal, Neu5Gc, and Sda epitopes to reduce the risk of antibody-mediated rejection. These pigs are known as 'triple-knockout' (TKO) pigs. Of considerable relevance to pig organ transplantation into human infants and children is the observation that these age groups show no or minimal serum antibody binding or cytotoxicity to TKO pig cells (Figure 4). Even adult humans have markedly less binding to, and cytotoxicity of, TKO pig cells as compared with WT pig cells (Figure 2).[98]

Introduction into pigs of protective human transgenes. Although deletion of expression of the 3 known carbohydrate xenoantigens is essential if pigs are to be organ donors for humans, other genetic manipulations may also contribute significantly to the success of xenotransplantation (Table 2).

Complement-regulatory proteins. Human cells use several mechanisms to protect themselves when the human immune system is activated by, and responds to, invading pathogens. One such example is protection from the complement cascade. When complement is activated to destroy invading pathogens, the presence of complement-regulatory proteins (CRPs) on the vascular endothelial cells prevents or limits the cytotoxic effects on the host.[30] Pigs express CRPs that are similar to those of humans, but pig CRPs are inefficient in protecting against human and NHP complement-mediated activity.[4,36] Therefore, human CRP transgenes have been introduced into pigs to protect their organs from complement-mediated injury after transplantation into human or NHP hosts.

Studies in the 1990s incorporated transgenic human CRPs, such as CD55 (decay-accelerating factor), CD46 (membrane cofactor protein), or CD59 (membrane attack complex inhibition factor) by microinjection of DNA directly into the pronucleus of a fertilized pig egg (Table 3). The introduction of these CRPs provided the pig with considerable protection from human antibody-mediated injury, and increased survival of pig heart and kidney grafts in NHPs from hours or days to weeks.[141,142]

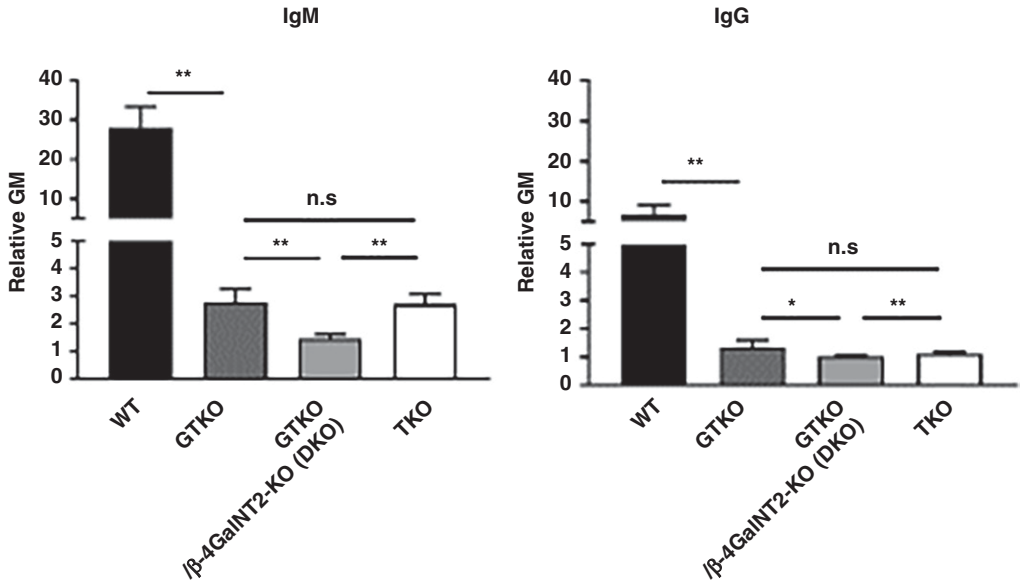

Figure 3. Baboon (an Old World monkey; $n = 14$) IgM (left) and IgG (right) antibody binding to WT, GTKO, GTKO/β4GalNT2-KO (DKO), and TKO pig RBCs. (Note that deletion of Neu5Gc [CMAH-KO] in pig cells appears to expose a fourth xenoantigen(s) against which baboons have natural antibodies.] These data support the observation that the deletion of expression of Gal has less effect in reducing antigenicity of human serum (70% reduction) (Figure 2), as compared with baboon serum (90% reduction). $^*P < 0.05$, $^{**}P < 0.01$; ns. (Used with permission from John Wiley & Sons, from reference 31; conveyed through Copyright Clearance Center)

Genetically engineered pig organs with transgenes of human CRPs combined with the deletion of expression of pig xenoantigens offered more robust protection against antibody-mediated rejection.[5]

Coagulation-regulatory proteins. Antibody and/or complement and/or inflammatory activation of the vascular endothelial cells of the pig graft may result in a change from a local anticoagulant state to a procoagulant state, resulting in the development of thrombotic microangiopathy in the graft and consumptive coagulopathy in the recipient.[52,78,125-127] As with complement regulation, pig coagulation-regulatory proteins are inefficient in maintaining a state of vascular endothelial anticoagulation in the pig organ after its transplantation into a human or NHP.

Transgenic expression of human thrombomodulin and/or endothelial protein C receptor (EPCR) works to provide an anticoagulant (and antiinflammatory) state that reduces the development of thrombotic microangiopathy and consumptive coagulopathy.[34,82,99] Platelet aggregation studies have indicated that pig cells that express thrombomodulin or EPCR are associated with significantly less platelet aggregation,[81] and in vivo studies have shown improved survival of pig kidney and heart transplants.

Other protective proteins. A NHP host develops a sustained systemic inflammatory response to a transplanted pig organ,[57,58] and the inflammation may be associated with potentiation of the immune response. This inflammation can be reduced to some extent by drug therapy (e.g., by blockade of IL-6). Transgenic expression of a human antiinflammatory (antiapoptotic) protein, such as hemeoxygenase-1 or A20, may provide local protection of the graft.[31]

Protection from the human adaptive immune response. The host adaptive immune response is a delayed, but important, response. Currently, it is largely controlled by exogenous drug therapy (e.g., agents that deplete T and B cells, and/or agents that block one of the T cell costimulation pathways), but in the future its effects are likely to be minimized by selective genetic engineering of the donor pig.

For example, macrophages link with the innate immune response to activate T cells that can mediate cellular xenograft rejection through direct cytotoxicity. Therefore, a method to limit the activation of host macrophages may prolong graft survival.[79,135,136] Human CD47, a cell-surface moiety, is recognized by macrophage signal-regulatory protein-α (SIRP-α), which inhibits macrophage activation and decreases macrophage-mediated phagocytosis of 'self' cells. However, NHP and human macrophages recognize pig CD47 as 'foreign,' and thus macrophage activity is *not* inhibited, possibly shortening graft survival.[31] The introduction of a transgene for human CD47 into the pig reduces phagocytosis by human and NHP macrophages, suppresses inflammatory cytokine production, and decreases T cell infiltration of pig xenografts.[79,134,135,160]

Swine leukocyte antigens. In pigs, swine leukocyte antigens (SLAs) correspond to the leukocyte antigens found in human leukocyte antigens (HLAs). SLAs are surface proteins on pig nucleated cells that modulate the adaptive immune response to intra- and extracellular microorganisms. Incompatibility between donor and recipient HLA types may impede graft survival in human allotransplantation. SLAs have similarities to HLA (e.g., their 3-dimensional structure), but there are some differences.[94] Like HLAs, the SLAs can be divided into class I and II (although SLA class II proteins lack a DP locus). After pig organ or cell transplantation in humans or NHPs, expression of SLA class I is associated with the presentation of intracellular peptides to host $CD8^+$T cells, whereas SLA class II presents extracellular peptides to $CD4^+$ T cells.[94]

Several studies have attempted to elucidate the humoral response of humans and NHPs to SLA. SLA has been confirmed to be a xenoantigen,[92] but less than 5% of HLA-nonsensitized persons have serum anti-SLA antibodies.[6,51,92,93,103] Even in HLA-sensitized subjects, crossreactivity between anti-HLA antibodies and SLA is uncommon,[71,144,161] suggesting that, in many patients, prior sensitization to HLA will *not* be detrimental to the survival of a pig xenograft. Nevertheless, these studies suggest a need to screen all potential recipients of a xenograft for the presence of anti-HLA antibodies that crossreact with SLA.

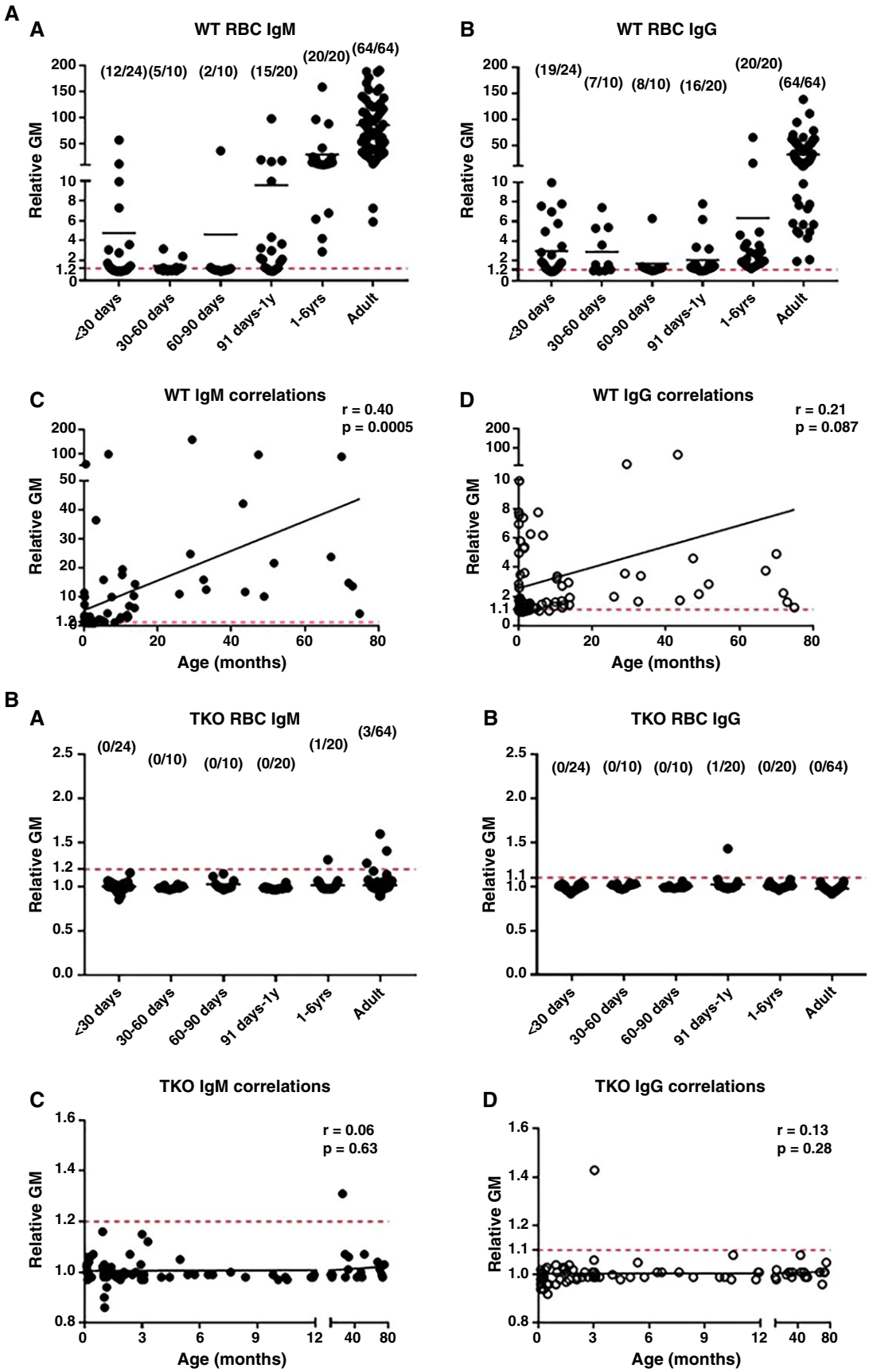

Figure 4. (A) GM binding and age correlation of human serum IgM (A, C) and IgG (B, D) antibodies to WT pig RBCs. The dotted lines indicate no IgM or IgG binding. (B) GM binding and age correlation of human serum IgM (A, C) and IgG (B, D) antibodies to TKO pig RBCs. The dotted lines indicate no IgM or IgG binding.) (Note the great difference in the scale on the *y* axis between A and B.) (Used with permission from Elsevier, from reference 98; conveyed through Copyright Clearance Center)

Table 2. Selected genetically modified pigs produced for xenotransplantation research

Antigen or deletion or 'masking'
Human H-transferase gene expression (expression of blood type O antigen)
Endo-β-galactosidase C (reduction of Gal antigen expression)
GTKO)
Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) gene knockout (NeuGc-KO)
β4GalNT2 (β1,4 N-acetylgalactosaminyltransferase) gene knockout (β4GalNT2-KO)
Complement regulation by human complement-regulatory gene expression
CD46 (membrane cofactor protein)
CD55 (decay-accelerating factor)
CD59 (protectin or membrane inhibitor of reactive lysis)
Anticoagulation and antiinflammatory gene expression or deletion
von Willebrand factor (vWF)–deficient (natural mutant)
Human tissue factor pathway inhibitor (TFPI)
Human thrombomodulin
Human EPCR
Human CD39 (ectonucleoside triphosphate diphosphohydrolase-1)
Anticoagulation, antiinflammatory, and antiapoptotic gene expression
Human A20 (tumor necrosis factor-α-induced protein 3)
Human heme oxygenase-1 (HO-1)
Inhibition of phagocytosis
Human CD47 (species-specific interaction with SIRPα inhibits phagocytosis)
Porcine asialoglycoprotein receptor 1 gene knockout (ASGR1-KO) (decreases platelet phagocytosis)
Human signal regulatory protein α (SIRPα) (decreases platelet phagocytosis by 'self' recognition)
Suppression of cellular immune response by gene expression or downregulation
CIITA-DN (MHC class II transactivator knockdown, resulting in swine leukocyte antigen class II knockdown)
Class I MHC knockout (MHC-I-KO)
HLA-E/human β2-microglobulin (inhibits human natural killer cell cytotoxicity)
HLA-G
Human FAS ligand (CD95L)
Human GnT-III (*N*-acetylglucosaminyltransferase III) gene
Porcine CTLA4-Ig (Cytotoxic T-Lymphocyte Antigen 4 or CD152)
Human TRAIL (tumor necrosis factor–α–related apoptosis-inducing ligand)
Programed death-ligand 1 (PD-L1)
Prevention of porcine endogenous retrovirus (PERV) activation
PERV siRNA
PERV-KO

Table 3. Timeline for application of evolving techniques for genetic engineering of pigs employed in xenotransplantation

Year	Technique
1992	Microinjection of randomly integrating transgenes
2000	Somatic cell nuclear transfer
2002	Homologous recombination
2011	Zinc finger nucleases
2013	Transcription activator-like effector nucleases
2014	CRISPR/Cas9[a]

[a]CRISPR/Cas9 = clustered randomly interspaced short palindromic repeats and the associated protein 9.

However, genetic-engineering techniques are being developed that will allow modification of SLA expression to ensure that HLA sensitization will not affect the outcome of a pig organ transplant.[93]

Organ xenotransplants from pigs that, through genetic engineering, do *not* express SLA would stimulate a much weaker human/NHP immune response, but may seriously hinder the survival of the organ-source pig because it would be more susceptible to infection. Recent approaches have therefore been focused on genetically engineering the pig to knockout expression of SLA class I antigens and/or to reduce expression of SLA class II antigens, as knockout of class II results in immunodeficiency and could be lethal to the developing pig.

One study used the CRISPR/Cas9 system to mutate the β2-microglobulin locus of SLA class I and found that proliferation of human peripheral blood mononuclear cells (PBMCs) was significantly lower in pigs with low expression of β2-microglobulin as compared with cells from WT pigs.[76] By reducing SLA class II expression, the introduction of a mutant dominant-negative transgene of the class II transactivator (CIITA) markedly impaired human $CD4^+$ T cell proliferation.[72] Studies in mice have shown prolonged survival of skin grafts from pigs that were $\beta2m^-/CIITA^-$.[61]

Although knockout or knockdown of SLA has potential in prolonging pig graft survival after xenotransplantation, these modifications may reduce survival of donor pigs, and, after xenotransplantation, the organ may have impaired protection against infection.[121] This approach, therefore, requires further exploration.

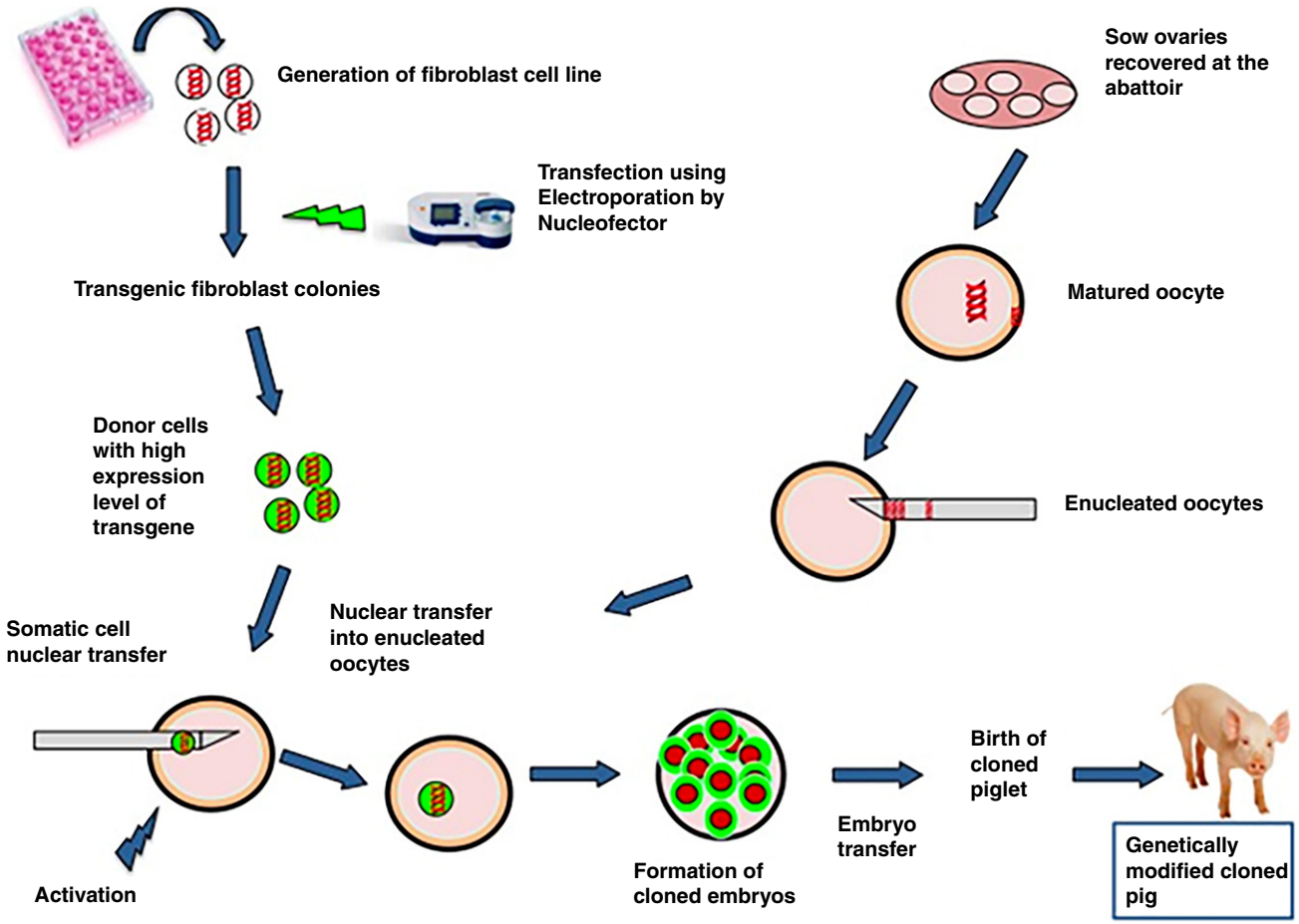

Figure 5. Steps involved in SCNT. (Reproduced with permission from Springer Nature, from reference 55)

The techniques of pig genetic engineering. ***Somatic cell nuclear transfer.*** The development of somatic cell nuclear transfer (SCNT) was critical to the successful production of gene-edited pigs[116,143] (Figure 5). In this process, the endogenous nuclei of porcine oocytes are replaced with gene-edited nuclei from donor pig fibroblasts. The newly constructed oocytes develop into pigs that express the genetic modifications made in the donor fibroblast nuclei. Using SCNT technology and homologous recombination, knockout of genes for enzymes (e.g., α1,3-galactosyltransferase) can be produced in the pig fibroblasts and then incorporated into pig oocytes.[35] Therefore, pigs derived from the oocytes lack the gene for α1,3-galactosyltransferase and phenotypically lack expression of Gal antigens (GTKO pigs) (Table 1).

Once the founder pigs have been produced by cloning technology, future generations can be bred naturally. GTKO pigs are recognized by the U.S. FDA as sources of food for consumption by humans and of tissues for transplantation.[137] Increasing sophistication of the techniques now allows multiple genetic manipulations (consisting of knockout and 'knockin' of genes), resulting in pigs with 10 or more genetic changes.

CRISPR/Cas9. The techniques by which pigs have been genetically manipulated have evolved over time (Table 3). The clustered regularly interspaced short palindromic repeats (CRISPR) technique is based on an RNA sequence that is homologous to the genomic target site. CRISPR carries a short guide nucleotide sequence that directs the Cas nuclease to induce a double-stranded DNA break at the target site. This break is then rejoined using the process of nonhomologous end joining, which is error prone and can result in the insertion or deletion of a few nucleotides (indels). If these indels occur within an exon, they can cause a frameshift mutation resulting in a truncated, nonfunctional protein, thus creating a knockout. Alternatively, if the DNA is repaired by the homology-directed presence of a DNA vector flanked with sequences homologous to the target region, an intervening sequence can be incorporated into the genome. CRISPR/Cas9 technology allows efficient gene targeting for creating knockouts and, with homology-directed repair, transgenes can be inserted into predetermined 'landing-pad' sites in the genome.[103]

CRISPR/Cas9 provides an opportunity to knockout multiple genes in cultured GTKO fibroblasts, followed by SCNT.[55] In an assessment of the effect of each knockout on human serum antibody binding and complement-mediated cell lysis (Figure 6),[55] GTKO alone reduced IgG binding by 68%, followed by 76% with GTKO+β4GalNT2KO, and 79% with GTKO+CMAHKO. Together, GTKO+β4GalNT2KO+CMAHKO (TKO pigs) reduced total human serum IgG binding by 92%.

Promoters and vectors. The use of bicistronic or multicistronic vectors allows 2 or more transgenes to be inserted under the control of one or more promoters (Figure 7). Incorporating several transgenes into a single vector incurs several benefits for mitigating the innate immune response to a pig organ.[133] A vector can be incorporated into a genome using homologous recombination specific to a landing pad or transgenic expression reading frame.[103] The landing pad can be vacated after knockout of a carbohydrate xenoantigen (e.g., Gal, Neu5Gc, and/or Sda).

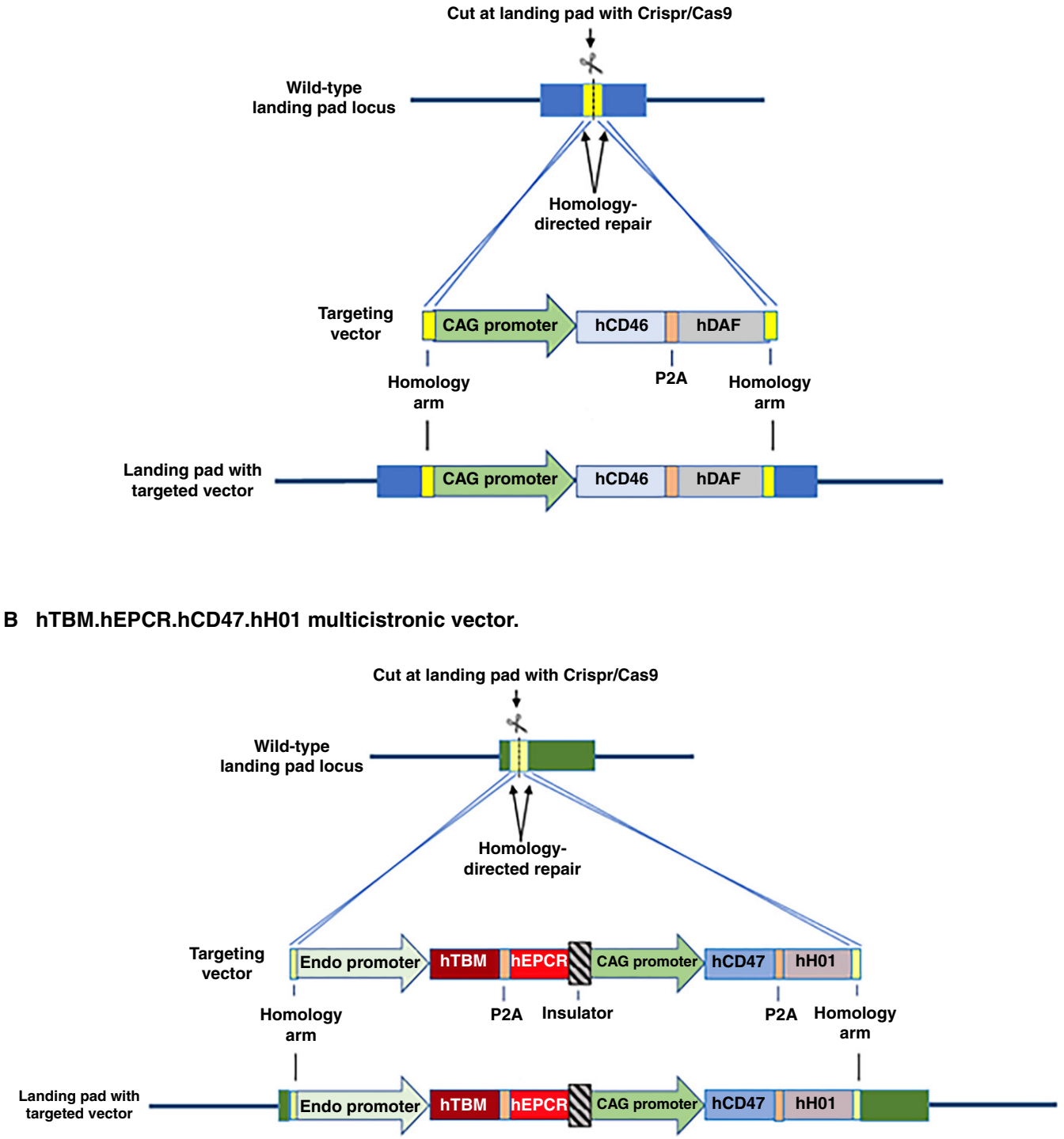

Figure 6. Design and targeting of multicistronic vectors (MCVs). CRISPR/Cas9 is designed to cut within an expression-permissive landing pad. Homology arms direct vector insertion to the landing pad by homology-directed repair. The CAG promoter was used to drive ubiquitous transgene expression (A, B), whereas one of several 'endo promoters' was used to obtain endothelial-specific expression (B). (Reproduced with permission from Springer Nature, from reference 55)

The type of promoter determines whether the transgenic protein will be expressed ubiquitously in all tissues of the pig (e.g., a CAG promoter) or endospecifically (i.e., only in the endothelial cells, for example, ICAM-2 or pig thrombomodulin promoters). For example, human CRPs (e.g., CD46, CD55, CD59) are more effective if widely expressed, whereas if human coagulation-regulatory proteins (e.g., thrombomodulin, EPCR) are widely expressed, the pig may develop bleeding tendencies. Therefore, coagulation-regulatory proteins are preferably expressed only in endothelial cells.

Judicious genetic engineering of donor pigs has resulted in significant prolongation of pig heart or kidney function in immunosuppressed NHP recipients, with delayed or absent rejection.[1,27,82,88,100,122]

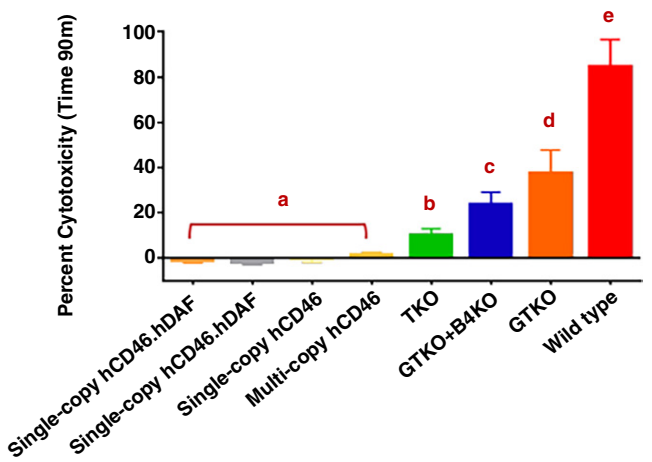

Figure 7. Effect of xenoantigen knockout and expression of complement inhibitors on serum cytotoxicity as measured by image-based complement-dependent cytotoxicity assay. Cytotoxicity decreased significantly from WT with each additional knockout (columns with different superscripts, $P < 0.05$). Cytotoxicity was nearly eliminated when CRPs were expressed as either hCD46 alone or multicopy hCD46, or from a single-copy bicistron composed of hCD46 and hCD55. All genotypes, except WT, include a GTKO background. (Reproduced with permission from Springer Nature, from reference 55)

Which type of donor pig is optimal for the initial clinical trials? Opinions vary on this topic, with some researchers believing that TKO pigs will suffice if the organ is transplanted into a patient who has no serum antibody binding or cytotoxicity to TKO pig cells.[2] Our own opinion is that the additional protection provided by expression of human 'protective' proteins, especially CRPs and coagulation-regulatory proteins, will provide resistance to immune-mediated injury associated with events such as ischemia-reperfusion injury, a systemic inflammatory response, and a systemic infection. The optimal combination of genetic manipulations requires further clarification. Nevertheless, TKO pigs (with or without added human transgenes) will almost certainly provide the organs for initial clinical trials.[31,98]

The testing of TKO pig organs in NHP models is complicated by the differences in serum antibody production between humans and NHPs (Figure 8).[54,159] Because NHPs express Neu5Gc, knockout of this carbohydrate xenoantigen in pigs exposes them to an unidentified carbohydrate xenoantigen (or antigens) against which NHPs (but not humans) have natural antibodies. Exposure to this xenoantigen(s) is associated with a high cytotoxicity (Figure 8).

A key issue is the determination of which genetic manipulations are essential for a successful clinical trial, and which may be beneficial, but perhaps are not essential. Reduction of expression of SLA class I and/or II can be considered nonessential at present because immunosuppressive agents can be administered to block the adaptive immune response, whereas no agents are available that can effectively prevent the innate immune response. National regulatory authorities (e.g., the FDA in the USA) will probably require some justification for the inclusion of each genetic manipulation made in pigs intended to be organ donors. Furthermore, each additional genetic manipulation risks a detrimental effect on the health of the pig or the viability or function of the organ graft, and thus will also require evaluation to produce the ultimate donor pig.

Potential complications of genetic engineering and SCNT of organ-source pigs. Some genetic mutations are lethal, and others are associated with physical defects or functional deficiencies in the pigs. These mutations include some of the manipulations discussed above (e.g., overexpression of one or more coagulation-regulatory proteins, knockout of SLA). Every novel and previously untested genetic manipulation entails some risk in this respect. However, if the optimal promoters and techniques are used, multiple genetic manipulations can be carried out successfully without detriment to the health of the pig.

Genetic engineering to prevent potential complications associated with the presence of porcine endogenous retroviruses. Another area in which genetic engineering of the pig could play a role is in minimizing or preventing potential complications related to the presence of porcine endogenous retroviruses or retroviral particles (PERVs), which are present in the DNA of every cell in the pig. These PERVs have been present in pigs for millions of years[110] and do not appear to harm the pig. Human cells contain similar human endogenous retroviruses (HERVs), which are generally believed to be largely innocuous, although some evidence indicates that they are overexpressed in some human tumors and may contribute to tumor development.[131] However, concern was raised[128] as to whether PERVs might be harmful after the transplantation of a pig organ into a human (e.g., by possibly causing immunodeficiency and/or tumerogenicity). The possibility was also raised with regard to whether fragments of PERVs might combine with fragments of HERVs to produce a problematic hybrid virus.

Although evidence indicates that under specific laboratory conditions, PERVs can be transmitted into human cells in vitro (specifically a PERV A/C recombinant virus), no evidence indicates transmission or any complication in an in vivo model in NHPs or in humans who have received porcine cells or tissues over decades of xenotransplantation research. However, this negative outcome may be in part associated with difficulties in transmitting the virus to NHPs.[38] The potential risk will be unknown until clinical trials are initiated.[37,60] What is perhaps of more concern than the fate of the patient (who may be very willing to accept this potential risk) is whether a patient who develops a complication associated with PERV will be a risk to his/her family and friends and other close contacts (e.g., healthcare providers), although this seems unlikely.

Although the risk of a complication is generally considered to be low, techniques have been developed to reduce or abrogate any potential risk. Activation of PERVs can be prevented,[39,120] and multiple copies of PERV have been knocked out, thus rendering the pig PERV free.[111] Whether the national regulatory authorities will consider this to be necessary is uncertain. At present, most research groups are proceeding with pigs that have been engineered or bred to be null for PERV-C, which eliminates the risk of potential PERV-A/C recombination events.[75] Drugs are available that are likely to successfully treat a PERV-related infection, if it should occur.[60,91]

Breeding and housing of organ-source pigs in a biosecure clean facility. To minimize the risk of the transfer of an exogenous infection (e.g., bacterial or viral) from the donor pig organ to the recipient, regulatory authorities will require the donor pig to be bred and housed under strict biosecure 'clean' conditions. The pigs will require careful screening and proof that they are negative for, or treated for, relevant microorganisms *before* they enter the facility. Several lists of microorganisms that should be excluded from the pigs have been published (e.g.,[91]). The regulatory requirements and means for their fulfillment have also been outlined[91] and will not be detailed here. The housing facility must be 'biosecure' to prevent any possible infection of the pigs by insects, humans, or other sources, and the staff maintaining the facility and caring for the pigs will require screening for

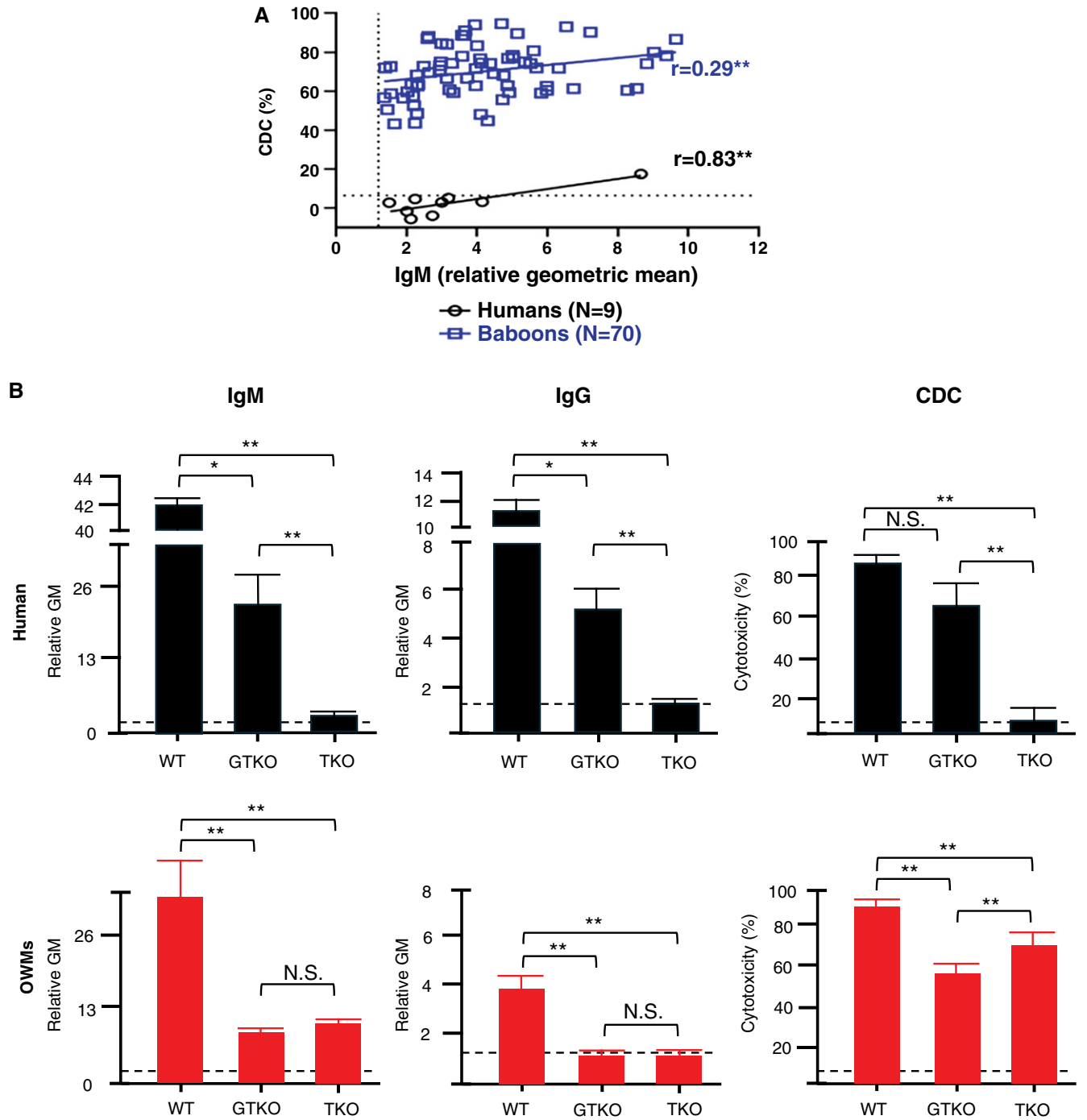

Figure 8. (A) Correlation of human (n = 9) and baboon (n = 72) serum IgM antibody binding with serum CDC (at 50% serum concentration) to TKO pig PBMCs. In both humans and baboons, there was a significant increase in cytotoxicity as IgM and IgG antibody binding to TKO pig PBMCs increased. In baboons, however, cytotoxicity was high whether IgM binding was high (e.g., 80% cytotoxicity at a rGM of 8) or relatively lower (e.g., 75% at a rGM of 2). (**P < 0.01). (B) Human and Old World monkey IgM (left) and IgG (middle) binding and complement-dependent cytotoxicity (CDC, at 25% serum concentration) (right) to WT, GTKO, and TKO pig PBMCs. Results are expressed as mean ± SEM (*P < 0.05, **P < 0.01; ns). On the y axis, the dotted line represents cutoff value of binding (rGM: IgM 1.2, IgG 1.1). For CDC on the y axis, the dotted line represents cutoff value of cytotoxicity (6.4%). Note the difference in scale on the y axis between IgM and IgG.

symptoms or signs of infection to prevent transfer to the pigs. If maintained in such a clean environment, it is anticipated that a pig herd will remain infection free.

Certain viruses are particularly important to eliminate from the pig herd. For example, porcine cytomegalovirus stimulates an immune response and may be associated with injury to a pig graft.[33,65,109,157]

Experts in treating the infectious complications associated with long-term immunosuppressive therapy in patients with organ allografts predict that patients with pig organ grafts will be susceptible to the same infectious complications.[60] Their many years of experience in managing such patients suggest that these complications will be equally manageable in patients with xenografts.

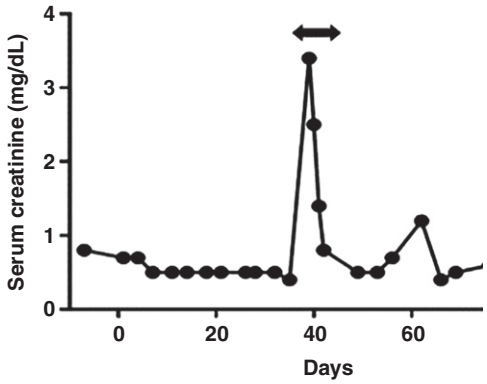

Figure 9. Example of a spontaneous increase in serum creatinine in a baboon with a life-supporting pig kidney transplant (with nephrectomy of the native kidneys at the time of pig kidney transplantation). The rapid reduction in creatinine to normal (human) levels was associated solely with an intravenous infusion of normal saline (arrow). A renal biopsy at the time showed no features of rejection.

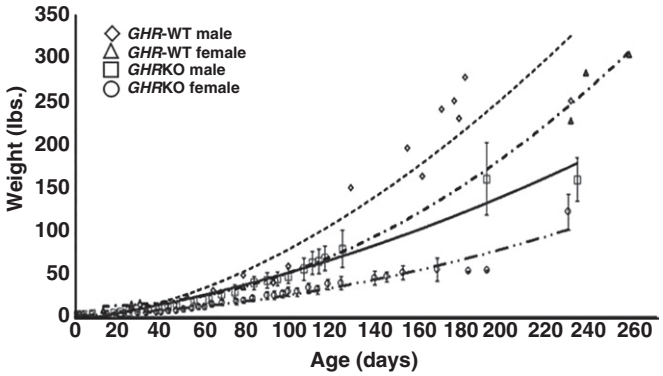

Figure 10. Growth curves of *GHR*KO and corresponding control (*GHR*-WT) pigs. *GHR*KO pigs produced by Revivicor showed reduced body weight compared with controls. Curves are best-fit lines for males and females. Means for *GHR*KO pigs are indicated by boxes and circles, and standard errors by vertical lines. Data for *GHR*-WT pigs are shown as individual data points. (Used with permission from Blackwell Publishing, from reference 85; conveyed through Copyright Clearance Center)

The function of a pig kidney in a primate recipient. Few detailed studies of pig kidney graft function after transplantation into an immunosuppressed NHP host have been carried out as yet,[68,70,86] but these studies indicate that life-supporting pig kidneys have maintained NHPs for many months or even greater than 1 y, demonstrating that renal function is adequate. Detailed studies (e.g., of glomerular filtration rate, and compatibility of the renin-angiotensin-aldosterone system) confirm that a pig kidney largely fulfills the functions of a NHP kidney[68,86] and is therefore likely to fulfill those of the human kidney.

Two observations have been made that warrant comment. First, baboons have been observed to develop an increase in serum creatinine that is associated with the development of hypovolemia/dehydration in the absence of histopathologic or other clinical features of graft rejection (Figure 9).[84] In our experience, an episode of rejection is associated with an increase in proteinuria, whereas hypovolemia/dehydration is not.

The baboon does not appear to be aware that it is becoming dehydrated, and we have not observed a measurable difference in fluid intake or urine output relative to those measures prior to the episode. The increase in serum creatinine can immediately be reversed by the intravenous or subcutaneous infusion of normal saline. Although alternative explanations are possible, these episodes could be because of the observation that primate angiotensinogen is a relatively poor substrate for porcine renin.[68,69] This difference may result in reduced vasoconstriction, which in turn leads to low arterial blood pressure and subsequent hypoperfusion of the pig kidney. If this becomes a serious problem, genetic engineering of the donor pig to produce human renin could solve the problem.

The second observation is that the pig kidney graft (whether from a WT or genetically engineered pig) grows rapidly, at least during the first few months after transplantation, as if it were still in the rapidly growing pig.[80,82,83,130] This rapid growth is likely associated with an innate factor in the graft. Similar observations have been made after orthotopic pig heart transplantation in NHPs.[100] In our experience, early rapid growth of the pig kidney within the abdomen has not been a problem, but others have reported compression of the kidney or even rupture of the abdominal surgical wound.[134] We suggest that the difference in reported outcomes may be associated with our use of rapamycin, which can restrict growth, as an immunosuppressive agent. However, rapid growth would be a greater problem after orthotopic heart transplantation in which the pig heart may be compressed in the more restricted confines of the pericardial and thoracic cavities.[100]

A suggestion has been made that this complication could be avoided if the pig were genetically engineered with knockout of growth hormone receptors.[77,85] Our group has tested this idea, with results indicating reduced growth of both the pig (Figure 10) and the pig kidney. However, we initially observed a significant rate of necrosis of the ureter in pigs with this genetic manipulation, and others have commented on the fragility of the ureter.[118]

The problem of rapid growth is resolved if the organ is taken from one of the many breeds of miniature swine, in which posttransplant growth of the kidney is much slower.[101]

Comment. Largely based on the advances that have been made in the genetic engineering of pigs, as well as the use of novel immunosuppressive agents[8] and greater experience in pig-to-NHP organ transplantation, clinical trials of pig organ xenotransplantation are drawing closer. However, several details need to be clarified before a trial can be appropriately initiated. Once proof of concept is obtained, xenotransplantation will open immense opportunities for patients with end-stage organ failure and with diseases such as diabetes mellitus, Parkinson's disease, and corneal blindness and possibly will provide a source of red blood cells for transfusion.

Acknowledgments

Work in DKCC's laboratory at the MGH/HMS is supported in part by NIH NIAID U19 grant AI090959 and in part by a Kidney X Prize from the US DHHS and the American Society of Nephrology. The baboons used in their studies are from the Michale E. Keeling Center M.D. Anderson Cancer Center, Bastrop, TX, which is supported by NIH grant P40 OD24628-01.

Conflict of Interest

WE and DA are employees of Revivicor, Blacksburg, VA, USA. DKCC is a consultant to eGenesis Bio, Cambridge, MA, USA. The other authors have no conflicts of interest.

Funding

This work was primarily funded by NIH.

References

1. **Adams AB, Kim SC, Martens GR, Ladowski JM, Estrada JL, Reyes LM, Breeden C, et al.** 2018. Xenoantigen deletion and chemical immunosuppression can prolong renal xenograft survival. Ann Surg **268**:564–573. https://doi.org/10.1097/SLA.0000000000002977.
2. **Adams AB, Lovasik BP, Faber DA, Burlak C, Breeden C, Estrada JL, Reyes LM, et al.** 2021. Anti-C5 antibody tesidolumab reduces early antibody-mediated rejection and prolongs survival in renal xenotransplantation. Ann Surg **274**:473–480. https://doi.org/10.1097/SLA.0000000000004996.
3. **Asaoka H, Matsuda H.** 1994. Detection of N-glycolylneuraminic acid-containing glycoproteins from various animal erythrocytes by chicken monoclonal antibody against Hanganutziu-Deicher antigens. J Vet Med Sci **56**:375–377. https://doi.org/10.1292/jvms.56.375.
4. **Atkinson JP, Oglesby TJ, White D, Adams EA, Liszewski MK.** 1991. Separation of self from non-self in the complement system: A role for membrane cofactor protein and decay accelerating factor. Clin Exp Immunol **86** (suppl 1):27–30. https://doi.org/10.1111/j.1365-2249.1991.tb06203.x.
5. **Azimzadeh AM, Kelishadi SS, Ezzelarab MB, Singh AK, Stoddard T, Iwase H, Zhang T, et al.** 2015. Early graft failure of GalTKO pig organs in baboons is reduced by expression of a human complement pathway-regulatory protein. Xenotransplantation **22**:310–316. https://doi.org/10.1111/xen.12176.
6. **Barreau N, Godfrin Y, Bouhours JF, Bignon JD, Karam G, Leteissier E, Moreau T, et al.** 2000. Interaction of anti-HLA antibodies with pig xenoantigens. Transplantation **69**:148–156. https://doi.org/10.1097/00007890-200001150-00025.
7. **Barth RN, Yamamoto S, LaMattina JC, Kumagai N, Kitamura H, Vagefi PA, Awwad M, et al.** 2003. Xenogeneic thymokidney and thymic tissue transplantation in a pig-to-baboon model. I. Evidence for pig-specific T-cell unresponsiveness. Transplantation **75**:1615–1624. https://doi.org/10.1097/01.TP.0000064335.50622.20.
8. **Bikhet M, Iwase H, Yamamoto T, Jagdale A, Foote JB, Ezzelarab M, Anderson DJ, et al.** 2021. What therapeutic regimen will be optimal for initial clinical trials of pig organ transplantation? Transplantation **105**:1143–1155. https://doi.org/10.1097/TP.0000000000003622.
9. **Binns RM, Calne RY, Millard PR.** 1970. Induced tolerance of a kidney allograft in a pig. Preliminary report. Eur Surg Res **2**:408–414. https://doi.org/10.1159/000127540.
10. **Bouhours D, Pourcel C, Bouhours JE.** 1996. Simultaneous expression by porcine aorta endothelial cells of glycosphingolipids bearing the major epitope for human xenoreactive antibodies (galα1-3gal), blood group h determinant and n-glycolylneuraminic acid. Glycoconj J **13**:947–953. https://doi.org/10.1007/BF01053190.
11. **Byrne GW, Du Z, Stalboerger P, Kogelberg H, McGregor CG.** 2014. Cloning and expression of porcine β1,4 N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. Xenotransplantation **21**:543–554. https://doi.org/10.1111/xen.12124.
12. **Calne RY, White HJ, Yoffa DE, Maginn RR, Binns RM, Samuel JR, Molina VP.** 1967. Observations of orthotopic liver transplantation in the pig. BMJ **2**:478–480. https://doi.org/10.1136/bmj.2.5550.478.
13. **Calne RY, White HJ, Yoffa DE, Binns RM, Maginn RR, Herbertson RM, Millard PR, et al.** 1967. Prolonged survival of liver transplants in the pig. BMJ **4**:645–648. https://doi.org/10.1136/bmj.4.5580.645.
14. **Calne RY, Yoffa DE, White HJ, Maginn RR.** 1968. A technique of orthotopic liver translantation in the pig. Br J Surg **55**:203–206. https://doi.org/10.1002/bjs.1800550310.
15. **Calne RY, White HJ, Herbertson BM, Millard PR, Davis DR, Salaman JR, Samuel JR.** 1968. Pig-to-baboon liver xenografts. Lancet **1**:1176–1178. https://doi.org/10.1016/S0140-6736(68)91869-2.
16. **Calne RY, White HJ, Binns RM, Herbertson BM, Millard PR, Pena J, Salaman JR, et al.** 1969. Immunosuppressive effects of the orthotopically transplanted porcine liver. Transplant Proc **1**:321–324.
17. **Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, et al.** 1969. Induction of immunological tolerance by porcine liver allografts. Nature **223**:472–476. https://doi.org/10.1038/223472a0.
18. **Calne RY, Sells RA, Pena JR, Ashby BS, Herbertson BM, Millard PR, Davis DR.** 1969. Toleragenic effects of porcine liver allografts. Br J Surg **56**:692–693.
19. **Calne RY, Davis DR, Pena JR, Balner H, de Vries M, Herbertson BM, Millard PR, et al.** 1970. Hepatic allografts and xenografts in primates. Lancet **1**:103–106. https://doi.org/10.1016/S0140-6736(70)90462-9.
20. **Calne RY, Sells RA, Marshall VC, Millard PR, Herbertson BM, Hadjiyannakis EJ, Dunn DC, et al.** 1972. Multiple organ grafts in the pig. Techniques and results of pancreatic, hepatic, cardiac, and renal allografts. Br J Surg **59**:969–977. https://doi.org/10.1002/bjs.1800591210.
21. **Calne RY, Dunn DC, Herbertson BM, Bitter-Suermann H, Davis DR, Smith DP, Reitter FH.** 1974. Preservation of the porcine liver. Transplant Proc **6**:289–294.
22. **Calne RY.** 1994. Immunosuppression in liver transplantation. N Engl J Med **331**:1154–1155. https://doi.org/10.1056/NEJM199410273311711.
23. **Calne RY.** 2000. Immunological tolerance–the liver effect. Immunol Rev **174**:280–282. https://doi.org/10.1034/j.1600-0528.2002.017419.x.
24. **Calne RY.** 1974. Clinical and experimental liver grafting. Guys Hosp Rep **123**:1–12.
25. **Calne RY.** 2008. Early days of liver transplantation. Am J Transplant **8**:1775–1778. https://doi.org/10.1111/j.1600-6143.2008.02315.x.
26. **Calne RY.** 1971. Kidney transplantation. Am Heart J **82**:838–839. https://doi.org/10.1016/0002-8703(71)90210-9.
27. **Cleveland DC, Jagdale A, Carlo WF, Iwase H, Crawford J, Walcott GP, Dabal RJ, et al.** 2022. The genetically engineered heart as a bridge to allotransplantation in infants: Just around the corner? Ann Thorac Surg. **114**:536–544.
28. **Cooper DKC, Koren E, Oriol R.** 1993. Genetically engineered pigs. Lancet **342**:682–683. https://doi.org/10.1016/0140-6736(93)91791-J.
29. **Cooper DKC, Satyananda V, Ekser B, van der Windt J, Hara H, Ezzelarab MB, Schuurman H-J.** 2014. Progress in pig-to-nonhuman primate transplantation models (1998–2013): A comprehensive review of the literature. Xenotransplantation **21**:397–419. https://doi.org/10.1111/xen.12127.
30. **Cooper DKC, Ezzelarab MB, Hara H, Iwase H, Lee W, Wijkstrom M, Bottino R.** 2016. The pathobiology of pig-to-primate xenotransplantation: A historical review. Xenotransplantation **23**:83–105. https://doi.org/10.1111/xen.12219.
31. **Cooper DKC, Hara H, Iwase H, Yamamoto T, Li Q, Ezzelarab M, Federzoni E, et al.** 2019. Justification of specific genetic modifications in pigs for clinical kidney or heart xenotransplantation. Xenotransplantation **26**:e12516. https://doi.org/10.1111/xen.12516.
32. **Cooper DKC.** 2022. Invited commentary: Initial reflections on the world's first clinical genetically-engineered pig heart transplant. Xenotransplantation. **29**:e12737. Doi:https://doi.org/10.1111/xen.12737.
33. **Cooper DKC, Yamamoto T, Hara H, Pierson RN 3rd.** 2022. The first clinical pig heart transplant—Was IVIg or pig cytomegalovirus detrimental to the outcome? Xenotransplantation **29**:e12771. https://doi.org/10.1111/xen.12771.
34. **Crikis S, Zhang XM, Dezfouli S, Dwyer KM, Murray-Segal LM, Salvaris E, Selan C, et al.** 2010. Anti-inflammatory and anticoagulant effects of transgenic expression of human thrombomodulin in mice. Am J Transplant **10**:242–250. https://doi.org/10.1111/j.1600-6143.2009.02939.x.
35. **Dai Y, Vaught TD, Boone J, Chen S, Phelps CJ, Ball S, Monahan JA, et al.** 2002. Targeted disruption of the α1,3-galactosyltransferase gene in cloned pigs. Nat Biotechnol **20**:251–255. https://doi.org/10.1038/nbt0302-251.
36. **Dalmasso AP, Vercellotti GM, Platt JL, Bach FH.** 1991. Inhibition of complement-mediated endothelial cell cytotoxicity by decay accelerating factor. Potential for prevention of xenograft hyperacute

rejection. Transplantation **52**:530–533. https://doi.org/10.1097/00007890-199109000-00029.
37. **Denner J.** 2017. Can antiretroviral drugs be used to treat porcine endogenous retrovirus (PERV) infection after xenotransplantation? Viruses **9**:213. https://doi.org/10.3390/v9080213.
38. **Denner J.** 2018. Why was PERV not transmitted during preclinical and clinical xenotransplantation trials and after inoculation of animals? Retrovirology **15**:28. https://doi.org/10.1186/s12977-018-0411-8.
39. **Dieckhoff B, Petersen B, Kues WA, Kurth R, Niemann H, Denner J.** 2008. Knockdown of porcine endogenous retrovirus (PERV) expression by PERV-specific shRNA in transgenic pigs. Xenotransplantation **15**:36–45. https://doi.org/10.1111/j.1399-3089.2008.00442.x.
40. **Dische FE, Neuberger J, Keating J, Parsons V, Calne RY, Williams R.** 1988. Kidney pathology in liver allograft recipients after long-term treatment with cyclosporin A. Lab Invest **58**:395–402.
41. **Dor FJ, Gollackner B, Cooper DK.** 2003. Can spleen transplantation induce tolerance? A review of the literature. Transpl Int **16**:451–460. https://doi.org/10.1111/j.1432-2277.2003.tb00332.x.
42. **Dor FJ, Cheng J, Alt A, Cooper DKC, Schuurman H-J.** 2004. Galα1,3Gal expression on porcine pancreatic islets, testis, spleen, and thymus. Xenotransplantation **11**:101–106. https://doi.org/10.1111/j.1399-3089.2004.00078.x.
43. **Dor FJMF, Tseng Y-L, Kuwaki K, Ko DSC, Cooper DKC.** 2004. Letters to the Editor. Xenotransplantation **11**:298–300. https://doi.org/10.1111/j.1399-3089.2004.00131.x.
44. **Dor FJ, Doucette KE, Mueller NJ, Wilkinson RA, Bajwa JA, McMorrow IM, Tseng Y-L, et al.** 2004. Posttransplant lymphoproliferative disease after allogeneic transplantation of the spleen in miniature swine. Transplantation **78**:286–291. https://doi.org/10.1097/01.TP.0000128342.64240.CF.
45. **Dor FJ, Gollackner B, Kuwaki K, Ko DSC, Cooper DKC, Houser SL.** 2005. Histopathology of spleen allograft rejection in miniature swine. Int J Exp Pathol **86**:57–66. https://doi.org/10.1111/j.0959-9673.2005.00414.x.
46. **Dor FJ, Tseng YL, Kuwaki K, Gollackner B, Ramirez ML, Prabharasuth DD, Cina RA, et al.** 2005. Immunological unresponsiveness in chimeric miniature swine following MHC-mismatched spleen transplantation. Transplantation **80**:1791–1804. https://doi.org/10.1097/01.tp.0000184625.27076.c8.
47. **Dor FJ, Cooper DK.** 2006. Immunologic benefits of spleen transplantation in the absence of graft-versus-host disease. Ann Surg **243**:710–711. https://doi.org/10.1097/01.sla.0000216774.20043.74.
48. **Dor FJ, Cooper DK.** 2006. Engraftment potential of adult spleen cells in vitro and in vivo. Transpl Immunol **16**:131. https://doi.org/10.1016/j.trim.2006.03.011.
49. **Dor FJ, Ramirez ML, Parmar K, Altman EL, Huang CA, Down JD, Cooper DKC.** 2006. Primitive hematopoietic cell populations reside in the spleen: Studies in the pig, baboon, and human. Exp Hematol **34**:1573–1582. https://doi.org/10.1016/j.exphem.2006.06.016.
50. **Dor FJ, Ramirez ML, Down JD, Cooper DK.** 2010. The spleen as a site for hematopoiesis. Transplantation **90**:1040–1041, author reply 1041–1042. https://doi.org/10.1097/TP.0b013e3181ef8a7b.
51. **Díaz Varela I, Sánchez Mozo P, Centeno Cortés A, Alonso Blanco C, Valdés Cañedo F.** 2003. Crossreactivity between swine leukocyte antigen and human anti-HLA-specific antibodies in sensitized patients awaiting renal transplantation. J Am Soc Nephrol **14**:2677–2683. https://doi.org/10.1097/01.ASN.0000088723.07259.CF.
52. **d'Apice AJ, Cowan PJ.** 2000. Profound coagulopathy associated with pig-to-primate xenotransplants: How many transgenes will be required to overcome this new barrier? Transplantation **70**:1273–1274. https://doi.org/10.1097/00007890-200011150-00003.
53. **Eddleston AL, Smith MG, Dominguez J, Williams R, Calne RY.** 1969. An immunological approach to the diagnosis of rejection of liver transplants. Gut **10**:1051–1052.
54. **Estrada JL, Martens G, Li P, Adams A, Newell KA, Ford ML, Butler JR, et al.** 2015. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/β4GALNT2 genes. Xenotransplantation **22**:194–202. https://doi.org/10.1111/xen.12161.
55. **Eyestone W, Adams K, Ball S, Bianchi J.** 2020. Gene-Edited Pigs for Xenotransplantation. p 121–140. In: Cooper DKC, Byrne GW, **editors.** Clinical xenotransplantation: Pathways and progress in the transplantation of organs and tissues between species. New York: Springer. https://doi.org/10.1007/978-3-030-49127-7_7.
56. **Ezzelarab M, Hara H, Busch J, Rood PPM, Zhu X, Ibrahim Z, Ball S, et al.** 2006. Antibodies directed to pig non-Gal antigens in naive and sensitized baboons. Xenotransplantation **13**:400–407. https://doi.org/10.1111/j.1399-3089.2006.00320.x.
57. **Ezzelarab MB, Cooper DKC.** 2015. Systemic inflammation in xenograft recipients (SIXR): A new paradigm in pig-to-primate xenotransplantation? Int J Surg. **23**:301–305.
58. **Ezzelarab MB, Ekser B, Azimzadeh A, Lin CC, Zhao Y, Rodriguez R, Echeverri GJ, et al.** 2015. Systemic inflammation in xenograft recipients precedes activation of coagulation. Xenotransplantation **22**:32–47. https://doi.org/10.1111/xen.12133.
59. **Feng H, Li T, Du J, Xia Q, Wang L, Chen S, Zhu L, et al.** 2022. Both natural and induced anti-Sda antibodies play important roles in GTKO pig-to-rhesus monkey xenotransplantation. Front Immunol **13**:849711. doi: https://doi.org/10.3389/fimmu.2022.849711.
60. **Fishman JA.** 2020. Prevention of infection in xenotransplantation: designated pathogen-free swine in the safety equation. Xenotransplantation **27**:e12595. https://doi.org/10.1111/xen.12595.
61. **Fu R, Fang M, Xu K, Ren J, Zou J, Su L, Chen X, et al.** 2020. Generation of GGTA1−/−β2M−/−CIITA−/− pigs using CRISPR/Cas9 technology to alleviate xenogeneic immune reactions. Transplantation **104**:1566–1573. https://doi.org/10.1097/TP.0000000000003205.
62. **Galili U, Mandrell RE, Hamadeh RM, Shohet SB, Griffiss JM.** 1988a. Interaction between human natural anti-α-galactosyl immunoglobulin G and bacteria of the human flora. Infect Immun **1988**:1730–1737. https://doi.org/10.1128/iai.56.7.1730-1737.1988.
63. **Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA.** 1988b. Man, apes, and Old World monkeys differ from other mammals in the expression of α-galactosyl epitopes on nucleated cells. J Biol Chem **263**:17755–17762. https://doi.org/10.1016/S0021-9258(19)77900-9.
64. **Gollackner B, Dor FJ, Knosalla C, Buhler L, Duggan M, Huang CA, Houser SL, et al.** 2003. Spleen transplantation in miniature swine: Surgical technique and results in major histocompatibility complex-matched donor and recipient pairs. Transplantation **75**:1799–1806. https://doi.org/10.1097/01.TP.0000063220.19441.5C.
65. **Griffith BP, Goerlich CE, Singh AK, Rothblatt M, Lau CL, Shah A, Lorber M, et al.** 2022. Genetically modified porcine-to-human cardiac xenotransplantation. N Engl J Med **387**:35–44. https://doi.org/10.1056/NEJMoa2201422.
66. **Gustafsson K, LeGuern C, Hirsch F, Germana S, Pratt K, Sachs DH.** 1990. Class II genes of miniature swine. IV. Characterization and expression of two allelic class II DQB cDNA clones. J Immunol **145**:1946–1951. https://doi.org/10.4049/jimmunol.145.6.1946.
67. **Hadjiyannakis EJ, Calne RY, Marshall VC, Davis DR.** 1971. Successful preservation of pig's liver for 5-8 hours by simple ice storage. Br J Surg **58**:835–836. https://doi.org/10.1002/bjs.1800581109.
68. **Hansen-Estruch C, Bikhet MH, Javed M, Katsurada A, Satou R, Shao W, Ayares D, et al.** 2023. Renin-angiotensin-aldosterone system function in the pig-to-baboon kidney xenotansplantaiton model. Am J Transplant **23**:353–365. https://doi.org/10.1016/j.ajt.2022.11.022.
69. **Hansen-Estruch C, Cooper DKC, Judd E.** 2022. Physiological aspects of pig kidney xenotransplantation and implications for management following transplant. Xenotransplantation **29**:e12743. https://doi.org/10.1111/xen.12743.
70. **Hansen-Estruch C, Bikhet MH, Shaik I, Vasudevan V, Zhao W, Javed M, Collins DE, et al.** 2023. Assessment of glomerular filtration and tubular secretion in baboons with life-supporting pig kidney grafts. Xenotransplantation **30**:e12795. https://doi.org/10.1111/xen.12795.

71. **Hara H, Ezzelarab M, Rood PP, Lin YJ, Busch J, Ibrahim Z, Zhu X, et al.** 2006. Allosensitized humans are at no greater risk of humoral rejection of GT-KO pig organs than other humans. Xenotransplantation **13**:357–365. https://doi.org/10.1111/j.1399-3089.2006.00319.x.
72. **Hara H, Witt W, Crossley T, Long C, Isse K, Fan L, Phelps CJ, et al.** 2013. Human dominant-negative class II transactivator transgenic pig—effect on the human anti-pig T-cell immune response and immune status. Immunology **140**:39–46. https://doi.org/10.1111/imm.12107.
73. **Hart AJ, Smellie WA, Calne RY.** 1971. Fate of kidney allografts from cadavers whose livers were also transplanted. Lancet **1**:103–105. https://doi.org/10.1016/S0140-6736(71)90839-7.
74. **Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, Foutz J, et al.** 2020. OPTN/SRTR 2018 Annual Data Report: Kidney. Am J Transplant **20** (suppl S1)j,l;j.:20–130. https://doi.org/10.1111/ajt.15672.
75. **Hector RD, Meikle S, Grant L, Wilkinson RA, Fishman JA, Scobie L.** 2007. Pre-screening of miniature swine may reduce the risk of transmitting human tropic recombinant porcine endogenous retroviruses. Xenotransplantation **14**:222–226. https://doi.org/10.1111/j.1399-3089.2007.00394.x.
76. **Hein R, Sake HJ, Pokoyski C, Hundrieser J, Brinkmann A, Baars W, Nowak-Imialek M, et al.** 2020. Triple (GGTA1, CMAH, B2M) modified pigs expressing an SLA class Ilow phenotype-effects onimmune status and susceptibility to human immune responses. Am J Transplant **20**:988–998. https://doi.org/10.1111/ajt.15710.
77. **Hinrichs A, Kessler B, Kurome M, Blutke A, Kemter E, Bernau M, Scholz AM, et al.** 2018. Growth hormone receptor-deficient pigs resemble the pathophysiology of human Laron syndrome and reveal altered activation of signaling cascades in the liver. Mol Metab **11**:113–128. https://doi.org/10.1016/j.molmet.2018.03.006.
78. **Houser SL, Kuwaki K, Knosalla C, Dor FJMF, Gollackner B, Cheng J, Shimizu A, et al.** 2004. Thrombotic microangiopathy and graft arteriopathy in pig hearts following transplantation into baboons. Xenotransplantation **11**:416–425. https://doi.org/10.1111/j.1399-3089.2004.00155.x.
79. **Ide K, Ohdan H, Kobayashi T, Hara H, Ishiyama K, Asahara T.** 2005. Antibody- and complement-independent phagocytotic and cytolytic activities of human macrophages toward porcine cells. Xenotransplantation **12**:181–188. https://doi.org/10.1111/j.1399-3089.2005.00222.x.
80. **Iwase H, Liu H, Wijkstrom M, Zhou H, Singh J, Hara H, Ezzelarab M, et al.** 2015. Pig kidney graft survival in a baboon for 136 days: Longest life-supporting organ graft survival to date. Xenotransplantation **22**:302–309. https://doi.org/10.1111/xen.12174.
81. **Iwase H, Ekser B, Hara H, Phelps C, Ayares D, Cooper DKC, Ezzelarab MB.** 2014. Regulation of human platelet aggregation by genetically modified pig endothelial cells and thrombin inhibition. Xenotransplantation **21**:72–83. https://doi.org/10.1111/xen.12073.
82. **Iwase H, Hara H, Ezzelarab M, Li T, Zhang Z, Gao B, Liu H, et al.** 2017. Immunological and physiologic observations in baboons with life-supporting genetically-engineered pig kidney grafts. Xenotransplantation **24**:e12293. https://doi.org/10.1111/xen.12293.
83. **Iwase H, Klein E, Cooper DKC.** 2018. Physiologic aspects of pig kidney transplantation in nonhuman primates. Comp Med **68**:332–340. https://doi.org/10.30802/AALAS-CM-17-000117.
84. **Iwase H, Yamamoto T, Cooper DKC.** 2018. Episodes of hypovolemia/dehydration in baboons with pig kidney transplants: A new syndrome of clinical importance? Xenotransplantation **e12472**:e12472. https://doi.org/10.1111/xen.12472.
85. **Iwase H, Ball S, Adams WT, Eyestone W, Walters A, Cooper DKC.** 2021. Growth hormone receptor knockout: Relevance to xenotransplantation. Xenotransplantation **28**:e12652.
86. **Jagdale A, Kumar V, Anderson DJ, Locke JE, Hanaway MJ, Eckhoff DE, Iwase H, et al.** 2021. Suggested patient selection criteria for initial clinical trials of pig kidney xenotransplantation in the USA. Transplantation **105**:1904–1908. https://doi.org/10.1097/TP.0000000000003632.
87. **Khan A, Sergio JJ, Zhao Y, Pearson DA, Sachs DH, Sykes M.** 1997. Discordant xenogeneic neonatal thymic transplantation can induce donor-specific tolerance. Transplantation **63**:124–131. https://doi.org/10.1097/00007890-199701150-00023.
88. **Kim SC, Mathews DV, Breeden CP, Higginbotham LB, Ladowski J, Martens G, Stephenson A, et al.** 2019. Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. Am J Transplant **19**:2174–2185. https://doi.org/10.1111/ajt.15329.
89. **Kobayashi T, Cooper DK.** 1999. Anti-Gal, α-Gal epitopes, and xenotransplantation. Subcell Biochem **32**:229–257.
90. **Kortz EO, Sakamoto K, Suzuki T, Guzzetta PC, Chester CH, Lunney JK, Sachs DH.** 1990. Mechanism of tolerance following class I–Disparate renal allografts in miniature swine. Cellular responses of tolerant animals. Transplantation **49**:1142–1149. https://doi.org/10.1097/00007890-199006000-00023.
91. **Kraebber K, Gray E.** 2020. Addressing regulatory requirements for the organ-source pig – A pragmatic approach to facility design and pathogen prevention. p. 141–153. In: Cooper DKC and Byrne GW, editors. Clinical xenotransplantation: pathways and progress in the transplantation of organs and tissues between species**.** New York: Springer.
92. **Ladowski JM, Reyes LM, Martens GR, Butler JR, Wang Z-Y, Eckhoff DE, Tector M, et al.** 2018. Swine leukocyte antigen class II is a xenoantigen. Transplantation **102**:249–254. https://doi.org/10.1097/TP.0000000000001924.
93. **Ladowski JM, Martens GR, Reyes LM, Hauptfeld-Dolejsek V, Tector M, Tector J.** 2019. Examining epitope mutagenesis as a strategy to reduce and eliminate human antibody binding to class II swine leukocyte antigens. Immunogenetics **71**:479–487. https://doi.org/10.1007/s00251-019-01123-y.
94. **Ladowski JM, Hara H, Cooper DKC.** 2020. The role of SLAs in xenotransplantation. Transplantation **105**:300–307. https://doi.org/10.1097/TP.000000000003303.
95. **Lambrigts D, Menard MT, Alexandre GP, Franssen C, Meurisse M, Van Calster P, Coignoul F, et al.** 1998. Creation of the "thymoheart" allograft: Implantation of autologous thymus into the heart prior to procurement. Transplantation **66**:810–814. https://doi.org/10.1097/00007890-199809270-00019.
96. **Lambrigts D, Sachs DH, Cooper DK.** 1998. Discordant organ xenotransplantation in primates: World experience and current status. Transplantation **66**:547–561. https://doi.org/10.1097/00007890-199809150-00001.
97. **Li Q, Shaikh S, Iwase H, Long C, Lee W, Zhang Z, Wang Y, et al.** 2019. Carbohydrate antigen expression and anti-pig antibodies in New World capuchin monkeys: Relevance to studies of xenotransplantation. Xenotransplantation. **26**:e12498. https://doi.org/10.1111/xen.12498.
98. **Li Q, Hara H, Banks CA, Yamamoto T, Ayares D, Mauchley DC, Dabal RJ, et al.** 2020. Anti-pig antibody in infants: Can a genetically engineered pig heart bridge to allotransplantation? Ann Thorac Surg **109**:1268–1273. https://doi.org/10.1016/j.athoracsur.2019.08.061.
99. **Li W, Zheng X, Gu J, Hunter J, Ferrell FL, Lupu F, Esmon NL, Esmon CT.** 2005. Overexpressing endothelial cell protein C receptor alters the hemostatic balance and protects mice from endotoxin. J Thromb Haemost **3**:1351–1359. https://doi.org/10.1111/j.1538-7836.2005.01385.x.
100. **Längin M, Mayr T, Reichart B, Michel S, Buchholz S, Guethoff S, Dashkevich A, et al.** 2018. Consistent success in life-supporting porcine cardiac xenotransplantation. Nature **564**:430–433. https://doi.org/10.1038/s41586-018-0765-z.
101. **Ma D, Hirose T, Lassiter G, Sasaki H, Rosales I, Coe TM, Rickert CG, et al.** 2022. Kidney transplantation from triple-knockout pigs expressing multiple human proteins in cynomolgus macaques. Am J Transplant **22**:46–57. https://doi.org/10.1111/ajt.16780.
102. **Marshall VC, Ashby BS, Hadjiyannakis EJ, Lines J, Calne RY.** 1970. Liver preservation in the pig. Br J Surg **57**:853.
103. **Martens GR, Reyes LM, Li P, Butler JR, Ladowski JM, Estrada JL, Sidner RA, et al.** 2017. Humoral reactivity of renal transplant-waitlisted patients to cells from GGTA1/CMAH/B4GalNT2, and SLA class I knockout pigs. Transplantation **101**:e86–e92. https://doi.org/10.1097/TP.0000000000001646.

104. **Metzger JJ, Gilliland GL, Lunney JK, Osborne BA, Rudikoff S, Sachs DH.** 1981. Transplantation in miniature swine. IX. Swine histocompatibility antigens: Isolation and purification of papain-solubilized SLA antigens. J Immunol **127**:769–775. https://doi.org/10.4049/jimmunol.127.2.769.
105. **Metzger JJ, Lunney JK, Sachs DH, Rudikoff S.** 1982. Transplantation in miniature swine. XII. N-terminal sequences of class I histocompatibility antigens (SLA) and beta 2-microglobulin. J Immunol **129**:716–721. https://doi.org/10.4049/jimmunol.129.2.716.
106. **Mezrich JD, Benjamin LC, Sachs JA, Houser SL, Vagefi PA, Sachs DH, Madsen JC, Yamada K.** 2005. Role of the thymus and kidney graft in the maintenance of tolerance to heart grafts in miniature swine. Transplantation **79**:1663–1673. https://doi.org/10.1097/01.TP.0000160679.04441.B7.
107. **Millard PR, Herbertson BM, Calne RY.** 1971. Morphological features of kidney and liver allografts in the pig. Transplant Proc **3**:505–508.
108. **Mohiuddin MM, Goerlich CE, Singh AK, Zhang T, Tatarov I, Lewis B, Sentz F, et al.** 2022. Progressive genetic modifications of porcine cardiac xenografts extend survival to 9 months. Xenotransplantation **29**:e12744. https://doi.org/10.1111/xen.12744.
109. **Mueller NJ, Kuwaki K, Dor FJ, Knosalla C, Gollackner B, Wilkinson RA, Sachs DH, Cooper DKC, Fishman JA.** 2004. Reduction of consumptive coagulopathy using porcine cytomegalovirus-free cardiac porcine grafts in pig-to-primate xenotransplantation. Transplantation **78**:1449–1453. https://doi.org/10.1097/01.TP.0000141361.68446.1F.
110. **Niebert M, Tonjes R.** 2005. Evolutionary spread and recombination of porcine endogenous retroviruses in the suiformes. J Virol **79**:649–654. https://doi.org/10.1128/JVI.79.1.649-654.2005.
111. **Niu D, Wei HJ, Lin L, George H, Wang T, Lee I-H, Zhao H-Y, et al.** 2017. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science **357**:1303–1307. https://doi.org/10.1126/science.aan4187.
112. **Pennington LR, Lunney JK, Sachs DH.** 1981. Transplantation in miniature swine. VIII. Recombination within the major histocompatibility complex of miniature swine. Transplantation **31**:66–71. https://doi.org/10.1097/00007890-198101000-00015.
113. **Pennington LR, Flye MW, Kirkman RL, Thistlethwaite JR Jr, Williams GM, Sachs DH.** 1981. Transplantation in miniature swine. X. Evidence for non-SLA-linked immune response gene(s) controlling rejection of SLA-matched kidney allografts. Transplantation **32**:315–320. https://doi.org/10.1097/00007890-198110000-00011.
114. **Pennington LR, Sakamoto K, Popitz-Bergez FA, Pescovitz MD, McDonough MA, MacVittie TJ, Gress RE, Sachs DH.** 1988. Bone marrow transplantation in miniature swine. I. Development of the model. Transplantation **45**:21–26. https://doi.org/10.1097/00007890-198801000-00006.
115. **Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen S-H, Ball S, et al.** 2003. Production of α1,3-galactosyltransferase-deficient pigs. Science **299**:411–414. https://doi.org/10.1126/science.1078942.
116. **Polejaeva IA, Chen SH, Vaught TD, Page RL, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares DL, Colman A, Campbell KH.** 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature **407**:86–90. https://doi.org/10.1038/35024082.
117. **Popitz-Bergez FA, Sakamoto K, Pennington LR, Pescovitz MD, McDonough MA, MacVittie TJ, Gress RE, Sachs DH.** 1988. Bone marrow transplantation in miniature swine. II. Effect of selective genetic differences on marrow engraftment and recipient survival. Transplantation **45**:27–31. https://doi.org/10.1097/00007890-198801000-00007.
118. **Porrett PM, Orandi BJ, Kumar V, Houp J, Anderson D, Cozette Killian A, Hauptfeld-Dolejsek V, et al.** 2022. First clinical-grade porcine kidney xenotransplant using a human decedent model. Am J Transplant **22**:1037–1053. https://doi.org/10.1111/ajt.16930.
119. **Ramirez ML, McMorrow IM, Sanderson TM, Lancos CJ, Tseng Y-L, Cooper DKC, Dor FJMF.** 2005. Lack of cardiac differentiation in c-kit-enriched porcine bone marrow and spleen hematopoietic cell cultures using 5-azacytidine. Cells Tissues Organs **180**:195–203. https://doi.org/10.1159/000088936.
120. **Ramsoondar J, Vaught T, Ball S, Mendicino M, Monahan J, Jobst P, Vance A, Duncan J, Wells K, Ayares D.** 2009. Production of transgenic pigs that express porcine endogenous retrovirus small interfering RNAs. Xenotransplantation **16**:164–180. https://doi.org/10.1111/j.1399-3089.2009.00525.x.
121. **Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F.** 2013. Genome engineering using the CRISPR-Cas9 system. Nat Protoc **8**:2281–2308. https://doi.org/10.1038/nprot.2013.143.
122. **Reichart B, Langin M, Radan R, Mokelke M, Buttgereit I, Ying J, Fresch AK, et al.** 2020. Pig-to-nonhumanprimate heart transplantation: The final step toward clinical xenotransplantation? J Heart Lung Transplant **39**:751–757. https://doi.org/10.1016/j.healun.2020.05.004.
123. **Rood PPM, Hara H, Busch JL, Ezzelarab M, Zhu X, Ball S, Ayares D, Awwad M, Cooper DKC.** 2006. Incidence and cytotoxicity of antibodies in cynomolgus monkeys directed to nonGal antigens, and their relevance for experimental models. Transpl Int **19**:158–165. https://doi.org/10.1111/j.1432-2277.2005.00252.x.
124. **Sakamoto K, Sachs DH, Shimada S, Popitz-Bergez FA, Pennington LR, Pescovitz MD, McDonough MA, MacVittie TJ, Katz SI, Gress RE.** 1988. Bone marrow transplantation in miniature swine. III. Graft-versus-host disease and the effect of T cell depletion of marrow. Transplantation **45**:869–875. https://doi.org/10.1097/00007890-198805000-00006.
125. **Shimizu A, Yamada K, Yamamoto S, Lavelle JM, Barth RN, Robson SC, Sachs DH, Colvin RB.** 2005. Thrombotic microangiopathic glomerulopathy in human decay accelerating factortransgenic swine-to-baboon kidney xenografts. J Am Soc Nephrol **16**:2732–2745. https://doi.org/10.1681/ASN.2004121148.
126. **Shimizu A, Hisashi Y, Kuwaki K, Tseng Y-L, Dor FJMF, Houser SL, Robson SC, et al.** 2008. Thrombotic microangiopathy associated with humoral rejection of cardiac xenografts from α1,3-galactosyltransferase gene-knockout pigs in baboons. Am J Pathol **172**:1471–1481. https://doi.org/10.2353/ajpath.2008.070672.
127. **Shimizu A, Yamada K, Robson SC, Sachs DH, Colvin RB.** 2012. Pathologic characteristics of transplanted kidney xenografts. J Am Soc Nephrol **23**:225–235. https://doi.org/10.1681/ASN.2011040429.
128. **Smith DM.** 1993. Endogenous retroviruses in xenografts. N Engl J Med **328**:142–143.
129. **Smith MG, Williams R, Doniach D, Calne RY.** 1971. Effect of orthotopic liver transplantation on serum-autoantibodies. Lancet **2**:1006–1009. https://doi.org/10.1016/S0140-6736(71)90325-4.
130. **Soin B, Ostlie D, Cozzi E, Smith KG, Bradley JR, Vial C, Masroor S, Lancaster R, White DJ, Friend PJ.** 2000. Growth of porcine kidneys in their native and xenograft environment. Xenotransplantation **7**:96–100. https://doi.org/10.1034/j.1399-3089.2000.00046.x.
131. **Stricker E, Peckham-Gregory EC, Scheurer ME.** 2023. HERVs and cancer—A comprehensive review of the relationship of human endogenous retroviruses and human cancers. Biomedicines **11**:936. https://doi.org/10.3390/biomedicines11030936.
132. **Suzuki T, Sundt TM, Kortz EO, Mixon A, Eckhaus MA, Gress RE, Spitzer TR, Sachs DH.** 1989. Bone marrow transplantation across an MHC barrier in miniature swine. Transplant Proc **21**:3076–3078.
133. **Szymczak-Workman AL, Vignali KM, Vignali DAA.** 2012. Design and construction of 2A peptide-linked multicistronic vectors. Cold Spring Harb Protoc **2012**:199–204. https://doi.org/10.1101/pdb.ip067876.
134. **Tanabe T, Watanabe H, Shah JA, Sahara H, Shimizu A, Nomura S, Asfour A, et al.** 2017. Role of intrinsic (graft) versus extrinsic (host) factors in the growth of transplanted organs following allogeneic and xenogeneic transplantation. Am J Transplant **17**:1778–1790. https://doi.org/10.1111/ajt.14210.
135. **Tena A, Kurtz J, Leonard D, Dobrinsky JR, Terlouw SL, Mtango N, et al.** 2014. Transgenic expression of human CD47 markedly increases engraftment in a murine model of pig-to-human hematopoietic cell transplantation. Am J Transplant **14**:2713–2722. https://doi.org/10.1111/ajt.12918.

136. **Tena AA, Sachs DH, Mallard C, Yang Y-G, Tasaki M, Farkash E, Rosales IA, et al.** 2017. Prolonged survival of pig skin on baboons after administration of pig cells expressing human CD47. Transplantation **101**:316–321. https://doi.org/10.1097/TP.0000000000001267.
137. **US Food and Drug Administration.** FDA Approves First-of-its-Kind Intentional Genomic Alteration in Line of Domestic Pigs for Both Human Food, Potential Therapeutic Uses. [Press release]. 2020 Dec 14. https://www.fda.gov/news-events/press-announcements/fda-approves-first-its-kind-intentional-genomic-alteration-line-domestic-pigs-both-human-food.
138. **Vagefi PA, Ierino FL, Gianello PR, Shimizu A, Kamano C, Sachs DH, Yamada K.** 2004. Role of the thymus in transplantation tolerance in miniature Swine: IV. The thymus is required during the induction phase, but not the maintenance phase, of renal allograft tolerance. Transplantation **77**:979–985. https://doi.org/10.1097/01.TP.0000116416.10799.C6.
139. **Varki A.** 2001. Loss of N-glycolylneuraminic acid in humans: mechanisms, consequences, and implications for hominid evolution. Am J Phys Anthropol Suppl **S33**: 54–69. https://doi.org/10.1002/ajpa.10018.
140. **Warr GW, Lascelles A, Calne RY, Coombs RR.** 1971. An autoantibody-like factor reactive with liver parenchymal cells in the serum of the pig. Clin Exp Immunol **9**:339–344.
141. **White DJ, Oglesby T, Liszewski MK, Tedja I, Hourcade D, Wang MW, Wright L, Wallwork J, Atkinson JP, et al.** 1992. Expression of human decay accelerating factor or membrane cofactor protein genes on mouse cells inhibits lysis by human complement. Transpl Int **5** (suppl 1):S648–S650. https://doi.org/10.1111/tri.1992.5.s1.648.
142. **White DJG, Langford GA, Cozzi E, Young VK.** 1995. Production of pigs transgenic for human DAF: A strategy for xenotransplantation. Xenotransplantation **2**:213–217. https://doi.org/10.1111/j.1399-3089.1995.tb00097.x.
143. **Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH.** 1997. Viable offspring derived from fetal and adult mammalian cells. Nature **385**:810–813. https://doi.org/10.1038/385810a0.
144. **Wong BS, Yamada K, Okumi M, Weiner J, O'Malley PE, Tseng Y-L, Dor FJMF, et al.** 2006. Allosensitization does not increase the risk of xenoreactivity to α1,3-Galactosyltransferase gene-knockout miniature swine in patients on transplantation waiting lists. Transplantation **82**:314–319. https://doi.org/10.1097/01.tp.0000228907.12073.0b.
145. **Wu A, Yamada K, Awwad M, Shimizu A, Watts A, Murphy M, Gojo S, et al .** 2000. Experience with porcine thymic transplantation in baboons. Transplant Proc **32**:1048. https://doi.org/10.1016/S0041-1345(00)01113-1.
146. **Wu A, Yamada K, Awwad M, Kitamura H, Wain J, Neville DM, Cooper DK, Sykes M, Sachs DH, et al.** 2001. Effects of xenogeneic thymic transplantation in baboons. Transplant Proc **33**:766. https://doi.org/10.1016/S0041-1345(00)02244-2.
147. **Wu A, Yamada K, Neville DM, Awwad M, Wain JC, Shimizu A, Gojo S, et al.** 2003. Xenogeneic thymus transplantation in a pig-to-baboon model. Transplantation **75**:282–291. https://doi.org/10.1097/01.TP.0000044137.97841.99.
148. **Yamada K, Gianello PR, Ierino FL, Shimizu A, Meehan S, Colvin RB, Sachs DH.** 1997. Influence of the thymus on transplantation tolerance in miniature swine. Transplant Proc **29**:1076. https://doi.org/10.1016/S0041-1345(96)00409-5.
149. **Yamada K, Gianello PR, Ierino FL, Lorf T, Shimizu A, Meehan S, Colvin RB, Sachs DH.** 1997. Role of the thymus in transplantation tolerance in miniature swine. I. Requirement of the thymus for rapid and stable induction of tolerance to class I-mismatched renal allografts. J Exp Med **186**:497–506. https://doi.org/10.1084/jem.186.4.497.
150. **Yamada K, Gianello PR, Ierino FL, Fishbein J, Lorf T, Shimizu A, Colvin RB, Sachs DH.** 1999. Role of the thymus in transplantation tolerance in miniature swine. II. Effect of steroids and age on the induction of tolerance to class I mismatched renal allografts. Transplantation **67**:458–467. https://doi.org/10.1097/00007890-199902150-00020.
151. **Yamada K, Ierino FL, Gianello PR, Shimizu A, Colvin RB, Sachs DH.** 1999. Role of the thymus in transplantation tolerance in miniature swine. III. Surgical manipulation of the thymus interferes with stable induction of tolerance to class I-mismatched renal allografts. Transplantation **67**:1112–1119. https://doi.org/10.1097/00007890-199904270-00005.
152. **Yamada K, Shimizu A, Ierino FL, Gargollo P, Barth R, Colvin RB, Sachs DH.** 1999. Allogeneic thymo-kidney transplants induce stable tolerance in miniature swine. Transplant Proc **31**:1199–1200. https://doi.org/10.1016/S0041-1345(98)01963-0.
153. **Yamada K, Shimizu A, Ierino FL, Utsugi R, Barth RN, Esnaola N, Colvin RB, Sachs DH.** 1999. Thymic transplantation in miniature swine. I. Development and function of the "thymokidney". Transplantation **68**:1684–1692. https://doi.org/10.1097/00007890-199912150-00011.
154. **Yamada K, Shimizu A, Utsugi R, Ierino FL, Gargollo P, Haller GW, Colvin RB, Sachs DH.** 2000. Thymic transplantation in miniature swine. II. Induction of tolerance by transplantation of composite thymokidneys to thymectomized recipients. J Immunol **164**:3079–3086. https://doi.org/10.4049/jimmunol.164.6.3079.
155. **Yamada K, Vagefi PA, Utsugi R, Kitamura H, Barth RN, LaMattina JC, Sachs DH.** 2003. Thymic transplantation in miniature swine. III. Induction of tolerance by transplantation of composite thymokidneys across fully major histocompatibility complex-mismatched barriers. Transplantation **76**:530–536. https://doi.org/10.1097/01.TP.0000080608.42480.E8.
156. **Yamada K, Yazawa K, Shimizu A, Iwanaga T, Hisashi Y, Nuhn M, O'Malley P, et al.** 2005. Marked prolongation of porcine renal xenograft survival in baboons through the use of α1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. Nat Med **11**:32–34. https://doi.org/10.1038/nm1172.
157. **Yamada K, Tasaki M, Sekijima M, Wilkinson RA, Villani V, Moran SG, et al.** 2014. Porcine cytomegalovirus infection is associated with early rejection of kidney grafts in a pig to baboon xenotransplantation model. Transplantation **98**:411–418. https://doi.org/10.1097/TP.0000000000000232.
158. **Yamamoto S, Lavelle JM, Vagefi PA, Arakawa H, Samelson-Jones E, Moran S, Teranishi K, et al.** 2005. Vascularized thymic lobe transplantation in a pig-to-baboon model: A novel strategy for xenogeneic tolerance induction and T-cell reconstitution. Transplantation **80**:1783–1790. https://doi.org/10.1097/01.tp.0000184445.70285.4b.
159. **Yamamoto T, Iwase H, Patel D, Jagdale A, Ayares D, Anderson D, Eckhoff DE, Cooper DKC, Hara H.** 2020. Old World monkeys are less than ideal transplantation models for testing pig organs lacking three carbohydrate antigens (triple-knockout). Sci Rep **10**:9771. https://doi.org/10.1038/s41598-020-66311-3.
160. **Yan JJ, Koo TY, Lee HS, Lee W-B, Kang B, Lee J-G, Young Jang J, et al.** 2018. Role of human CD200 overexpression in pig-to-human xenogeneic immune response compared with human CD47 overexpression. Transplantation **102**:406–416. https://doi.org/10.1097/TP.0000000000001966.
161. **Zhang Z, Hara H, Long C, Iwase H, Qi H, Macedo C, Mangiola M, et al.** 2018. Immune responses of HLA-highly-sensitized and non-sensitized patients to genetically engineered pig cells. Transplantation **102**:e195–e204. https://doi.org/10.1097/TP.0000000000002060.